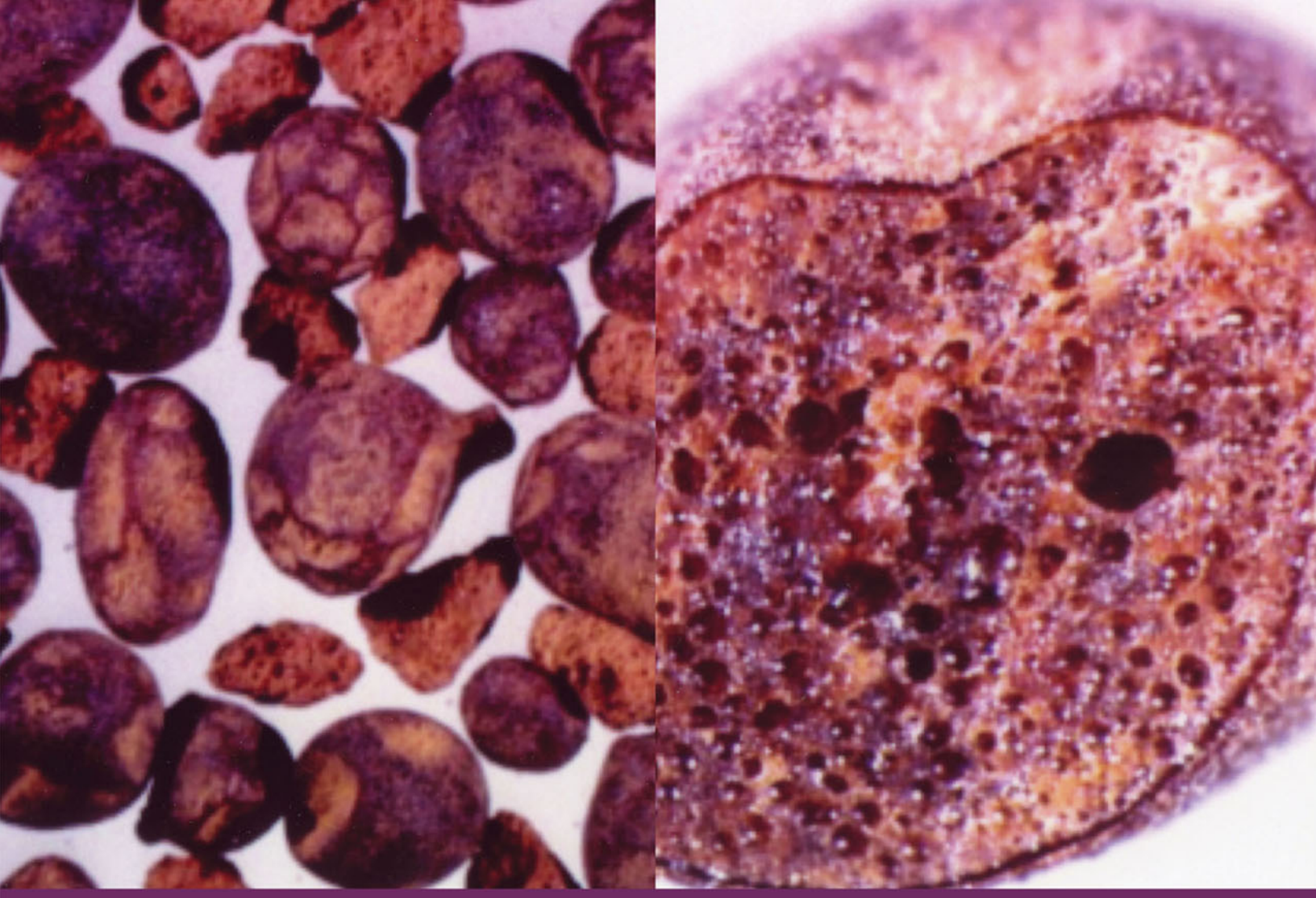

Microencapsulation in the Food Industry

A Practical Implementation Guide

Edited by

Anilkumar Gaonkar, Niraj Vasisht,
Atul Khare, and Robert Sobel

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Dedicated with love to
our Parents, Wives,
and Children

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AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD • PARIS
SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
32 Jamestown Road, London NW1 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-404568-2

Printed and bound in the United States of America

14 15 16 17 18 10 9 8 7 6 5 4 3 2 1

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Foreword

Over 60 years have passed since the invention and implementation of industrially significant microencapsulation processes by NCR for carbonless paper applications. During this time, thousands of patents and academic papers have been written and published along with over 50 books on this topic. Yet, with all this effort, there is a tremendous interest in this field. Every year conferences and short courses are offered that are well attended. Every year at my company we field over 300 calls from companies that are excited and intrigued about how microencapsulation can provide their product or idea intellectual and physical protection in their markets. The use of microencapsulation methodologies for markets as diverse as the food, pharmaceutical, industrial, personal and home care, and agribusiness sectors, has made this area diverse, broad, and challenging to understand. Furthermore, the many techniques that are used in this field tend to have very specific limitations, advantages, and trade secret modifications that compound the challenges to both new and experienced practitioners.

Ten years ago, Dr. Anilkumar Gaonkar, with the help of many contributors to this book, organized the Pre-IFT meeting short course on microencapsulation especially targeted for the food industry professionals. This short course continued as a yearly offering from the IFT. Five years ago some of the contributors to this book and I realized that a new short course on microencapsulation could be useful to the general public. Dr. Marc Meyers spearheaded the organization of that course and it included many of the authors that are in this book. What was unique about these courses was the focus on a wide range of microencapsulation methods combined with applied examples and some of the practical issues that are easy to overlook considering the breadth and variety of the process solutions. This book addresses these tremendously important issues such as the economics of microencapsulation, scale-up issues, and a whole section on the materials that are used in microencapsulation. Through overviews, process descriptions, materials that can be used to provide the encapsulation, production and regulatory issues, and finally interesting application examples, the editors and contributors of this book have provided a clear and complete reference to this complicated area for both the microencapsulation producers and the users of microencapsulation.

For over half a century, microencapsulation has been an exciting and industrially significant area to work in. New materials, better process controls, and a continuing need to protect and deliver active ingredients on demand will push this field to new heights. This book will provide you, the reader, with a reference to the technology and challenges and, hopefully, expand your thinking and, ultimately, the breadth of the technology.

Good reading, good dreaming, and good innovation.

Willie Hendrickson, Ph.D.
CEO
AVEKA Group

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Preface

The world of Microencapsulation has seen exponential growth over the past fifty years, and particularly more so over the last decade. We, the authors, have held hands over the years, providing our scientific insight to evolving product development demands as attendees at international conferences and workshops. It was June 2011 when one of us, Anilkumar, had an epiphany – it would be quite helpful for the attending audience if we captured our collective learning, experiences, and practices, along with our microencapsulation colleagues around the world, and created a handbook of practical wisdom all in one place. The rest was history, and this was the genesis of our adventure together. A team of qualified experts was sought with a core team to create, compile, edit, and format each selection. We thank Elsevier for seeing our vision and appreciate their patience in getting the book ready for print.

We recognized that the fundamental researchers and scientists were seeking technology nuances. Finding these nuggets in this much-evolved field of science is difficult. Honestly, one would be misleading the reader if we state that what is presented in the chapters is simply science. Microencapsulation is an art and a science, and we hope that the book has done justice in balancing the two acts.

A few earlier pieces of literature in the field of microencapsulation have tapped in basic concepts, and fundamentals of understanding of the process technologies. We understand that today's reader is well versed in such fundamental concepts and basics. We felt that a focus on industrial applications pertinent to the 21st century would yield greater value to the reader. This involves exploring novel excipients, process technologies, analytical and characterization methods, and regulatory roadmaps. We believe that this extends our understanding of the functionality of the final microencapsulated product.

The thirst of today's learners relates to finding unique solutions to some very specific product needs. As research and development is not a static practice, the field of encapsulation has traversed from the micro-scale level to new developments in the area of nano-scaled encapsulation. These developments have found many applications in health foods, nutraceuticals, dietary and consumer health, and wellness products.

This book is comprised of 7 parts and 42 chapters. We have attempted to bridge fundamental concepts to the knowledge of new and novel processing techniques, choice of materials along with the selection process, testing and evaluation of materials, regulatory aspects, scale-up, packaging, economics, and application-specific uses of microencapsulation in the food industry. We have chosen to include the expired and active intellectual property highlighting this field of research as it applies to the food and nutraceutical industries. The content and format allows us to create an Industrial Application Handbook capturing the art and science of this field to the food industry.

The book provides a single source consolidation of information pertaining to new challenges in the food industry that can potentially be solved by microencapsulation technology. The projected audience encompasses college students, scientists, engineers, professionals, business managers, technical leaders, marketing personnel, and small business owners serving the food and nutraceutical industries. Most of the contributors to this book are from industry and a few are from academia. Hundreds of years of collective experience of the authors from several countries have shaped this book. We hope that the readers of this book will be enriched with knowledge of the current and emerging trends on microencapsulation of food ingredients.

The Editors

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About the Editors

Anilkumar G. Gaonkar is currently working as a VP, R&D Asia Pacific at Mondelēz India Foods (formerly Cadbury India Ltd.) in Mumbai, India on an expat assignment. He has been a Mondelēz International Fellow at Mondelēz International's (Legacy Kraft Foods) R&D Center in Glenview, IL, USA. Anil has 27 years of R&D experience with Kraft and Mondelēz. Prior to joining Kraft, he worked at the University of Minnesota, St. Paul, MN, Auburn University, Auburn, AL., and Institute for Chemical Research, Kyoto University, Kyoto, Japan for about 8 years. His areas of research interest include microencapsulation, controlled delivery, emulsions, foams, colloids, surface chemistry, fats and oils, egg science, and salad dressings.

Anil received his B.Sc. degree in chemistry and M.Sc. degree in physical chemistry from the Karnatak University, Dharwad, India and Ph.D. degree in chemistry from Poona University, Pune, India.

Anil has authored or co-authored over 60 journal articles, proceeding papers and abstracts. He is an inventor and co-inventor of more than 30 US and foreign patents and many patents are pending. Anil received numerous awards for innovation, productivity, and leadership. He has organized and co-chaired several national and international symposia as well as lectured at numerous conferences, symposia, and short courses. He has edited four books in the area of food characterization, interactions, and processing. Anil is planning to retire in December 2014, be a consultant, and dedicate majority of his retired life helping the underprivileged people in India.

Niraj Vasisht is currently serving as Chief Technical Officer and Senior Vice President of Product Development at Bio Delivery Sciences International, Inc. He heads the chemistry, manufacturing and control operations and oversees the product design, formulation development, quality control, process engineering,validation and stability testing of the drug product and CTM and commercial manufacturing operations at our vendor sites worldwide. In his current role, he is responsible for technical suitability of drug delivery platforms and candidate molecules suitable for the technology. Dr. Vasisht serves as BDSI's product development representative for FDA interactions for NDA and MAA filings.

He is known worldwide for his expertise in microencapsulation based controlled release and drug delivery technologies. He has 20 years of industry experience and has taught microencapsulation courses in profession al society meetings, and courses all around the world.

From 1994 to 2005, Dr.Vasisht held positions of increasing responsibility at Southwest Research Institute where he ultimately served as the Director of Microencapsulation, Pharmaceutical Development and Nanomaterials and was responsible for leading the group that provides research and development and product development services to food, nutriceutical, pharmaceutical, and consumer health companies.

He is an inventor on multiple patents in the field of microencapsulation. Dr.Vasisht received a Bachelor's in Chemical Engineering from the Indian Institute of Technology at Kanpur, a Master's of Science from the University of New Hampshire and a Doctorate in Chemical Engineering from Rensselaer Polytechnic Institute. He resides in Cary, North Carolina, with his wife and three sons.

Atul Khare is Principal, Khare Innovation Consulting, with 21 years research and development management experience in food and pharma/medical device industry with expertise in developing and commercializing new technologies. His research competencies are in the development of encapsulated delivery systems for controlled release of flavor and functional ingredients in different food applications such as chewing gum, cereals and beverages. Atul has held leadership positions at Akorn Inc., FONA International Inc. and Baxter Healthcare Corporation. Atul is a professional member of IFT. Atul received a Ph.D. in Chemical Engineering from Purdue University and his MBA from The University of Chicago Booth School of Business. Atul has authored or co-authored over 50 journal articles, proceeding papers and abstracts, and 5 patents.

Robert Sobel is currently serving FONA International as a Vice President of Research and Innovation in the development of new and novel flavor encapsulation delivery systems and taste modification technologies. He has 15 years of industrial flavor R&D experience at FONA International, located in Geneva, Illinois, USA. Prior to joining FONA International, Robert was an educator within both secondary and undergraduate settings teaching chemistry and physics.

Robert received a B.S. in chemistry with a minor in physics from Northern Illinois University, his M.A. in educational leadership from Aurora University, and his Ph.D. in chemistry from Northern Illinois University. Graduate research emphasis includes physical chemistry and unified separation science.

Robert has published manuscripts in *Analytical Chemica Actica*, *The Journal of Chemical Education* and *Perfumer & Flavorist*. He is cited as an inventor on many patents and patents pending in the art of microencapsulation. He has given a variety of technical symposia on analysis of flavor encapsulates, challenges of microencapsulation of flavors, proteins and polysaccharides as microencapsulating agents, real-time reaction monitoring and flavor analysis using surface acoustic wave microsensor arrays and artificial neural networks, and design of experiments for scalability and quality improvement of spray drying flavorants. Robert resides in Elburn, Illinois, with his wife and two daughters.

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Part I

Introduction

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Chapter 1

Introduction to Microencapsulation and Controlled Delivery in Foods

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1.1 INTRODUCTION

This introductory chapter provides the background, including the necessary theory and impetus, for the art and science of microencapsulation techniques found within the food industry. The spirit of this communication is to provide a background of encapsulation techniques and procedures that are contemporary to the field of encapsulation technology, thus giving the reader a clearer understanding of the science and an improved ability to apply this technology in their environment.

This chapter is divided into nine key areas consisting of: (1) microencapsulation defined, (2) reasons for microencapsulation, (3) types of microcapsules, (4) a historical account of encapsulation, (5) materials used for encapsulation purposes and their regulatory aspects, (6) microencapsulation techniques used within the food industry and examples thereof, (7) trends in microencapsulation, (8) challenges in microencapsulation of food ingredients, and (9) the future of microencapsulation of food ingredients. The culmination of these nine areas becomes the foundation of material presented herein.

This introductory chapter is the first of seven parts of this book. Part 2 describes concepts related to factors, mechanisms, mass, and heat transfer. Different process technologies using microencapsulation of food ingredients are the subject matter of Part 3. Part 4 deals with various materials (matrix, coating, excipient, etc.) used for microencapsulation of food ingredients. Testing and quality control are the subjects for Part 5. Regulatory, quality, scale-up, packaging, and economics are addressed in Part 6. Finally, applications of microencapsulation technologies are described in Part 7.

1.2 MICROENCAPSULATION DEFINED

Microencapsulation (as it applies to the food industry) is the process whereby various food ingredients can be stored within a microscopic size shell or coating for protection and/or later release. More specifically, microencapsulation is the process of enclosing small particles, a liquid, or a gas within a layer of coating or within a matrix. Traditionally, microencapsulation does not utilize capsules greater than 3 mm in length. Encapsulations that fall within the range of 100 nm to 1000 nm are classified as microencapsulations. Components that are between 1 nm and 100 nm are classified as nanocapsules or nanoencapsulations (Thies, 1996).

The common nomenclature used to define the various parts of the encapsulate includes terminology for the shell as well as the ingredient to be encapsulated. The ingredient that is to be encapsulated is usually called an active, core, payload, internal phase, encapsulate, or fill. The material that envelopes the active is commonly called shell, wall, coating, external phase, support phase, or membrane. The shell material is usually insoluble and nonreactive with the core. It accounts for 1 to 80% of the microcapsules by weight. The microencapsulant's shell can be made of sugars, gums, proteins, natural and modified polysaccharides, lipids, waxes, and synthetic polymers (Gibbs et al., 1999).

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00001-7

1.3 REASONS FOR MICROENCAPSULATION

Microencapsulation—considered both an art and a science—is a versatile technology used in a variety of industries. Examples include the pharmaceutical, chemical, food, and agricultural industries. One reason for using microencapsulation technologies is ingredient protection, that is, to avoid degradation resulting from exposure to environmental factors such as water, oxygen, heat, and light. Traditionally, this is done to improve the shelf-life of the active material. In some cases, encapsulation can be used to mask undesirable taste, odor, and color, thus preventing interference with product performance. Ease of handling is another reason for microencapsulating, as it can be used as a simple method for converting a liquid food ingredient into a solid (free-flowing powder). Microencapsulation can be used to prevent reactions and undesirable interactions between active food ingredients and those between actives and food components. Considering the ease of handling, microencapsulation also provides the opportunity to reduce the flammability and volatility of various food ingredients. Finally, microencapsulation can be used to control the delivery of a food ingredient. This is known as controlled release or controlled delivery.

Controlled release of food ingredients by microencapsulation can be achieved through understanding the mechanism by which the food ingredient is to be released. Various release mechanisms, also known as release triggers or signaled release, include temperature (thermal: heat, cold); moisture or solvent release via dissolution; shear or pressure release (mechanical, chewing (mastication)); pH; and enzymatic release. Triggered release is usually fine-tuned to a specific target release point. This also includes delayed release, or sustained release, over time. Targeted release seeks delivery of the food ingredient at a specific processing or storage stage, the specific consumption stage of the consumer good, or a specific location within the body (i.e., gastrointestinal tract).

The microencapsulation system must be designed with the release mechanism (trigger) in mind. As indicated earlier, an active food ingredient (payload) can be delivered at different stages of the processing, storage, and consumption cycle. The release trigger used will largely depend on the type of the food/beverage product and the location at which the payload needs to be released. Water (moisture) is used as the release trigger for releasing an active during rehydration and dissolution of a food powder when the water is added. The same is applicable when saliva dissolves a ready-to-eat food product during mastication. Water-soluble or -dispersible matrix/coatings (e.g., carbohydrates and/or proteins) are employed when the active needs to be released with water as a release trigger. Heat is used as the release trigger (thermal trigger) to release an active after warming, cooking, baking, roasting, steaming, or microwaving a food product. Examples include an addition of hot water to the food powder used to prepare hot drinks (coffee, tea, cocoa drink, chocolate drink, etc.) and soups. When heat is the preferred release trigger, lipids, fats, and waxes are used as matrix/shell materials. Mechanical shear (mastication) is used as the release trigger during chewing of a ready-to-eat (RTE) food. Enzymes and pH are used as release triggers when an active has to be delivered in a specific part of the gastrointestinal tract (i.e., mouth, stomach, small intestine, or the colon). The matrix/coating made up of a starch (sensitive to amylase in the mouth) is ideal for release in the mouth. When the matrix/coating is made up of protein, it would disintegrate in the presence of proteases in the stomach. Enteric food polymers such as zein, shellac, and denatured proteins are stable (insoluble) in the stomach (high acid environment; low pH) and soluble (disintegrates) at the pH environment existing in the small intestine. Hence, enteric polymers are employed for stabilizing the active until it exits the stomach and releases in the small intestine.

It is clear from these examples and applications of encapsulation that the food processing and consumption cycle is critical to the development of the trigger release mechanism used.

At the start of the cycle, consideration must be given to the ingredients and how they will interact with the processing step: this applies to both the creation of the microencapsulation system and the development and production of the finished food product. The processing step in the manufacture of a food product may involve shear, temperature (heat or cold), pressure, aeration, and addition of moisture. How these parameters affect the integrity of the microcapsules must be taken into account while developing the system itself. Once the food product is processed, its storage and handling must also be considered: this is done to ensure that the previously encapsulated food ingredients will be able to withstand the shelf-life and storage condition of the material. The final step in the cycle is the preparation of the food by the consumer. The microencapsulation must be able to perform and deliver the food ingredient at the appropriate time, that is, during cooking, at hydration, or when the finished product is consumed (i.e., mastication).

Information on microencapsulation of aroma, acidulant, bacteria, base/buffer, coloring agents, fatty acid, flavor, salt, leavening agents, lipid, mineral, salt, nutraceutical, oxidizer, protein, peptide, and so on, can be found in many patented art and literature references.

1.4 TYPES OF MICROCAPSULES

The morphology (form and structure) of the microencapsulation falls into two categories—microcapsules and microspheres. The grouping is based on the method used to manufacture the material. The first type, the microcapsule, is so named because it has well-defined core−shell morphology. Microcapsules are traditionally created solely by a chemical means, formed in a liquid-filled tank or tubular reactor (Thies, 1996). The second type, the microsphere, is mechanically formed through either an atomization process or milling process, whereby the active ingredients are disbursed within the matrix. This microsphere encapsulate is sometimes referred to as a matrix encapsulation. There are also hybrids of this technique in which a matrix particle can be coated with a shell material resulting in a core−shell morphology, whereby the core is a matrix encapsulation. There are a variety of structures associated with these different morphologies.

Microcapsules come in a variety of shapes—the shape being dependent on the method used to form the particle. The simplest shape is a well-defined core−shell morphology, described as a microcapsule. Another shape is known as a multinuclear morphology. Previously, this was described as a matrix encapsulation, or microsphere. In some processes, bulk matrix encapsulation must be milled or ground to the microscale, which results in the formation of an irregularly shaped microparticle, which is not a sphere. Finally, the hybridization (previously described) is known as a multiwall encapsulation.

The merits of a matrix encapsulation versus a microcapsule depend on how the particle will be used and the economy of scale for that food ingredient encapsulation. Matrix particles do not have a specific outer coating, which results in having some of the food ingredients, or actives, exposed near the surface. This leads to an incomplete encapsulation, which may not be well suited to all applications. An example of where this technology is not well suited can be seen with the encapsulation of various nutraceutical ingredients—more specifically, omega-3 fatty acids (Kolanowski et al., 2005). Later chapters will discuss methods for optimizing matrix particles to minimize the exposure of food ingredients at the surface. Matrix particles also give the ability to contain by weight less than 30% of a food active ingredient. This technique tends to be a rather inexpensive form of encapsulation.

Microcapsules, on the other hand, tend to have very little exposure of the active food ingredient at the surface, and are considered to be a complete form of encapsulation. This is because they have a well-defined core−shell morphology. The formation of microcapsules tends to be a more expensive processing technique. This will be demonstrated in future chapters. In many instances, there are more unit operations needed to create microcapsules than to create microspheres. Even though they are more expensive to process, a higher level of food ingredients can be encapsulated.

1.5 HISTORICAL ACCOUNT OF MICROENCAPSULATION

Microencapsulation technology mirrors the progress and development of many other contemporary technologies today. As with most innovations, component technologies are produced for a particular application or industry, then they are merged to create an innovative new product. This trend can also be seen with the microencapsulation field, where various technologies from unrelated industries have been combined to create a new product, for example, a microsphere or a microcapsule used for the controlled delivery of a food ingredient. Another trend in the microencapsulation field has been a reduction in particle size. Some of the first encapsulations occurred at the macro-scale level and were later reduced to the microscale level. Continuing that trend, many innovative companies in the encapsulation field today are seeking new ways to explore, develop, and commercialize encapsulation at the nanoscale level.

Microencapsulation technology can be traced back to the inception of one of its core techniques—spray drying. Spray-drying technology was first patented in 1872 by Samuel Percy as a way of preserving of milk solids (Percy, 1872). Percy outlined a "simultaneous atomizing and desiccating technique" for the improvement of desolvating liquid substances. His new invention demonstrated that atomized liquid could be mixed with air (heated or at ambient temperature), whereby rapid desiccation would occur, resulting in the production of powder with low moisture content. Drying applications for dextrin, starches, and gelatin are outlined in the patent. Although Percy's patent is not specific to encapsulation technology, it does form the basis for many future encapsulation technologies.

Shortly after the publication of Percy's patent, William Cains (Cains, 1875) received a patent titled "Improvement in Apparatus for Sugar Coating Confectionary, Pills, etc." This patent described a form of macroencapsulation that could be used for the coating of food and confection ingredients, as well as the coating of pills. This coating technique is commonly referred to as "pan coating." While this technology does not directly scale to the micro level, it created the fundamental basis by which many food ingredients are coated and enrobed today. In fact, Cains does not suggest in his patent that there are chemical and physical property enhancements as a result of his technology. These enhancements would be developed and exploited in the years to come as new discoveries were made in microencapsulation

techniques. The application of a coating material on the outer surface of an active food ingredient is imperative for the enhancement of chemical and physical properties of the newly coated material, as well as the delivery and controlled release of the protected ingredient. Later techniques such as bottom spray fluid-bed coating have improved Cains' design, and have provided the ability to coat microscale particulates (Cains, 1875).

The manufacture of fillable hard gelatin capsules was first reported in 1890. There were, however, many manufacturing issues—issues that were still around in the 1930s. These issues included leaky capsules and inaccurate metering of active ingredients because of the inclusion of air bubbles during the filling sealing process. As cited by Scherer, "The formation of the capsules in this manner is a relatively laborious procedure inasmuch as it entails careful manipulation by the operator of the gelatin plates, and the resulting product is not only lacking in uniformity by the reason of the human element involved, but is very apt to be inferior by reason of the fact that it is almost impossible to prevent the entrance of air into some of the capsules formed during each of the die stamping operations due to careless manipulation by the operator" (Scherer, 1934).

From this citation it becomes apparent that pre-1930s state-of-the-art capsule making had many difficulties metering specific, discrete quantities of an active ingredient. In 1934, Scherer patented a process by which these problems could be addressed (Scherer, 1934). This technique utilized a system of plates whereby one film of gelatin would be placed on each plate with the insertion of liquid active residing in the middle and then sealed. Scherer introduced the world to a new way of mass-producing actives that could be coated and protected from the environment. The other advantages of this technique are the improved metering of actives and the avoidance of both air bubbles and the potential for leaky seams.

A few years earlier, a process was developed whereby emulsions in mixtures were spray dried first. This led the development of early forms of matrix microencapsulation (Rappold and Volk A.G., 1926).

The technology and chemistry illustrated by Scherer, however, could not be translated to the microscopic level. It was not until 1957 that Barrett K. Green and Lowell Schleicher of the National Cash Register Company invented a technique for the manufacture of oil-containing microscopic capsules (Green and Schleicher, 1957). Green and Schleicher described a technique that strayed away from mechanical application of a shell in an effort to exploit the surface and film chemistry for shell applications. Their technique would later go on to be called coacervation, and would be used in the first generation of carbonless paper. The invention of microencapsulation by coacervation provided the intellectual stimulus for the invention of other methods known as urea-formaldehyde, melamine-formaldehyde, and interfacial polymerization.

Later on in 1957, Horton E. Swisher explored the viscoelastic properties of polysaccharides to be used as shell material for the preparation of solid flavorings. In Swisher's patent, he described a technique whereby a molten mass of polysaccharides is created and blended with liquid flavorings. This blended melt composition is then extruded through a die plate and chilled in a cooled solvent bath (Swisher, 1957). The particulates formed from this technique exhibit improved product performance by inhibiting ingression of atmospheric gases into the encapsulation material. This technique inhibits interactions with the environment, but does not halt chemical processes that can take place internally within the substrate.

In the mid-1960s, another area of microencapsulation found it roots in liposome structures that acted like cell walls. Initial studies of liposomes were pioneered by Alec Bangham and his group of researchers who were exploring the functionality of various phospholipids. Bangham's group was able to characterize the surface chemistry, as well as determine the relative interior diameter of self-assembled phospholipids known as "Banghamites" (Bangham and Horne, 1962, 1964; Glauert et al., 1962; Horne et al., 1963).

Dale E. Wurster developed a coating technique that utilized a spouted bed dryer in conjunction with an upward spraying nozzle positioned at the bottom of the fluid bed (Wurster and Lindlof, 1965). This technique would be called fluid bed spray coating or the "Wurster coating," named after its inventor. On a macroscale, this technique could be used for encapsulating various food ingredients, pharmaceutical tablets, confectionary items, fertilizer particles, chemical prills, grain, and seed. For macroscale materials, this technique would establish itself as a peer to pan coating technology as previously described in "pan coating"—Cains' invention. The benefit of "Wurster coating" is that it provides an avenue for coating micro-scale materials, whereas its predecessor, pan coating, does not provide this capability.

The next technique that furthered the art of encapsulation is known as organic phase separation. It is based on the principle of polymer−polymer incompatibility. This technique was highlighted in a 1968 patent describing a process of "forming minute capsules en masse" (Powell et al., 1968). This technique outlined the ability to create "seamless protecting walls surrounding the core" demonstrating a process to create well-defined core−shell morphology similar to the aforementioned coacervation technique invented by Green and Schleicher. In some cases, workers in the field even consider organic phase separation as a form of coacervation.

Coacervation was widely used for carbonless transfer paper applications. This technique was improved on by Matson in 1970 with the introduction of an aminoplast encapsulation technique (Matson, 1970). Coacervation uses a urea-formaldehyde pre-condensate that is mixed with actives, and then condensed to form a capsule morphology. According to Matson's claims, this technique is superior in comparison to its coacervation counterpart because it delivers better control and reproducibility. Matson also argues that coacervation-based encapsulations are unsatisfactory because of the gelatin raw material containing too much variation, which leads to quality control issues. This urea-formaldehyde technique has found its way into many industries with the exception of the food industry because these ingredients are not permissible for use in foods. There is a lack of agreement that the mechanism of urea-formaldehyde is being classified as coacervation or as "*insitu* polymerization."

An additional argument embracing encapsulation as an "art" can be found with the introduction of interfacial polycondensation, a form of polymerization (Vandegaer, 1971). This patent provides another pathway by which a liquid-dispersed active can be coated through the use of polycondensate:

> *Procedure for encapsulation of materials initially embodied, contained or carried in liquid is affected by interfacial polycondensation between coacting intermediates respectively in immiscible liquids, droplets or one liquid which is to be encapsulated and which contains one intermediate, being first established in a body of the other liquid. Thereafter the second intermediate is incorporated in the other liquid to produce minute capsules of the first liquid having a skin of polycondensate, e.g., polyamide, polysulfonamide, polyester, polycarbonate, polyurethane, or polyuria. (Vandegaer, 1971)*

This technique expanded its utility into the food ingredient landscape when compared to its urea-formaldehyde peer.

The patent arena for coacervation expanded to include liquid−liquid phase separation. This technique sought to improve the retention of volatile materials while also improving the shell properties of the microcapsule (Hart et al., 1973). This process overcame the limitations of the urea-formaldehyde encapsulation process in that the shell material was much stronger than, and not as brittle as, urea-formaldehyde. The inventors mirrored their manufacturing technique—unlike their predecessors—to create a stable dispersion of hydrophobic active in an aqueous phase. Like other techniques, the dispersion approach ensured that particle size could be reduced to the microscale. The aqueous and hydrophobic active dispersions were established in solution with the following materials: polyhydroxyl phenolic material (that is, resorcinol); an aldehyde; and polyvinyl alcohol (PVA). After modification of the pH and temperature and the introduction of chemical strains to the system, a polyhydroxyl phenolic-aldehyde deposition occurs at the interface of the two liquid phases. Hart et al. (1973) claim that this technique can be used for the encapsulation of a variety of food ingredients, including olive oil, fish oils, vegetable oils, and cocoa butter. This process is known as VARFAC for vinyl alcohol resorcinol formaldehyde acid complex. In practice, VARFAC is not food grade, but may be used in packaging.

Prior to the mid-1980s, encapsulation techniques were primarily developed to protect ingredients with a very limited ability to control release of the ingredient. Many of the techniques were dependent on mechanical crushing for the release of the active. This was common for techniques that arose during the 1960s and 1970s. Other techniques developed during this time depended heavily on the dissolution of the shell material, resulting in active release. During the mid-1980s a series of patents taught the use of shell materials to control and sustain the release of food ingredient actives. One form of controlled release uses a porous shelled encapsulant (Lim and Moss, 1982; Won, 1987). This form of encapsulant slowly diffuses the active over a period of time. This technique can be used to extend the release time of a vitamin, pharmaceutical, or other functional food ingredients.

Lattice-entrapped active ingredients were used to expand controlled release and sustained release of active ingredients. This technique, highlighted in a 1989 patent, explores the entrapment of an active via thermodynamic mechanisms within a lattice of shell material. The release of the functional ingredient can be mechanical or diffusion, permeation, or degradation limited. The inventors suggest that this technique can be used for the encapsulation of flavors, sweeteners, and other food ingredients (Abrutyn et al., 1989).

Novel ways of using controlled release techniques progressed into the 1990s with the use of liposomal release by irradiating with microwaves. A 1992 patent titled "Microwave Browning Composition" cites the use of a liposome-encapsulated Maillard browning reagent that is released during the cooking process. Once released, the Maillard reagent is able to engage in browning reactions with the food matrix, which cause the evolution of flavor and aroma (Haynes et al., 1992).

Flavor encapsulation came with the addition of a new processing technology for the creation of a glassy-matrix-encapsulated flavor. This technique is similar to the Swisher technique (1957) with the exception that it utilizes an extrusion system in place of a large pressurized vessel. This changed the manufacture of glassy encapsulated flavors from being a batch process to being a continuous process.

In summary, the ongoing development of microencapsulation as a viable science in the food industry began with Samuel Percy's creation of spray drying in 1872. Percy's invention resulted in the production of powder with low moisture content. William Cains followed in 1875 with his innovative "pan coating" process to coat food, confection ingredients, and pills. In 1890, the manufacture of fillable hard gelatin capsules was first reported. In 1926, a process was developed in which emulsions in mixture were spray dried first. In 1934, Scherer introduced the world to a new way of mass-producing an active that could be coated and protected from the environment. In 1957, Barrett K. Green and Lowel Schleicher of the National Cash Register Company invented a technique for the manufacture of oil-contained microscopic capsules—a process that would later be known as coacervation and would be used to develop carbonless paper. Later in 1957, Horton E. Swisher's patent described a technique whereby a molten mass of polysaccharides is created and blended with liquid flavorings. In the mid-1960s, A.D. Bangham and his group of researchers discovered a method for characterizing the surface chemistry as well as determining the relative interior diameter of self-assembled phospholipids (Bangham and Horne, 1964). The "Wurster coating," named after Dale E. Wurster, was developed in 1965 as a method for encapsulating various food ingredients, as well as other materials. In 1968, Powell and others defined a technique that outlined the process for creating "seamless protecting walls surrounding the core." In 1970, Matson improved the coacervation process with the introduction of an aminoplast encapsulation technique that improved coacervation by delivering better control and reproducibility. Furthering the "art" of encapsulation, Vandegaer introduced a new way in which a liquid-dispersed active can be coated through the use of polycondensate. The patent arena for coacervation expanded to include liquid−liquid phase separation, with Hart and others introducing a method for improving the retention of volatile materials while also improving the shell properties of the microcapsules. Fulger and Popplewell (1997) invented a process for incorporating a volatile component into a matrix and solidifying the mixture under pressure sufficient to prevent substantial volatilization of the volatile active. In the mid-1980s, a series of patents taught the use of shell materials to control and sustain the release of food ingredient actives. In 1989, Abrutyn's patent explored the entrapment of an active via thermodynamic mechanism within a lattice of shell material. In 1992, Haynes et al. introduced "Microwave Browning Composition," which releases a browning reagent during the cooking process.

Learning from the history of encapsulation has led to the exploration and development of innovation in both encapsulation methodologies and processes and these will be outlined further in subsequent chapters.

1.6 MATERIALS USED FOR MICROENCAPSULATION PURPOSES

Encapsulation materials are somewhat limited for the food industry. This limitation is based on allowable ingredients for use in foods. Traditionally, the formation of a microencapsulation requires that there be incompatibility between the shell and the active so that a coating will exist at the surface of the active ingredient. For hydrophobic actives, a hydrophilic material must be used to encapsulate. An example of a hydrophobic active is edible oil and fat. A wide variety of polysaccharides, proteins, and polymers have been used for encapsulation. Table 1.1 outlines these various ingredients.

TABLE 1.1 Materials Used for Microencapsulation of Hydrophobic Actives

Polysaccharides (unmodified)	Polysaccharides (modified)	Polysaccharides (gums)	Proteins (vegetable)	Proteins (animal)	Polymers
Sugar	Dextrin	Gum arabic	Soy	Gelatin	PEG
Starch	Cyclodextrin	Alginate	Wheat	Casein	PVA
Glucose syrup	OSA starch	Carageenan	Corn (zein)	WPC	PVP
Maltodextrin	Cellulose	Pectin		WPI	Cellulose derivatives
				Caseinate	Chitosan

Abbreviations: OSA, octenyl succinate; WPC, whey protein concentrate; WPI, whey protein isolate; PEG, polyethylene glycol; PVA, polyvinyl acetate; PVP, polyvinyl pyrrolidone.

TABLE 1.2 Materials Used for Microencapsulation of Hydrophilic Actives

Lipids	Waxes	Polymers
Hard fat	Beeswax	Shellac
Hydrogenated fat	Paraffin wax	Ethyl cellulose
Glycerides	Microcrystalline wax	
Phospholipids	Carnuba wax	
Fatty acids		
Plant sterols		
Sorbitan esters		

TABLE 1.3 Methods Used in Microencapsulation

Physical Methods	Chemical Methods
Spray drying	Phase separation
Spray cooling/chilling	Solvent evaporation
Spinning/rotating disc	Coacervation
Fluidized bed (drying, granulation, and coating)	Interfacial polymerization
Extrusion	Liposome
Coextrusion	Coextrusion
Molecular encapsulation	Nanoencapsulation
Multiple emulsions	

In systems where a hydrophilic active requires encapsulation, a hydrophobic material is usually used as a matrix or coating material. These hydrophobic materials include lipids, waxes, and polymers. Various hydrophobic materials used for encapsulation of hydrophilic actives are outlined in Table 1.2. A detailed account of the materials used as a matrix and/or coating in the process of microencapsulating an active is presented in Part 4 of this book.

1.7 MICROENCAPSULATION TECHNIQUES USED WITHIN THE FOOD INDUSTRY

As previously mentioned, there are two classifications of microencapsulation based on the formation of the microspheres or microcapsules. Microspheres are typically formed by physical means, and the techniques associated with this include spray drying, spray cooling, spray chilling, spinning disk, fluid-bed coating, extrusion, coextrusion, molecular encapsulation; and multiple emulsions. Chemical processes associated with the formation of microcapsules include phase separation, solvent evaporation, interfacial polarization, coextrusion, coacervation, nanoencapsulations, and liposomes. The various methods used for microencapsulation are presented in Table 1.3. These methodologies are further explored in greater detail in Part 3 of this book.

1.8 TRENDS IN MICROENCAPSULATION

As presented in both the introductory materials and the historical account of microencapsulation, there have been a variety of new techniques added to this field over the past 30 years. In the mid-1950s, coacervation and spray drying were

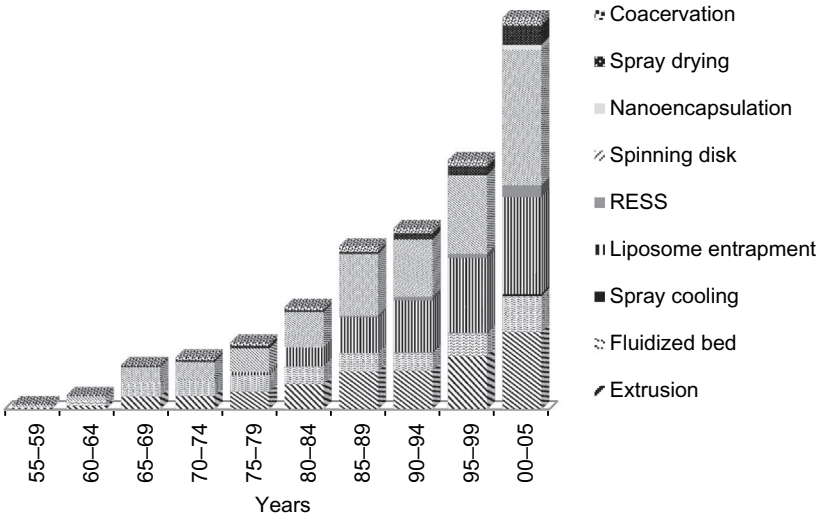

FIGURE 1.1 Trends in microencapsulation—number of papers. Abbreviations: RESS, rapid expansion from supercritical solutions. *Courtesy of Danisco.*

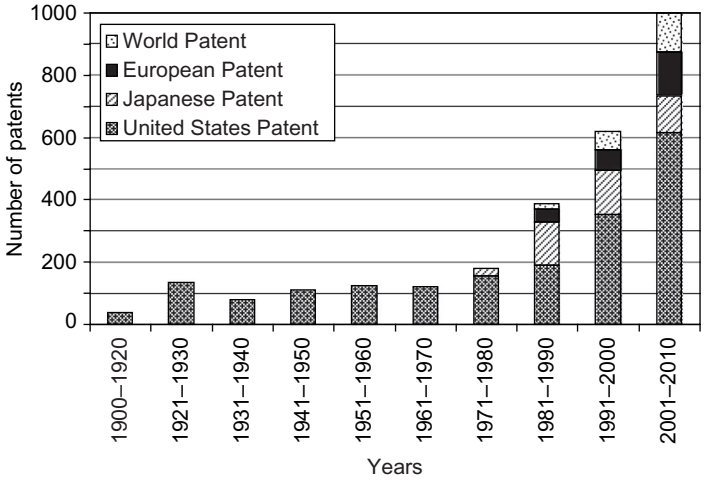

FIGURE 1.2 Trends in microencapsulation—number of patents in food/beverage arena.

the primary techniques: unfortunately, few publications were offered on the subject. It was not until the mid-1980s that these techniques were being exploited for encapsulation purposes, as shown in Figure 1.1. In the figure it can be seen that the fields of extrusion, fluidized bed, liposome entrapment, spinning disk, and spray drying are growing significantly.

Trends relating to patents in the area of microencapsulation for the food/beverage industries are shown in Figure 1.2. It is clear that the patents in the area of microencapsulation have grown exponentially over the years. The growth rate in the number of patents is significantly higher in the United States than in other regions. Boh and Sumiga (2008) have also reported an exponential increase in number of patents and scientific articles on microencapsulation over the years. With the demand for encapsulation technology to protect, mask, and control delivery of food ingredients, it is anticipated that the number of papers and patents in this area will continue to grow.

1.9 CHALLENGES IN MICROENCAPSULATION OF FOOD INGREDIENTS

There are no all-encompassing techniques or approaches that apply to the microencapsulation of food ingredients. A tailor-made solution is required in each situation because of a wide variation in types of food, storage and transportation requirements, and the necessity for different release triggers and consumption requirements. Harsh processing conditions can negatively impact the integrity of the microencapsules, thus the process must be designed around those conditions. Another challenge is that only a very limited number of matrix/coating/excipient materials have been

approved for use in food. Regulatory compliance narrows the selection of generally regarded as safe (GRAS) materials for use in the encapsulation of food ingredients. In addition, narrow profit margins may not support the additional cost (e.g., capital, process, and materials) of microencapsulation. Review chapters pertaining to microencapsulation of food ingredients exist elsewhere (Balassa and Fanger, 1971; Jackson and Lee, 1991; Shahidi and Han, 1993; Risch and Reineccius, 1995; Dziezak, 1998; Gibbs, 1999; Vilstrup, 2001; Uhlemann et al., 2002; Ubbink and Schoonman, 2003; Lakkis, 2007; Garti, 2008; Zuidam and Nedovic, 2010).

1.10 THE FUTURE OF MICROENCAPSULATION OF FOOD INGREDIENTS

In spite of all the challenges mentioned, the use of microencapsulated ingredients in food is steadily growing because consumers are now ready to pay extra for the use of microencapsulation technology to successfully incorporate sensitive, health-promoting ingredients in food. The report titled "Food Encapsulation: A Global Strategic Business Report" by Global Industry Analysts, Inc. (GIA) provides a review of noteworthy market trends, growth drivers, and challenges (Global Industry Analysts, Inc., 2010). This report analyzes markets for food encapsulation in the United States, Canada, Japan, Europe, Asia-Pacific, Latin America, and the rest of the world. Annual estimates and forecasts are given for each region for the period of 2006–2015. The report profiles 45 companies, including many key and niche players. According to the report, the global food encapsulation market is projected to reach about $39 billion by 2015. Growth in the market is especially driven by factors such as changing dietary habits; increased popularity of exotic flavors, cuisines, and the gourmet food segment; the rising emphasis on preservation of food quality; and the speed and agility of new product innovation. Venture capital investments are expected to be especially high in the functional foods segment because of the growing demand for foods with disease prevention benefits for a rapidly aging population.

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Part II

Concept of Microencapsulation

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Chapter 2

Factors and Mechanisms in Microencapsulation

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2.1 INTRODUCTION

Microencapsulation technology serves two key applications in the food industry. The technology is used extensively for providing stability of the active ingredient in food products, which may otherwise render undesirable functionality. Whether the product relates to flavors, chewing gum, candies, coffee, probiotics, health foods, vitamins, minerals, or enzymes, the governing principles to achieving desirable product stability can be managed by controlling the structural design of the microcapsule that offers improved performance in the food products.

Another primary application of microencapsulation technology is to bring a desired physicochemical change in sensory perception in the food product at the desired time or by using a suitable triggering mechanism. Having an improved understanding of the molecular and physicochemical interaction of the active ingredient and material composition is critical to creating such a *dynamic* system.

This chapter describes the factors and the theoretical mechanisms that impact the stability, mass transport, and offer triggered release. The major building blocks for developing food-grade delivery systems are given. This section is followed by design concepts for the microcapsule structure. Because food products are complex, multicomponent systems, a discussion of multivariant characteristics of the factors is presented. The chapter concludes with a comparison of the theory and industry practice with regards to release mechanisms.

2.2 STRUCTURAL DESIGN OF THE MICROCAPSULE

A microcapsule comprises many different components of which the active ingredient and the matrix polymer are the two key components that control the rate of diffusion. Understanding the morphology, physicochemical compatibility, and thermodynamics of both the active and matrix polymer is important.

In food systems, the microcapsule shell can provide several different functionalities for stability:

- Protection of sensitive active ingredients such as flavors, vitamins, minerals, unsaturated lipids, essential oils, and salts from oxygen, water, and light.
- Processing convenience by converting difficult-to-handle liquids to powdered food systems.
- Separation of two components during storage hold time.

From a morphological or structural standpoint, the factors affecting the stability and release are type, size, shape, and payload of the microcapsules. Also, molecular weight of the active, functional moiety and surface charge, concentration, solubility, wettability, and temperature are important parameters and they will be discussed in the following sections.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00002-9

2.3 MICROCAPSULE OR MICROSPHERE TYPE

Morphological configuration in which the active ingredient is configured into a microcapsule with a distinct matrix wall around the active ingredient, or in microsphere morphology where the active ingredient is contained in a number of small discrete droplets or particles that are dispersed in the matrix material, can significantly impact stability and release of the active ingredient. One may also envision that the small droplets in a microsphere are so small that the active may be inherently soluble in the matrix polymer. Figure 2.1 shows the different structural configurations of microencapsulated systems and presents how the active ingredient is distributed in the matrix polymer, however,both microcapsule and microsphere morphologies must be free of defects, pin holes, or high curvatures to provide enhanced stability. The presence of defects can cause oxidative or hydrolytic degradation over longer periods of time.

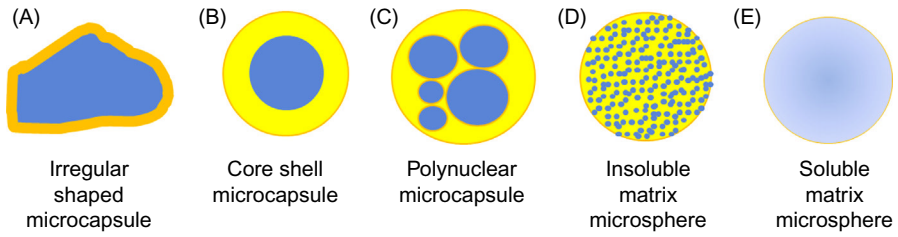

FIGURE 2.1 Microcapsule (A, B, and C) versus microsphere (D and E) morphology.

2.4 MICROCAPSULE SIZE, SHAPE, AND PAYLOAD

Particle size is one of many parameters that may be adjusted to control release rates of encapsulated ingredients, as shown in Figure 2.2.

The size of the microcapsule (or microsphere) and its corresponding active payload are interrelated. For a defined microcapsule size, a lower active loading in a microcapsule will offer better protection than a high loading, because the thickness of the matrix wall increases dramatically with a decrease in loading. Table 2.1 shows how the volume of the thickness of the shell wall is impacted by the size of the microcapsule and loading. This is also described in a plot in Figure 2.3. As the microcapsule shell thickness increases, the ability of the microcapsule to provide barrier properties increases.

Note that as the microcapsule dimensions become smaller and smaller, the thickness approaches infinitesimal values (i.e., nanoscale). In other words, large microcapsules of the same active loading offer the advantage of greater stability because the surface area per unit mass of the active is significantly higher as the surface area of transport increases as a square of the radius of the microcapsule. Figure 2.4 describes the relationship between the microcapsule size and the

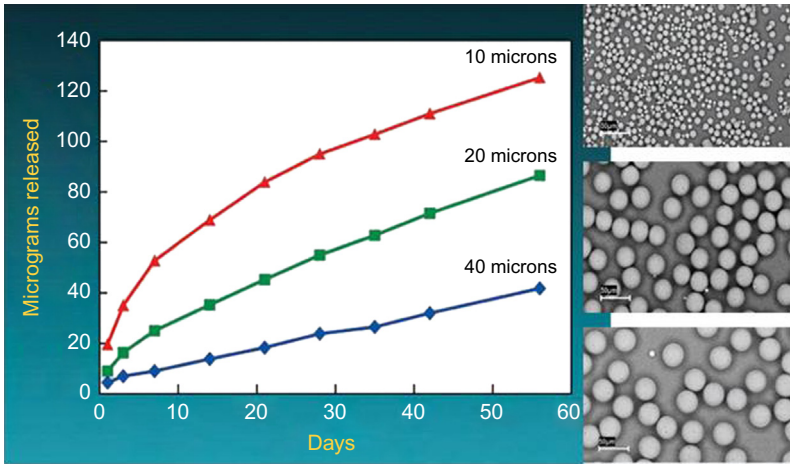

FIGURE 2.2 Impact of size of the microsphere in controlled release.

TABLE 2.1 Effect of Thickness of the Shell Wall on the Microcapsule Volume and Loading

Diameter (μm)	Vol. Microcapsule (μm^3)	Thickness (μm) @ 90% Load	Thickness (μm) @ 50% Load	Thickness (μm) @ 10% Load
1000	523,600,000	17.3	103.1	267.9
500	65,450,000	8.6	51.6	134.0
250	8,181,250	4.3	25.8	67.0
100	523,600	1.7	10.3	26.8
50	65,450	0.9	5.2	13.4
25	8181.25	0.4	2.6	6.7
10	523.6	0.2	1.0	2.7
5	65.45	0.1	0.5	1.3
2.5	8.18125	0.0	0.3	0.7
1	0.5236	0.0	0.1	0.3

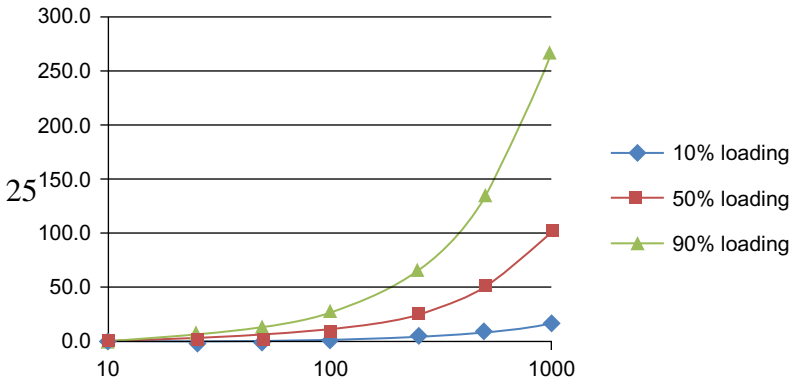

FIGURE 2.3 Microcapsule thickness as a function of microcapsule diameter.

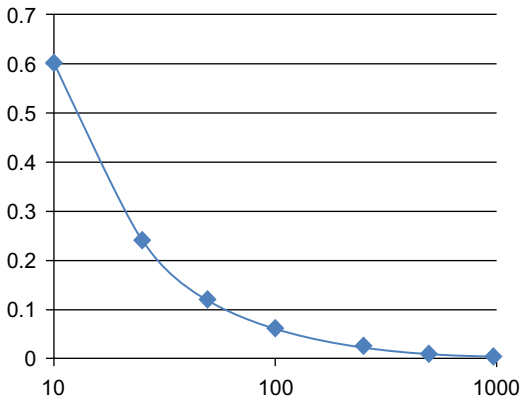

FIGURE 2.4 Microcapsule size and the surface area relationship.

total surface area (as demonstrated by number of microcapsules) at the same payload. The figure shows that when the size of the microcapsules is small, the surface area is significantly high as compared to the size of the microcapsules. In such a situation, it is inherently difficult to design a sustained release formulation. As a result, sustain release formulations are often designed with surface area/volume ratios less than approximately 0.2.

2.5 PHYSICOCHEMICAL FACTORS

From a physicochemical compatibility perspective, the factors affecting the microcapsule stability and release are as follows.

2.5.1 Molecular Weight of the Active Agent

Often one cannot alter the size once the active ingredient for the food product is ascertained. In this context, it is simply important to know that size of the active ingredient is important. Typical food active ingredients have molecular weights that are less than 500 Da. Because of the small molecular dimension, these molecules can slip through tortuosity of the matrix polymer interstitial space or through the polar heads of the phospholipids in the case of a liposome. As the molecular size increases, the diffusion decreases exponentially. This means that larger molecules, such as proteins and peptides, cannot diffuse quickly.

2.5.2 Functional Moiety and Surface Charge

Glucose enters cells much faster than other sugars, facilitated by a carrier protein specific for glucose. This concept is also known as facilitated diffusion and has applications in the pharmaceutical drug industry, although its use in the food industry is rare. In contrast, the ionic surface charge on the active ingredient can play a significant role in retarding the rate of diffusion by electrovalent binding to the matrix polymer moieties. Changing the ionic properties often results in a change in solubility of the active ingredient in the matrix phase. Thermodynamics also affects microcapsule stability and release. Thermodynamic properties such as concentration, temperature, solubility, and interfacial properties are all key factors contributing to the performance and stability of the microcapsule.

2.5.3 Concentration

Molecules are in constant motion and tend to move from regions where they are in higher concentration to regions where they are less concentrated. Microcapsules are no exception. The active ingredient moves from areas of high concentration of that material into areas of low concentration of that material. As the concentration gradient between the inside of the microcapsules increases as compared to the surrounding food outside, the rate of diffusion increases. This is important from two standpoints. First, because the initial concentration gradient in a microcapsule is high, this is a contributing cause for a burst effect. Also, as the concentration gradient decreases, the driving force associated with release decreases and, therefore, such a system exhibits a first order release.

2.5.4 Solubility

In most product development efforts, one often needs to make a decision whether the active ingredient must be in a solubilized state or a dispersed state. When the active is in a solubilized state, one can expect faster release. The concepts for such a release and its kinetics are presented in the next chapter. Microcapsules with active ingredients in a dispersed state require a balance between the stability and release rate. This is particularly relevant when the active has low solubility in the food product in which the microcapsule is placed.

As the solubility of the active ingredient increases in the matrix, the active ingredient can move through the matrix more easily. The solubility is characterized by partition (P) or distribution (D) coefficients. These coefficients are a measure of the difference in solubility of the active ingredient in an aqueous versus oil phase. Normally, one of the solvents used is water, while the second is hydrophobic, such as octanol. Hence, both the partition and distribution coefficients are measures of how hydrophilic (“water loving”) or lipophilic (“oil loving”) an

active ingredient is. The logarithm of the ratio of the concentration is called log *P*. The log *P* value is also known as a measure of lipophilicity.

$$\text{Log } P = \log[(C \text{ in octanol})/(C \text{ in water})]$$

2.5.5 Wettability

The wettability of the active ingredient impacts dissolution and subsequent release. If the aqueous media cannot wet the surface of the active ingredient, no release will occur. To quantitate the wettability, Griffin in 1954 defined the term hydrophilic−lipophilic balance (HLB) and a useful scale between 0 and 20 that allows one to explore how wettable the active would be with water. Griffin proposed a mathematical equation as follows:

$$\text{HLB} = 20 * \text{Mh}/\text{Mw}$$

where Mh is the molecular mass of the hydrophilic portion of the active ingredient molecule and Mw is the molecular mass of the entire molecule, giving a result on a scale of 0 to 20 (Garti, 1996). An HLB value of 0 means completely lipophilic, while a value of 20 corresponds to completely hydrophilic. Using this scale, the HLB value can be used to predict the surfactant properties of a molecule, as presented in Table 2.2.

In addition to these properties, scientists also use solubility parameters to take into account hydrogen bonding between water and the hydroxyl groups.

TABLE 2.2 Griffin's HLB Guidance Chart

Ingredient	HLB Guidance
Oil soluble	<10
Water soluble	>10
Antifoaming agents	>4 and < 8
W/O (water-in-oil) emulsifier	>7 and <11
O/W (oil-in-water) emulsifier	>12 and <16
Wetting agent	>11 and <14
Detergent	>12 and <15
Solubilizing agent	>16 and <20

2.5.6 Temperature

Temperature is the most critical thermodynamic property that influences the release of the active ingredient. In most cases, increase in temperature causes molecules to move faster, therefore enhancing diffusion. The temperature also allows the matrix to undergo entropic relaxation from a metastable state to an equilibrium state. As the density increases, the molecule undergoes fewer collisions; this allows for faster diffusion. Similarly, lowering the temperature will lower the diffusion rate by lowering the energy of each particle. As a result, microcapsules stored at room temperatures or under refrigeration offer greater stability than those kept at elevated temperatures.

Because temperature is a controllable property, its impact on matrix polymers cannot be undermined. Polymer matrices typically undergo phase transition with respect to temperature, thus changing from a crystalline to amorphous state, glassy to rubbery state, or solid to molten state, and sol to gel state. In each of the phase transition states, the product release profiles differ. Obviously, the selection of the matrix material becomes a key factor in microencapsulation design. The selection criteria for the matrix polymer are described in Chapter 16.

TABLE 2.3 Critical Process Control Parameters That Influence Release and Stability

Process	Critical Process Control Parameter
Atomization	Mixing time, mixing energy, evaporation rate, thermal profile, feed rate, air flow
Ionic gelation	Mixing time, mixing energy, salt content, zeta potential, cooling temperature, cool rate, gel point, feed ratio
Coacervation	Mixing time, mixing energy, pH control/zeta potential, cooling temperature, cool rate, gel point
Liposome	Mixing energy, pH, ionic charge, thermal conditions, freezing cycling
Fluid bed	batch size, evaporation rate, thermal profile, feed rate, air flow rate, feed ratio

2.5.7 Process Factors

Each microencapsulation process uses its specific critical control variable, and so a detailed description of all processes that can lead to a change in release profile is not possible. However, common pitfalls for an ill-designed microcapsule structure that result in high porosity or lower density are described in Table 2.3.

2.6 MECHANISM OF DIFFUSION

The mechanism of diffusion or mass transport has been a topic of extensive research over the past 70 years (Crank, 1975). Despite years of history, one skilled in the state-of-the-art must construct an assessment for the target food product. Because food products are complex, diffusion mechanisms are controlled by several attributes. Diffusion entails several steps:

- Surface wetting
- Hydration or swelling of the matrix composition or layer
- Disintegration or erosion of the matrix
- Dissolution of the active ingredient to induce molecular diffusion or mobility
- Permeation of the active ingredient in the matrix phase
- Permeation of the active ingredient through the matrix phase into the bulk food phase

Each of the steps has the potential to control the rate and amount of release, and depending on the release duration, the rate controlling step may change over time. As an example, the microencapsulation of a water-insoluble food active ingredient in a poorly erodible matrix may provide the stability protection one needs, but the rate of release will be significantly compromised because the rate and amount of the active release will undergo two critical rate controlling steps: rate of erosion followed by solubilization of the active ingredient. Fundamentally, the rate controlling step in a complex food product ultimately defines the release kinetics.

However, in most engineering practices, the standard approach is to consider the rate controlling step, which can potentially dominate over the others. The rate controlling step depends on the choice of matrix material, morphology, physicochemical properties of the active and matrix polymer, and system in which the microcapsule is placed. With respect to practical considerations, one must recognize that using a single rate controlling step as a basis may not mimic a superimposition of several kinetics. A number of methods are described to determine the kinetic model of diffusion (Benita, 1984). Some of the tools used include gradients of chemical potential (Dash et al., 2010), equilibrium analyses (Donbrow et al., 1988), cell dynamics models (Ghosal et al., 1992), Monte-Carlo simulation (Jalsenjak, 1992), and non-regression analyses (Higuchi, 1963; Thies, 1982; Nixon, 1984).

An excellent starting point to understand the different kinetic release profiles is to consider concepts of zero order diffusion, Fickian diffusion, first order diffusion, Higuchi's diffusion model, and case II diffusion (Figure 2.5).

2.6.1 Zero Order or Pseudo-Zero Order Diffusion Model

A zero order diffusion rate is independent of the concentration of the active ingredient. Increasing the concentration will not speed up the rate of release, nor does the reduction in concentration slow down the diffusion. This is

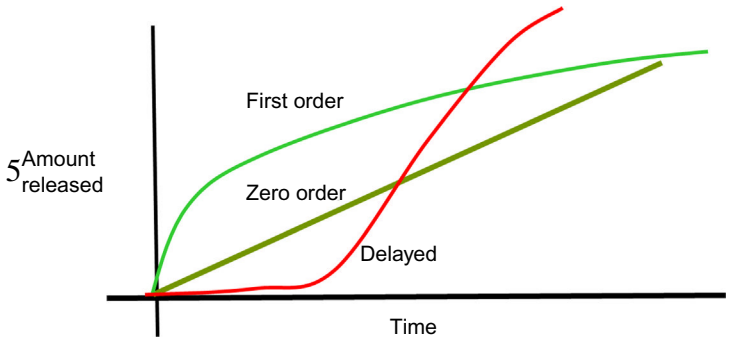

FIGURE 2.5 Schematic release profiles for microencapsulated systems.

counterintuitive. In a zero order diffusion model, the hypothesis is that the amount of active loading is infinite. In other words, one can achieve zero order diffusion when the release amount is extremely slow as compared to the amount of active loaded in the microcapsule. Zero order release in diffusion is described as the amount released is directly proportional to time. As shown in Figure 2.5 and mathematically:

$$C \alpha t \tag{2.1}$$

$$dC_t/dt = k_0 \tag{2.2}$$

where C_t is the amount of active ingredient released in time t, and k_0 is a zero order constant.

By integration of Eq. 2.2:

$$(C_t - C_0) = k_0 \cdot (t - 0) \tag{2.3}$$

where C_0 represents the initial release at $t \rightarrow 0$ for a fixed volume in which the release is measured:

$$C_t = C_0 + k_0 \cdot t \tag{2.4}$$

This equation is called the integrated zero order rate law.

It must be pointed out that a true zero order kinetics is often rare in the food industry, because of the short desirable release time, solubility of the active agent in the matrix, surface activity, and desirability for a burst release from the microcapsule.

2.6.2 Fickian Diffusion Model

Fickian diffusion postulates that the diffusive flux, J, goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the spatial concentration gradient. In terms of one-dimensional spherical coordinates relating to a microsphere morphology, *Fick's first law* is:

$$J = (1/A) \cdot dC/dt = -D\, dC/dr \tag{2.5}$$

where J is the diffusion flux or mass flow of the active ingredient under the assumption of steady state, D is the diffusion coefficient, r is the radius of the designed microcapsule, and A is the surface area of the microcapsule. The negative sign in Eq. 2.5 is because diffusion occurs in the direction opposite to that of increasing concentration. It must be emphasized that the equation is consistent only for an isotropic medium, where the diffusion properties do not change in other spherical coordinates.

Equation 2.5 can be simplified with the knowledge that the concentration difference between the inside and outside of the microcapsule is:

$$dC/dt = -D \cdot A \cdot (\Delta C)/R \tag{2.6}$$

where $(\Delta C) = C_{oM} - C_{IM}$, and where C_{oM} is the concentration of the active agent on the outside of the microcapsule while C_{iM} is the concentration of the active agent on the inside of the microcapsule. R is the thickness of the microcapsule.

Comparing Eqs. 2.2 and 2.6, one can show that Fickian diffusion will approximate zero order diffusion when:

$$k_0 = -DA(\Delta C)/R \tag{2.7}$$

In essence, for constant release, a pseudo-zero order rule can be used for a practical approximation. The higher the k_0, the faster the rate of diffusion. One also concludes that the rate of diffusion can be increased by an increase in diffusion coefficient, surface area, and concentration driving force, while it can be decreased by increasing the thickness of the microcapsule wall.

2.6.3 First Order Diffusion Model

First order diffusion depends on the concentration of the active agent. The rate law for a first order diffusion is:

$$dC/dt = k_1 C \tag{2.8}$$

where k_1 is the first order rate constant, which has units of 1/s.

The integrated first order rate gives:

$$\ln C - \ln C_0 = -k_1 t \tag{2.9}$$

A plot of ln C versus time t gives a straight line with a slope of $-k_1$.

2.6.4 Higuchi's Diffusion Model

In 1961, Higuchi published the derivation of an equation that allowed for an active agent to diffuse from a matrix phase when the active is a finely dispersed drug into a perfect sink. He based his equation on a pseudo-steady state where the cumulative amount released is proportional to the square root of time. His model is based on the hypotheses that:

1. Initial active ingredient concentration in the matrix is much higher than the solubility in the matrix.
2. Diffusion takes place only in one dimension.
3. There is no surface contamination with the active agent (edge effect must be negligible) and the active agent particles are much smaller than the microcapsule system.
4. No swelling or dissolution of the matrix occurs.
5. Diffusivity of the active agent is constant.
6. Perfect sink conditions are always attained in the release environment.

Initially conceived for planar systems, it was then extended to different geometrics and porous systems. For a one-dimension system, the following equation can be used:

$$C_t = -[DA(2C_0 - C_s)C_s t]^{1/2} \tag{2.10}$$

where C_s is the solubility of the active in the matrix, D is the diffusion coefficient, C_t is the concentration of the active released at time t, and A is the surface area through which the release occurs.

The major benefit of this equation is that a formulator could be used to facilitate microsphere optimization, where the active agent is well dispersed in the microsphere matrix rather than using a microcapsule morphology to understand the release mechanism.

2.6.5 Case II Diffusion

In many cases, the diffusion mechanism of an active ingredient cannot be described adequately. This is particularly true when diffusion of the active agent occurs in a glassy polymer matrix in contrast to the rubbery state. In essence, the phase transition point, such as glass point, plays a significant role in the type of diffusion mechanism the active agent will exhibit from the microsphere. Such form of diffusion is known as non-Fickian case II diffusion.

Unlike Fickian diffusion, which is controlled by diffusion coefficient, in case II diffusion the parameter is a constant velocity front, which marks the boundary of the swollen gel and the unswollen glassy matrix. Korsmeyer et al. (1983) derived a simple relationship based on power law that allows for the release of the active agent from a polymeric matrix. They presented the diffusional release in a power law relationship, as shown in Table 2.4.

2.6.6 Osmosis

Osmosis is a specialized case of diffusion that involves the passive transport of water. In this case, water moves through a selectively permeable membrane from a region of its higher concentration to a region of its lower concentration.

TABLE 2.4 Interpretation of Diffusional Release Mechanisms from Polymeric Films

Transport Mechanism	Power Law Release Exponent (n)	Release Rate as a Function of Time
Fickian diffusion	0.5	$t^{-0.5}$
Non-Fickian transport	$0.45 < n = 0.89$	t^{n-1}
Case II transport	0.89	Zero order release
Super case II transport	Higher than 0.89	t^{n-1}

The key to designing an osmotic release system is to select a polymer membrane that selectively allows passage of certain types of molecules while restricting the movement of others. Although each molecule moves down its own concentration gradient, from a region of its high concentration to a region of its low concentration, the rate of release is impacted because of backpressure exerted by other molecules such as water. Changing the ionic solute concentration changes the way the membrane behaves.

Osmosis as a mechanism of release can be used for triggered release (Deasy, 1984). In an ill-designed microcapsule product, which has defects and holes, osmotic pressure can be used to provide sustained release by virtue of the osmotic pressure. The pressure required to stop osmosis is called the osmotic pressure. Osmotic pressure arises when two solutions of different concentrations, or a pure solvent and a solution, are separated by a semipermeable membrane.

In dilute solutions, osmotic pressure (Π) is directly proportional to the molarity of the solution. The Van't Hoff equation provides osmotic pressure calculations as:

$$\prod = MRT \tag{2.11}$$

where:

Π = osmotic pressure
M = molarity = moles ÷ volume (L)
R = 8.3145 J/k mol is the normal gas constant (R' = 0.0821 L atm/K mol is the gas constant expressed in terms of liters and atmospheres)
T = temperature (K)

A schematic that demonstrates osmotic release being triggered by the absorption of water into the microcapsule core is presented in Figure 2.6. At the pressure where osmotic pressure is exceeded, subsequent swelling ruptures the microcapsule shell.

In this chapter, while most of the discussion is focused on the physics of mass transport versus diffusion concepts, almost any mechanism that induces a structural change to the microcapsule can be used as a triggering mechanism. Figure 2.7 depicts many such triggered release concepts. The most common triggering mechanisms are temperature, pH, use of salts, osmotic pressure, and light.

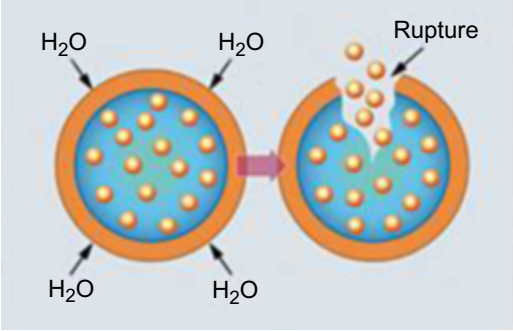

FIGURE 2.6 Concept of burst release by osmotic pressures.

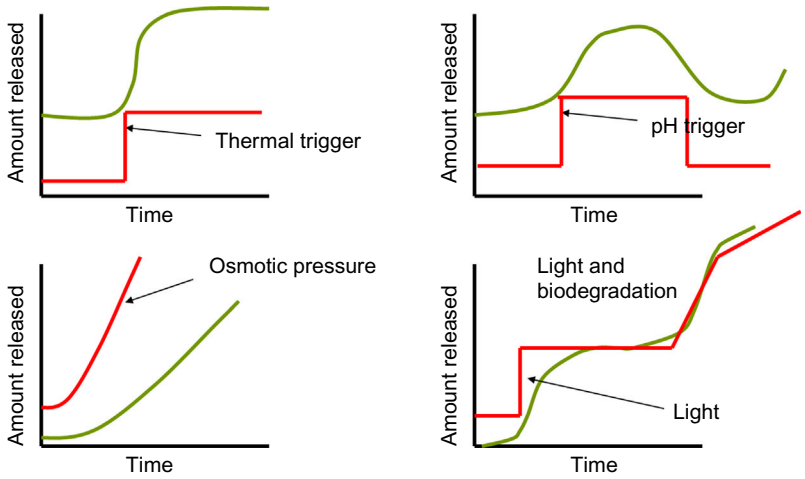

FIGURE 2.7 Nondiffusion release mechanisms.

Depending on the triggering and the morphological difference in the microcapsules, the response release curve may be sigmoidal, waveform, or asymptotic in nature. Release profiles such as these are unique to different food products in the food industry.

2.7 CONCLUSION

In this chapter, the different rate controlling factors were presented. The mechanisms of release are subject to how the microencapsulation is conducted, that is, method or process, shell composition, physicochemical state, and morphological properties. The versatile technologies that have resulted from the evolution of this technology can improve product performance and provide better controlled delivery of ingredients. Understanding of the molecular and physicochemical interaction of the active ingredient and material composition is critical to creating such a *dynamic* system. Selection and development of microencapsulated food ingredients for controlled delivery require a careful balance of physical and chemical properties and processing of ingredients, available techniques, and regulatory guidelines. Finally, there are no all-encompassing techniques or approaches that apply to the microencapsulation of food ingredients.

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Chapter 3

Applications of Mass and Heat Transfer in Microencapsulation Processes

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3.1 INTRODUCTION

The stability and release properties are different for microcapsules depending on the morphology. The morphology of microcapsules depends mainly on the core material and the coating attributes of the shell. Both microcapsules and microspheres may have regular or irregular shapes, or may be classified as mononuclear, polynuclear, and matrix types, as shown in Figure 3.1.

Core−shell morphologies of mononuclear microcapsules contain the shell around the core, while polynuclear capsules have many cores enclosed within the shell. Alternatively, in a microsphere, the core agent is distributed homogeneously into the matrix polymer. While the schematic in Figure 3.1 shows a spherical geometry, this may be irregular, as shown in Figure 3.2.

For purposes of simplicity, we consider two morphological structures—microspheres and core−shell microcapsules—to understand how the concept of mass and heat can be used. The microsphere embodiment assumes that the active ingredient is fully solubilized in the matrix material, and the microcapsule, which is envisioned as a core−shell morphology, has a distinct wall surrounding the active material from the surrounding media. Obviously, core materials in microcapsule morphology may exist in the form of a solid, liquid, or gaseous state.

To gain a better insight into mass transfer problems of microencapsulated food product systems, a combination of experimental investigations and mathematical modeling is required. This allows the use of a model as a predictive tool to decide on gross difference rather than in lieu of a well-designed experiment to assess storage stability or to assess the release profile. While unsteady-state diffusion is important, from a practicality standpoint, this chapter keeps away from unsteady-state diffusion, and therefore the governing partial differential equations are not discussed.

3.2 MECHANISM OF DIFFUSION

As described in Chapter 2, the diffusion entails several steps:

- Surface wetting
- Hydration or swelling of the matrix composition or layer
- Disintegration or erosion of the matrix
- Dissolution of the active ingredient to induce molecular diffusion or mobility
- Permeation of the active ingredient in the matrix phase
- Permeation of the active ingredient in the bulk food phase

Each of the steps has the potential to control the rate and amount of release. Fundamentally, the rate controlling step in a complex food product ultimately defines the release kinetics. This chapter assumes that the diffusion is the rate controlling step and as a result we focus on the permeation of the active ingredient in the matrix.

The release profiles through matrices are classified into two key types of orders. Figure 3.3 provides a schematic to the types of order in microencapsulated systems: zero order diffusion and Fickian diffusion.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00003-0

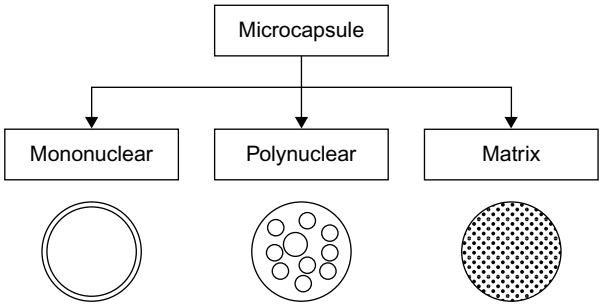

FIGURE 3.1 Morphology of microcapsules.

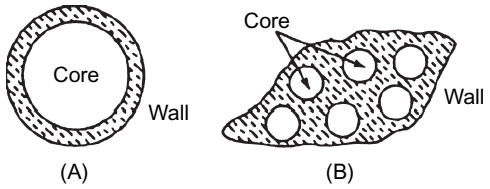

FIGURE 3.2 Geometric and irregular shapes of microcapsules. (A) Microcapsule morphology, (B) Microsphere or microparticlate morphology.

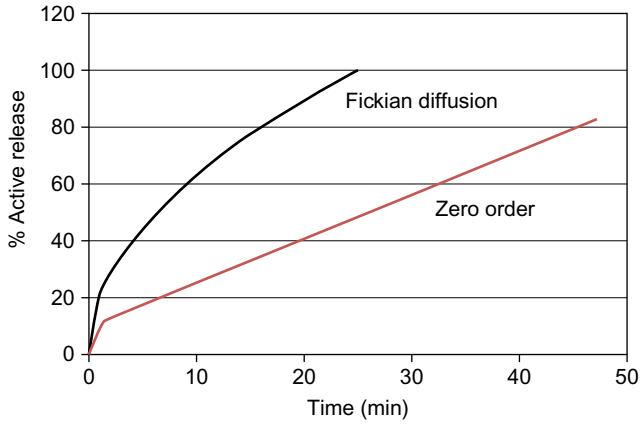

FIGURE 3.3 Schematic release profiles for microencapsulated systems.

3.3 ZERO ORDER OR PSEUDO-ZERO ORDER DIFFUSION MODEL

A zero order diffusion rate is independent of the concentration of the active ingredient. Increasing the concentration will not speed up the rate of release, nor does the reduction in concentration slow the diffusion down. This is counterintuitive. In a zero order diffusion model the hypothesis is that the amount of active loading is infinite. The release amount is so slow as compared to the system that the microcapsule behaves effectively as an unlimited drug source with only a limited rate of release. Zero order release in diffusion is described as the amount released directly proportional to time. As shown in Figure 2.4 (see Chapter 2) and mathematically:

$$C \alpha t$$

$$\text{or } \mathrm{d}C_t/\mathrm{d}t = k_0$$

where C_t is the amount of active ingredient released in time t, and k_0 is a zero order constant. By integration of equation:

$$(C_t - C_0) = k_0 \cdot (t - 0)$$

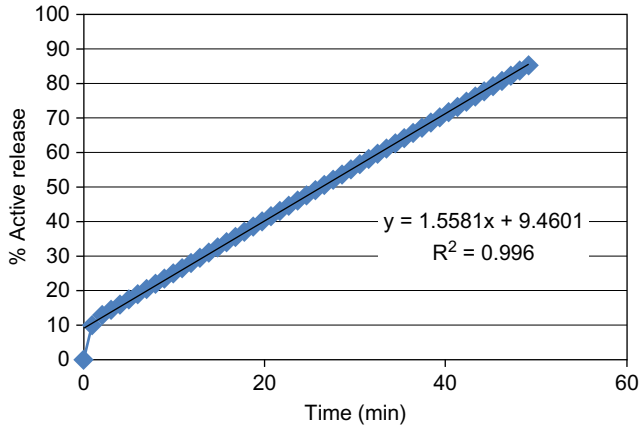

FIGURE 3.4 A practical representation of zero order kinetics of an active releasing from a hydrogel.

where C_o represents the initial release at $t \to 0$ for a fixed volume in which the release is measured:

$$C_t = C_0 + k_0 \cdot t$$

This equation is called the integrated zero order rate law.

Equation (3.3) which represents the zero order kinetics, is presented graphically in Figure 3.4. The figure describes a hypothetical release of an active ingredient by zero order kinetics. One may assume such kinetics in these different scenarios (1) an active insoluble ingredient entrapped in an eroding polymer, (2) ingredient or an active ingredient in hydrogel that requires a mobile phase (hydrating front), or (3) an osmotic release necessary for the active to leave the hydrogel matrix. The *y*-intercept which represents "initial burst," is defined by C_0. As one can see, the percent release, which is presented as $\frac{C_t}{C_{t\to\infty}}$, is a linear function. The common practice is to consider zero order release up to 80%. In most cases, the profile then flattens out as there is inadequate driving force to continue the zero order kinetics.

It must be pointed out that true zero order kinetics is often rare in the food industry, because of the short desirable release time, solubility of the active agent in the matrix, surface activity, and burst release from the microcapsule.

3.4 FICKIAN DIFFUSION MODEL

Fickian diffusion postulates that the diffusive flux J goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the spatial concentration gradient. In terms of one-dimensional spherical coordinates relating to a microsphere morphology, *Fick's first law* is:

$$J = (1/A) \cdot \mathrm{d}C/\mathrm{d}t = -D\,\mathrm{d}C/\mathrm{d}r$$

where J is the diffusion flux described as the amount transferred per unit area per unit time or mass flow of the active ingredient under the assumption of steady state, D is the diffusion coefficient, r is the radius of the designed microcapsule, and A is surface area of the microcapsule. The negative sign in the equation is because diffusion occurs in the direction opposite to that of increasing concentration. It must be emphasized that the equation is consistent only for an isotropic homogeneous medium, where the diffusion properties do not change in other spherical coordinates.

The equation can be simplified with the knowledge that the concentration difference between the inside and outside of the microcapsule is:

$$\mathrm{d}C/\mathrm{d}t = -D \cdot A \cdot (\Delta C)/R$$

where $(\Delta C) = C_{oM} - C_{IM}$, and where C_{oM} is the concentration of the active agent on the outside of the microcapsule while C_{iM} is the concentration of the active agent on the inside of the microcapsule. R is the thickness of the microcapsule.

Comparing equations, one can show that Fickian diffusion will approximate zero order diffusion when:

$$k_0 = DA(\Delta C)/R$$

Thus, for steady state under constant reservoir conditions represented by a high loading, Fickian diffusion can be represented by zero order kinetics. For constant release, a pseudo-zero order rule can be used for a practical approximation. The higher the k_0, the faster the rate of diffusion.

However, as unsteady-state diffusion is most common in microencapsulated systems, the mass transport and diffusion kinetics can be presented by using two concepts: Fick's law and conservation of mass.

In the following subsections, mass transfer in microspheres and microcapsules are considered with different boundary conditions that mimic practical possibilities for industrial systems.

3.4.1 Mass Transfer in a Microsphere Morphology

The mathematics of mass transfer from a solid microsphere into an unbound fluid of infinite sink can be characterized by a simple model of diffusive transport, which occurs between the solid microsphere and the surrounding fluid. For purposes of simplicity, the model can help us calculate the rate of mass transfer, and eventually the rate of change of the radius of the microsphere with time. Such a system can be envisioned as a flavor microsphere that dissolves in saliva in the mouth.

Lewis (1984) presented a simple model of the diffusive transport, which can be constructed in Figure 3.5. The figure describes a microsphere as a radius "a," where the microsphere contains a pure component A. Because there is no concentration gradient inside the microsphere, we only need to consider the mass transport process in the surrounding fluid. We can assume that the problem is spherically symmetric. This means that in a spherical polar coordinate system, described by r, θ, φ-coordinate system, there are no gradients in the polar angular coordinate θ, or in the azimuthal angular coordinate φ.

After, an initial transient steady state is assumed to prevail. Di Matteo et al. (2003), in the example defined in Figure 3.5 and studied here, assume that the rate of dissolution is slower than the rate of diffusion. This implies that the change in size of the microsphere due to mass transfer occurs on a time scale that is very large compared with the time scale for the diffusion for a given radius of the microsphere to reach steady state.

Using steady-state mass transfer through a thickness of Δr:

$$4\pi r^2 J(r) - 4\pi(r+\Delta r)^2 J(r+\Delta r) = 0$$

Dividing by $4\pi(\Delta r)$ and rearranging with limits $\Delta r \to 0$, we get:

$$\frac{d}{dr}(r^2 J) = 0$$

Using Fick's law:

$$J = -D\,\mathrm{d}c/\mathrm{d}r, \quad \text{and where} \quad c = C_0 x$$

$$\frac{d}{dr}x = -K1\frac{1}{C_0 D}\frac{1}{r^2}$$

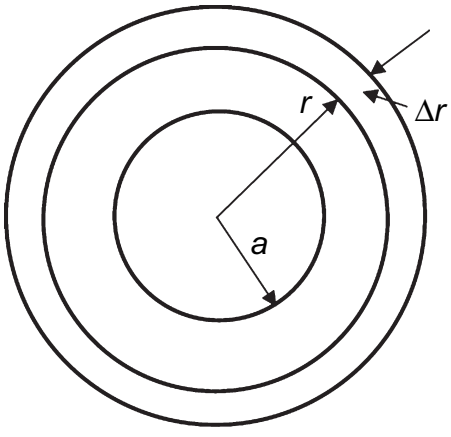

FIGURE 3.5 Diffusion of active in a microsphere morphology.

Integrating:

$$x = -K1\frac{1}{C_0 D}\frac{1}{r} + K2$$

For the microsphere dissolving in the surrounding liquid:

$$x(a) = xo \text{ and } x(r \to \infty) = 0$$

Therefore:

$$K1 = C_0\ D\ a\ x_0 \text{ and } K2 = 0$$

Furthermore, the concentration at $C(r)$ will be:

$$x(r) = x_0\frac{a}{r} \text{ or } C(r) = C_0\frac{a}{r}$$

Or flux can be calculated to be:

$$J = C_0\ D\ x_0\frac{a}{r^2}$$

And the steady-state mass transfer can be calculated as:

$$= 4\pi C_0\ D\ a$$

3.4.2 Unsteady-State Diffusion From a Microsphere

For one-dimensional mass transfer at $0 \le r \le R$ with no angular dependence, the differential equation that defines the mass balance in an unsteady state can be described as:

$$\frac{\partial u}{\partial t} = D\ \frac{\partial^2 c}{\partial r^2}$$

Caretto (2009) considered two cases with the following initial and boundary conditions that allow us to represent a simplified practical case in food applications using microspheres. In a semi-infinite medium:

For a constant surface concentration:

Case 1: Initial condition: $t = 0,\ c(r,\ 0) = c_i$
Boundary condition: $c(0,\ t) = C_s$ and $c(r \to \infty,\ t) = c_i$

For a constant surface flux condition:

Case 2: Initial condition: $t = 0,\ c(r,\ 0) = c_i$
Boundary condition: $-D\frac{\partial c}{\partial r}|_{r=0} = J_o$ and $c(r \to \infty,\ t) = c_i$

The solution to the differential equation can be solved to yield the following solution:

For Case 1, the constant surface concentration boundary condition yields:

$$\frac{(c - c_s)}{(c_i - c_s)} = erf\left(\frac{x}{2\sqrt{Dt}}\right) J_o = D\frac{(c_s - c_i)}{\sqrt{\pi Dt}}$$

For Case 2, the constant surface flux, $J(r = 0) = J_o$:

$$c(r,t) - c_i = 2J_o\sqrt{\frac{t}{\pi D}}\exp\left(\frac{-r^2}{4Dt}\right) - \frac{J_o r}{D}\ erfc\left(\frac{r}{2\sqrt{Dt}}\right)$$

where $erfc(W) = 1 - erf(W)$.

The solution for constant surface concentration (Case 1) can find applications where the flavor releases from a typical spray-dried microsphere as a flavor microsphere dissolving in saliva in the mouth. Constant surface flux (Case 2)

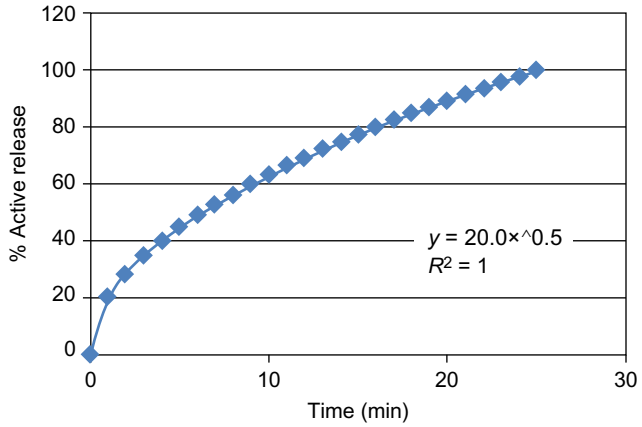

FIGURE 3.6 A practical representation of Fickian diffusion of an active releasing from a matrix microsphere in a one-dimension system.

can find applications in food packaging, where an antimicrobial active is required for release at a constant flux to maintain antimicrobial effectiveness.

Subramanian's (2010) assessment of a constant surface concentration condition—Case 1—can be plotted as a representation of a practical example of Fickian diffusion of an active ingredient released from a solid matrix microsphere. Figure 3.6 shows the shape of the curve where significant active is released early, and as the concentration of the active in the microsphere reduces, so does the driving force. This results in a flattening of the profile as it approaches complete release.

3.4.3 Mass Transfer in a Microcapsule Morphology

Mass transfer from a microcapsule into an unbound fluid of infinite sink can be characterized by a simple model of a reservoir and a surrounding sink media separated by a wall of spherical geometry.

For purposes of simplicity, the model can be calculated using Fick's law, where the net particle flux is:

$$J(x) = -D \cdot \mathrm{d}c/dx$$

based on conservation of mass.

In spherical coordinates, consider a microsphere with inner and outer radii r_1 and r_2, respectively. Using Fickian diffusion and the mass equation (Figure 3.7) in spherical coordinates where azimuthal and polaroid symmetry becomes one dimension, a steady state equation can be written as:

$$\frac{d}{dr}\left(r^2 \frac{dc}{dr}\right) = 0$$

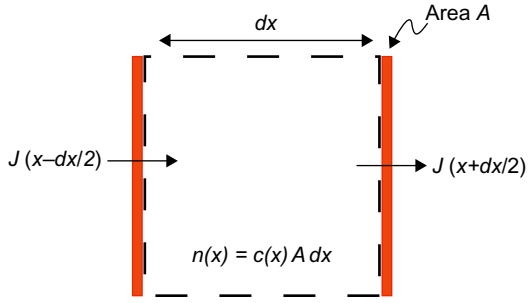

FIGURE 3.7 Diffusion of active in a microsphere morphology.

Integrating:

$$c(r) = A\frac{1}{r} + B$$

Using concentration inside the microcapsule to be c_1 at $r = r_1$ and the concentration outside the microsphere is c_s, the differential equation can be solved with the boundary conditions to yield:

$$c(r) = J_1 r_1^2\left(\frac{1}{r} + \frac{1}{r_1}\right) + c_s$$

Similar to the Case 2 condition at a constant surface flux in an unsteady-state solution, the above equation can be used to determine the size of the reservoir microcapsules of radius r_2, where the antimicrobial active needs to maintain an effective concentration for a minimum required constant flux J_1 (Ruiz-Lopez et al., 2011). One also concludes that rate diffusion can be achieved by increasing the diffusion coefficient, surface area, and concentration driving force, while it can be decreased by increasing the thickness of the microcapsule wall.

3.4.4 Analogy to Heat Transfer

Heat transfer analogies can be derived similarly:

$$\frac{d}{dr}\left(r^2\frac{dT}{dr}\right) = 0$$

Integrating:

$$T(r) = A\frac{1}{r} + B$$

Using flux inside the microcapsule to be J_1 at $r = r_1$ (Neumann boundary condition) and the concentration outside the microsphere is T_2 at $r = r_2$ (Dirichlet boundary condition), the differential equation solved by Roy and Sengupta (1991) can be solved with the boundary conditions to yield:

$$T(r) = J_1 r_1^2\left(\frac{1}{r} + \frac{1}{r_2}\right) + T_2$$

Thus, the unknown temperature inside the microcapsule can be calculated as:

$$T_1(r) = J_1 r_1^2\left(\frac{1}{r_1} + \frac{1}{r_2}\right) + T_2$$

Obviously, this equation is designed for wall thicknesses that are reasonably large and when r_1 approaches r_2, T_1, and T_2.

Let us look at practical applications of such a boundary condition. Say we have active material that exists in a solid form but melts at temperature T_1, which is desired for release by diffusion where the surrounding media temperature is T_2. In essence, the heat J_1 can be calculated when r_1, r_2, T_1, and T_2 are close. In such a case, we can envision a triggered melt release system where the wall melts at temperature T_1.

Heat transfer problems are also used in terms of creation of microcapsules. For example, quenching of the microspheres at a fixed temperature in a cooling tower or refrigerated system can be expressed as an unsteady-state solution as follows:

$$\frac{1}{\varphi}\frac{\partial T}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial T}{\partial r}\right)$$

where φ is the thermal heat coefficient of the wall materials and the surrounding temperature is set below $T \leq T_0$, and $\frac{\partial T}{\partial r} = 0$ at $r = 0$.

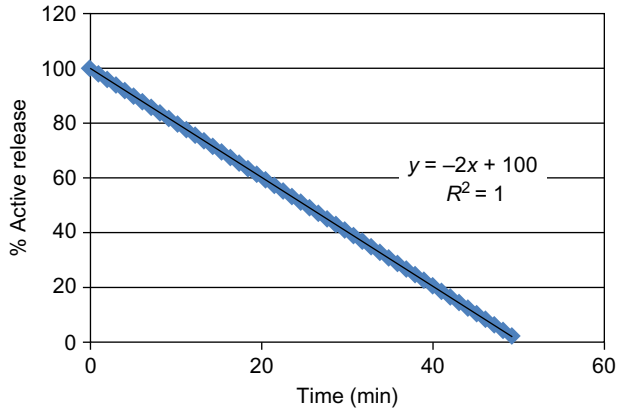

FIGURE 3.8 Concentration gradient in first order diffusion.

3.5 FIRST ORDER DIFFUSION MODEL

First order diffusion depends on the concentration of the active agent alone, where other competing mechanisms are zero order or do not impact the release of the active agent. The rate law for a first order diffusion is:

$$dC/dt = k_1 C$$

where k_1 is the first order rate constant, which has units of 1/s.

The integrated first order rate gives:

$$\ln C - \ln C_0 = -k_1 t$$

A plot of ln C versus time t gives a straight line with a slope of $-k$ in Figure 3.8.

3.6 CONCLUSION

Because the concept of diffusion is fundamental to the part of the release system for which we plan to design our microencapsulated formulations, we have singled out mass transport from the concept of heat transfer to establish an understanding of complex systems. To gain a better insight into mass transfer problems of microencapsulated food product systems, a combination of experimental investigations and mathematical modeling is needed. The model used in this chapter should be used as a predictive tool and should not be in lieu of experiments to assess the release profile or product stability. Establishing a foundation in mass transfer concepts will allow the engineering intuition needed to design microencapsulated systems to be built.

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Part III

Process Technologies in Microencapsulation

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Chapter 4

Overview of Microencapsulation Process Technologies

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4.1 INTRODUCTION

During the twentieth century, encapsulation methods and techniques evolved to produce capsules with varying size, morphology, payload, and materials. Dozens of processes are now available for preparing microcapsules, while no single process exists to capture the wide variety of capsule permutations and core−shell combinations. Processes are available to prepare matrix particles and core−shell capsules, capsules with sizes from tens of nanometers to millimeters, and capsules with solid, liquid, or gas cores. The process chosen is highly dependent on the capsule design and function. Common processes often encountered in the food industry are spray drying, fluid-bed coating, and complex coacervation. While these processes account for a large number of the encapsulated products on the market, they do not fully represent the complete array of available technologies (Kondo, 1979; Sparks, 1989; Gibbs et al., 1999; Vilstrup, 2001; Lakkis, 2007; Garti and McClements, 2012).

The availability of multiple processes offers capsule engineers options for each aspect of the capsule. Small microspheres can be prepared via spray drying, while large liquid-filled core−shell capsules may be prepared using annular jet atomization. An even coating may be applied to solid particles with fluid-bed coating to form a core−shell morphology, while complex coacervation is employed to generate small liquid-filled core−shell capsules. The variety of processes also accommodates a range of production capacities from milligrams per hour to tons per hour, with contract or toll manufacturers available for several of the processes. For development support, several of the processes are also available on a lab scale with commercially available specialized equipment.

The numerous processes available may also be challenging for capsule development. A particular process may prove fruitful in the development stage on a laboratory scale, while capital equipment costs, contract manufacturer availability, or maturity of the process may be cost prohibitive. Alternatively, microcapsule engineers may only have access to one or two processes, limiting the scope of size, materials, payload, and production capacity.

Despite the challenges associated with process selection, the numerous process technologies provide a wide spectrum of options for designing and producing microcapsules. Knowledge of each process is recommended to support informed decisions for selecting the appropriate process. This chapter and the subsequent chapters provide introductory information for the most common processes.

4.2 PROCESS COMPONENTS

The primary components of a microcapsule are shown in Figure 4.1 and include the core material and shell materials. In a microsphere, the shell material is also referred to as the matrix. The process components are core formation, shell or matrix formation, and shell or matrix solidification. Core formation can occur prior to or during the encapsulation process. For the encapsulation of solid particles, core formation typically takes place prior to encapsulation. Grinding,

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00004-2

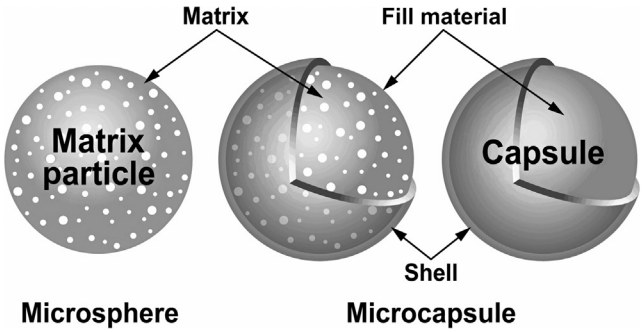

FIGURE 4.1 Encapsulation morphologies. (Copyright © 2014 James Oxley.)

milling, or granulation may be required to generate a core material that is suitable for encapsulation. If the core material is soluble in the encapsulating matrix or solvent used for depositing the encapsulating material, preprocessing may not be required for the core material. When encapsulating a liquid, core material preparation typically consists of emulsification to form droplets in a two-phase system. If the physical properties of the core material and process permit, a solid core material may be melted and processed as a liquid. Viscosity modifiers or surfactants can be added to a liquid core to facilitate the incorporation of a core material into a microcapsule.

The encapsulating matrix or shell material is generally applied as a liquid to permit enrobing of the core material. The interfacial tension between the core and shell material must favor the wetting of the core material by the shell material. The shell material may be a molten coating material, a solution of dissolved shell material, a suspension of shell material and binder, or a composition of multiple dissolved and suspended components. Viscosity must be low enough for the shell material to flow around the core material. Furthermore, the miscibility of the core and shell material must be minimized to prevent blending of the two phases prior to solidification of the shell material. Some of the encapsulation processes, such as coextrusion or spray coating, can tolerate some miscibility between a shell material solvent and core material. However, the encapsulated product stability and shell integrity will be degraded if the solidified shell material has some solubility in a liquid core.

Once the core material is surrounded by the shell material, the liquid shell material must be solidified to form the microcapsule. Solidification mechanisms include solvent evaporation, gelation, freezing, or chemical reactions. Solvent evaporation is one of the most common shell solidification mechanisms, especially when using water as the solvent. Shell material is dispersed or dissolved into an aqueous solution, followed by the application of heat to remove the water once the solution has encapsulated the core material. The resulting precipitation of dissolved solids results in the formation of a solid shell. Common examples include starch or gum acacia. Other volatile solvents can be used when appropriate.

Thermal solidification is used with gelling or freezing shell materials. A core material is dispersed, dissolved, or emulsified into an aqueous phase containing a gelling agent heated above the gelling temperature. After surrounding the core material and after cooling, the gel solidifies and encapsulates the core material. Additional water removal may be required depending on the final application of the microcapsule. An alternative gelation mechanism is ionic gelation. Rather than using heat to induce the formation of a gel, ion exchange is the trigger for solidification of the gelling material. Alginate is the most common example of this system, where water soluble sodium alginate is hardened with calcium ions to form a water insoluble calcium alginate gel.

Freezing a shell material is a common technique for solidifying a shell material without the use of water. A core material can be dissolved, suspended, or emulsified in a molten shell material, followed by exposure to a cooler environment to solidify the molten shell material. Common examples include fats, waxes, or lipids where the melt temperature is above 50°C for processing. Lower melting coating materials can be used, but may require climate controlled storage after formation of the encapsulated products.

The final shell solidification mechanism is chemical, requiring the formation of bonds between shell material components or noncovalent intermolecular interactions to form a solid shell. The lipid bilayers of liposomes are formed through hydrophobic and hydrophilic interactions of lipid amphiphiles within aqueous solution (Reineccius, 1995). Polyanions and polycations, such as gum acacia and gelatin, interact to form a complex coacervate shell. A third example is the crosslinking of proteins, such as gelatin, with gluteraldehyde or transglutaminase (Reineccius, 1995; Fuchsbauer et al., 1996).

4.3 PROCESSES

There are multiple encapsulation processes available to combine the shell and core materials into microcapsules. The four general categories are atomization, spray coating, coextrusion, and emulsion-based processes. Each general technology offers a unique method of forming an encapsulated product, with a variety of advantages and disadvantages. The existence of each technique is required to produce the range of particle sizes and morphologies that are commercially available. The first three general processes rely on physical changes to form particles, primarily through drying, gelling, or freezing of a shell material. The fourth category, emulsion-based processes, utilizes chemical-based solidification methods as a major shell solidification method.

4.3.1 Atomization

Atomization is the formation of an aerosol or suspension of small droplets in a gas phase. For the purposes of encapsulation, the gas phase is generally air and the small droplets contain a mix of shell material and core material. Figure 4.2 shows an illustration of the process where a homogeneous mixture of core and shell materials is atomized through a nozzle. This process is the foundation of several more specific processes including spray drying, spray chilling, spray congealing, rotating disc atomization, electrospraying, and prilling (Masters, 1991). The atomized mixture may be core material dispersed in a solvent (spray drying), typically water for food applications, with a dissolved or suspended shell material. Alternatively, the mixture may consist of core material dispersed in a molten shell material (spray congealing, prilling) or thermally gelling mixture (spray chilling). Additional details about atomization are available in Chapters 5 and 6.

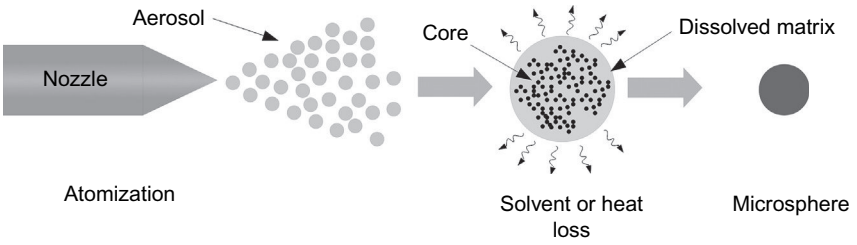

FIGURE 4.2 Basic illustration of atomization process. (Copyright © 2014 James Oxley.)

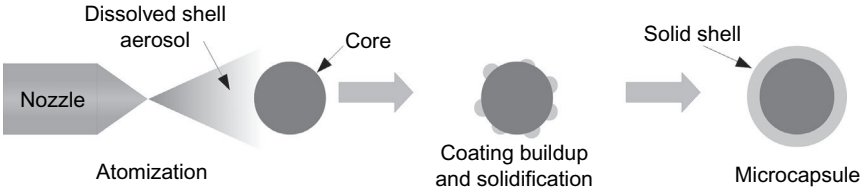

FIGURE 4.3 Illustration of spray-coating process. (Copyright © 2014 James Oxley.)

4.3.2 Spray Coating

Spray coating is the deposition of atomized droplets onto a solid particle. This process is practiced in many forms and under many names, including fluid-bed coating, Wurster coating, air suspension coating, granulation, and pan coating (Teunou and Poncelet, 2002; Sahni and Chaudhuri, 2012). A general illustration of the process is shown in Figure 4.3. Solid core particles are suspended in air (fluid-bed coating) or rotated in a drum (pan coating), followed by the introduction of an aerosol containing shell material. The aerosol may be solvent based or a melt material. The droplets wet and spread around the solid core particles, followed by solidification through drying or congealing. Subsequent droplets wet the surface and dry, resulting in a buildup of shell material around the solid core. The spray-coating technique is further described in Chapters 7 and 9.

4.3.3 Coextrusion

An alternative form of atomization is annular-jet atomization, also referred to as coextrusion. An illustration of this process is shown in Figure 4.4. Using a concentric nozzle system, core–shell droplets are first formed through axisymmetric breakup of an annular jet. The outer shell droplet then solidifies to form a microcapsule shell. Multiple

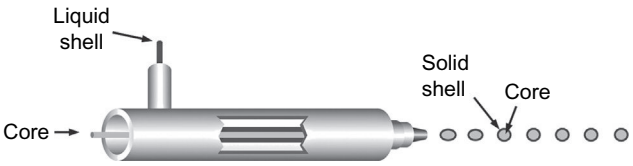

FIGURE 4.4 Illustration of coextrusion process. (Copyright © 2014 James Oxley.)

configurations are available for coextrusion, including stationary coextrusion, vibrating nozzle coextrusion, centrifugal coextrusion, submerged nozzle coextrusion, electrohydrodynamic (EHD) coextrusion, or flow focused coextrusion (Brandau, 2002; Loscertales et al., 2002; Berkland et al., 2004). More information about this technique is available in Chapters 10 and 11.

4.3.4 Emulsion Based

The first three processes just defined all require atomization to form droplets for the formation of microspheres, formation of microcapsules, or coating solid particles. An alternative method of droplet formation is the use of emulsions, forming droplets in a two-phase liquid system. The first part of this process is the emulsion formation, dispersing a core material liquid phase into an immiscible liquid phase that may contain dissolved shell material. Adjustments are then made to the two-phase system to induce shell formation around the dispersed phase droplets. Oil-in-water is the most common two-phase system used for encapsulation. Variations of this process include simple coacervation, complex coacervation, *in situ* polymerization, interfacial polymerization, liposomes, layer-by-layer emulsions, micelles, solvent evaporation, in-water drying, solid lipid nanoparticles, and sol-gel chemistry (Kondo, 1979; Reineccius, 1995; O'Donnell and McGinity, 1997; Scher, 1999; Hench and West, 2002; Brown et al., 2003; Guzey and McClements, 2006). Not all of these systems are suitable for use in food, but may find applications in relevant areas such as agriculture or food packaging. A general illustration of the emulsion-based process is shown in Figure 4.5. Additional information about emulsion-based techniques is available in Chapters 12–15.

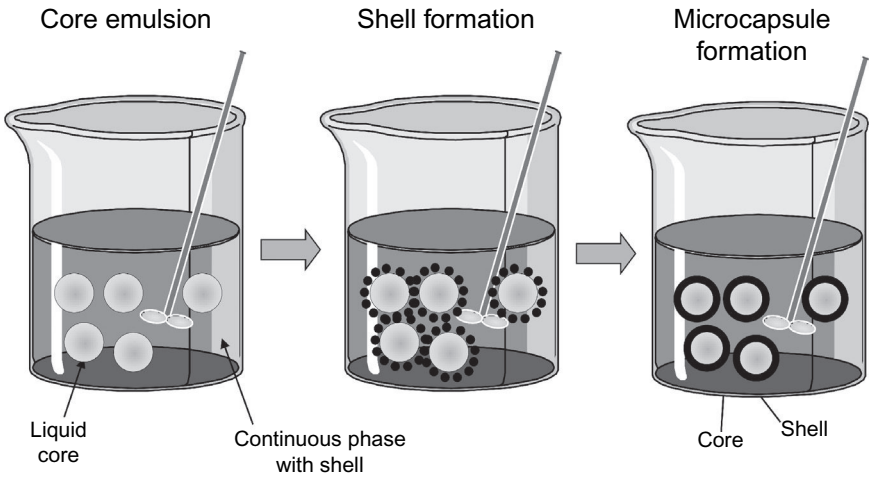

FIGURE 4.5 Illustration of emulsion-based process. (Copyright © 2014 James Oxley.)

4.3.5 Other

A variety of encapsulation processes exist beyond the four general categories described. Some of the common processes include extrusion, molecular complexation, and absorption/desorption systems. Extrusion, described further in Chapter 8, is the processing of a hot melt through a single or twin screw system to extrude a formed shape of the cooling melt (Porzio, 2004). Core material is dispersed into the hot melt prior to extrusion for matrix encapsulation. Subsequent milling of the extruded material results in the formation of matrix particles, as illustrated in Figure 4.1. Molecular complexation is commonly associated with cyclodextrins (Reineccius et al., 2003). Cyclodextrins are cyclic oligosaccharides with a hydrophobic pocket capable of reversibly binding (e.g., molecular complexation) hydrophobic molecules for sequestration and

controlled release. Finally, inorganic materials (matrix) may be used as sorbents for the delivery and controlled release of active ingredients (core material). Common sorbents include clay (e.g., montmorillonite, kaolin) and other aluminosilicates (e.g., zeolites). Further information about microporous materials for ingredient delivery is described in Chapter 25. The process of encapsulation using cyclodextrins or inorganic absorbents generally consists of exposing the sorbent to the active ingredient followed by removal of excess unabsorbed material.

4.4 COMPARISONS

The multiple processes available for encapsulation offer a wide range of capabilities for producing particles with various sizes, morphologies, payloads, materials, and costs. Each process has a unique set of strengths and weaknesses related to these variables, justifying the need for multiple encapsulation processes. Selection of the proper process begins with understanding the objectives for developing a microcapsule (Chapter 2) and the specifications for the available processes. Feasibility of encapsulated product development is then determined by matching the objectives with potential processes and materials (Part IV).

4.4.1 Size

Encapsulation is a proven technology for preparing particles from approximately 10 nm to 10 mm. The size of the particle is crucial to both the final application of the encapsulated material and the product in which it will be incorporated. Criteria such as mouth-feel, beverage suspension, product homogeneity, and mechanical stability of the particle all contribute to selection of a suitable final encapsulated product size. Additionally, the wide range of potential food and beverage products using encapsulated materials requires that multiple technologies be available to meet the various particle design requirements. The breadth of this size range relative to common encapsulation techniques is illustrated in Figure 4.6. The illustration is an approximate guide to the size range limits achievable with the techniques on a commercial scale. The size ranges may shift higher or lower depending on material properties, operating conditions, equipment specifications, and other process parameters. Furthermore, ongoing research and development for each process continues to expand the feasible size range limits.

The physical processes for atomization, spray coating, and coextrusion are listed in the top half of the graph, while the emulsion-based techniques are listed in the bottom half. With the exception of EHD coextrusion, a trend is evident that physical-based encapsulation techniques are best suited for the formation of capsules over 10 μm. This is due to the physical limitations of the equipment used to form the coextruded or aerosolized droplets for each process. The emulsion-based techniques are better suited for capsule sizes below this range, stretching into submicron sizes with the proper emulsification techniques. High shear processes, such as high pressure homogenization and ultrasound, combined with surfactants and viscosity modifiers, can readily generate submicron droplets for encapsulation (Donsi et al., 2012).

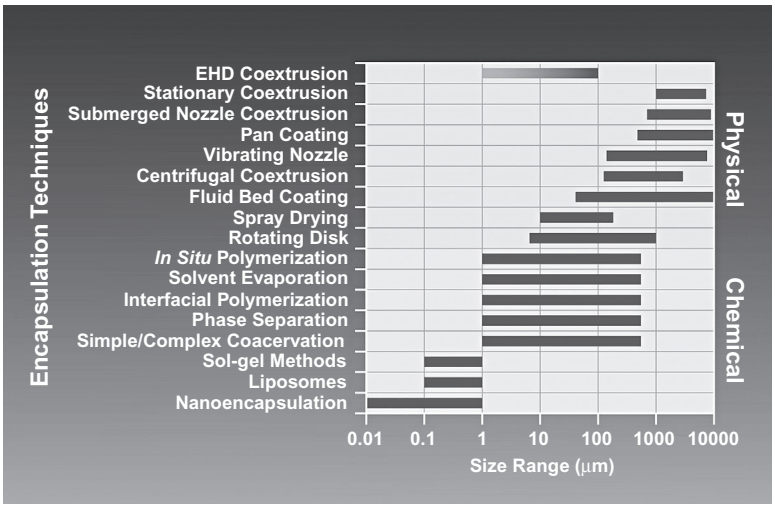

FIGURE 4.6 Size comparison of common encapsulation processes. (Copyright © 2014 James Oxley.)

4.4.2 Morphology

In addition to size options, each process offers variation in the encapsulated product morphologies that can be produced. Morphology, as shown in Figure 4.1, can influence release rate, core material protection, and payload capacity. Microspheres commonly generate a first order release, provide less protection for core material located at or near the surface of the microsphere, and carry less core material. Microcapsules can provide a burst release mechanism, uniform protection for a core material, and can carry a higher payload with less shell material. Table 4.1 summarizes the processes, their ability to prepare microspheres or microcapsules, and the core material phase. While gases can be encapsulated using many of the processes described, solids and liquids are the primary phases encapsulated for food products. Atomization and most of the emulsion-based processes are capable of encapsulating both solid and liquid core materials. Spray coating and coextrusion are limited to core materials that are solid and liquid, respectively. However, liquids can be encapsulated using spray coating if the liquid is first encapsulated within a solid matrix. Similarly, solids can be encapsulated using the coextrusion techniques if the solids are first dispersed into a liquid carrier phase. The disadvantage of these approaches is the dilution of core material and lower final payload concentration.

The atomization processes are best for preparing microspheres. The main exception to this is the use of rotating disc atomization to overcoat solid particles (Johnson et al., 1965). A more rare exception is the use of unique formulations

TABLE 4.1 Process Morphology Comparisons

Process	Microsphere	Microcapsule	Phase
Atomization	Yes	No	Solid/liquid
Spray drying	Yes	No[a]	Solid/liquid
Spray chilling/congealing/prilling	Yes	No[a]	Solid/liquid
Rotating disc	Yes	Yes	Solid/liquid
Spray coating	No	Yes	Solid
Fluid-bed/Wurster coating	No	Yes	Solid
Pan coating	No	Yes	Solid
Granulation	Yes	No	Solid
Coextrusion	Yes	Yes	Liquid
Stationary nozzle	Yes	Yes	Liquid
Vibrating nozzle	Yes	Yes	Liquid
Centrifugal nozzle	Yes	Yes	Liquid
Submerged nozzle	Yes	Yes	Liquid
EHD nozzle	Yes	Yes	Liquid
Emulsion	Yes	Yes	Liquid
Simple/complex coacervation	No	Yes	Solid/liquid
In situ polymerization	No	Yes	Solid/liquid
Interfacial polymerization	No	Yes	Liquid
Solvent evaporation	Yes	No[a]	Solid/liquid
Liposomes	No	Yes	Liquid
Sol-gel	Yes	Yes	Solid/liquid
Extrusion	Yes	No	Solid/liquid
Molecular complexation	Yes	No	Liquid

[a]*Some rare unique formulations capable of forming core–shell capsules.*

capable of forming discrete core−shell droplets, and subsequent capsules, after atomization (Liu et al., 2011). Spray coating is used to prepare core−shell microcapsules with a solid core. An exception is the use of spray coating to facilitate granulation, or agglomeration of smaller particles into a larger particle. Coextrusion was originally developed as an alternative for spray drying to prepare core−shell morphology capsules and remains the primary function of this technique. However, the outer nozzle of a coextrusion system can be disabled to allow for the preparation of microspheres. All of the emulsion methods are suitable for the encapsulation of immiscible liquids, and most can also be used for the encapsulation of solid particles. Similar to the atomization processes, extrusion can encapsulate both solids and liquids. Finally, molecular complexation requires the core material to be liquid or, more preferably, dissolved in a liquid to promote complexation.

4.4.3 Payload

The payload resulting from an encapsulation process is the amount of core material encapsulated within a shell or matrix. A payload amount can range from less than 1% to approximately 99%, depending on the particle morphology, encapsulation materials, and process. Particle morphology has the greatest influence on payload. As the morphology becomes more complex, such as combining a microsphere within a microcapsule, the loading decreases. A microsphere, as shown in Figure 4.1, is the simplest morphology and will commonly accommodate payloads of 10 to 30%. As seen in the figure, the payload is dispersed homogeneously through the encapsulating matrix. Lower loadings are feasible, but may be cost prohibitive as more encapsulated material is required to achieve a set concentration of active ingredient within the finished product. Higher loadings, while feasible, have significant disadvantages. The primary problem associated with higher loadings in a microsphere is decreased protection of the encapsulated ingredient. As the concentration of core material increases, the amount of protective matrix material decreases. Furthermore, the amount of core material at or near the surface of the microsphere will increase, resulting in faster degradation from, or release into, the surrounding environment. As the payload in a microsphere surpasses 50%, the risk of inverting the morphology also increases. Depending on the phase of the core material and the interfacial tension between the core and matrix phase, the intended core material may encapsulate the shell material resulting in an inverted microsphere and no protection or release control of the core material. Despite the disadvantages of increasing payload in a microsphere, higher payloads may be desirable for situations such as granulation, quick release, or minimal environmental protection of a core material.

The core−shell microcapsule morphology can accommodate a significantly larger payload than the microsphere morphology. Payloads up 99% are feasible, while lower payloads can be more difficult to achieve depending on the encapsulation process. The payload is directly related to the shell thickness of a microcapsule. Higher payloads require a thinner shell, as shown in Figure 4.7, while lower payloads will have a thicker shell. Although a thinner shell affords a higher payload, it also provides less mechanical strength and a thinner diffusion barrier against the surrounding environment. Also evident from Figure 4.7 is the observation that small linear changes in shell thickness have a more significant impact on microcapsule payload. For example, a 100 μm capsule with 90% payload will have a 2 μm thick shell, approximately. Increasing the shell thickness to 10 μm, which appears as a small 8% change relative to the diameter of the microcapsule, reduces the payload to 50%.

The combination of a microsphere and microcapsule is shown as the middle morphology in Figure 4.1. The addition of a shell to a microsphere can alleviate some of the problems encountered with incorporating higher loadings into a

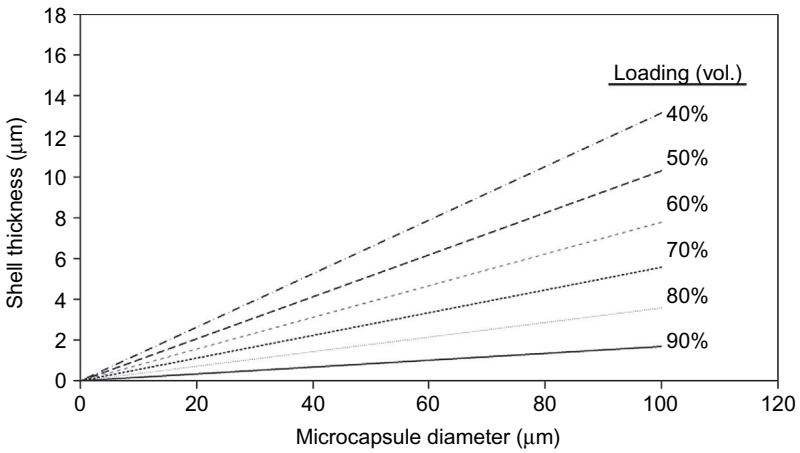

FIGURE 4.7 Payload comparison: loading versus shell thickness. (Copyright © 2014 James Oxley.)

microsphere. This continuous shell can also act as a triggered release barrier prior to enabling the release properties of the encapsulated microsphere. However, the addition of an outer shell can negate the payload gains and further reduce the payload depending on the thickness of the shell. Additional payload reductions occur as the core material is diluted with additives, such as antioxidants or plasticizers.

The relationship between payload capabilities and processes follows the possible morphologies for each process. The atomization techniques will generally yield lower payloads of 10 to 30%. Spray drying can accommodate higher loadings through adjustment of the core/shell ratio in the spray-drying solution. Spray congealing or prilling of hot melts is limited to lower loadings, especially with solid core materials, because the feed slurry for atomization must remain at a low viscosity to form an aerosol. Spray coating offers a wide range of payloads, using the exposure time of the solid core material to the spray coating to adjust the shell thickness and corresponding payload to the desired levels. Similarly, coextrusion can be tuned to generate capsules with a wide range of payloads, limited by the nozzle geometries and shell materials. A lower critical amount of shell solution is required to ensure axisymmetric breakup of a coaxial jet into core−shell droplets. The use of a solvent-based shell material will facilitate the formation of a thin shell through evaporation of the solvent while affording sufficient volume to first form the core−shell droplet. Finally, the emulsion-based processes offer the highest potential payloads. Shells can be prepared only a few molecules thick, resulting in capsules with loadings over 99%. Micelles or capsules prepared with layer-by-layer shells are examples of emulsion-based systems that can accommodate the highest loading (Guzey and McClements, 2006).

4.4.4 Materials

Each encapsulation process has limitations related to the potential shell and core materials used in a capsule formulation. The limitations of each process are related to the use of solid or liquid core materials and were previously summarized in Table 4.1. Further limitations arise from the properties of the core and shell materials, including melting points, boiling points, glass transition temperatures (T_g), interfacial tension, solubility, and reactivity. Atomization can utilize the matrix solidification mechanisms of evaporation, gelling, or congealing for the shell material. Therefore, shell materials can be used that are gelling agents, hot melts with a high melting point and T_g, or materials that are soluble or suspended in volatile solvents. The core material must have lower volatility than the solvent to prevent evaporation during the shell hardening stage of the process, or loss during the gel or hot melt preparation process.

Similarly, spray coating uses the evaporation or congealing mechanisms to deposit shells onto solid particles. Additional properties to consider are wettability of the shell material onto the core particles and solubility of the core material in the shell material solution, which may result in some extraction of the core material into the final dried shell (Sundberg and Durant, 2003). Due to long exposure times of the core material to heat during the spray-coating process, especially when using evaporation to deposit a coating, the use of core materials with volatile components should be avoided.

Building on the shell material considerations for atomization and spray coating, coextrusion adds the consideration of reactivity between the core and shell materials. While reactivity can be a concern during atomization and spray coating, potential interference with the coextrusion encapsulation process is more likely to occur. An initial reaction between components during spray atomization will occur when the initial components are mixed prior to atomization. The core material for spray coating is in a solid state, which will slow potential reactions between the core and shell material. Because the core material for the coextrusion process is liquid, and likely remains liquid after encapsulation, precautions must be taken during shell material selection to avoid problematic chemical reactions between the two capsule components.

The emulsion-based encapsulation processes present the greatest restrictions for materials used in the encapsulation process. The most prevalent restriction applies to the common use of an oil-in-water emulsion. Reverse phase or double emulsion systems can be used to encapsulate aqueous solutions, but their stability, payload, or difficulty of preparation often negates the benefits of having an aqueous core. Therefore, an oil core is the most widely used with emulsion-based processes for techniques such as complex coacervation. Aqueous cores are more often used with liposomes or solvent evaporation capsules. The shell materials for emulsion-based processes are closely tied to the particular process. For example, complex coacervation is associated with the use of gelatin and gum arabic for formation of the shell (Kondo, 1979). *In situ* polymerization results in the formation of a poly(urea-formaldehyde) or poly(melamine-formaldehyde) shell, while interfacial polymerization can be used to form polyamide, polyamine, polyurea, or polyurethane shells (Scher, 1999). Liposomes are limited to phospholipid materials, and micelles are limited to amphiphilic molecules for formation. Solvent evaporation can accommodate a larger number of materials, comparable to the atomization techniques, presuming they are insoluble in the continuous phase used for the process.

4.4.5 Production Scale

The production scale of a process is an important factor when considering an encapsulation process. The major processes described are available on an industrial scale with varying production capacities. A key difference in the processes on a large scale is their operation in continuous production or batch production. Atomization and coextrusion can continuously produce encapsulated material on a large scale. While process limitations may exist that require batch processing, such as preparing the feed stocks or postproduction processing, the fundamental encapsulation step of atomization and coextrusion will continue as long as core and shell materials are fed into the equipment. Atomization processes are capable of producing material at rates up to 30 ton/h (Niro, 2012). Large-scale coextrusion systems are capable of producing capsules up to at least 1 ton/h (Brandau, 2002). Conversely, spray coating and emulsion-based processes are traditionally batch processes, requiring starting and stopping of the process to produce and collect microcapsules. Spray coating has evolved to include industrial-scale continuous fluid-bed coating, though a large portion of the commercial-scale units continue to operate as batch processes. Batch spray coaters can have capacities up to 1 ton/batch, while continuous spray coaters can operate at a capacity of 1 ton/h (Teunou and Poncelet, 2002; Wysshaar, 2012). Of the emulsion-based processes, solvent evaporation and liposomes are the two processes with continuous production operations (Barnadas-Rodriguez and Sabes, 2001).

4.4.6 Cost

The cost of operating an encapsulation process can vary significantly, from tens of cents per kg to tens of dollars per kg. Multiple parameters influence cost, such as the formulation, quantities, particle size, payload, and collection. In general, the physical processes and, more specifically, the continuous processes are the least expensive to operate. Cost of encapsulation production rises as particle size decreases, payload decreases, and morphology complexity increases. Figure 4.8 illustrates a comparison between some of the common encapsulation process, approximately placing each process on a scale of complexity versus capacity. Potential cost of an encapsulation process rises as the capacity decreases and complexity increases. The atomization processes offer the highest capacity (approximately 30 ton/h) with minimal complexity, while a process such as EHD coextrusion is currently a slow process (approximately 5 g/h) with multiple components to consider. Each process can move throughout the graph depending on the specifics of the formulation and process parameters. For example, the production capacity of spray drying may be significantly diminished if preparing small particles, $<10\,\mu m$, using a very dilute solution of shell and core materials, such as $<5\%$ solids. Complexity is also increased due to the difficulties involved with solvent reclamation and collection of small particles. Conversely, a spray-congealing application for preparing larger particles, $>500\,\mu m$, from a hot melt will have greater production capacity and simplicity. When using a hot melt, all of the feed material is incorporated into the final

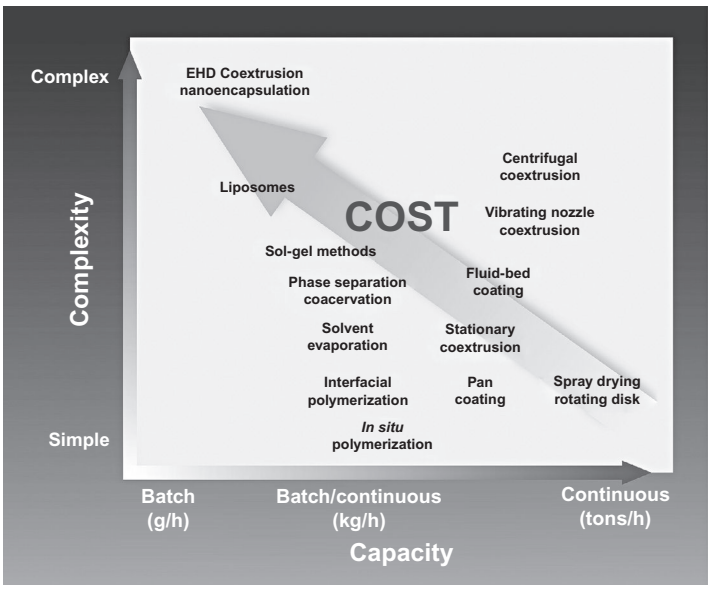

FIGURE 4.8 General process comparison. (Copyright © 2014 James Oxley.)

product, which translates to a higher production capacity for the same flow rates when comparing dissolved spray-dried formulations. The process is also simplified through the use of an ambient temperature collection chamber and simplified particle collection technique, as particles >500 μm are easier to collect.

4.5 EMERGING PROCESSES AND TRENDS

The major encapsulation processes of atomization, spray coating, coextrusion, and emulsion-based systems were developed and commercially implemented many decades ago. These processes now serve as the foundation for the development of new processes to solve emerging encapsulation challenges. Market and product trends are pushing the limits of encapsulation processes, leading scientists to modify existing processes or develop new processes. The trends of encapsulation processes are focused on reduced capsule size, reduced production costs with higher capacity, reduction in the use of solvents or other potentially harmful chemicals, and generation of narrow size distribution particles. The academic and patent literature contains numerous examples of encapsulation process development. Four examples follow for each major process category.

As the application of nanoparticles becomes more prevalent, the ability to readily produce nanoparticles and nanocapsules is increasingly important. Standard atomization techniques are not feasible for producing nanoparticles due to limitations of both the traditional atomization processes and collection techniques (Masters, 1991). To meet current demands, a laboratory-scale benchtop spray dryer was developed by BÜCHI Labortechnik AG and made commercially available as the B-90 Nano Spray Dryer (Li et al., 2010). This equipment is suitable for making nanoparticles down to 300 to 500 nm with recovery over 90%, depending on the formulation. While the production rates are low and scale-up is not yet fully realized, the public availability of this technology for research purposes will accelerate nanocapsule development and transition to a larger scale.

Spray coating, and specifically fluid-bed coating, was initially developed as a batch process. Recent advances in processing technology have resulted in the broad adaptation of continuous fluid-bed coating for higher production capacities (Teunou and Poncelet, 2002). Additional space for advancement of this technology is available for overcoating smaller particles, less than 50 μm in diameter.

The future of the coextrusion process is focused on preparing smaller microcapsules. Current large-scale commercial coextrusion processes have an approximate lower capsule size limit of 150 to 200 μm. The emulsion-based processes can produce core−shell capsules below this size range, but at the expense of a continuous technique and more limited shell material choices. Therefore, multiple research and development efforts have focused on the modification of the coextrusion process to produce smaller core−shell microcapsules. The most successful developments include the use of EHD, vibration, and flow focusing (Loscertales et al., 2002; Berkland et al., 2004; Martín-Banderas et al., 2005). These developments have evolved into the commercial companies Yflow, Orbis Biosciences Inc., and Ingeniatrics Tecnolgías, respectively.

While emulsion-based processes can be used to prepare low micron or submicron capsules, size distribution is often difficult to control. Several recent advances have focused on the formation of monodisperse or designer emulsions. Membrane emulsification was recently commercialized by Nanomi and Micropore Technologies Ltd. for the preparation of monodisperse droplets, which can then be encapsulated using one of the numerous emulsion-based techniques previously described (Kosvintsev et al., 2005; Wagdare et al., 2010). Alternatively, the use of microfluidic systems can be employed to produce more complex emulsions shown in Figure 4.9 (Chu et al., 2007; Shah et al., 2008).

4.6 PROCESS SELECTION

Considering the number and variety of encapsulation processes, selection can be a challenging task. Multiple factors must be considered to help narrow the list of possible processes, including core material, shell material, particle morphology, particle size, size distribution, production quantities, budget, and process availability. If more than one process remains feasible after considering the initial parameters for encapsulate product development, the advantages and disadvantages of each process can be compared further to determine the best process for a particular application. In some instances, original encapsulation development objectives and expectations may need to be adjusted to accommodate a process that has more favorable characteristics that outweigh the sacrifice in specification adjustment. For example, expectations of a specific particle size or distribution may need to be adjusted to accommodate a process that is more economically favorable for the end application. As a second example, a microsphere with a lower payload may need to be considered in place of a core−shell capsule with higher loading if the desired shell material is not suitable for use with a core−shell formation process.

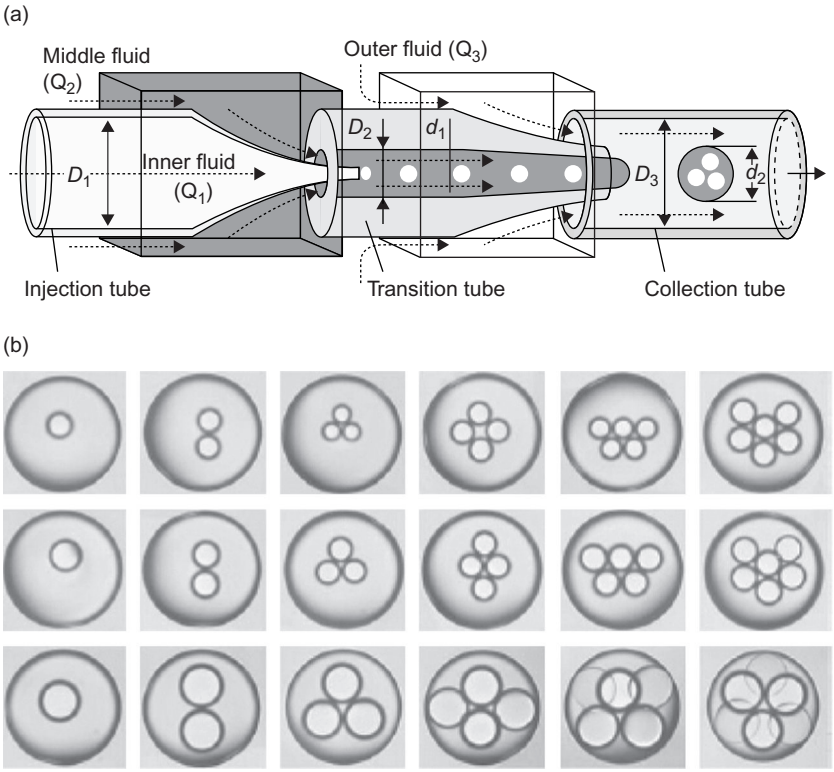

FIGURE 4.9 Fabrication of double emulsions in microfluidic devices. (a) Schematic of a device that employs two sequential coflow emulsion generators. (b) Optical micrographs of monodisperse double emulsions showing controlled increase in the diameter of the inner droplets in each column, while the number of inner droplets is constant. *Adapted from Shah et al. (2008).*

The information in this chapter is set forth to provide a comparative overview of the multiple processes available in the encapsulation industry. Specific values provided, such as particle size limits and production capacities, are guides and not absolute values for each process. While the encapsulation processes are supported by numerous engineering and scientific studies, the selection and operation of an encapsulation process remains empirical. The subsequent chapters provide more detailed information about each process and should serve as an informative guide for further understanding of each process, their benefits, and their limitations.

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Chapter 5

Atomization and Spray-Drying Processes

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5.1 INTRODUCTION

Spray drying is a method by which a liquid or slurry is converted to a dry powder by atomization into a flowing stream of hot gas. Air is generally the heated gas, although if the liquid contains flammable solvents such as ethanol or toxic solvents such as methylene chloride, nitrogen is generally used as the drying gas (Mujumdar, 2007). This is the preferred method for drying emulsions such as flavor oil emulsions in the food industry.

A common configuration for spray drying is shown in Figure 5.1. Here the liquid is atomized into drops at the top of the chamber. The drops travel into the turbulent flow of heated air in the top of the chamber with both the heated air and drops flowing in the same direction, which is known as cocurrent flow. The liquid phase is rapidly heated and molecules of the liquid move to the surface of the drop and transfer to the gas phase. As the drops solidify, they are entrained in the heated gas flow and move to a cyclonic flow chamber where the solids move out of the chamber and the gas is exhausted to atmosphere.

All spray dryers use these basic components although there are variants in chamber configuration, atomizers used, cyclone design, solids recycle, gas conditioning or recirculation after condensation and/or cooling, air flow designs, and attached devices such as fluid beds, air sweeps, post filtering or washing, horns, rattlers, or other vibration devices.

Spray dryers come in sizes from evaporating less than a liter per hour to thousands of liters per hour. The dryer shown in Figure 5.2 is specifically designed to evaporate small quantities for the collection of very valuable substances at high efficiencies. Figure 5.3 is a bench-top spray dryer from Buchi. Figure 5.4 shows a laboratory spray dryer from Niro that is capable of evaporating about 3 L per hour of water.

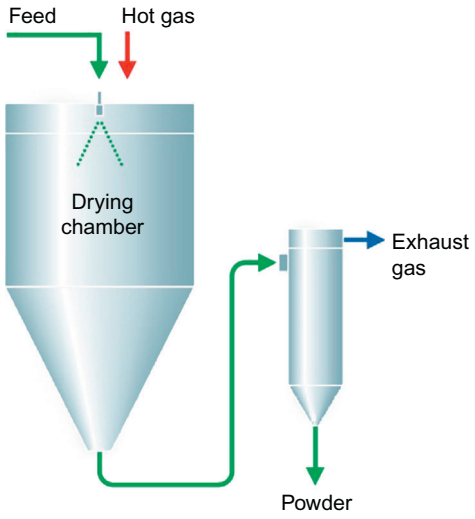

FIGURE 5.1 Spray dryer in simplest form. *Courtesy of GEA Process Engineering, Inc.*

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00005-4

FIGURE 5.2 Spray dryer from ProCepT.

FIGURE 5.3 Buchi B-290 spray dryer.

FIGURE 5.4 Niro Mobil Minor spray dryer.

FIGURE 5.5 Stream of fluid breaks up into smaller packets.

5.2 ATOMIZATION

The Plateau–Rayleigh instability, often just called Rayleigh instability (Rayleigh, 1878), explains why and how a stream of fluid breaks up into smaller packets with the same volume but less surface area. This is evident in Figure 5.5. The driving force of the Rayleigh instability is that liquids, by virtue of their surface tensions, tend to minimize their surface area. One might note that as the surface perturbation is resolved in the stream, a large drop is formed as well as a smaller drop forming from the thread connection between the larger diameters.

All spray dryers use what can be termed as an atomizer to transform the solution, dispersion, or emulsion into a spray with a prescribed drop size. Different techniques for atomization vary based on how energy is applied to the bulk liquid. The atomizers generally used in spray drying are often one of several types: rotary, single-fluid high pressure nozzles, and two-fluid nozzles that use air or nitrogen gas to atomize the liquid into droplets. More recently, ultrasonic nozzles have become available when only smaller volumes are needed to be atomized, although with the added limitations of viscosity or solids in suspension. The amount of liquid to be atomized, the drop size, narrowness or breadth of desired size distribution, viscosity of the liquid, size and amounts of particles in suspension, and chamber design all are factors that can weigh into the decision as to atomizer type, size, and design. With higher volume applications, multiple nozzles can be placed in a chamber in such a way so as not to interact and allow constant evaporation conditions.

Pressure nozzles, as represented in Figure 5.6, are generally designed using a swirl chamber to provide the drops leaving the orifice with a swirl pattern, with the drops often defining a hollow cone as shown in the figure. The drops have a fairly broad range of particle sizes depending on the orifice size and the liquid flow rate to the nozzle. The capacity (flow rate) of the nozzle is approximately directly proportional to the square root of the pressure and inversely proportional to the square root of liquid density (Masters, 1979). Pressures of liquid to the nozzle are often in the 1500 to 5000 psi range. Higher viscosity, higher solids content or liquid density, and lower atomization pressure all result in larger particles. Conversely, increases in feed rate will decrease the droplet size up to the rated capacity of the nozzle. With varying orifice sizes and applied pressures, drops can be achieved in the range of 50 microns up to hundreds of microns with a typical orifice size of 0.5 to 4 mm. In other words, to produce smaller drops for a given feed rate, one would increase the pressure drop across an orifice by going to a smaller orifice. In very high volume applications, multiple pressure nozzles can be used with 3 to 7 nozzles in chambers as large as 5 to 7 meters in diameter. Typical feed rates per nozzle are on the order of 200 to 1000 kg liquid/hour. A hollow cone pressure nozzle is shown in Figure 5.7. Nozzles of different orifice sizes are shown in Figure 5.8.

Two-fluid atomizers consist of an orifice and an air cap, which allows interaction of the liquid stream with the mass flow of an atomizing gas. The gas, generally air, but sometimes nitrogen, can interact with the liquid stream external to the liquid stream, or by exiting the cap as a sheath of air surrounding the liquid as in the cap shown in Figure 5.9. In larger units, the cap orifice may be external to the liquid jet orifice, forcing air to interact in a chamber. This type is known as an internal mix nozzle. Two-fluid nozzles are generally used in lower volume applications and when the feed solution or suspension is more viscous or with abrasive feeds, and also in situations where smaller particles cannot be achieved with the pressure nozzles. Two-fluid nozzles do require a compressed gas source. The liquid is pumped at near atmospheric pressure although the internal mix nozzles may require pressures on the order of 20 to 75 psi. Gas pressures to the nozzles are in the range of 15 psig to approximately 150 psig. Two-fluid atomizers can carry solids, although the solids content may be a lower percentage than with rotary atomizers and also generally with more uniform

FIGURE 5.6 Pressure nozzle.

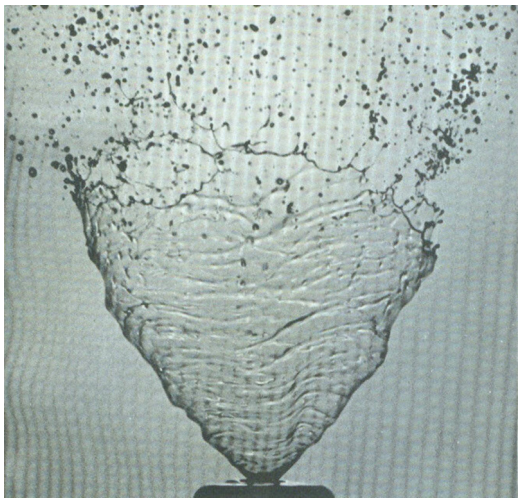

FIGURE 5.7 Hollow cone pressure nozzle.

FIGURE 5.8 Assorted pressure nozzles of varying size.

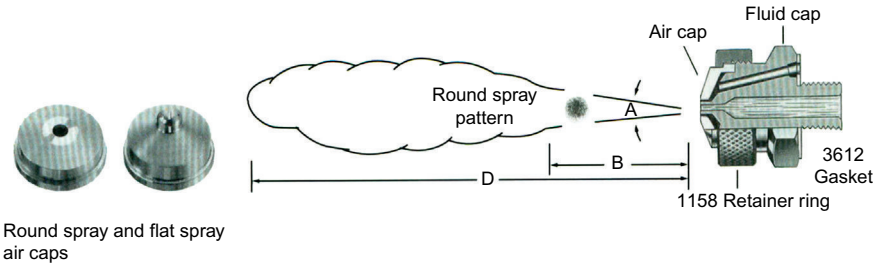

FIGURE 5.9 Two-fluid atomizers from spraying systems.

fine particle slurries as these nozzles are more subject to blockage. The particle size range of the generated drops is often narrower than that of the pressure nozzle, although not quite as narrow as the rotary atomizer. One big advantage of 2-fluid atomizers is that these nozzles can achieve much finer average particle size distributions than other types of atomizers, with average particle sizes of 10 microns and lower and at or below 1 micron with very dilute solutions and at very high pressures as shown in Figure 5.10. In general, the particle size is proportional to the mass ratio of the flows of atomizing gas to liquid flow. The design of a type of 2-fluid atomizer is shown in Figure 5.9.

Liquid feed solutions or suspensions can by centrifugally accelerated using a rotary atomizer. Velocities of greater than 300 ft/sec can be achieved with the production of very fine droplets. The degree of atomization depends on feed rate, viscosity of the liquid feed, and the peripheral speed of the wheel or disc. The liquid feed is delivered to the center of the wheel or disc in varying designs and the liquid spreads over the surface as a thin film, which varies in thickness based on the speed, viscosity of the liquid, and the feed rate. The fluid tends to slip and so is flung from the edge at a lower velocity than the peripheral speed. To prepare smaller drops, one can roughen the surface of the disc or provide radial vanes to minimize this slippage.

For flat, smooth, sharp-edged discs and at slow disc speeds when viscosity and surface tension dominate the mechanism of atomization, drop formation can occur at the edge of the rotating disc to provide quite uniform size distributions with very few satellite drops formed. With increased speed and feed rate, the droplet formation mechanism changes to that of ligament break-up as shown in Figure 5.11. Further increase in feed rate or increase in viscosity may result in sheets of liquid leaving the disc edge, which can further break into drops giving a very broad droplet size distribution.

Rotary atomizers, as designed by a number of companies, Figures 5.12 and 5.13, have been found to provide narrower size distributions than other types of nozzles and can reduce losses of volatile components on drying or to achieve specific final product characteristics.

More recently, ultrasonic atomizers have found use in dryers designed for small quantities such as the ProCepT spray dryer and the unit from Buchi. The nozzle shown in Figure 5.14 is from Sono-Tek.

5.3 DRYING CONFIGURATIONS

The most common spray-dryer configurations can be termed *single effect* in that the drying air enters the top of the drying chamber and flows cocurrently with the incoming atomized drops of the liquid feed. An example of this

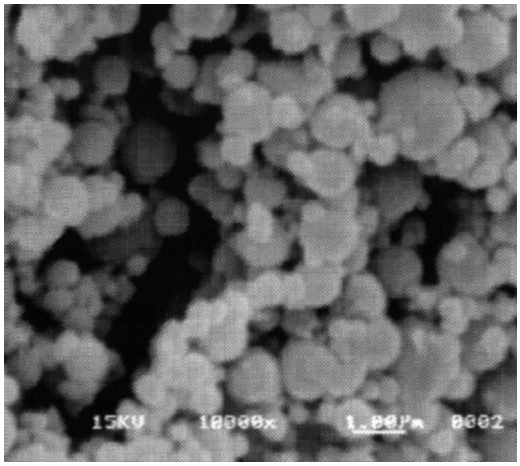

FIGURE 5.10 Spray-dried particles of about 1 micron diameter (photomicrograph and size distribution).

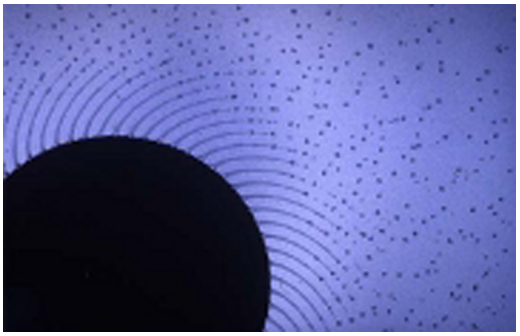

FIGURE 5.11 Rotary atomizer droplet formation.

FIGURE 5.12 Rotary atomizer designs. *Courtesy of GEA Process Engineering.*

FIGURE 5.13 Rotary atomizer designs. *Courtesy of Drytec Contract Processing.*

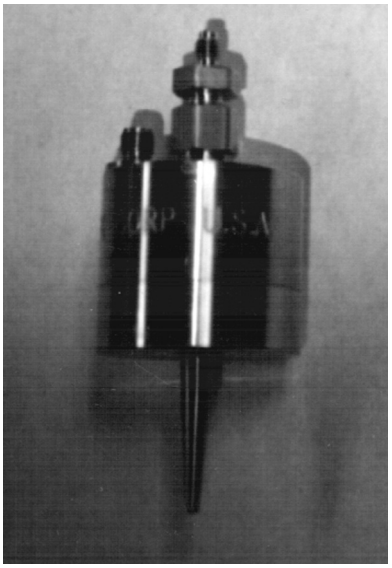

FIGURE 5.14 Ultrasonic atomizer (note electrical connection on left and liquid feed port at top).

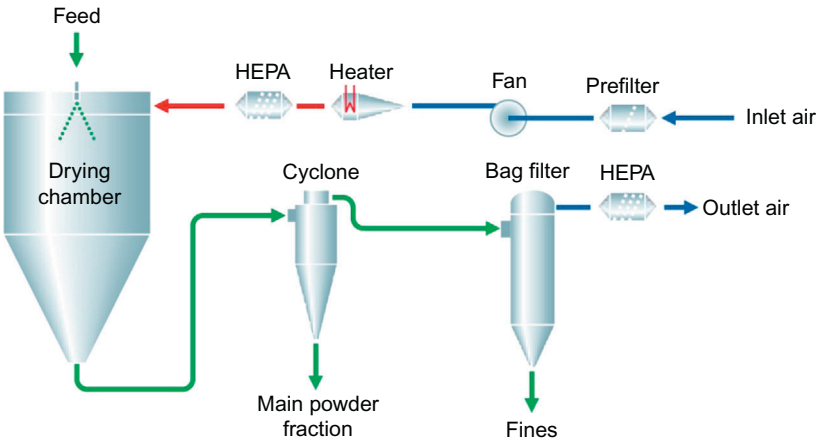

FIGURE 5.15 Spray-drying chamber with cyclone and bag filter in a once-through configuration.

design is shown in Figure 5.15. The powders obtained with such types of dryer are fine with a lot of dusts and poor flowability. To reduce the dusts and increase the flowability of the powders by formation of agglomerates, a newer generation of spray dryers termed *multiple effect* spray dryers has been designed. Instead of achieving the finished product in one stage, the drying is done in two steps. One step dries as normal in the chamber with the larger particulates passing into an integrated vibratory fluid bed at the bottom of the chamber. Heated air is delivered into the fluidized bed (Step 2 of the drying), forcing fines upward to be combined with fines coming from the drying chamber to pass into a cyclone separator. This combined stream of fines, now separated from the main air flow, exits the cyclone and is conveyed to the top of the chamber to interact with incoming atomized droplets. In an alternate design, these fines enter into the lower part of the chamber to be combined with fines recently dried in the top of the chamber where they enter into the initial stage of the fluid bed where they can be rewetted. The integration of this fluidized bed provides the production of agglomerated particles within a size range of approximately 100 to 300 μm depending on chamber size and air flows. Because of this large particle size, these powders are free flowing and much less dusty. The produced granules are sometimes known as "instantized" powders. Also, this type of dryer has been used to achieve better particle flow and granule compressibility, which can be used in directly compressible tablet operations.

Another dryer configuration has the hot gas entering the chamber in a counter-current flow to the atomizer direction. The counter-current flow enables the particles to have a longer residence time within the system for more complete utilization of heat energy; this kind of design is more appropriate for larger atomized drops. This type of dryer is not used as often in the food or pharmaceutical industries because of the longer thermal exposure of the product particles.

For spray drying of food ingredients concerned with the recovery of volatile components or for spray drying where flammable or toxic solvents such as ethanol or dichloromethane are in use, a closed cycle system is used. This is shown in Figure 5.16. Nitrogen from either a gas separation system or liquid nitrogen storage is added to purge the closed cycle system. Gas only needs to be input to the system as required such as at the bag house pulsers for bag cleaning. In this system, pressure nozzles or rotary atomizers are generally used. If solvents are used, vented gas is generally scrubbed further using charcoal filter beds. Condensers can use glycol systems or nontoxic solvents such as limonene as the circulating subzero liquid. Heaters are generally electric in this type of system rather than the direct or indirect gas-fired systems more typical in food plants. This type of system has found utility in the pharmaceutical industry for the spray drying of solutions of active ingredient with polymers or other additives for the formation of amorphous solids needed to improve the overall oral bioavailability of the formulation.

Drying chambers can be of several basic designs. Cylindrical flat-bottom dryers are of a fairly old design and were used for egg products, toppings, and juices or pulps. A pneumatic powder discharge was used to remove the product. Conical bottom chambers are more commonly used for many food products, including maltodextrins, flavors, and nutritional products such as dried oil emulsions. The angle of the cone bottom would be based on the flow requirements of fine particles down the chamber walls.

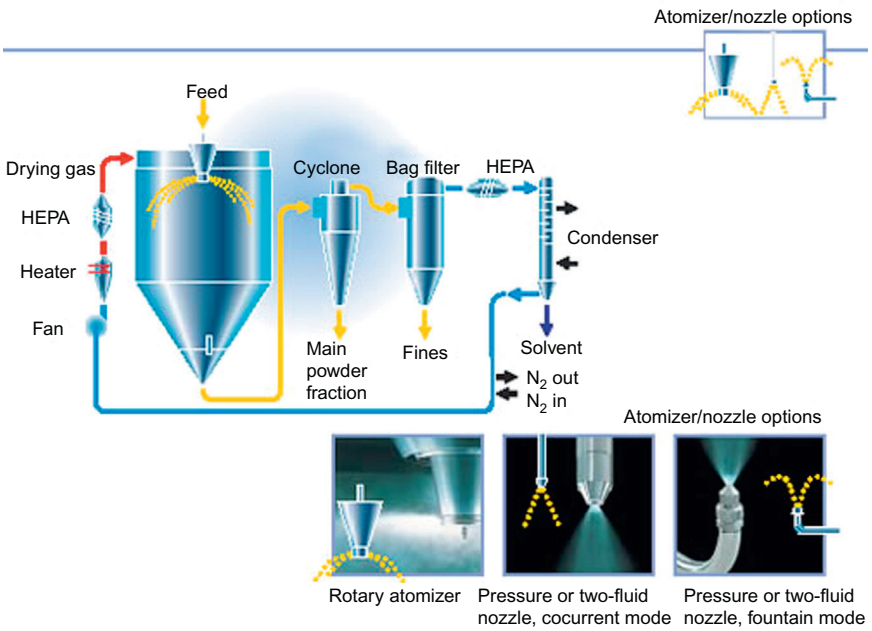

FIGURE 5.16 Closed cycle design. *Courtesy of GEA Process Engineering.*

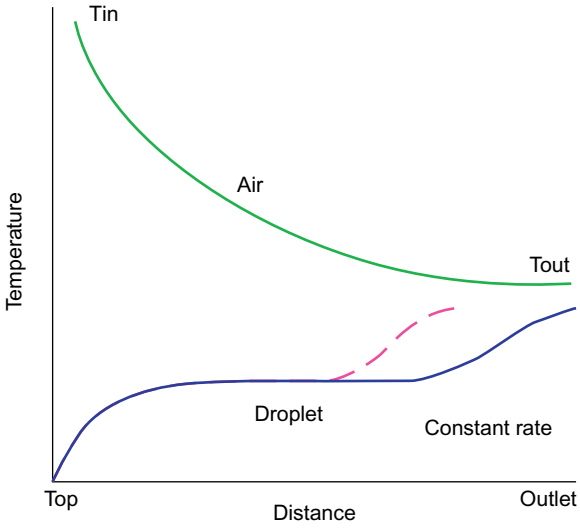

FIGURE 5.17 Temperature versus droplet travel in spray-drying chamber.

5.3.1 Mass Transfer and Heat Transfer Considerations

The evaporation of volatiles from atomized droplets involves both heat transfer and mass transfer. Heat is transferred from the heated gas in the dryer to the droplets and converted to latent heat within the liquid. Mass transfer occurs as molecules transfer from the surface of the drops to the boundary layer of gas surrounding each drop. The rates of heat and mass transfer are a function of the Δt between drop and chamber gas and the humidity within the chamber. The rates of transfer are also a function of droplet diameter and relative velocity between the droplet and the chamber gas. For each product there is a representative curve that describes drying properties for that product at specific temperature and gas velocity within the chamber. Figure 5.17 shows an example of such a curve. The drop initially gains in

temperature over a relatively short period of time in the top of the chamber by transfer of what is known as sensible heat. Then, as molecules of liquid move into the gas phase at the surface of the drop, a period of constant rate drying occurs where most of the free moisture of the drop is removed. The wet-bulb temperature represents the droplet temperature. If drying occurs too rapidly, an outer layer of dry material forms as molecules of liquid cannot reach the surface rapidly enough and the dried layer presents a formidable barrier to moisture transfer. The inner temperature of the drop can rise until the liquid vapor pressure can burst through the surface layer and cause a change in overall particle morphology and what might be termed "blast holes" or burst balloons to be evident on the product surface. This may sometimes be corrected by proper dryer design or changes in matrix coating formulation (Thijssen, 1972). The control of the temperature of this constant rate drying regime can sometimes assist in reducing the overall temperature exposure of the product and thereby decrease losses of volatile components or thermal degradation of the product.

The last stage of spray drying is the separation of the dried solids from the gas stream, and with proper design this can be accomplished with high efficiency. The product is removed from the cyclone where it is collected into intermediate or final packaging. The exhaust gas stream for open cycle dryers must meet local, state, and national standards. For closed cycle dryers, all solids have to be removed to prevent scorching or other degradation on reheating of the gas. Separation equipment generally involves a high efficiency cyclone and/or a bag filter assembly. Occasionally, wet scrubbers are used downstream of the cyclone separators. Also, in the case of difficult-to-handle materials where there may be surface stickiness or cohesive powders, a cyclone separator may not be needed and the chamber product is sent directly to a bag filter assembly equipped with bag pulsing to remove product from the filters. Efficiency of collection in cyclones can be adjusted by the design with an understanding of air velocities and particle sizes to be removed. Other options to improve collection efficiencies include chamber air sweeps and quenching gas streams introduced either in the lower part of the chamber or between chamber and cyclone.

5.4 OPERATIONAL PRACTICE

The controlled operation of once-through, single-effect spray dryers involves the maintenance of the parameters of the liquid feed system as well as the conditions of temperature and air flow within the dryer. Modern control systems allow for the setting of any two of the three main operational parameters: inlet temperature, outlet temperature, and feed rate (keeping air flow constant). More often, a feed rate is set and a desired outlet temperature is set to obtain a moisture specification, and the controls adjust inlet temperature to achieve the appropriate outlet temperature. It is also possible to have set a desired inlet temperature and then either manually or have a control to adjust feed rates to achieve a desired outlet temperature. Manual control is more often used on smaller laboratory or pilot plant equipment. For example, if a dryer is started at an initial pumping rate of liquid and the desired outlet temperature is too high and needs to stay at the maximum inlet temperature for efficient operation, it is simple to increase the feed rate to achieve the desired outlet temperature. However, if the moisture content is too high in the product, a decision has to be made whether to increase the inlet temperature and the subsequent outlet temperature to achieve the lower moisture, or to lessen the feed rate so as to bring up the outlet temperature or to possibly change the atomization conditions to achieve smaller particles, which will dry faster and to a lower moisture content.

Integral fluid beds add a level of complexity to the setting of drying conditions. It is generally desired to achieve only partial drying in the chamber and to do some drying in the fluid bed. Also it is necessary to return fine particles to the drying zone in the upper part of the chamber to interact with atomized droplets for the formation of aggregates. There are added variables of incoming temperature and velocity of gas to the fluid bed as well as the need to convey air from the bottom of the cyclone to the top of the chamber. Besides moisture control in the product, parameters have to be set to achieve specified agglomerate friability and particle size.

There are a number of ways to address problematic materials during the spray-drying process. Fine particles do not necessarily flow well in chambers. Cone bottoms can be designed with sharper angles to promote movement down without excessive wall buildup and larger radius curves can be used to prevent buildup in angled piping. However, it is often necessary to apply various vibration methods to prevent powder layers forming on chamber walls or in curved sections of piping at the bottom of the chamber, the entrance to the cyclone, or other places in the equipment. There are quite a variety of devices known to assist in removal of solids from chambers and piping surfaces, which include rattlers, sonic horns, and pneumatic hammers. If other methods do not solve these issues, air lances or air sweeps have been used in chambers to assist in sweeping the product toward the bottom outlet.

Flow aids can be introduced to the chamber during spray drying for the improved powder flow of tacky materials. Because a spray-drying chamber is operated under a slight negative pressure, most often with fans pulling air or nitrogen through the chamber, a small port can be opened in the top of the chamber to allow controlled feeding of flow aid

materials such as micron to submicron particulate silica. The silica can greatly improve the flow of the product and decrease chamber buildup, generally increasing product collection efficiencies.

Safety in operation is most important especially when related to prevention of dust explosions and air over layer exothermic degradation. Determination of dust explosion pressure rise (Kst), the maximum dust explosion pressure (Pmax), and minimum ignition temperature (MIT) may be important to measure before operations commence.

5.5 FEED PREPARATION

The preparation of the feed solution, suspension, or emulsion is often overlooked in considering the successful spray drying of a product. Proper addition of additives so as not to cause foaming, the use of surface active materials for the wetting out of solids to get uniform dispersions, proper use of agitators or high speed rotor/stator or high pressure homogenizers with circulating loops can all be major factors to achieve homogeneous feed suspensions or uniform emulsions. Proper tank design to maintain the homogeneity of the suspensions is often important.

Emulsions can often be quite tricky. Flavor oils or nutritional oils may need to be homogenized uniformly into very fine drops to be microencapsulated into a matrix particle with the continuous carrier being gum acacia or possibly a succinylated starch. Other hydrophilic polymers such as maltodextrin or gelatins can be used. Addition of emulsion stabilizers such as lecithins can also improve the quality of the emulsion and thus the improved performance in a product. A very fine emulsion drop is generally important in producing dry particles with little surface oil. There is generally a limit to the amount of active oil in the product at approximately 40 to 50%. If higher amounts are attempted, there may be too much surface oil as the drop dries. Any surface oil runs a much greater risk of fire in the chamber or bag house. Flavors often have volatile components, which can be lost. Besides adjusting the film-forming material, minimizing the amount of loss can sometimes be controlled with addition of less volatile components thus diluting out the volatile component and reducing its partial vapor pressure by Raoult's law. Other additives to improve oxidative stability of oils may be added as well as components that can affect the film strength as the drop begins to dry.

An interesting paper describes the various morphologies of particles that can be achieved with spray drying. Particles can be engineered by the use of varying drying conditions or feed suspensions, which can lead to some unusual particle morphologies and product functionality (Vehring, 2008).

Emulsion droplet size is a critical parameter for reducing the loss of precious high value volatile flavor oils or food ingredients during spray drying. Increasing emulsion droplet size led to reduction in the flavor retention. The emulsion droplet size was optimized by the use of high shear mixing followed by high pressure homogenization (Soottitantawat et al., 2003). The optimum emulsion droplet size for the highest flavor/food ingredient encapsulation and lowest surface oils needs to be found with experimentation and appropriate combination of wall material with surfactants and viscosity modifiers (Jafari et al., 2008).

5.6 RECENT ADVANCES IN ATOMIZATION AND SPRAY-DRYING PROCESSES

ZoomEssence, Inc. (www.zoomessence.com/index.html) has claimed that it has a patent pending DriZoom™ technology that eliminates the use of heat and thereby improves volatile flavor retention and reduces the loss to the atmosphere. This results in an improved quality of spray-dry powder (higher density powder) that quickly dissolves into liquids. This spray-drying technology is applicable for encapsulation of highly volatile aromatic flavors.

Orbis Biosciences (www.orbisbio.com/precision-particle-fabrication.html) has a unique atomization process that makes monodispersed microscapsules based on coaxial nozzle technology. This precision particle fabrication technology may enable superior control over the release rates of the food actives.

5.7 CONCLUSION

The design and operation of spray dryers can vary greatly, with these units being used to process many types of food products and ingredients used in the marketplace today. With varying characteristics of particulates in suspension, fats, and oils contained in dispersions or fine emulsions, each product may require unique processing conditions to generate free-flowing dry powders with the desired product characteristics. Safety, energy conservation, and economics all can weigh into the design of spray-drying equipment and formulation design.

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Chapter 6

New Advances in Spray-Drying Processes

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6.1 INTRODUCTION

Today, spray drying is the most widely used technique for microencapsulation in the food industry (Desai and Park, 2005). The reason for this is the possibility of continuous production of tailor-made particles for very specific functions and needs of the food industry. Each particle in the final powder can be customized not only in its physical (size, shape, density, etc.), chemical, nutraceutical, and organoleptic (e.g., smell, taste) properties but also the release mechanism (in time and place) of the shell material and/or microencapsulated material. The microencapsulated systems can be multilayered to serve several purposes at once or in several steps when eaten or used.

The range of products that are microencapsulated by spray drying is vast, including aromas, flavors, oils, probiotic cultures, vitamins, nutraceuticals, and much more. The shell materials are also numerous, and some examples are carbohydrates (e.g., starch, maltodextrins, chitosan, corn syrup solids, modified starch, or cyclodextrins), cellulose (e.g., carboxymethylcellulose, methylcellulose, or ethylcellulose), gum (e.g., gum acacia, agar, sodium alginate, or carrageenan), lipids (e.g., wax, paraffin, beeswax, diacylglycerols, oils, or fats), protein (e.g., gluten, casein, gelatin, albumin, or peptides), and mixtures thereof (Desai and Park, 2005; Murugeasan and Orsat, 2012).

Spray drying can be combined with other techniques, such as fluid beds (integrated or external), for further drying (Pisecky, 2012), agglomeration, granulation, multicore encapsulation (Jafari et al., 2008), coating, and/or functionalization of the powder. Often the agglomeration option is a great advantage because the granules can be produced instead of the traditionally often dusty products.

6.2 TECHNOLOGIES

Spray drying is a method of drying liquids, solutions, and liquid suspensions. The spray drying system is described in general in Chapter 5, so in the following sections only the recent advances are described. In recent years there has been quite a development within optimization of the chamber design and dimensions, optimization of air dispersers, inlet air control, energy optimization, temperature optimization for sensitive products, and the sanitary aspects of the entire system. The spray-drying systems have also been further developed with adaptive means as well as online and inline measurements of the powder quality.

6.3 COMPUTATIONAL OPTIMIZATION

To optimize the spray dryer performance and design, computational fluid dynamics (CFD) can be used. CFD can be used to design vital parts of a spray-drying system (see Ullum, 2005; Ullum et al., 2008, 2010), such as the air disperser, drying chamber, cyclone, bag filter setup, and much more. In a spray dryer, the dimensions and geometry are vital for a successful drying of a specific product to avoid chamber deposits while keeping the dimensions as small as possible to minimize the productions costs.

Conventional CFD simulation programs assume that all feeds are essentially alike, but in reality they are not. Some feeds take longer to dry than others, and some are also sticky, which can lead to deposits on dryer walls, significantly impairing system performance. However, without empirical data, it is difficult to predict whether a given feed would pose such problems or where deposits might form. The reliability of this simulation technique has been vastly improved

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00006-6

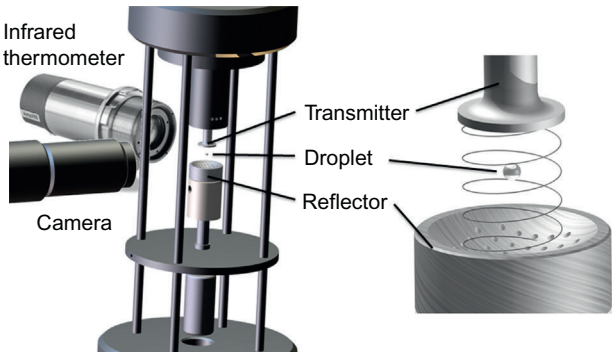

FIGURE 6.1 Left: DRYING KINETICS ANALYZER. Right: Zoom on the levitated droplet where the helix illustrates the ultrasonic field. Note the holes in the reflector used to inject conditioned drying gas.

using a method called DRYNETICS™,[1] which puts the software on a more scientific footing. In DRYNETICS the drying rate is calculated based on actual measurements (as described in this chapter) through advanced mathematical modeling, and implemented in computer simulations of full-scale spray dryers.

6.4 ANALYZING THE DRYING PROCESS OF A DROPLET

Full-scale spray drying experiments are expensive in terms of materials consumption, operational costs, and man-hours. This may be a limiting factor when developing new formulations for microencapsulated food systems as only a small number of formulation candidates can be tested experimentally. Furthermore, often only a few process parameters (e.g., drying temperature) are evaluated, although these have significant influence on final spray-dried powder properties (Walton, 2000).

Single droplet drying experiments provide a fast and inexpensive method for testing new formulations and optimizing process parameters (Hecht and King, 2000; Liu et al., 2000). Single droplet drying experiments are often based on the principle of suspending a droplet from a filament and monitoring the drying process (El-Sayed et al., 1990; Fu et al., 2012). However, this method has the obvious drawback that the filament affects the particle morphology formation. Alternatively, the droplet may be suspended in an ultrasonic field, which is the case for the DRYING KINETICS ANALYZER™ (Sloth et al., 2006, 2010).[2]

In the DRYING KINETICS ANALYZER, a high power ultrasonic horn (58 kHz) is placed 5/2 wavelengths above a concave reflector to generate an acoustic field. Due to the forces of this acoustic field, a small droplet ($d \approx 1000$ μm) of feed may be inserted and held constant against the gravity, as shown in Figure 6.1. While the droplet is drying, it is monitored with a CCD camera and an infrared thermometer. The former is used to record a video of the drying process and morphology formation while the latter is used to record the development in surface temperature.

The levitation unit is encapsulated in a small drying chamber (not shown in Figure 6.1) so that the drying gas (nitrogen) temperature and humidity may be set arbitrarily. The drying gas is injected into the drying chamber through a number of small holes in the reflector below the particle, which gives a relative velocity between the drying gas and the droplet, like in a conventional spray dryer. When the particle is dry it may be extracted from the acoustic field and subjected to different laboratory analyses. These could, for example, be optical or scanning electron microscopy for instigating the particle morphology in detail.

Experiments (Figure 6.2) have shown that if the drying gas conditions in the DRYING KINETICS ANALYZER are the same as the corresponding outlet conditions from a conventional spray dryer, the particle morphology in the two drying systems matches. Thus, one major advantage of single droplet drying is the option of screening different feed formulations for morphology using this system instead of a full-scale dryer (Sloth, 2010). The single droplet drying experiments can reduce the consumption of valuable feed and help narrow down the number of formulation candidates used in pilot and full-scale tests (Brask et al., 2007).

A simple example of food microencapsulation is shown in Figures 6.3 and 6.4. Here a droplet of homogenized low fat milk has been dried in the DRYING KINETICS ANALYZER. Before drying, the milk is water/fat emulsion with fat droplets in the size range of 0.1 to 3 μm as the discrete phase. Figure 6.3 shows an optical image of a droplet of

1 DRYNETICS™ is a trademark of GEA Process Engineering A/S.
2 DRYING KINETICS ANALYZER™ is a trademark of GEA Process Engineering A/S.

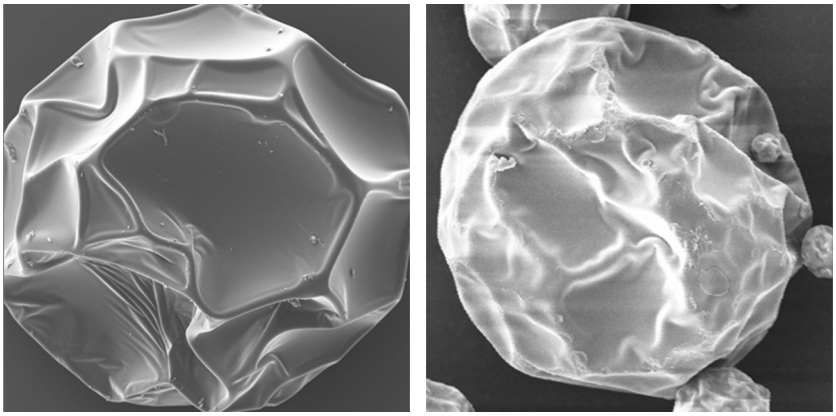

FIGURE 6.2 SEM images of particles dried in the DRYING KINETICS ANALYZER (left) and in the spray dryer (right). The diameter of the DRYING KINETICS ANALYZER particle is $d = 720\,\mu m$ while the spray-dried particle has a size of $d = 32\,\mu m$. The feed dried was a solution containing 29% maltodextrin DE18 solids.

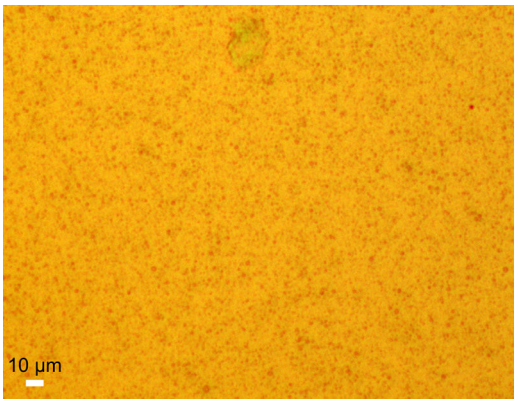

FIGURE 6.3 An optical image of a droplet of low fat milk with staining of the fat particles.

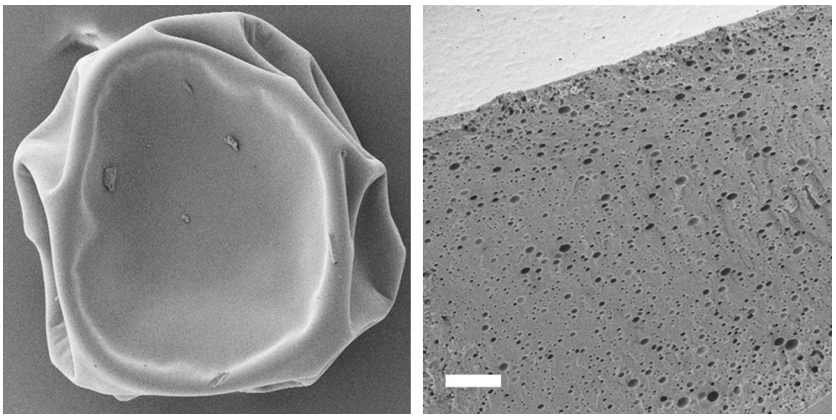

FIGURE 6.4 Low fat milk particle produced using the DRYING KINETICS ANALYZER. (a): A whole particle of size 480 µm. (b): A cut-through particle—the scale bar indicates 10 µm.

milk where there is coloring bound to the fat droplets. Figure 6.4 shows electron microscope images of the cut-through particle and it can be seen that the emulsion fat droplets remain after the drying process. It is noted that the fat droplets can be seen only as concavities due to the sample preparation procedure for the electron microscope. By comparing the images of the milk before and after drying, it can be seen that the size and shape of the fat droplets are unaffected by the drying process.

The experiment described does not represent a widely used food microencapsulation system and the approach taken does not resemble that of spray-drying milk of any type. However, the experiment serves well as an example of how the DRYING KINETICS ANALYZER can be used for evaluation purposes during the design phase of food microencapsulation systems.

6.5 DRYING KINETICS AS INPUT FOR CFD

Using the DRYING KINETICS ANALYZER, it is also possible to find the drying rate for a given feed. During an experiment, data for the development in particle size and surface temperature are logged along with the position of the particle in the ultrasonic field: as the particle dries it gets lighter and rises a bit in the field. Based on these data, the drying curve (i.e., the particle residual moisture content as a function of the drying time) is found through advanced mathematical modeling and used in CFD simulations of spray dryers (see Figure 6.5). That is, the specific drying kinetics for the product in question may be found and used in the CFD simulations, which are important for achieving high accuracy results (Ullum et al., 2008). The results can also be used together with CFD to produce state-of-the art simulations of spray dryers (Thybo et al., 2008).

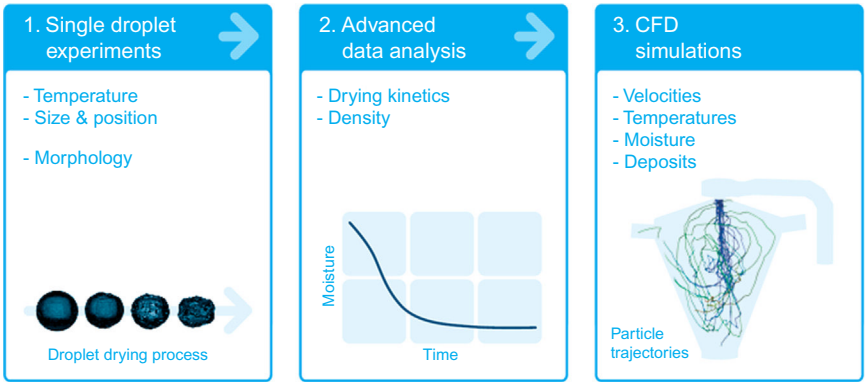

FIGURE 6.5 The three steps in the DRYNETICS method.

6.5.1 Spray Drying Equipment and Controls

As already mentioned, spray drying systems consist of several components. Each component can be optimized on its own or in a system perspective. These components, as well as spray drying in general, were described in Chapter 5. Therefore, in the following, only new developments on selected topics are addressed.

6.5.2 Temperature Control

Many products in the food industry are sensitive to high temperatures. Therefore, there has been quite an intense effort as to how to lower the temperatures in the spray dryers. There are several ways of doing this, but one of the key elements has been the dehumidification and temperature control of the inlet air for it to run at stable conditions independent of the season, outside temperature, outside humidity, and so on. By dehumidifying the inlet air, lower temperatures are possible than traditionally used. It is, for example, possible to spray dry with inlet temperatures as low as 80°C and outlet temperatures as low as 40°C. These temperature ranges open up spray drying to many new products that have otherwise been spoiled by the process conditions.

Figure 6.6 shows the temperature profile in an industrial size spray dryer cut through the center axis. The hot drying gas is quickly cooled due to the evaporation resulting in a low temperature in the majority of the drying chamber.

6.5.3 Flexible Spray Drying, Agglomeration, and Granulates

In modern spray dryers, it is possible to have several built-in functionalities in the same setup. It is, for example, possible to integrate a fluid bed for further agglomeration (e.g., by adding a nozzle) and/or drying of the product. This makes it possible to run several different products and a large span of particle sizes on the same drying system.

Figure 6.7 shows examples of spray drying chambers with integrated fluid beds for agglomeration and/or further drying.

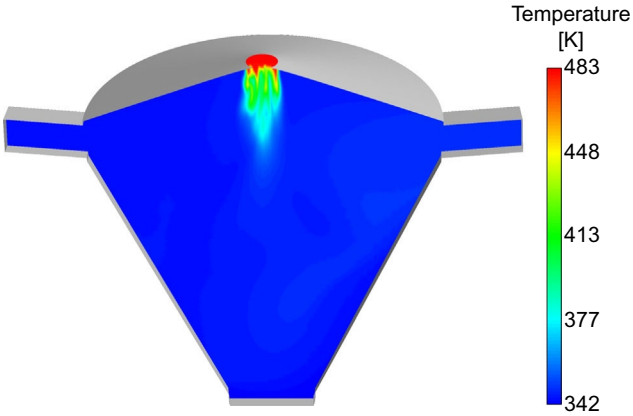

FIGURE 6.6 Temperature plot of a spray dryer.

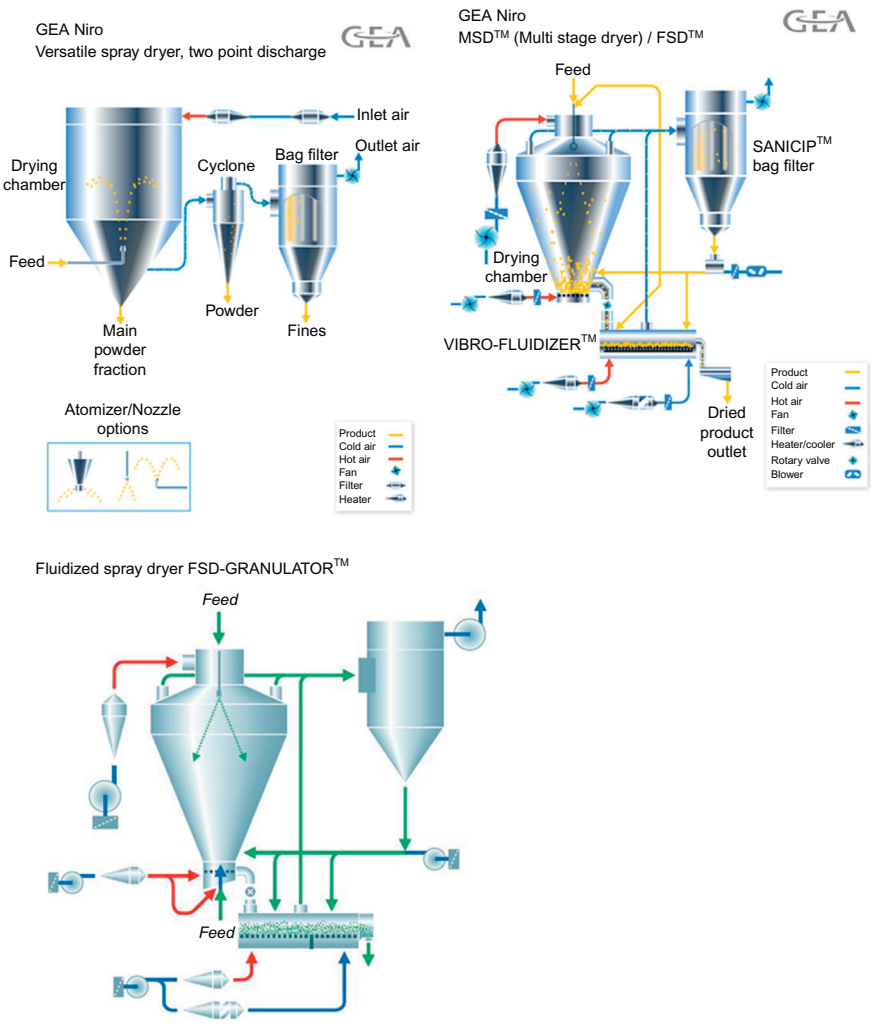

FIGURE 6.7 Three examples of spray dryers. (a) A flexible spray dryer, (b) a spray dryer with an internal as well as external fluid bed, and (c) a spray dryer with a granulator.

6.5.4 Cleaning-In-Place

The very nature of, for example, dairy and food products means that there is a risk of product deposits on the surface of the process equipment. The products' fat, sugar, and protein content gives hygroscopic and sticky characteristics at the temperature and humidity conditions present during processing. To meet today's strict sanitary requirements, drying plants must satisfy the highest design standards to avoid product degradation and contamination during plant operation. For drying plants in general, it is often relevant and profitable to equip the plants with automatic cleaning in place (CIP) to reduce the downtime of the plant.

Cleaning requirements for well-designed liquid processing equipment are achieved by circulating cleaning solutions and water under well-controlled conditions. Cleaning requirements for the drying and powder handling equipment—the dry areas—can be met by either dry or wet methods, or a combination of both. Dry methods involve the manual sweeping of surfaces in contact with the product, or air sweeping by allowing a high velocity air stream to pass over the surfaces in question. Wet methods can involve either manual hosing of surfaces or more effectively by use of automated CIP systems.

The longer the plant operates, the greater is the risk of deposit formation. Spray drying plants have been developed for minimizing deposit formation and to permit plant operation over longer periods of time. However, occasionally the plant has to be shut down for cleaning, and cleaning-in-place has become essential to minimize cleaning time and thereby maintain high plant productivity.

Systems that are suitable for cleaning individual items or complete process plants are already on the market. The cleaning process involves integrating cleaning nozzles into the plant components together with their associated pipework and instrumentation. The CIP operation is computer controlled and documented. A program controls pump and valve functions, and cleaning sequences are given the optimum timing for efficient cleaning of all parts of the plant. The system is flexible and different washing programs can be selected according to the needs. The program software includes the flexibility to alter and adapt the washing programs according to experience and need when new products are processed or new operating conditions are applied. Further, individual programs can be developed for specific products for which cleaning efficiency, cleaning time, or the amount of chemicals used can differ.

6.5.5 Sanitary Bag Filters

One of the components that have to be especially sanitarily CIP designed is the bag filters. The SANICIP™[3] bag filter is designed in accordance with the strictest sanitary requirements given by the leading international agencies in the European Union and the United States.

The exhaust air containing fine powder particles is passed through the SANICIP filter for powder separation. The bag filter consists of a cylindrical bag housing with a spiral-shaped or radial air inlet, clean air plenum on top, and a conical bottom with fluidized powder discharge. During operation, the product collected on the outside of the filter material is periodically blown off by a compressed air jet stream from the inside of each bag by means of a specially designed reverse jet air nozzle positioned above the bag.

This results in a very even discharge of powder. The frequency and duration of the blowing sequence can be adjusted to suit actual running conditions. The result is a low pressure drop across the filter, that is, reduced energy consumption and noise emission. Another special feature of the SANICIP bag filter is that the bags are wet cleaned from the inside toward the dirty outside by means of a clean water spray, reducing the overall amount of CIP water considerably.

All the fines discharged from the bag filter are returned to the process. This means that no powder is lost as waste, but is sold as first-class product. It is also possible to upgrade old plants equipped with cyclones and traditional bag filters or wet scrubbers with the new sanitary bag filter design.

6.5.6 Process Controls and Adaptive Feedback

The individual components as well as the entire drying systems can be regulated by a number of process and component controls. For example, a spray dryer may be controlled by regulating the outlet gas temperature, or the inlet gas temperature, or an inlet air flow rate, or by inline or online measurements of the product.

The SPRAYEYE™[4] (patent pending (Jensen et al., 2009)) nozzle monitoring system provides better monitoring of the spray drying process by giving a highly detailed, unimpeded, real-time view of the spray nozzles inside the drying

3 SANICIP™ is a trademark of GEA Process Engineering A/S.
4 SPRAYEYE™ is a trademark of GEA Process Engineering A/S.

chamber. Infrared cameras measure and survey temperatures to the control system. This allows operators to check for a wide range of faults before they become a problem and take corrective action in due time and only when needed.

In the nozzle monitoring system, cameras show a real-time continuous picture of the spray zone allowing operators to ensure that there are no leaks or any product buildup on the nozzles and that the spray pattern of the nozzles into the drying chamber remains at an optimum level. Furthermore, it is possible with cameras to check whether the high pressure shut-off valves are leaking. This helps avoid unnecessary shutdowns, improves product quality, and reduces the risk of heat buildup within the chamber, which can, in extreme cases, cause fire or an explosion. In an emergency, the control can be set to shut down the spray dryer if there is a risk of a fire or an explosion.

6.6 CONCLUSION

POWDEREYE™[5] is a system for inline powder analysis made specifically for spray dryers. The system allows producers of food and dairy powders to perform all the most important product quality tests during spray drying in a single operation.

Other technologies are available, but POWDEREYE has taken the process one level up. The samples are taken in a timed frequency and followed by real-time analysis. The analysis includes powder moisture content (e.g., by near-infrared technology), powder bulk density (loose and tapped), powder temperature, and scorched particles (by digital image analysis). All data are displayed as trend curves, and alarms in the plant human machine interface (HMI) system allow swift, corrective action to be made, thus minimizing both loss of product and process capacity.

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5 POWDEREYE™ is a trademark of GEA Process Engineering A/S.

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Chapter 7

Fluid Bed Coating-Based Microencapsulation

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7.1 INTRODUCTION

Many dry foods or food ingredients are particulate materials such as powder, crystals, or granules. Fluidization is often used in upstream or downstream processing of these materials. Fluidization is a process where air is drawn or pushed upward through a bed of particulate material at a rate that lifts and suspends particles. Lifted and suspended particles are free to move about and the particle bed behaves like a fluid. Fluidization can be used for a variety of purposes including particle size classification, heat treatment, evaporation of volatile components from wet material, or particle transfer. In most fluidizing systems, the air is drawn through the bed, which creates a negative pressure compared to the atmosphere and helps confine material to the process.

Fluidization can be interfaced with spray processes to apply coatings to particles and/or agglomerate particles to form larger granules. These processes are often referred to as fluid bed coating or fluid bed granulating processes. In these systems, process air used to fluidize particles also evaporates and removes the volatile vehicle of the coating/granulating system or congeals the molten spray on particle surfaces. These systems are designed to pass or cycle the particles through the spray region in a manner that achieves the desired coating or granulating goals most economically. Fluid bed coating/granulating is used for a variety of purposes in the food industry including the following:

1. Reduction of fine particles or dust
2. Improved handling properties
3. Improved homogeneity
4. Color or flavor addition
5. Reduction of undesired off flavors or bad taste
6. Protective barrier coat applications to shield from oxygen, moisture, or other reactive ingredients
7. Introduction of sustained, delayed, burst, or enteric release properties

Most fluid bed coating/granulating systems in common use today have evolved from the work of Dr. Dale E. Wurster at the University of Wisconsin in the 1950s and 1960s (Wurster, 1963, 1964, 1965a,b,1966a,b). Patents from his work disclosed a variety of ways to cycle particles through the spray region of a nozzle or nozzles. Commercially viable variations of nozzle design and equipment configuration have been disclosed since then (Kishibata and Sawaguchi, 1989; Imanidis et al., 1990; Huttlin, 1990, 1991, 1992, 2000, 2002, 2004, 2005, 2009, 2010a,b; Littman et al., 1993; Jones, 1995, 2009; Walter, 2001; Jones et al., 2004; Schneidereit and Pritzke, 2006; Jacob et al., 2007; Mehta et al., 2007). The most widely used processes today are Wurster (bottom spray), top spray, and tangential spray (rotary) systems. Process selection and comparisons of products produced with these processes have been discussed (Jones, 1985; Mehta and Jones, 1985; Rubino, 1999). "Static" tangential spray designs involving use of a diagonally slotted plate are also in use today. These designs are detailed in Table 7.1 and Figures 7.1 through 7.4. Other variations of these designs may provide similar processing capabilities, but they have not realized broad practical use and will not be discussed in this chapter. There are also continuous process fluid bed configurations discussed later in this chapter that have found a commercial niche (Wurster, 1966b; Jacob et al., 2007).

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00007-8

TABLE 7.1 Fluid Bed Processes

Process	Nozzle Position	Process Type	Typical Process Goal
Wurster (bottom spray)	At the fluidization plate with spray directed upward concurrently with low density particle flow	Batch	Coating or granulating
Top spray	Above fluidizing bed with spray directed downward countercurrent to particle flow	Batch	Granulating
Tangential spray (rotary)	In side positions with spray directed tangentially with high density particle flow	Batch	Granulating
Tangential spray (static)	At the fluidization plate with spray directed diagonally concurrent with high density particle flow	Batch	Granulating

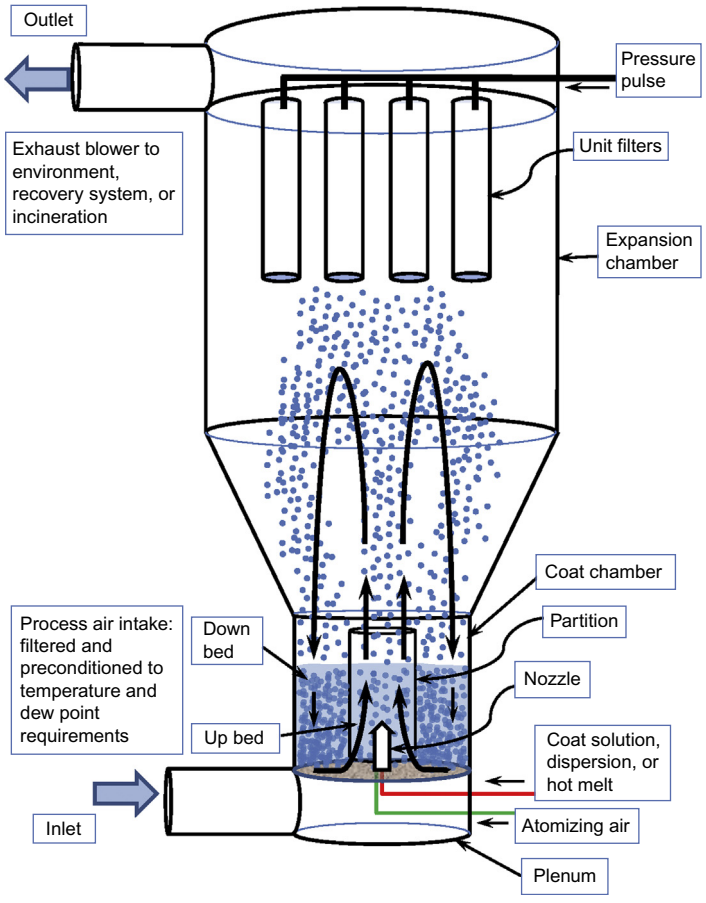

FIGURE 7.1 Wurster (bottom spray) fluid bed coater.

Although fluidization requires solid particulate materials, liquid or volatile components can be coated or granulated if they are confined in or to a solid particulate. This can be achieved by either absorbing the liquid or volatile components in or on a solid core material through a mixing or coating process, or encapsulating the material in some manner prior to application of a fluid bed process. If components are volatile such as flavor or fragrance components, selective evaporative losses are likely during fluidization due to the relatively high volume of air moving through the system.

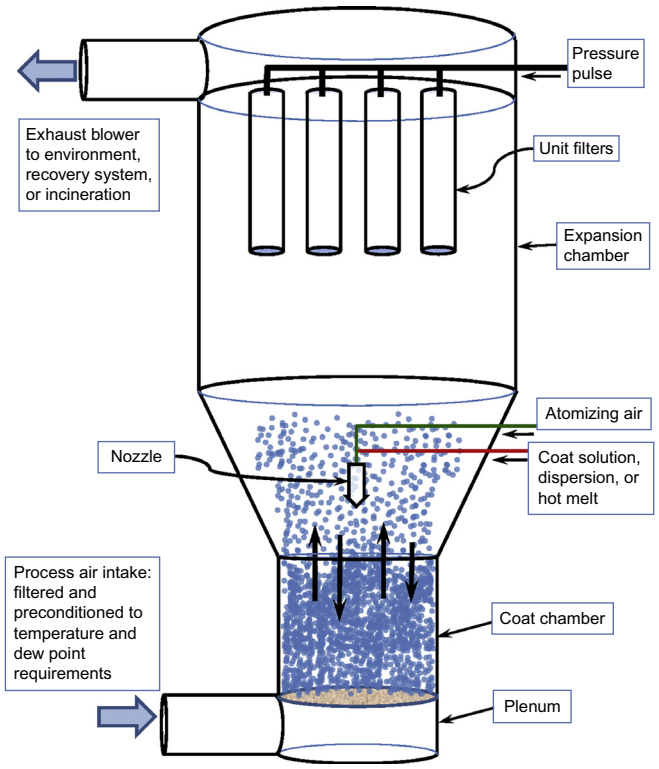

FIGURE 7.2 Top-spray granulator.

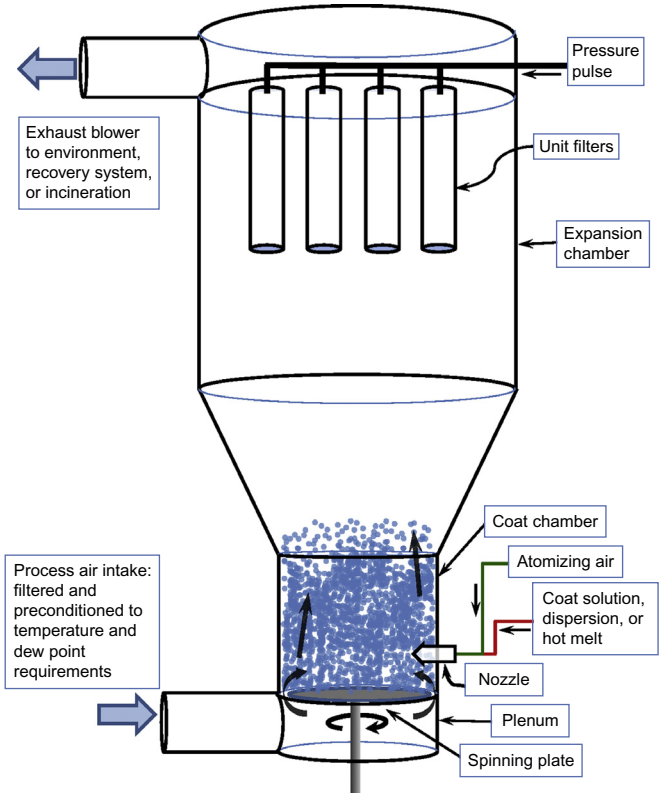

FIGURE 7.3 Tangential spray granulator (rotary).

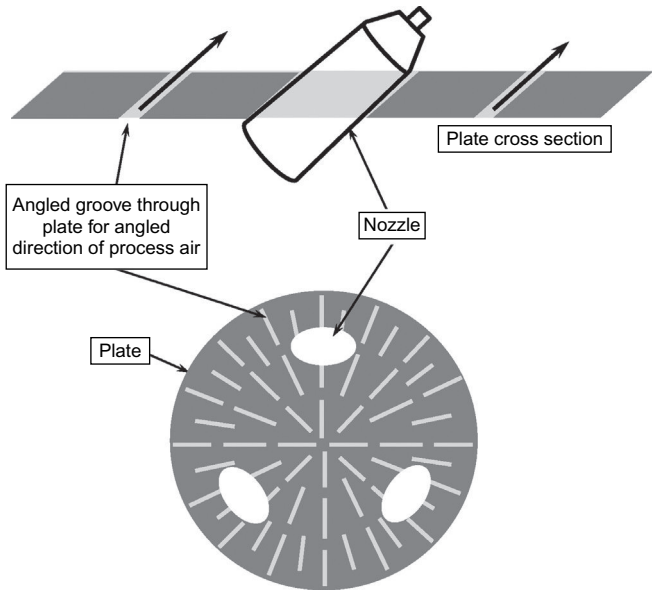

FIGURE 7.4 Tangential spray granulator (static).

Use of the Wurster process in the food industry is often limited by the potentially higher costs associated with Wurster equipment or the longer process time commonly required for coating individual particles without agglomeration. Processing costs are typically based on process time and process times for agglomeration processes such as those performed in a top spray system are linked to process air flow (drying capacity). Process air needs for the Wurster process are comparable to those for top spray; thus, process times and costs can be comparable for granulation work on these systems.

This chapter first reviews the details of particle movement in each process and follows with a discussion of technical aspects of fluid bed particle granulating and film coating. A general overview of fluid bed coating and granulation applications in the food industry is provided to outline the practical scope of its use.

7.2 WURSTER (BOTTOM SPRAY) FLUID BED COATING

The Wurster process is recognized as the best of the fluid bed processes for coating individual particles (Mehta and Jones, 1985). The upward movement of particles at low population density concurrently with spray direction and rapid drying minimize the potential for particle agglomeration and attrition. In addition to this coating capability, this system can also be configured and operated to granulate material and provide products comparable to those produced by other fluid bed processes.

The Wurster fluid bed coating system illustrated in Figure 7.1 is characterized by a differential air flow induced by the fluidization plate at the base of the particle bed. This differential induces rapid upward movement of air in the "up bed" or "coating zone" concurrently with the upward spray direction. The air velocity in this region is significantly higher than the minimum requirement to fluidize particles; thus, particles are carried rapidly upward concurrently with spray. The nozzle is mounted at the base of the bed in the center of this up bed region. Particles decelerate as they pass from the up bed region into the expansion chamber due to the increase in cross-sectional area. After decelerating, they fall into the "down bed" region that surrounds the up bed region. In a properly designed and run system, the process air is sufficient to both accelerate particles though the up bed region and maintain fluidization in the down bed region. At the base of the fluidizing down bed region, the path of least resistance for air flow is toward the up bed region due to lower particle population; thus, particles near the base of the down bed are carried inward to feed into the up bed region. Particles complete a cycle about once every 6 to 10 seconds in an optimal configuration and batch loading. The process is carried out until the desired coating or properties are achieved.

The physical partition shown in Figure 7.1 around the up bed region creates a physical barrier between the up and down bed regions for more ordered particle flow; however, a similar particle flow pattern is realized in the absence of this partition. Although a partition is described in the original Wurster patent, it is the differential air flow that was the

novel aspect of the patent. Given sustained release, formulations applied with and without the partition have been shown to provide identical controlled release products. Use of a partition is dependent on equipment design, particle characteristics, and personal preference.

Removal of the partition and reduction of the fluidizing air differential allows the bottom spray process to be used for granulating. In general, reduced differential promotes more random, turbulent particle flow in the spray region. Sufficient differential remains to maintain a cyclic particle flow and ensure optimal uniformity.

More detailed discussion of the fluidization and coating dynamics in a Wurster fluid bed coating process can be found in a book chapter authored by Dr. Wurster (Wurster, 1990). A recent general article on application of the Wurster process in the pharmaceutical industry has been published (Jones, 2009). Detailed modeling and imaging of the flow in Wurster systems have also been attempted (Mafadi et al., 2003; Heng et al., 2006; Palmer et al., 2007; Gryczka et al., 2008; Fries et al., 2011).

7.3 TOP SPRAY GRANULATION

A top spray configuration is shown in Figure 7.2. This process incorporates one or more nozzles in each of several potential spray positions spraying downward into a fluidizing bed. As coating or granulating solution or dispersion is sprayed into a fluidizing bed, the relatively high population density of the bed promotes agglomeration. As granulated particles form, their larger size requires more air to fluidize; thus, they remain lower in the bed and allow smaller particles to wet and agglomerate. This size stratification in the bed provides a leveling tendency to bring granules to a similar size.

The top spray process is not well suited to coat individual particles (Mehta and Jones, 1985). Attempts to reduce agglomeration and promote coating in a top spray system typically involve slower application rate, more fluidization air to reduce particle population density in the spray region, and/or raising the nozzle(s). Such changes lead to both reduced coating efficiency and poor coating uniformity due to spray drying and less ordered particle movement.

7.4 ROTARY TANGENTIAL SPRAY GRANULATION

The rotary tangential spray process illustrated in Figure 7.3 uses a rotating nonperforated base plate with process air flowing upward around the outer edge of the plate into the moving particle bed. Variations of this process were disclosed in patents issued in 1990 and 1989 (Kishibata and Sawaguchi, 1989; Hirschfeld and Weh, 1994). The fluidizing process air and plate rotation impart an outer circulating, rolling motion of the bed. Nozzle(s) are oriented to spray in a tangential position into the circulating bed. The relatively high particle population in the spray region coupled with the drying and physical action promotes agglomeration to a uniform size. Granules produced with the rotary process tend to be denser compared to other fluid bed granulations with a smoother surface due to significant physical interactions between particles.

7.5 STATIC TANGENTIAL SPRAY GRANULATION

A static tangential spray design is illustrated in Figure 7.4. This design uses a base plate that is perforated with slots that are cut diagonally through to the plate to angularly direct process air flow as it passes through the plate. Nozzles imbedded in the plate spray concurrently with the process air and particle movement. The process is described in greater detail in a 1990 patent (Huttlin, 1990).

7.6 DISCUSSION

The following discussion is focused on critical process parameters associated with fluid bed coating or granulating. Subjects of this discussion include fluidization requirements, temperature, drying capacity, nozzle and spray, scale-up, and continuous processing.

7.6.1 Fluidization

Process air (the air used to fluidize particles and carry solvent vapor away) is often set based on past experience and visual observation of the fluidizing bed. Depending on several factors, this can lead to less than optimal results and

conclusions, which could easily compromise applications. A good knowledge of fluidization is important to the proper application of these processes.

Fluidization is achieved through the application of adequate air velocity. Although the pressure differential across a given bed of material will vary with bed depth (material mass), the velocity required to fluidize the given material is the same regardless of bed depth; thus, if 500 scfm (cubic feet per minute at standard conditions) of air in a given coating unit is adequate to fluidize 20 kg of material, it is also adequate to fluidize 50 kg in the same unit. Air velocities higher than the minimum requirement will lift particles higher, but the differential pressure from the base of the bed to above the upper particles will remain relatively constant for a given amount of the material when fluidizing. Required velocity will vary with particle size, shape, and absolute density.

Table 7.2 indicates approximate velocities required to reach minimum fluidization for various materials. These velocities are based on observed bed behavior at varying velocities in a 6 or 9 inch diameter chamber with uniform air distribution. The error associated with these numbers is estimated to be within $\pm 5\%$. Be aware that these are minimal values and rates might be set up to three times or more above the minimum for small particles to ensure consistent fluidization through a coating process for the entire distribution of particle sizes in the bed. Also, less spherical and less dense materials will typically require less air to lift, taking into account that the surface area of a fluidization plate allows extension of this information to the volume of air flow needed to reach this minimum fluidization point. For example, a given weight of 25/30 sugar spheres on 0.5 ft^2 area of fluidization plate would require ≈ 46 cfm (cubic feet per minute) of air to reach minimum fluidization (92 ft/min $\times$ 0.5 $ft^2 = 46$ cfm). This information can be helpful in establishing process air flow requirements and adjusting it for particle growth throughout a process. This information can be easily translated to a top spray system with a uniform air distribution across the entire surface of a plate.

Translation to a Wurster coating process is a bit more challenging. The air distribution plate for a Wurster process creates separate zones for the differential air flow. A limiting factor is the down bed area where air flow should be adequate to at least minimally lift or fluidize particles. The need in this region must be factored up appropriately to determine overall process air need. The air distribution realized from a given plate is related to the amount of open space in each region of plate and the diameter of the holes used to create that open space. A basic assumption is that the pressure drop across the entire surface of the plate is equal. For this to be true, air must pass faster through the more open space due to less resistance. In addition, air passes more freely through larger holes; thus, air drawn through portions of a plate in which each portion contains equivalent open space, but each open space is created with different hole sizes, will distribute with a higher percentage going through the large hole region. Proper circulation in a Wurster process is realized by maintaining adequate fluidization in the down bed region with sufficient up bed region air to dilute and pass particles completely through the up-bed zone without excessive velocity. This creates a path for material at the bottom of the fluidizing down bed zone to feed into the up bed. Material flow is influenced by the amount of air entering the up bed region through the plate, the length of the partition, the gap between the plate and the partition (which gates down bed air and particles into the up bed), and the amount of material in the down bed. More material in the down bed will speed the feed to the up bed region.

TABLE 7.2 Minimum Fluidization Velocities

Material	≈Absolute Density (g/cc)	≈Bulk Density (g/cc)	≈Minimum Fluidization Air Velocity (ft/min)
550 mg tablet	1.6	0.8	458
2 mm × 3 mm pellet	0.8	0.4	169
18/20 sugar sphere (≈850 to 1000 μm)	1.6	0.8	132
25/30 sugar sphere (≈600 to 710 μm)	1.6	0.8	92
20/60 KCl	1.98	1.0	34
Bakers' special sugar (≈75 to 300 μm)	1.59	0.8	26

cc = cubic centimeter.

In the tangential spray (rotary) system, the air flow need is dependent on particle size and the gap at the outer edge of the plate.

Table 7.2 indicates some practical limits associated with fluid bed coating processes. Larger particles require more air to fluidize. Eventually, an upper limit is realized where a given blower reaches its limit and cannot provide enough air to fluidize the material. More air can always be supplied with a bigger blower; however, the large amount of air needed to move large particles results in coating inefficiency as the coating material has greater potential to dry or congeal before contacting particles. The limit associated with these concerns creates a practical application limit close to 2 to 3 cm in particle size for common particle shapes and densities.

A fine particle limit is realized due a number of factors including the low air flow requirement needed to fluidize. This low air flow requirement reduces the drying capacity, which lengthens process time.

Core particle size distributions should remain relatively consistent for a coating process to perform equivalently each time it is run. This is less critical for granulation work in top and tangential spray systems as the process is designed to tie fine particles up into bigger agglomerates. In a Wurster coating system where particles must not only fluidize but also pass upward through the up-bed region, particle size distribution must be relatively narrow. If distribution becomes too wide, the air flow needs for the extremes of the distribution become too wide and particle circulation is compromised. An ideal distribution for Wurster coating is one where the largest particles are not more than four or five times the size of the smallest; however, it will work for distributions where the largest is up to 10 times the smallest.

An often overlooked aspect of fluidization is the volume change of the air as temperature shifts through the process air path. The general gas equation can be used to estimate the volume change:

$$V = \frac{nRT}{P}$$

where:

V = volume in liters
n = number of moles
$R = 0.0821 \frac{(L)(atm)}{(mole)(^\circ K)}$
T = temperature in K = °C + 273
P = pressure in atmospheres

Assuming 1 atmosphere pressure (or a constant pressure), the volume shift from one temperature to another can be determined from the K temperature ratio:

$$V_2 = V_1 \frac{T_2}{T_1}$$

where:

V_1 = volume at temperature 1
V_2 = volume at temperature 2
T_1 = temperature 1 (K)
T_2 = temperature 2 (K)

This volume change can be significant as indicated in Figure 7.5 showing the relationship of volume versus temperature for a quantity of air equal to 1 ft^3 at 77°F and 1 atmosphere pressure. Knowledge of the equipment and conditions of process air flow and temperature can be critical to achieving desired outcomes. For example, 500 cfm of air at 77°F is equivalent to 550 cfm at 130°F (500 cfm × (1.1 ft^3 at 130°F)/(1.0 ft^3 at 77°F) = 550 cfm).

Process air is typically drawn from the atmosphere, filtered, and treated to meet the temperature and humidity requirements of the process. Exhausted process air and volatile materials (such as evaporated solvent vehicle) pass through the unit filters that retain particles in the process and either exhaust to atmosphere or, in the case of organic solvent or odor concerns, pass through an incineration or recovery system to minimize environmental impact.

7.6.2 Temperature

The inlet process air temperature for fluid bed coating in food applications generally ranges from near ambient to ≈210°F. The inlet temperature is measured in the plenum area immediately below the product chamber and base plate. The inlet temperature setting depends on a variety of concerns including the solvent vehicle (volatility), ingredient stability factors, process concerns (such as electrostatic cling), and core or coating properties such a glass transition

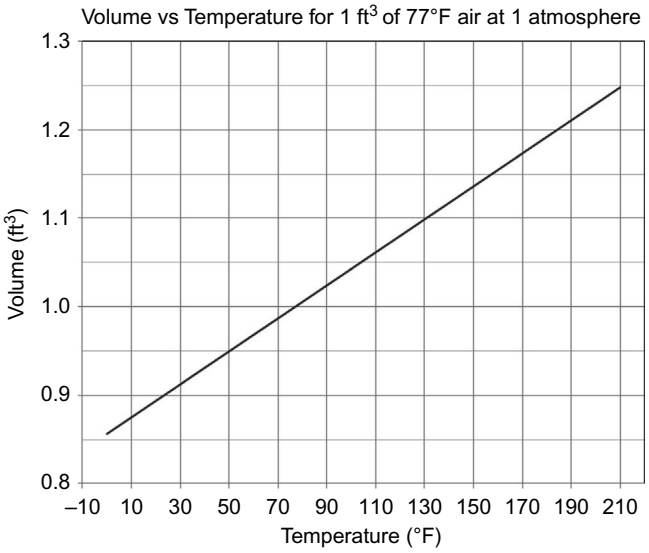

FIGURE 7.5 Graph indicating the air volume change with temperature.

temperature or melt point. Fluidized beds are only exposed to the inlet temperature near the base plate because the temperature rapidly shifts after passage through the plate to temperature conditions within the coating unit. Temperature within the unit is influenced by the surrounding environment temperature and the losses or gains from it, the volume of air flow, and evaporative cooling or hot-melt warming from the sprayed coating material. In the absence of other limiting factors, inlet temperatures for evaporative processes are set to provide an optimal drying capacity without spray drying coating before particle contact. For hot-melt coatings, inlet temperature is typically set below the melt point of the melt material to congeal coating on particle surfaces.

Other critical temperatures are the bed (product) and outlet temperatures. Bed temperature is measured in the outer fluidizing region and outlet temperature at a point above the fluidizing bed near the transition into the expansion chamber. The difference between bed and outlet temperature is typically within a few degrees of one another and dependent on equipment configuration and process conditions.

7.6.3 Drying Capacity

Drying capacity is the amount of "space" in the process air that is available to carry solvent vapor away. It is related to air quantity, temperature, dew point, solvent vapor pressure, and current baseline quantity of the solvent in the air. The graph displayed in Figure 7.6 indicates the saturation point of various solvents in air at atmospheric pressure versus

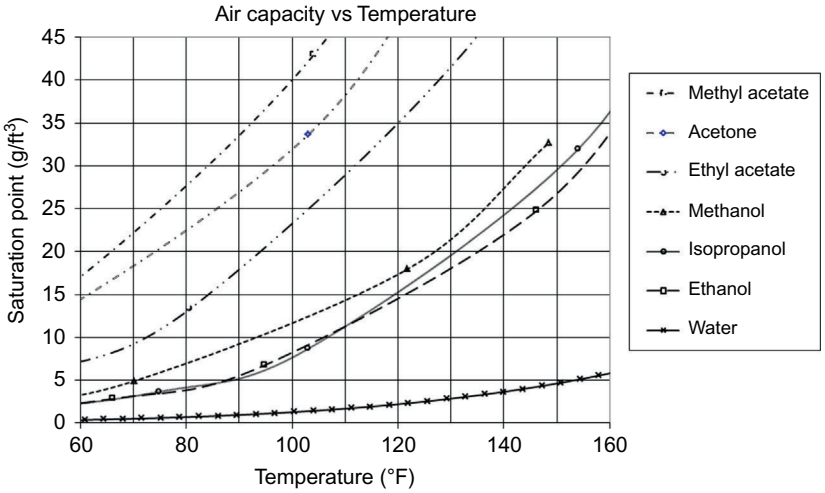

FIGURE 7.6 Air capacity for organic solvent vapor.

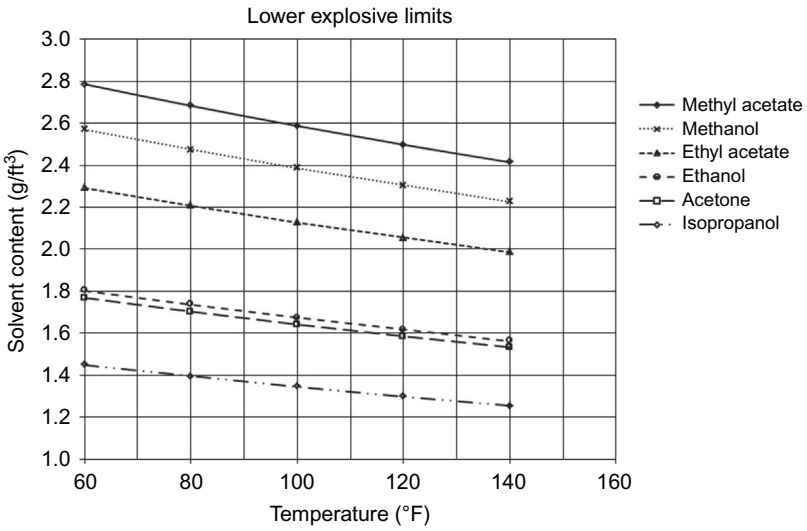

FIGURE 7.7 Lower explosive limits (LEL) for various organic solvents.

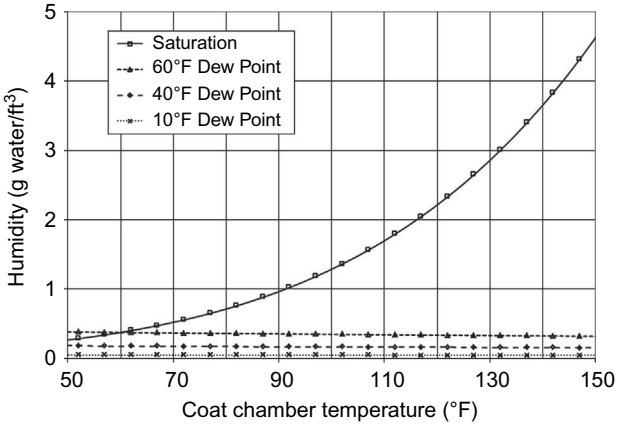

FIGURE 7.8 Aqueous system drying capacity. Graph shows humidity limits.

temperature. Below each line is the air capacity available for that solvent. For example, the capacity for acetone in air is ≈ 32 g/ft^3 at 100°F; thus, a fluidized bed operating at 500 cfm and 100°F bed temperature has the capacity to carry acetone vapor away at a rate of 16,000 g/min (32 g/ft^3 × 500 ft^3/min = 16,000 g/min).

In actual practice for organic solvent processes, systems must be operated at conditions below the lower explosive limit (LEL). Figure 7.7 indicates the LEL for the selected organic solvents for a range of temperatures. Spray rate must be maintained safely below the LEL level. A generally safe rate would be at or below ¼ to ½ of the LEL. For acetone at 100°F bed temperature, the LEL is near 1.65 g/ft^3; thus, an upper spray rate of 0.825 g/ft^3 would be at ½ the LEL. At 500 cfm of air flow, this equates to $\leq \approx 412$ g acetone/minute (0.825 g/ft^3 × 500 cfm = 412 g/min). Note that this is solvent rate and if a solution contained 10% solids (90% acetone), the solution rate would be $\leq \approx 457$ g solution/minute [(412 g acetone/min)/(0.9) = 457 g solution/min].

Precautions are taken in commercial systems to prevent or arrest an explosive condition with organic solvent use. Such precautions include either a pressure relief system or interlocks to ensure pumps shut down in the event of inadequate process air flow. Because process air is pulled through fluid bed systems, they are typically under a negative pressure compared to atmosphere and relatively open to quickly reduce pressure associated with an explosive condition.

Aqueous systems are not restricted by LEL concerns; however, drying capacity is reduced by the amount of water already in the process air. The water line from Figure 7.6 has been expanded in Figure 7.8. The amounts of water that would already present in the process air at dew points of 10, 40, or 60°F have been added to the chart. The difference between the saturation point and water content at the process air dew point is the available drying capacity. Higher temperatures provide higher drying capacity. Ideally, process air dew point is controlled to a consistent level; however, this can add significantly to equipment and operational costs depending on geographic location and the treatment

requirements to provide that consistent dew point. As a result, process air dew point is often left to the whims of "Mother Nature" or, at a minimal level of control, reduced to an acceptable level during more humid times of the year with a dehumidifying chiller or desiccant to remove water. A varying dew point can be problematic for processes due to the shifting drying capacity. Processes can be built with spray rate, temperature, or process air parameter ranges that allow adjustment to the dew point conditions to provide a consistent product.

Most aqueous fluid-bed granulation processes are applied at spray rates near and above the available drying capacity. When spraying above the drying capacity, a portion of the sprayed water is left in the fluidizing material. This water builds up through the process to promote granule formation. The rate of water buildup can be estimated from the graph in Figure 7.8. For example, at 65°F, the saturation point is ≈ 0.44 g/ft^3 and the water content at a 40°F process air dew point is ≈ 0.18 g/ft^3; thus, the difference is 0.26 g/ft^3. At 500 cfm process air, 130 g/min water will saturate the air (0.26 g/ft^3 × 500 cfm = 130 g/min). If water is sprayed at 180 g/min, ≈ 50 g/min would build up in the product (180 g/min − 130 g/min = 50 g/min). After 1 hour of spray time, ≈ 3000 g of water would be present in the product bed. At the conclusion of the spray process, spray is stopped and parameters are set to dry to the required moisture content. Dry time can be estimated from the water content and the drying capacity of the process air (drying capacity is the difference between the saturation point and dew point water content at the bed temperature). Actual dry time may be longer than such an estimate because the process air will not likely be saturated as the drying progresses. The end of a drying process can often be detected by the increase in bed or outlet temperature as cooling effects of evaporation lessen.

7.6.4 Nozzles and Spray

Two-fluid nozzles are typically used to spray solvent, solution, dispersion, or hot-melt material into the fluid bed. A standard two-fluid nozzle is illustrated in Figure 7.9. Other designs including an annular, radial spray pattern have been disclosed and configured into fluid-bed systems (Huttlin, 1991, 1992, 2000, 2002, 2004, 2005, 2009, 2010a,b), but these will not be discussed here due to limited application in the food industry.

With the standard two-fluid nozzle, sprayed material is pumped through the tip of the nozzle as one of the fluids. The second fluid is typically air and it is supplied by pressure from a desiccated, compressed air supply through the coaxial channel surrounding the nozzle tip to atomize the liquid as it emerges. Nozzle design, air velocity, and air volume govern the extent of atomization. Nozzle tips vary by the inside and outside diameter of the tip, the inside diameter of the cap orifice, extension of the tip above the cap, and directional channeling of the air prior to exiting the cap.

Atomized droplet diameters typically range from 5 to 100 μm. Parameters are generally adjustable to provide a relatively narrow droplet size distribution ranging from a median diameter near 8 μm or higher. The actual distribution depends on spray rate, viscosity, and nozzle design limits. Droplet size is not always critical in granulation work where the goal is to build larger agglomerates; however, it can be a critical parameter when coating individual particles in a Wurster coater. The critical concerns are related to drying speed, and core particle size and integrity. Figure 7.10 is a

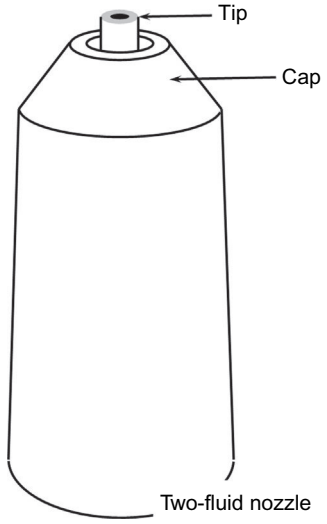

FIGURE 7.9 Two-fluid nozzle.

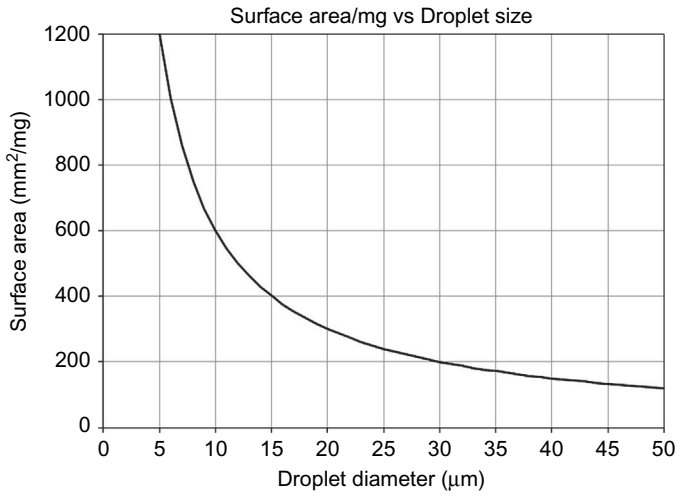

FIGURE 7.10 Theoretical assessment of water droplet size.

heoretical assessment of the surface area provided at water droplet sizes from 5 to 50 μm. Smaller droplets create more surface area per given amount of a fluid. Note that reducing droplet diameter in half doubles the overall surface area. Because drying is a surface phenomenon, this could potentially halve the drying time and allow faster spray rate and improved process efficiency.

Smaller droplets can be achieved through higher atomizing air pressure (higher air velocity and volume), reduced spray rate, or reduced liquid viscosity. Higher pressures impart energy not only to droplet formation but also to fluidizing particles. If particles are not robust enough to withstand that energy, they can fracture into smaller fragments, which can compromise the coating goals. In addition, the faster drying achieved with smaller droplets can result in spray drying where droplets dry before contacting a particle to deposit coating. This can also result in nozzle capping where coat material dries on the nozzle tip and obstructs atomization and liquid flow.

Droplet size also imparts a limit on the range of particle sizes that can be coated in a Wurster coater. In Section 7.6.1, a lower particle size limit associated with the low fluidization air requirement and low drying capacity was mentioned. Particle sizes that can be coated by this process are also limited by droplet size. When particles are near or smaller than spray droplets, overwetting of particles and the potential for multiple particles to adhere to droplets can lead to particle agglomeration. The extent of agglomeration can be related to these phenomena as well as the characteristics of the coating system. Successful Wurster coating of individual 50 μm diameter particles, without significant agglomeration, has been achieved with acceptable yield.

Coating solution and suspension viscosity is typically less than 300 centipoise. Nevertheless, viscosities up to 3000 centipoise are potentially applicable depending on coating/granulating goals. As solution viscosity increases, more atomizing air energy is needed to break it up; thus, droplet size increases. Also at higher viscosity, droplets do not wet the particle surface as well, which promotes agglomeration. In general, lower viscosity is needed for individual particle coating and viscosities as low as 5 to 10 centipoise may be required for particles below 200 μm depending on coat formulation properties.

A nozzle shield has been employed in some Wurster systems to help insure more complete spray pattern development before particle contact (Jacob et al., 2007; Jones, 1993, 1995, 2009). This need may be related to the physical dimension elements of the system and resulting particle flow dynamics. The added gap has potential to promote spray drying due to added distance to reach particles.

For hot-melt systems where molten wax is sprayed and congealed on particle surfaces, solution, liquid lines, and nozzles must be maintained at temperatures that keep the wax in a liquid state. Process air temperature is maintained appropriately to congeal the wax on particle surfaces after it exits the nozzle tip.

7.6.5 Scale-Up

Because fluid bed processes are batch processes, scale-up generally involves increasing the size of the unit. Size increases can involve vertical increases in bed depth and/or increases in the base plate area. Small research-scale Wurster equipment includes coat chamber sizes ranging from 2 inch diameter and higher and can accommodate batches as small as 10 to 20 grams. As base plate area is increased, process air must be increased to maintain required air velocity for fluidization. Spray rate is typically increased in proportion to air flow to maintain consistent drying conditions. Eventually, a spray limit is reached where the nozzle either cannot reach required spray rates with adequate atomization or can no longer cover the bed or coating zone with adequate uniformity. In the Wurster system, a limit is reached where the outer perimeter of the up bed region exceeds the outer limit of spray droplet travel within the particle flow. Although this limit may vary with configuration and design, it generally falls at a 4 to 5 inch radius from the nozzle tip. This limit defines the optimum upper capacity for a Wurster coating unit.

Regardless of the limiting factor in the single nozzle design, further scale-up is achieved by introducing multiple nozzles and coating zones. Top spray systems can be configured with multiple nozzles on a supply manifold and/or multiple spray locations. It is relatively inexpensive to increase unit size for top spray due to the relatively low cost and flexibility of adding size. The lower equipment cost is one reason top spray may be preferred in some situations over Wurster (bottom spray) for granulation work.

Properly designed multiple-nozzle Wurster systems ensure complete circulation of material and feed coating zones in a manner consistent with the single nozzle. Figure 7.11 illustrates various coating zone partitioning layouts for varying multinozzle configurations. Manufacture of the air distribution plate is relatively expensive due to the precise air distribution needs across the plate. Note the areas outside any of the down bed regions but still within the outer circumferences of the multinozzle configurations. Proper handling of these zones to ensure particles remain circulating in the system is critical to a successful scale-up. Also, process air must be properly balanced to ensure that all zones

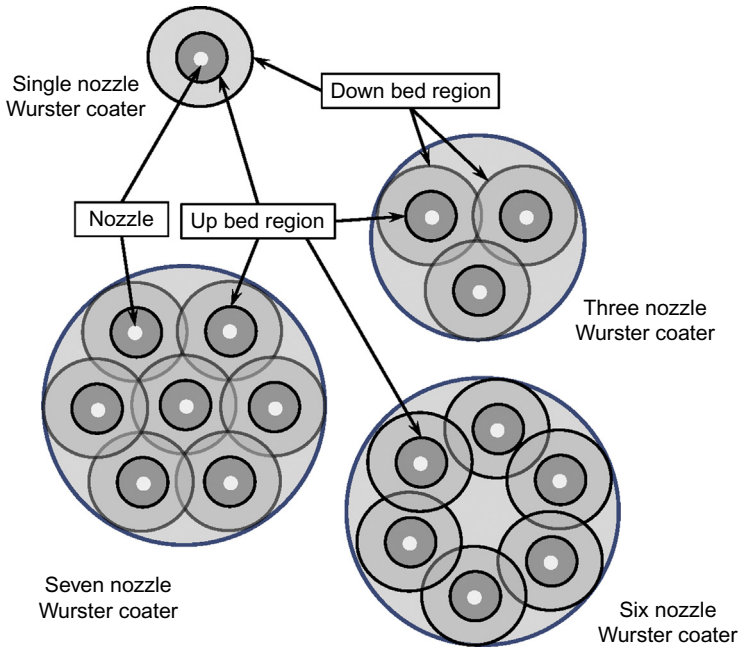

FIGURE 7.11 Wurster scale-up illustration.

receive an equivalent amount of process air to prevent "plugging off" a zone or zones due to excess buildup of the particle bed in one area of the plate. Some multiple nozzle systems are designed with linear scalability from the basic single nozzle design to allow relatively easy scale-up. A linear scale-up includes scaling the charge and process air flow linearly with zone number. For example, a 50 kg charge at 500 scfm process air in a single nozzle unit would scale to a 350 kg charge and 3500 scfm in a seven nozzle unit; each nozzle sprays at the same rate as the single nozzle unit.

7.6.6 Continuous Processes

Continuous processes have been developed by arranging material flow through a set series of spray nozzles to a discharge point. The continuous bottom spray coater configuration illustrated in Figure 7.12 was disclosed in a patent authored by Wurster (Wurster, 1966b). This configuration is similar to a multinozzle Wurster coater; however, the coating zones are arranged in series. Core material is fed in at the feed point on the left and jumps from one chamber to the next chamber and eventually to the discharge on the right. Although particles may spend varying amounts of time in each chamber, the time each particle spends in the entire system is somewhat uniform. This means that, acceptable product can be achieved depending on the goal. Variations of this continuous concept are commercially available.

7.7 FORMULATION CONSIDERATIONS

Coating or granulating formulations are generally created from food-grade materials that are generally regarded as safe (GRAS). As mentioned in Section 7.6.3, organic solvent and aqueous systems are applicable; however, organic solvent systems require that residual solvent following a fluid bed process falls within allowed limits. Organic solvent systems generally involve the use of acetone or the lower alcohols such as methanol, ethanol, and isopropanol. Aqueous systems are preferred and make up a majority of applications.

Sprayed material can be solutions, dispersions/suspensions of small particles with binding ingredients or film forming properties, or hot-melt waxes. A critical concern is that the sprayed material can reliably be passed through the nozzle tip.

It is possible to add dry ingredients to a process while it is running. This is typically done by opening a feed port to the bed of the unit and allowing the negative pressure within the unit to draw the material in.

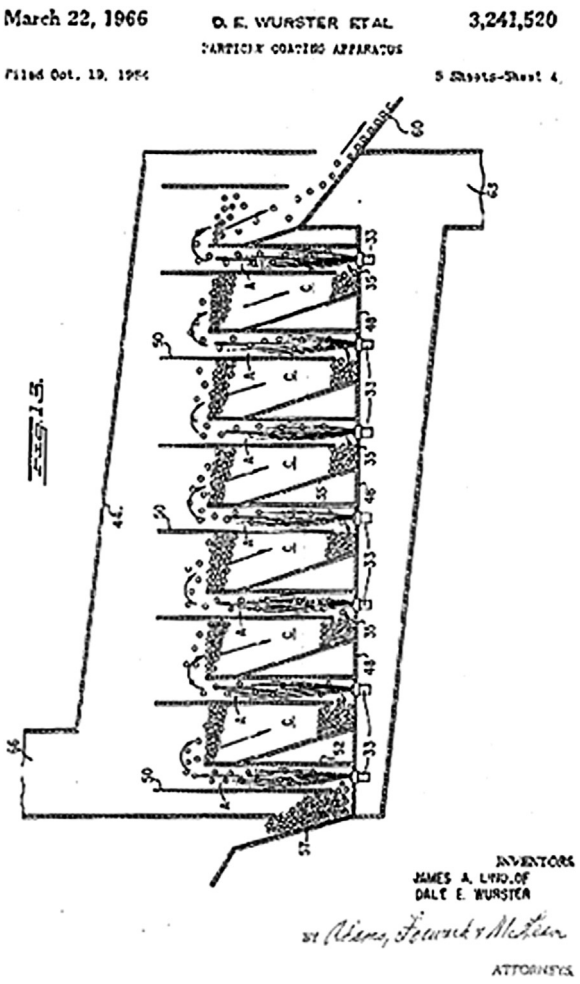

FIGURE 7.12 Wurster continuous coater conceptual design.

Granulations rely on one or more components in the formulation to have binding properties that help hold agglomerates together. Potential binding materials include maltodextrins, gum arabic, starches or starch derivatives, methylcellulose, hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), protein materials (zein, whey or soy protein isolates, caseinates, or gelatin), and waxes. Binding ingredients can either be incorporated into the coat solution (to be fully hydrated and sprayed to take full advantage of the binding capacity) or added to the dry fluidized bed charge and sprayed with water alone or binder solution to wet the dry binder during processing and "activate" the binding properties.

Binding materials are also useful for uniformly incorporating color, flavor, or other low-level ingredients into a bulk material. If dry mixed, such ingredients may segregate or stratify in a dry blend. If they are bound to the surface of the bulk ingredients in a fluid bed process, the integrity of the mix is maintained throughout storage and handling.

7.8 CONCLUSION

Nutritional supplement incorporation into food has been an area of continuous growth. Often these ingredients impart a bad taste or a taste incompatible with the food it is in. This bad taste can be masked by the addition of a protective coating on the bad tasting ingredient. The coating is formulated to remain relatively intact until it has passed the mouth. Release is realized due to eventual permeation through the coating shell or elimination of the shell through dissolution, enzymatic degradation, or erosion processes. Any food-grade coating material has some capacity to mask taste and the selected ingredients depend significantly on the intensity of the bad taste.

Some ingredients are prone to instability due to oxidation, hydrolysis, or incompatibility with other formulation ingredients. Addition of a protective coating around the ingredient or the coreactive ingredient will extend shelf-life. One example is starch or a starch derivative applied as an oxygen barrier layer on a core particle. Another example is a physical/structural layer on iron or a protein ingredient to prevent protein degradation accelerated by their contact.

High temperature instability can be realized in downstream processing or during a cooking process. Incorporation of a wax coating with an appropriate melting point can moderate temperature sufficiently to locally reduce heat exposure and improve stability.

Extended release coatings are useful for some applications such as nutritional supplements. This is often approached with a strategy similar to that of taste masking. These coatings generally involve the use of water-insoluble materials that release slowly with a semipermeable coating layer. Coating materials that can be used in this capacity include hot-melt materials such as food-grade waxes or fatty acids, shellac (confectioners' glaze), and zein. Material releases by either permeation through the layer and/or slow dissolution, degradation, or erosion of the layer. Dissolution or degradation can be influenced by pH, hydrolysis, and enzymes.

Coatings are used to provide enteric release (release in the intestinal region). Enteric release is best achieved by application of a neutral to base soluble coating that remains intact at the low pH of the stomach but dissolves at the higher pHs of the intestinal region. Enteric coatings typically involve the use of weakly acidic coating polymers that dissolve after deprotonation at neutral to basic pH. The enteric polymers commonly used in the pharmaceutical industry are not approved for use in foods; thus, materials such as alginic acid, fatty acids, or shellac products with a suitable acid value are incorporated.

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Chapter 8

Extrusion-Based Microencapsulation for the Food Industry

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8.1 INTRODUCTION

Encapsulating flavor oils, omega-3 fatty acids, and enzymes commercially through extrusion technology is relatively new: it represents only 2 to 3% (Porzio, 2008) of all the technologies for encapsulating active or sensitive materials. This is largely because the extrusion process is quite complex and much less explored than traditional encapsulation processes.

Besides the extrusion process conditions that will be discussed further in this chapter, the composition of the matrix, properties of the encapsulated material, and their interaction with each other play a significant role in the success of the extruded encapsulate. Other authors discuss these topics in detail in the appropriate chapters of this book. Here, we will refer to an encapsulate as a certain carbohydrate matrix, or a mixture of starch and protein matrix, that is stable in the glass state at room temperature. The main goal for the extrusion process in this application is to produce a hard, dense, glassy pellet that will protect the well-mixed, encapsulated material from evaporation and oxidation for an extended period of time, but will still easily release the active material when desired. A high-density pellet shows greater stability and better protects the active material. However, an expanded, low-density extrudate will be prone to releasing the encapsulated materials prematurely and may considerably reduce its shelf-life.

Different extruders are used in the food industry: single-screw; twin-screw, co-rotating; and twin-screw, counter-rotating. To encapsulate active materials, developers and manufacturers typically use twin-screw, corotating extruders because they provide mixing and kneading efficiency, good pumping capability, flexibility to use different ingredients, and the ability to reproduce results.

An example of the twin-screw, corotating extruder is shown in Figure 8.1. A twin-screw extruder consists of a frame, motor, gearbox, process section or barrels, extrusion head, and cutter. The extruder has a modular design, with a process section as short as three or four barrels or as long as 14 barrels. The length of the process section will depend on the application and what functions and residence time are required to achieve the desired product quality. Within the process section are two shafts with screw elements that are rotating in the same direction—corotating—and those elements, which can be configured in an almost infinite number of ways, are responsible for conveying, mixing, melting, and cooking the product inside the extruder. The extruder head consists of the distribution plate and cone that guide the product flow from the screw channels to the dies, ensuring homogeneous and even flow through all die holes. At the end of the extruder head is the die plate, which contains holes that provide desired shape to the product. The finished product, or extrudate, can be cut with fast-rotating knives immediately after exiting the die, or later downstream.

The process for manufacturing encapsulated products commercially can be as follows. Powders are metered by a gravimetric feeder into the feed throat of the extruder's first barrel. Water or another plasticizer is added in the same barrel to plasticize the powder. Flavoring oil can be added right after the water injection or later in the process. The main goal is to create a melted carbohydrate matrix and evenly distribute flavoring oil within the matrix.

To accomplish this, starch gelatinization or "matrix melt" is conducted at relatively gentle extrusion conditions, with low mechanical energy input and low temperatures. It is also important to cool the melt prior to its exiting the die so that volatile components are not lost as it flashes at the die, and to ensure a high-density, tight pellet. For this

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00008-X

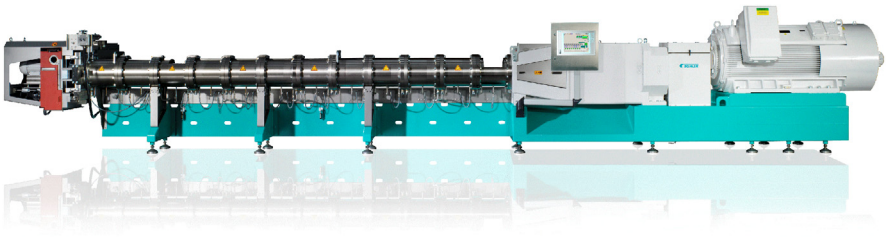

FIGURE 8.1 Twin-screw extrusion equipment.

FIGURE 8.2 Samples of the extruded pellets with encapsulated flavor oils.

purpose, the last barrels are chilled to maintain a die temperature below 100°C. However, the temperature should not be so low as to prevent the carbohydrate melt recrystallizing before it exits the die, thus clogging the die holes.

To melt the matrix at low temperature, disperse the oil, and cool the homogeneous mass, this extrusion process requires a longer residence time and thus a longer machine, about 7 to 11 barrels long.

Extrusion die can be tempered to better control the viscous material flow through the die holes. Shear rate through the die results in the generated heat that could be removed through active cooling or tempering. Liquid heating is preferred to electrically heated dies, but it is more complicated in design. Coat-hanger dies are unique kinds of dies that allow strands to be formed onto a belt where the product is oriented in parallel. Product strands are subjected to cooling from top or bottom and cut with a remote pelletizer. It may be challenging to keep the individual strands separated from each other, especially when cooling air is blown from the top.

The size of the extruded, encapsulated product that is formed and cut directly at the die is usually in the range of 0.5 to 1.5 mm. As the pieces become smaller it is more difficult to separate them during cutting. The pellets are quite sticky and tend to adhere to each other, creating a chain of pellets rather than separate pieces. The extrudate can also be smeared, rather than cut, by the knives. To improve cutting at the die, hot air can be blown through the pelletizer housing. This helps to maintain favorable temperature at the die while avoiding significant cooling and melt crystallization in the die hole. It also allows volatile aroma to be more easily evaporated from the outer surface of the pellet resulting in a less sticky product and fewer lumps. Samples of the extruded pellets with encapsulated flavor oils are shown in Figure 8.2.

High pelletizer speed is required to cut the strands into small beads directly at the die. Typically, the pelletizer motor turns in the range of 2500 to 4500 rpm, depending on the number of knives installed and the amount of die holes. Thin knives of 0.5 to 1 mm thickness are made from flexible steel, and are sometimes Teflon® coated to prevent beads sticking to the blades.

Depending on the product application, this relatively large size of the pellet can be viewed as a benefit or a limitation of the extrusion process. It is a benefit in the applications where visible pieces are desired. For example, in particles with flavor substance for tea bags, and in reconstituted rice with embedded vitamins. However, extruded pellets cannot compete with spray-dried materials where the smaller particles are desired.

In some cases, product is extruded as a continuous rope or sheet, cooled and dried, and then milled to the desired particle size; however, this process can potentially damage the integrity of the pellet and, thus, propagate the oxidation

of the oils (van Lengerich, 2001). One has to carefully investigate the impact of the milling process on the encapsulated material's shelf-life.

Several flavor companies have developed their own encapsulated, volatile flavor oils or active ingredients using extrusion technology. These include McCormick's FlavorCell®, Caplock® from International Flavors & Fragrances, Evoglass® from Symrise, Durarome® from Firmenich, as well as many other commercial products. All these products are proprietary and patented. It is worthwhile to review at least some of these patents to get a broad overview on this subject.

8.2 EVOLUTION OF EXTRUSION TECHNOLOGY

One of the earliest examples of oil encapsulation by extrusion is described in the US patent granted to Sunkist in 1957 (Swisher, 1957) and referred to through the years in many other patents. This method includes several process steps to produce encapsulated essential oil that can be used in the food, soaps, cosmetics, and other industries. First, the corn syrup-based mixture is heated to a molten state while moisture content is adjusted to a low rate between 3 and 9%. Then the mixture of essential oil, heat-stable antioxidant, and emulsifier are agitated to disperse them evenly in the melted corn syrup matrix. The oil-soluble antioxidant is used to protect the essential oil from degradation in the hot molten corn syrup, while the emulsifier is used to ensure a finely dispersed emulsion. Sunkist found that adding the emulsifier, with its ability to assist in distributing small droplets of oil in the molten matrix, increased the oil load from 6 to 12%. In this case, the extruder is used to shape solid particles. After the extruder, these particles are washed with organic solvent to eliminate surface oils. Finally, the solvent is dried and the particles are ready for storage.

Sunkist described other interesting findings in this work as well. For example, it proposed to mount the extruder vertically. When the melted mass exits the die, the strands will not touch or stick together, instead becoming stretched under the force of gravity. The extrudate will fall directly into a solvent bath, creating thin, hardened cylinders. These cylinders are further reduced in size by impact breaking, which creates uniform particles and results in a reduced new open area for oxidizing the oil compared to the traditional irregular products of grinding.

Other patents describe using solvent baths. For example, US Patent 4,707,367 (Miller and Mutka, 1987) describes the process of mixing and melting a carbohydrate mixture to a molten state, together with an emulsifier, followed by adding flavor oil to the closed vessel under certain pressure to form a homogeneous mixture. This melted mixture is then extruded through a die into a cool solvent bath. The melt is extruded as a continuous rope and broken into small particles through fast agitation action in the solvent bath. Hardened particles are separated from the solvent, dried, dusted with anticaking agent, and packaged.

A solvent bath requires additional process steps. Also, increasing capacities to make this process more economical is a great challenge. Because of this, many producers have eliminated this step and perform cooling within the extruder.

US Patent 5,183,690 (Bagley et al., 1993) describes an extrusion process for encapsulating biologically active agents, such as bacteria and viruses, in the carbohydrate matrix. The carbohydrate matrix was mixed with water, melted, and cooled to 25°C prior to introducing the active agent into the extruder. No die was installed at the end of the extruder. The product was air dried to 10% moisture, and then ground to the desired particle size.

In some cases, cooling the melted matrix is enhanced by evaporating steam, as described in US Patent 6,416,799 (Porzio and Popplewell, 2002). Here the powder and plasticizer were mixed in the extruder barrel, powders were melted, and the plasticizer was vented to the atmosphere. After that, the flavor oil was added to the relatively cool process. The plasticizer, in many cases water, plays an important role in melting and gelatinizing the matrix, as well as protecting product from being oversheared. However, reducing the plasticizer through venting reduces or completely eliminates the need for a postextrusion drying step, which, in turn, reduces the capital investment, as well as lowers energy costs.

US Patent 6,190,591 (van Lengerich, 2001) describes very similar process where first a matrix material, such as starch, is extruded under low shear mixing conditions and plasticized at high moisture content. Water addition rates in the range of 20 to 50% based on the weight of the matrix material are specified. While the material is conveyed and mixed, the barrel temperature of the extruder is raised above the gelatinization temperature to at least partially gelatinize the starch. Once the material is plasticized, the patent describes removal of some of the water by evaporation to the atmosphere through an open barrel. As a result, the plasticized hot mass will lose some of the heat and temperature will decrease to some extent. Further barrel cooling and/or the direct injection of inert gas such as carbon dioxide or nitrogen is suggested to efficiently cool down the temperature of the mass before the heat-sensitive ingredient is admixed into the matrix.

The same patent outlines how the cooled, plasticized mass is extruded through dies having openings of about 0.5 to 5.0 mm allowing pellet size to be adjusted. The inventor also describes different cutting methods: by either cutting directly at the die plate or by cutting with a remote cutter installed away from the extruder. The latter method allows for additional surface cooling and for surface drying. This procedure makes the ropes less sticky which improves cutting into small particles.

US Patent 7,803,413 (van Lengerich et al., 2010) describes a process of encapsulating omega-3 fatty acid in the carbohydrate and protein mixture matrix, incorporating the antioxidant to protect the encapsulated oil against rancidity. In this case, glycerol was used as a plasticizer, allowing the antioxidant to be mobile in the glassy state. The antioxidant has to stay mobile within the matrix to react with the penetrating oxygen before this oxygen can reach and react with oil. In this patent, the matrix consisted of the carbohydrate and protein mixture. Matrix powder was metered into the extruder with a gravimetric feeder; oil-in-water emulsion was pumped into the extruder with one liquid addition system; glycerol with solubilized antioxidant and water was added through a second liquid addition system; and supplementary water was added through a third system as an aid to control the melt's viscosity. Within the extruder the dry powder was intensively mixed with all liquids to produce a homogeneous dough, to disperse oil into tiny droplets, and to evenly distribute these droplets within the dough. Multiple liquid components create a significant lubrication effect, which promotes a low-temperature, low-shear, and low-pressure extrusion process. Through the continuous mixing and conveying action of the extruder, dough is moved through the extruder to the die. The extruder barrels are chilled to increase viscosity of the dough such that it can be cut after exiting the die. The die holes in this case are round, with diameter of 0.5 to 1 mm. These freshly extruded pellets claimed to have oil loads of 10 to 25% by weight. At the cutter, the pellets can be dusted with anticaking agent to prevent them from clumping.

One of the limiting factors in using extrusion technology for encapsulation of volatile components is that oil load is limited to 10 to 15% (using the spray-drying process, oil load is about 20%). With higher oil addition to the matrix in the extrusion process, excess oil will start separating at the die from the product mass, creating a spitting effect of oil out of the die. It seems as though the matrix cannot hold more oil in its medium. Free surface oil is expensive waste, because it will evaporate, but at the same time it is also a potential hazard as these volatile components are combustible and can be dangerous to transport.

8.3 CONCLUSION

Extrusion technology has great potential for usage in the flavor and other sensitive materials encapsulation industry. It is a continuous and robust process and a plant can produce products 24/7, using very little water and thus requiring minimal drying. As in other applications of the extruders in the food industry, encapsulation mechanism and interaction between different components of the system within the twin-screw extruder are very little explored and understood. There is still a lot of art involved in this process. However, as water and energy become more expensive in the developed countries, the more interesting and valuable extrusion technology will become for the encapsulation industry.

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Chapter 9

Spheronization, Granulation, Pelletization, and Agglomeration Processes

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9.1 INTRODUCTION

A number of different process principles can be used to manufacture products with compact structures (Jacob, 2007; Glatt Ingenieurtechnik GmbH, 2010a). In process development, the most suitable process and equipment must be selected.

Factors to be considered are, for example:

- raw materials (powder, liquids) and their properties (solubility, viscosity)
- formulation (use of additives, composition)
- final product specification (mass, flow, moisture, size)
- flexibility of plant (number of products, frequency of product changes)
- regulations and guidelines (food safety, FDA, GMP)
- interfaces (up- and downstream process steps)
- operation (batch, continuous)
- health and safety (explosion risks, emissions)

In any case, as much specification as possible of the project is required to evaluate different process designs and options. Specific aspects of different industries such as pharmaceutical or food must be taken into account. The process needs to be discussed and clarified in the context of the huge number of options in industry.

The "spheronization" option describes processes where structured and typically wet materials with plastic properties are transferred to a more spherical shape. To do this, the preforms must undergo mechanical forces to create plastic deformation and compaction of particle surfaces. Figure 9.1 shows the basic principle of spheronization for two typical applications.

On the left in Figure 9.1 is an example of a spheronization process using wet granules as feed material. Such raw materials can be produced by means of different types of mixers or high-shear granulators. Depending on the level of acting forces, the wet mass can be soft (low density), more compact (higher density), and compact in the case of a preforming process step such as extrusion. Essential for all options is that the materials to be spheronized have to be plastic and deformable over the required processing time and there should not be too much stickiness during processing. Figure 9.2 shows two typical samples of products manufactured using spheronization.

Agglomeration is one of the principles used to manufacture coarser particles. It is a process where small particles or powders were stuck together to form coarser particle structures (Figure 9.3, left). This is typically done by means of a liquid binder that is applied to the powder. The process can be done in batch-wise or continuous operation.

When the apparatus cannot apply drying energy, the agglomerates remain wet and plastic (Figure 9.3, right). Such products can be spheronized in an additional process step. High-shear granulators are frequently used to produce wet granules. The agglomerates are compact and dense, as shown in Figure 9.4 (left).

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00009-1

Another processing option for agglomeration is fluidized bed processing. Figure 9.3 (left) shows the principle of particle formation. In fluidized bed processing, the drying step is part of the overall mechanism of particle growth. Due to the heated fluidization of air (or other gases such as nitrogen, etc.), the binder liquid (supplied as solution, suspension, etc.) dries by evaporation of water (or organic solvent) and the remaining solid forms liquid bridges between individual particles.

Agglomerates produced using fluidized bed technologies are less compact and more porous compared to high-shear products (Figure 9.4, right).

A second option to produce granules is spray granulation. This process is based on a layering principle and produces denser particle structures than agglomeration using fluidized beds or high-shear equipment (Figure 9.5).

Spray granulation has to be carried out in fluidized beds. Here, different technical options are available. These variations show different process flexibilities and final granule properties. Continuous processing has advantages in spray granulation applications.

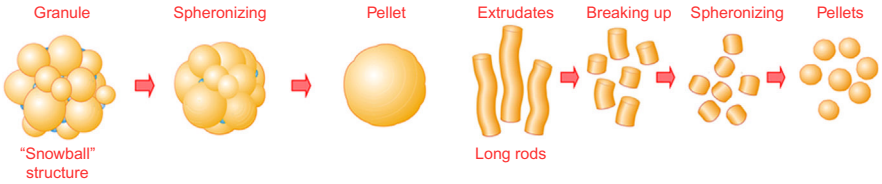

FIGURE 9.1 Principle of spheronization.

FIGURE 9.2 Product samples produced using spheronization of a wet agglomerate (left) and an extrudate (right).

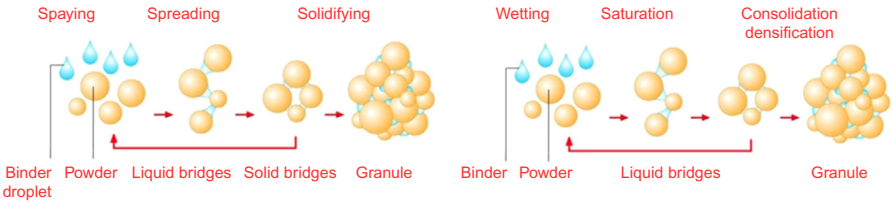

FIGURE 9.3 Principle of agglomeration in fluidized beds (left) and high-shear (wet) granulators (right).

FIGURE 9.4 Product samples produced by agglomeration using high-shear processing (left) and fluidized bed technology (right).

In the case of spray granulation processes, a liquid raw material (e.g., solutions, suspensions, emulsions, melts) is sprayed on fluidized particles. The solvent (typically water, organic solvents) evaporates from the particle surfaces and the remaining solid builds a new layer that increases the particle size (see Figures 9.5 and 9.6).

In the following section, the different processing options are explained in more detail in relation to the available technical concepts of equipment that can be utilized.

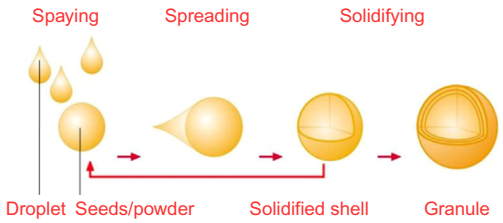

FIGURE 9.5 Principle of spray granulation.

FIGURE 9.6 Product samples produced by spray granulation from suspension (left) and solution (right) using fluidized bed technology.

9.2 BASIC EQUIPMENT

A wide range of technical principles are available to carry out processes such as spheronization, granulation, pelletization, and agglomeration. As mentioned in the previous section, different executions of fluidized bed equipment, high-shear processors, or pelletizers can be used. Moreover, special equipment was developed for customized needs and specialty products.

Figure 9.7 shows a summary of technical options for fluidized bed apparatuses for batch operation. These options are mainly used for drying, agglomeration, and coating. If complex operations must be realized, the process is divided into separate sequences, which are carried out successively.

For optimized energy efficiency, minimal operator activity, higher capacity, and requirements for long-time automatic operation continuous fluidized bed technologies were developed. Some examples for continuous fluidized bed configurations are shown in Figure 9.8 (Glatt Ingenieurtechnik GmbH, 2006).

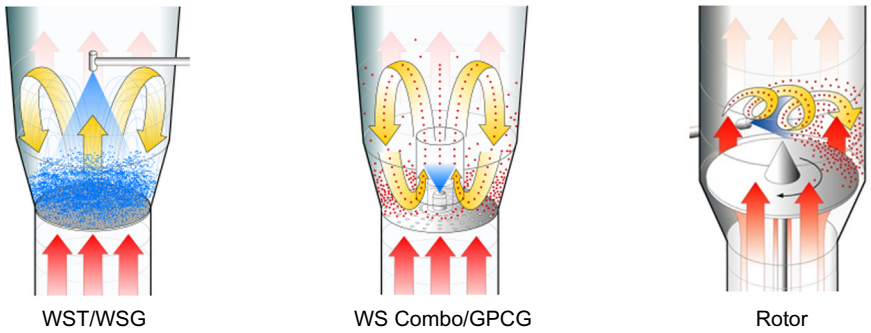

FIGURE 9.7 Summary of batch fluidized bed equipment.

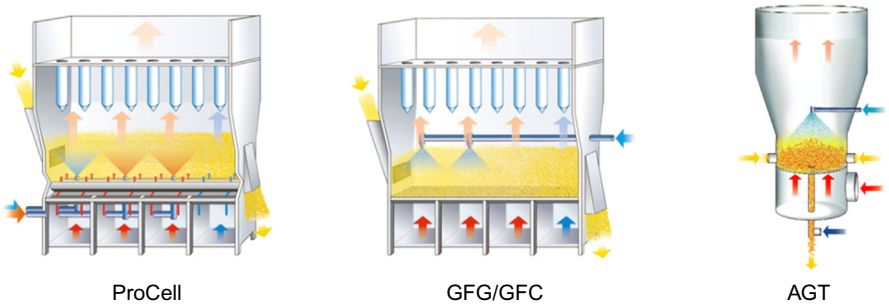

FIGURE 9.8 Summary of continuous fluidized bed equipment.

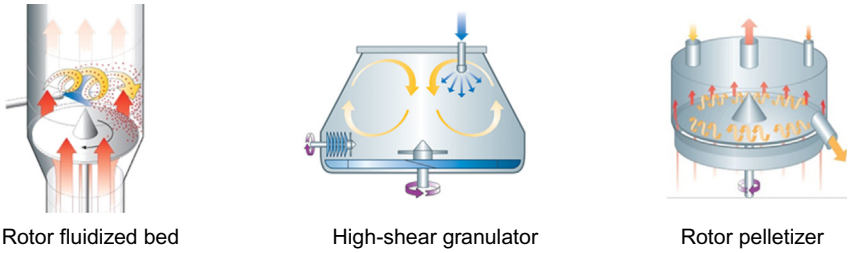

FIGURE 9.9 Summary of pelletization and high-shear granulation equipment.

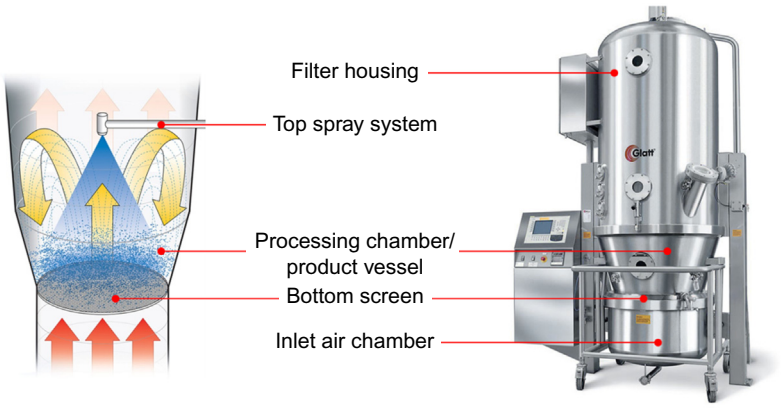

FIGURE 9.10 Glatt batch fluidized bed system for drying and agglomeration: process principle (left) and industrial equipment (right).

In all fluidized processing principles, particle movement is established by the acting fluidizing medium, where typically air or nitrogen is used. Due to that basic principle, the level of mechanical forces in fluidized bed processors is limited compared to equipment designs where mechanical tools are additionally integrated into the process chamber. Rotor fluidized beds is one of the options where advantages of fluidized beds are combined with the mechanically supported particle mixing by a rotation disc. Figure 9.9 is a summary of technical concepts using mechanical agitation to handle the product in the processing chamber.

9.3 BATCH FLUIDIZED BEDS FOR DRYING, AGGLOMERATION, AND COATING

Batch fluidized beds have been used for decades for a wide range of applications. Typically, the pharmaceutical and food industry uses these processors in different applications. Figure 9.10 shows the basic principle of a batch fluidized bed unit. The circular design of the bottom screen is the fundamental principle. In most cases, the process chamber has a conical design to slow down the velocity of process air steam in the upper part of the apparatus. Depending on the application, spray nozzles can be integrated in the process chamber. For agglomeration, top-spray nozzles can be

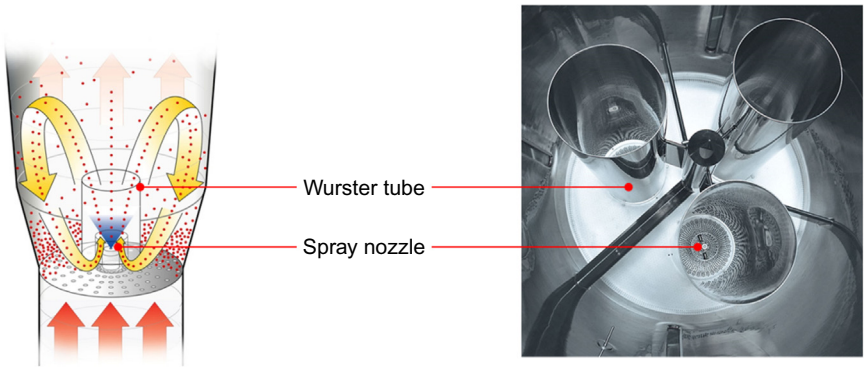

FIGURE 9.11 Glatt Wurster processor: process principle (left) and impression from industrial equipment (right).

installed above the fluidized bed. They atomize the binder liquid against the process air flow and distribute the spray liquid on the fluidized particles. The same concept can also be used for some coating applications (e.g., hot-melt coating). Especially for agglomeration, filter systems are mounted above the process chamber. Different concepts are available such as bag filters, cartridges with blowback purge, or shacking filters designed as one or more filter chambers.

If a very uniform distribution of spray liquids on the particles is needed, the so-called Wurster process is preferred over top-spray processing. This can be the case when dense pellets have to be built up in a batch operation starting from powder or small particles using the layering principle. In a Wurster processor, the bottom-spray principle is always used. Here, the injection of spray liquid is in the direction of the process air flow (Figure 9.11).

A specially designed bottom plate is used and a Wurster tube is installed above the bottom-spray nozzle. Due to the design of the bottom plate, a higher air flow is created inside the Wurster tube compared to the outer fluidized bed region. All particles entering the Wurster tube will be pneumatically transported upwards and will fall down to the outer fluidized bed in the upper part of the apparatus. This principle ensures a very uniform and controlled particle movement and a well-defined passage of all particles through the spray zone, which makes optimized layering and pellet buildup possible.

9.4 CONTINUOUS FLUIDIZED BEDS FOR DRYING, AGGLOMERATION, SPRAY GRANULATION, AND COATING

Continuous processing is a need in some fields of application. This can be the case due to capacity reasons, needs from upstream and downstream processes, or due to the process itself. In principle, there is no real limitation from the product output point of view. Continuous fluidized bed systems are available from small scale (a few grams per hour) to large commercial scale (a few tons per hour) (Glatt Ingenieurtechnik GmbH, 2006, 2010b; Jacob, 2007).

This concept has significant advantages in industrial-scale production if 24-hour operation is possible for at least a few days without product changeover. In smaller-scale production, continuous processing can be used to manufacture a wide range of lot sizes by keeping a small unit running for a long time at steady-state conditions. Here, the operation time defines the size of a production lot. Also, for continuous fluidized bed processing, different design concepts are available.

From the process flexibility point of view, the most suitable concept is horizontal fluidized bed equipment, which is characterized by a rectangular design of the bottom screen. Figure 9.12 shows the overall technical design of such a continuous fluidized bed unit.

The typical design of the process chamber with rectangular cross-section ensures a uniform movement of particles from the inlet (solid feed at one end of the chamber) to the outlet of the processing chamber (at the opposite end of the chamber). On its way from solid feed point to the product discharge, the product can be treated in different zones of the processing chamber. For instance, in drying applications different inlet air temperatures or flow rates can be supplied depending on the process needs. So, multistep processing is possible in one apparatus. Figure 9.13 illustrates the variability of a horizontal fluidized bed. Different colors of the vertical arrows represent different air temperatures. The sketches show various nozzle setups including top and bottom spray. Also, different liquids can be sprayed simultaneously.

When different processes have to be done in a continuous apparatus, the residence time distribution has to be as narrow as possible to avoid undesired back mixing between the process zones. This distribution can be influenced directly

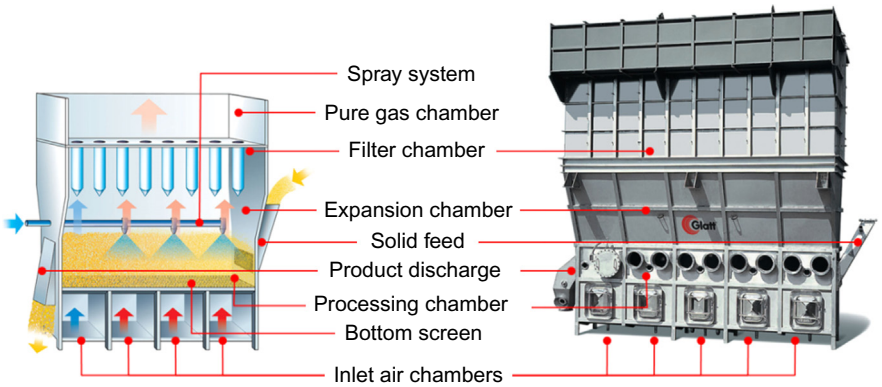

FIGURE 9.12 Glatt GF processor: process principle (left) and industrial equipment (right).

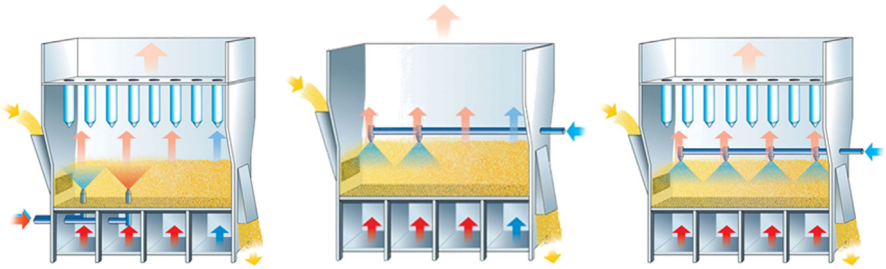

FIGURE 9.13 Glatt GF processor: variability of the process principle (differences in air temperature distribution, nozzle orientation, internal or external filter setup).

by design (length-to-width ratio) or indirectly by integration of partition plates or weirs in the process chamber (Jacob, 2010).

In contrast to horizontal fluidized bed concepts, designs with circular cross-section of the bottom screen are more suitable for single-step processes such as spray granulation or agglomeration. Figure 9.14 shows the principle of the Glatt AGT continuous fluid bed granulator.

Such granulators have been in use for decades for applications in spray granulation in a wide range of industries. Typically, the AGT type produces products with dense and compact particle morphology. This type of equipment is characterized by a unique classifying product discharge (Figure 9.14). To separate dust or fines from the final product, a vertical tube is integrated into the inlet air chamber up to the bottom screen level. A classifying air stream, which is additional to the main process air stream, enters the discharge tube and creates an upwards flow into the process chamber. Any fluidized particle can enter the discharge tube downwards. Here, a separation process takes place where light and small particles will be blown back to the process chamber by the counterflow of classifying air. The size of continuously discharged granules can be controlled by setting the flow rate of classifying air. This principle allows direct control of the granulation process only by changing process conditions.

Depending on process needs or customer requirements, the technical setup of an AGT processor can differ from case to case. As in all fluidized bed granulation processes, nozzle orientation has a direct influence on product properties. Additionally, the choice of filter concept depends on factors such as particle distribution in the fluidized bed, fluidization velocity, particle density, cleaning mode, and so on. As a few examples, Figure 9.15 illustrates different technical setups.

9.5 PROCELL TYPE OF CONTINUOUS SPOUTED BEDS FOR DRYING, AGGLOMERATION, SPRAY GRANULATION, AND COATING

The market and demands on product properties in various industries are changing all the time. Because of this, new innovative products have to be designed. These new products need unique and efficient process technologies (Glatt Ingenieurtechnik GmbH, 2010a, 2010b).

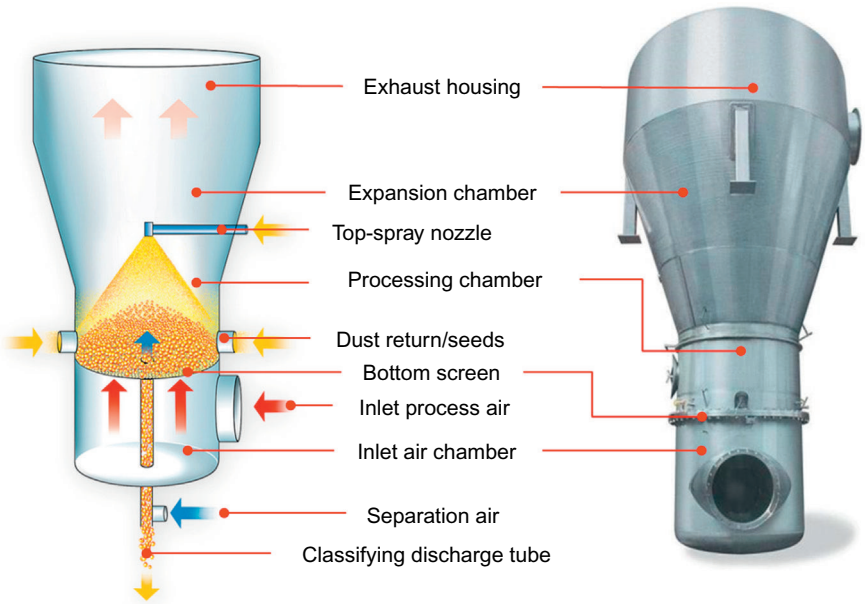

FIGURE 9.14 Glatt AGT processor: process principle (left) and industrial equipment (right).

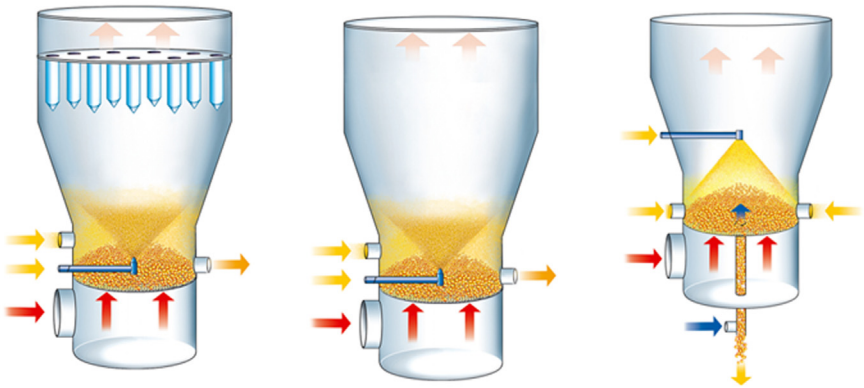

FIGURE 9.15 Glatt AGT processor: variability of the process principle (classifying or nonclassifying product discharge, nozzle orientation, internal or external filter setup).

To fulfill these requirements, Glatt has developed the ProCell apparatus based on a patented air distribution principle (Figure 9.16).

In general, spouted bed processing can be considered a special processing option in particle technology. In contrast to standard fluidized beds, particles are fluidized by a gas jet in a strongly conical or prismatic process chamber. The innovative ProCell processing unit was developed to carry out various processes such as spray granulation, coating, and agglomeration.

Typical of this apparatus is that the fluidizing air enters the process chamber through two parallel gaps (Figure 9.16 (right)). The two flows are diverted upwards and combine. This produces a central air flow in which particles will move upwards. The particles separate from the central air spout in the upper part of the apparatus, and then circulate back into the outer zones. There they return toward the point of air entry slipping down the walls. Due to the flow characteristics, the spout zone provides optimal conditions for the injection of fluids. All particles return to the nozzle with a very high frequency and pass the nozzle with high velocity. This is why spraying conditions are similar to batch Wurster coating technology (see also Figure 9.17).

In industrial-scale production, the ProCell units are designed based on the GF type of horizontal fluidized beds (Figures 9.12 and 9.13). This allows all the technical options (filter configurations, nozzle orientations, and positions) of that standard equipment to be used.

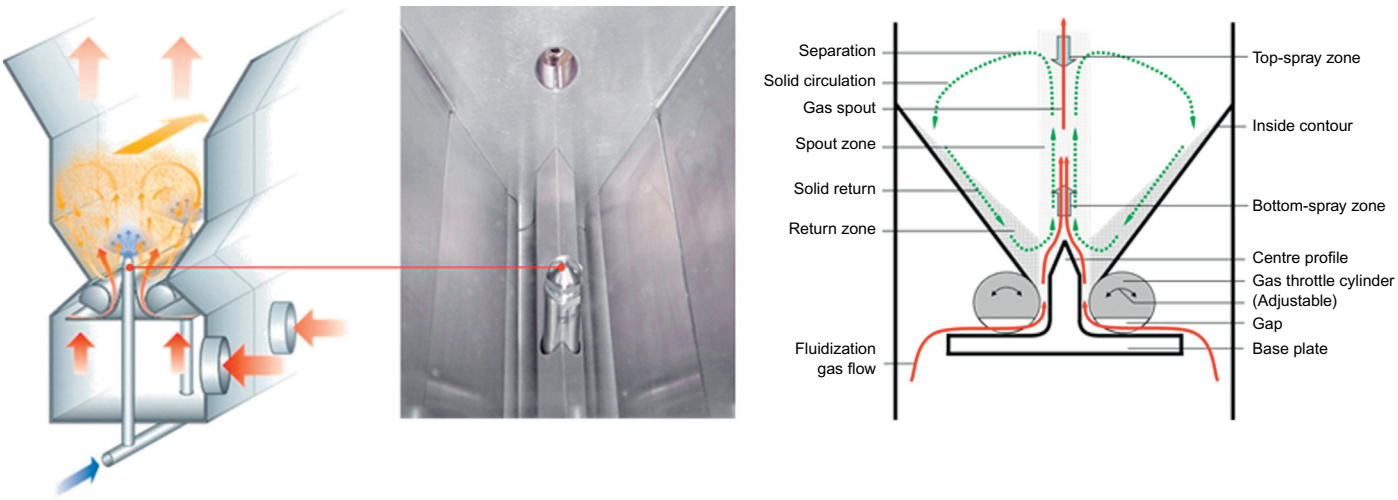

FIGURE 9.16 Glatt ProCell unit: process principle (left), detailed view of bottom-spray nozzle and air distributor (center), and principle of fluidization (right).

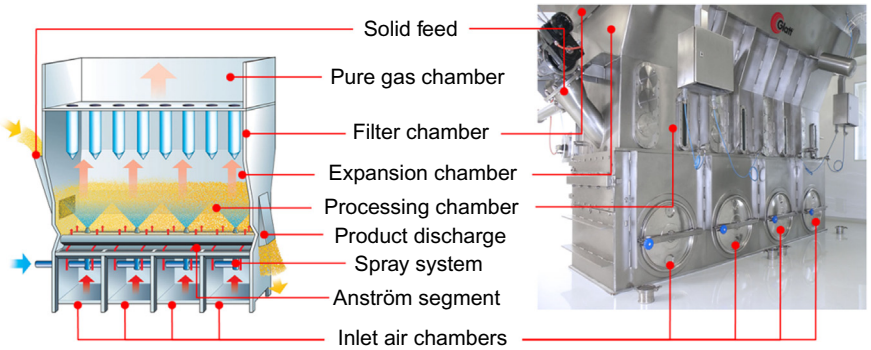

FIGURE 9.17 Glatt ProCell industrial-scale unit: process principle (left) and industrial equipment (right).

9.6 TECHNICAL OPTIONS FOR PELLETIZATION

As explained before, different options are suitable to produce pellets. The most used option is the combination of a wet-state process step to produce the plastic preform (high-shear processes, extruders) and subsequent mechanical processing in a rotor-pelletizer to create the homogeneous and dense pellet structure. In Figure 9.18, the basic principle of such a rotor-pelletizer is shown. Here, a rotating disc is integrated at the bottom of a circular process chamber. The nonperforated disc is speed adjustable and supplied with smooth or structured surfaces. Between the rotating disc and the housing, a circular gap is designed where a process air steam can enter the process chamber. Due to the rotating energy, centrifugal forces are created and the particles move to the outside where they are pushed upwards by the process air steam. This combination of forces is responsible for the special (helix) type of particle movement and due to that an intensive treatment of the plastic raw material is ensured (Neuwirth et al., 2013). The outer forces soothe the plastic particle surface and change the particle shape to the typical spherical pellet form.

As long as the product shows plastic behavior, the pelletizer can form the required product shape. In this state, an incorporation of dust is possible. Dust can come from attrition or be supplied with the wet preform. After a defined processing time (batch time), the pellets are discharged for further treatment in a dryer, and so on.

Sometimes continuous processes such as extrusion are used to manufacture the preforms. In such cases, continuous pelletization is required to avoid intermediate buffering or storing of wet materials. Therefore, pelletizer cascades can be used as shown in Figure 9.19. The control of residence time in the individual pelletizers is realized by level adjustment in each of the process chambers. Here, adjustable overflow weirs can be used.

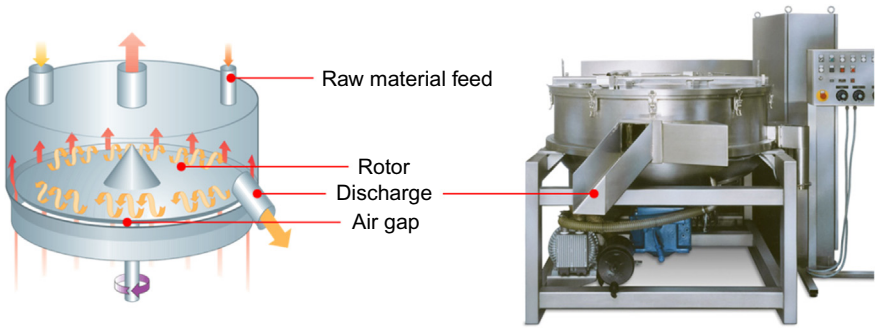

FIGURE 9.18 Glatt pelletizer: process principle (left) and industrial equipment (right).

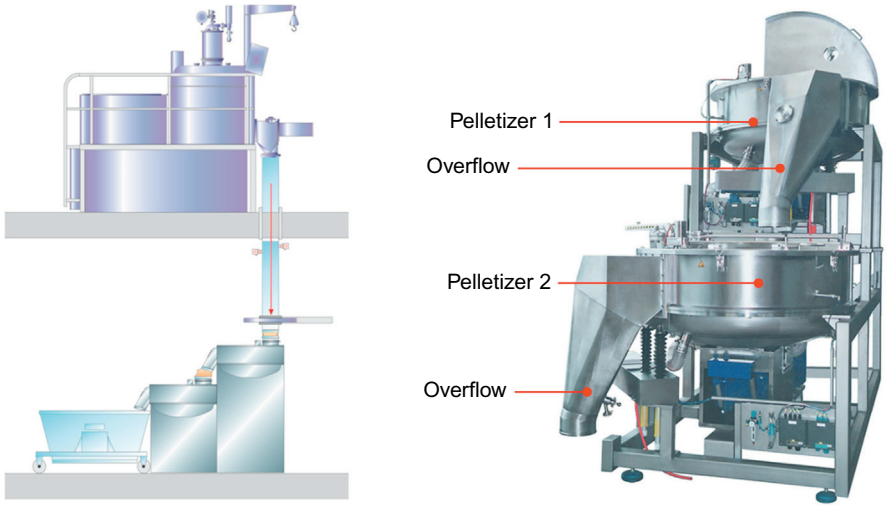

FIGURE 9.19 Glatt pelletizer cascade: process principle (left) and industrial equipment (right).

9.7 TECHNICAL OPTIONS FOR HIGH-SHEAR GRANULATION

For any pelletization process starting from powder as raw material, high-shear granulation (also known as wet granulation) is one of the options to manufacture wet granules. The process principle is agglomeration, where a binder liquid is used to stick small particles together to produce granules. These granules are not dried in process and are still plastic when discharged. The products obtained by high-shear granulation have to be dried afterwards.

Figure 9.20 illustrates the process principle of a batch type of equipment. Here, a mixing tool is used to move the material inside the product bowl. The binder liquid is added by a spray nozzle from the top of the bowl. To avoid local overwetting and lump formation, choppers are installed next to the mixing tool. These fast rotating tools are essential for uniform particle growth due to their positive effect on binder liquid distribution.

Other types of high-shear granulators are designed to be top driven. This concept has advantages regarding cleaning and easy sealing of the mixer shaft. Moreover, continuous granulation systems are also available for high throughputs (Jacob, 2007).

9.8 TECHNICAL OPTIONS FOR EXTRUSION

Extrusion using various principles (single or double screw, basket) can be used to densify wet masses produced by mixing or high-shear granulation (wet granulation).

In Figure 9.21, the basics of a continuous basket extruder are shown. This equipment is suitable to treat wet granules produced by a premixing or wet-granulation step. Basket extrusion is preferred over screw extrusion when heat- or pressure-sensitive materials have to be handled.

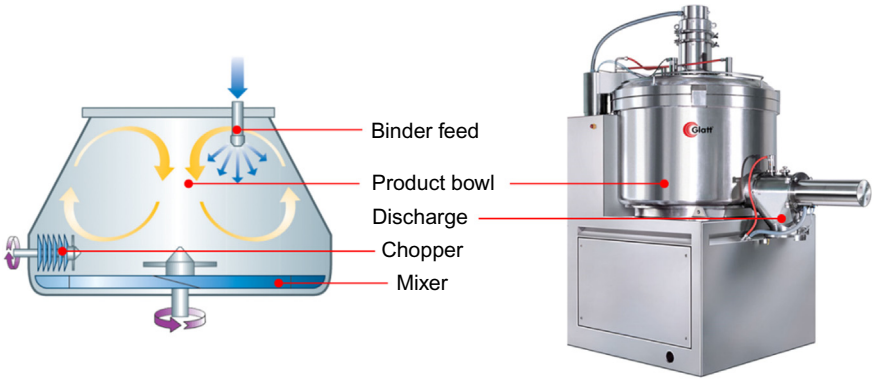

FIGURE 9.20 Glatt VG wet granulator: process principle (left) and industrial equipment (right).

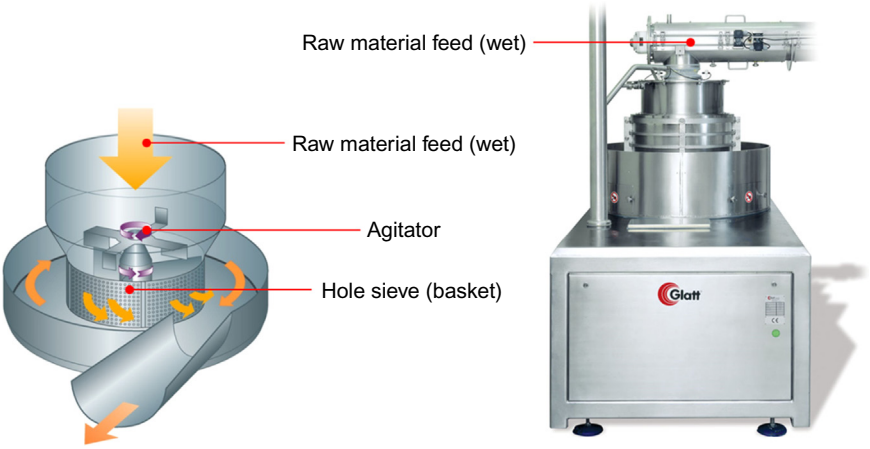

FIGURE 9.21 Glatt GBE basket extruder: process principle (left) and industrial equipment (right).

9.9 APPLICATION CASE STUDIES

Product development requires a wide flexibility from the process engineering point of view. Additional experimental studies have to be carried out in different scales. Usually, lab-scale investigations start from gram scale and end at a scale of a few kilograms.

For such feasibility studies and process developments, the modular designed ProCell LabSystem was developed (Figure 9.22) (Glatt Ingenieurtechnik GmbH, 2010b). This equipment offers a maximum operation range from a temperature and flow rate point of view as well as from process flexibility.

Figure 9.23 shows different process inserts available for the ProCell LabSystem. All processing options (besides Rotor 7) can be operated in both batch and continuous mode.

All case studies explained in the following sections were carried out using modules of this type of lab equipment.

9.10 FORMULATION OF ENZYMES

Formulations containing enzymes are of significant importance in a wide range of industries. For instance, suppliers of feed, food, and detergent additives use different types of enzymes for their innovative products.

Because of their sensitivity to heat and shearing forces, the formulation of these products must be done very carefully. One of the topics in formulation is the protection of the enzyme using additives to increase stability.

To fulfill the requirement, different processing options can be utilized. Two examples will demonstrate the options.

FIGURE 9.22 Glatt ProCell LabSystem (Glatt Ingenieurtechnik GmbH, 2010b).

FIGURE 9.23 Glatt ProCell LabSystem.

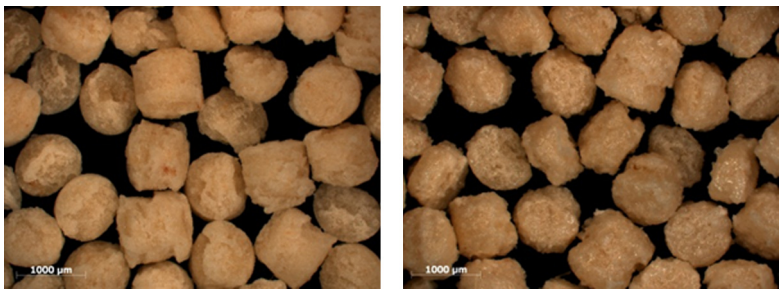

FIGURE 9.24 Formulation of enzymes: pellet produced by GBE basket extrusion and rotor pelletization (left); Wurster-coated pellets (right).

Figure 9.24 shows samples of a formulation manufactured by the following process sequence:

- spray drying of the liquid enzyme formulation
- agglomeration of the enzyme powder by wet granulation using a binder liquid and additional base powders when needed
- basket extrusion of the wet mass
- rotor pelletization of the extrudates
- fluidized bed drying of the pellets
- Wurster fluidized bed coating of the pellets with protective additive

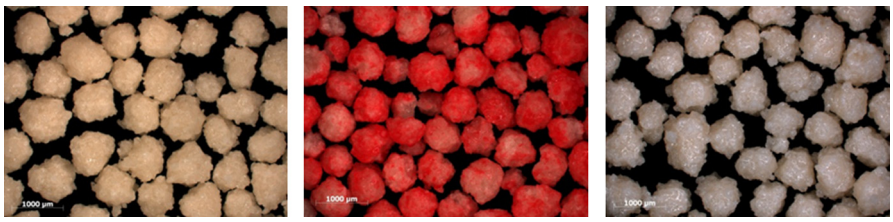

FIGURE 9.25 Formulation of enzymes: granules produced by spray granulation (left); Wurster-coated pellets (center), and matrix product produced by combined spray granulation of enzyme and additive (right).

This approach is frequently used for feed enzymes. For example, the adjustment of the final product enzyme activity is easily possible by changing the ratio of enzyme and additional base powders. Depending on composition, moisture content, and processing conditions, the pellets can be more spherical or more irregular. Special attention must be taken on the final shaping step. Particle shape should be as spherical shaped as possible to support easy coating due to a low specific surface.

Another possibility for enzyme formulation is based on spray granulation using fluidized bed technology. The product was produced using:

- continuous spray granulation of the liquid enzyme formulation using fluidized bed
- batch-wise Wurster fluidized bed coating of the pellets with protective additive

Figure 9.25 left shows typical granules produced by spraying the liquid enzyme in a fluidized bed granulator. The granules need no additional drying step and are very compact, dust free, and free-flowing. A Wurster processor is used to apply a protective coating on the granules.

In some cases, the overall process can be simplified further. Depending on the functionality of the protective additive, a matrix structure of the final product is also an option. Therefore, the additive is mixed into the liquid enzyme formulation. The complete formulation can then be spray granulated in a continuous fluidized bed granulator. This processing option is optimal regarding operational costs and process stability. Additionally, any handling of enzyme dust or powder can be avoided.

9.11 FORMULATION OF VITAMINS

The different types of vitamins are considered very sensitive during processing. There is more than one option to formulate vitamins to free-flowing and stable solid product forms.

In the case study, the vitamins were supplied in liquid form. To test different options, experimental studies were carried out in lab-scale equipment. The concentration of vitamin in the final product was adjusted by recipe of the formulation. Different approaches were compared:

- continuous spray granulation of the liquid enzyme formulation using fluidized bed
- continuous spray granulation of the liquid enzyme formulation using spouted bed technology (use of ProCell apparatus)
- agglomeration of the enzyme powder by wet granulation using a liquid vitamin and additional base powder
- mixing of base powder with liquid vitamin; basket extrusion of the wet mass; rotor pelletization of the extrudates; and fluidized bed drying of the pellets

All process options can produce free-flowing and mechanically stable products (Figure 9.26). The differences in particle growth principles have a direct influence on the mechanical properties of the granules or pellets. Additionally, the behavior of the different formulations in application is different. This situation is quite normal and can be used in product development studies. Ultimately, the most suitable process version can be selected depending on the field of application and required product specification.

9.12 ENCAPSULATION OF VOLATILE INGREDIENTS

The process of continuous spray granulation (Figure 9.5) is suitable for a lot of different applications. A special case of product formulation is matrix encapsulation of etheric oils or of volatile substances. In this case, the active ingredient

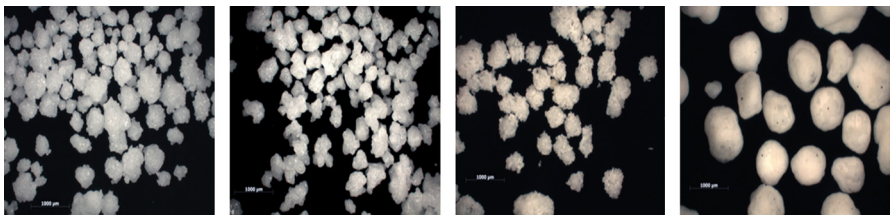

FIGURE 9.26 Formulation of vitamins: granules produced by spray granulation in fluidized bed, in spouted bed; agglomerated in rotor-fluidized bed; pelletized extrudates (from left to right).

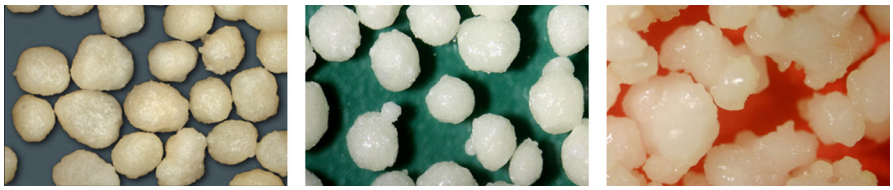

FIGURE 9.27 Formulation of vitamins. Granules produced by encapsulation in spouted bed: lavender oil (left), orange oil (middle), PUFA (right).

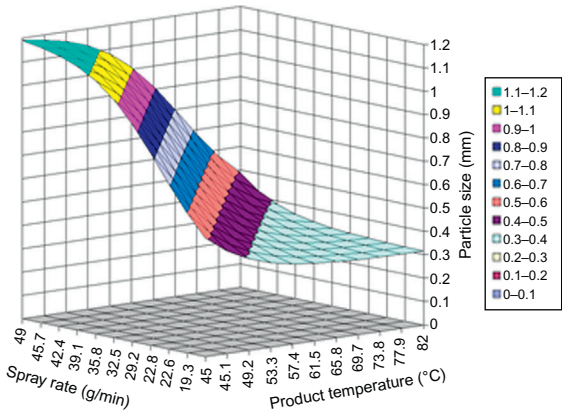

FIGURE 9.28 Surface plot of particle size. Three dimensional profiles for particle optimization (Jacob, 2012).

(e.g., the oil) is emulsified in a carrier liquid (e.g., starch type, maltodextrin, etc.) and then spray granulated. For such processes, where heat-sensitive materials are processed, treatment at low temperatures and at minimum residence time is needed; the spouted bed principle has significant advantages compared to standard types of fluidized bed units.

Some product examples produced by encapsulation using spray granulation in the ProCell apparatus are shown in Figure 9.27 for illustration.

In any encapsulation process, the process conditions (e.g., temperature, air or nitrogen, residence time) and type of equipment (e.g., nozzle position) have to be taken into consideration as well as the formulation of the emulsion (e.g., concentrations, additives, droplet size distribution) (Jacob, 2012).

In product and process development, these factors must be investigated to find an optimal solution (Figure 9.28).

9.13 CONCLUSION

Spheronization, granulation, pelletization, and agglomeration can be used to transfer powders or liquids to free-flowing granules with defined properties.

This chapter provided practical examples of the particle formation process using operation principles of different types of equipment. Finally, practical examples of food formulations and processes were summarized.

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Chapter 10

Annular Jet-Based Processes

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10.1 INTRODUCTION

The technologies described in the previous chapters are very well suited to producing powders, or in some cases granules, that have properties that either correspond to their liquid precursor or supersede it by delayed release. Delayed release, in this case, meaning that those powders or extrudates lose their precious content while in storage and exposure permanently. This leads to certain economical inflictions, as the active content is typically a valued one and changes in the composition can reduce the product quality quickly.

A search for alternative delivery systems and production methods was mainly driven by food technology, namely, fermentation as initial industrialization efforts, but in the course of the development led to further benefits for many other fields of application.

The use of a single nozzle was well known in the technologies used, such as in spray drying, dripping, and even vibrational drip casting methods to yield particles of varying qualities. In all cases, only matrix materials, so-called microspheres, consisting of a mixture of all ingredients, could be accessed. One drawback in such morphologies is that, in most cases, actives, especially hydrophobic and liquid actives as flavors, could only be added in small amounts. The matrix material was still a main part of the resulting particle and furthermore some of the desired properties of the liquid flavor were lost altogether.

Researchers in the field tried to use a multitude of concentric nozzles. The shell material was now used in the outermost nozzle and the active was used in the core. This allowed for not only a much higher load of the particle of up to 90%, but also maintained many of the desired properties of the liquid flavors. Some of the early applications include tobacco products, fermentation of alcoholic beverages, and chewing gum. Nowadays, many products contain visible or invisible capsules made by the technologies described here to enhance product quality and protect the active agents.

The use of flavors in food that were encapsulated goes back in the 1960s and 1970s where tobacco manufacturers tried to enhance cigarette filters with various devices that encapsulated liquid flavors (Boukair, 1969; Leake and Cogbill, 1969; Neals, 1970).

10.2 PROCESS TECHNOLOGIES

10.2.1 Laminar Flow Breakup

Laminar flow breakup is the general term for a number of processes based on the same theoretical background, but lead to different apparatus and units, with different fields of applications and scalability.

In the beginning, the experiments of Savart (Savart, 1833; Eggers, 2006) showed that a laminar flow of a liquid breaks up into droplets. Savart could verify that a circular flow of liquids separates into droplets (and satellite droplets). It was, however, not yet realized that the surface tension was the reason for the breakup, which was found later only by Plateau (Plateau, 1849). Later, Rayleigh (Rayleigh, 1880; Eggers, 2006) was able to enhance this theory. Nowadays, experimental aids such as high-speed camera techniques make it possible to investigate and exploit the breakup of laminar flows in detail.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00010-8

By extruding a liquid through an orifice with no more than gravitational force, droplets, the diameters of which are determined by viscosity and surface tension of the liquid, are formed. The resulting droplets in that case (gravitational dripping) have a diameter x_t, which is only related to those material properties and the gravitational force, and not related to the diameter of the orifice (Brandau, 2002).

If the flow rate is accelerated, there is a resulting minimum flow rate for the transition of "dripping" to "flow," the latter meaning that a constant flow of liquid is extruded from the nozzle. This minimum flow or critical flow rate is a function of the surface tension, the viscosity, and the nozzle orifice.

If a liquid flow reaches the critical speed of v_{crit}, the surface tension tries to compress the liquid thread and an instability with a minimal wavelength of $x_t = 3.3\sqrt{(\sigma/\rho g)} \neq f(D)$ results.

This instability disrupts the thread and forms liquid cylinders that subsequently form (due to the surface tension) spherical droplets. Assuming the identical volume for droplet and cylinder, an ideal droplet diameter that only corresponds to the nozzle diameter results ($x_T = 1.89D$), always assuming that the flow is laminar and is within the laminar flow breakup range of the Ohnesorge–Reynolds plot (Figure 10.1). Recalling that the flow rates are dependent on material properties, resulting in a droplet size related to the nozzle diameter only, it is necessary to understand that this is a two-step process. Initially, a laminar flow is generated—the properties of which are determined by the material—and second, same-sized droplets are formed, which is determined by the nozzle diameter.

The disruption of the flow can lead to so-called satellite droplets, which have a diameter of $x_S \geq 0.1x_T$. Their quantity is comparably small (0.1–1%) but the total surface of the spray increases. Because droplets of different diameters have different falling speeds, the distance of the droplets is not constant anymore and droplets can merge once again. This leads to the typical "noseforming" that can be found on unoptimized processes. The forming of the satellite droplets is determined mostly by the surface tension σ of the liquid. The viscosity has only a minor role (because $p_{stat} = (4\pi D\rho)/(D^2\pi) = 4\sigma/D$, $p_{dyn} = \rho v^2/2$, ρ = density, D = nozzle diameter, p = pressure), but for all practical purposes the viscosities η can range between 10^{-3} and 10^4 N/m. Due to this, the Ohnesorge number (Oh) [$\mathrm{Oh} = \eta/\sqrt{\sigma\rho D}$] can range over seven magnitudes. Therefore, the influence of the viscosity is in fact quite high. Materials that have a higher viscosity produce a longer wavelength and therefore larger droplets.

The maximum viscosity usable for the laminar flow breakup processes is determined by the fact that a laminar flow has to be obtained. In practical terms, upper limits (not absolute limits, only a guideline) of 5000 mPa · s are reasonable.

To avoid these problems, an external vibration is introduced in the flow. The vibration can be either in the direction of the flow or perpendicular to it. With that vibration, the flow breakup is supported and leads to uniform droplets determined by the frequency of the external vibration and the reduction of satellites. When using optimized frequencies, satellite forming no longer exists.

It is not important how the vibration is brought into the liquid (vibration of the nozzle or the liquid, vibration in the flow axis or perpendicular to it), but due to technical reasons, a vibrating nozzle with a vibration in the flow axis performs better.

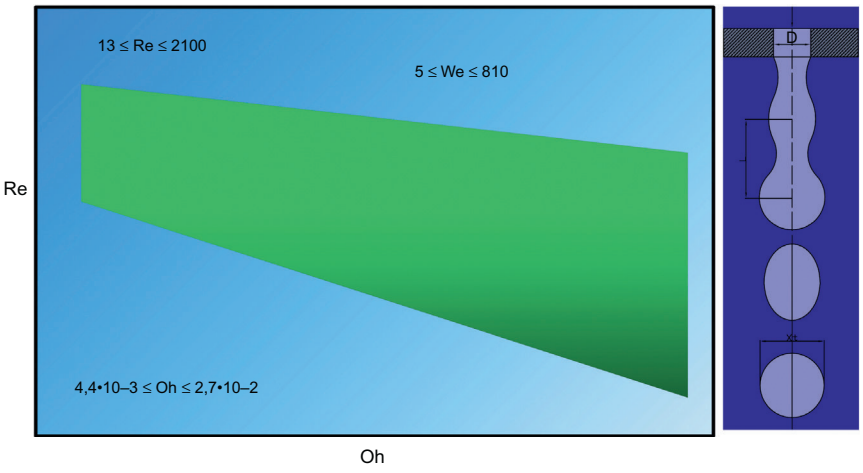

FIGURE 10.1 Ohnesorge–Reynolds plot showing the region where liquid laminar flow can be broken into regular droplets.

10.2.2 Vibrational Drip Casting

Vibrational drip casting is one of the most scalable technologies to produce core–shell encapsulated microcapsules. There is a wide range of equipment sizes available, starting from small or very small units up to large-scale equipment for the production of more than 20,000 kg products per hour. Of course, there are not many products in food applications yet that use such large-scale equipment, but large products such as encapsulated products can already reach several thousand tons per year (Burger et al., 1992).

The principle of those technologies has been known for some time but their industrialization took a considerable time as there were, despite technical problems in scaling up, also the need and acceptance of such products to be overcome. In fact, the first commercialization took place in nuclear fuel production in Germany for the high temperature reactor (pebble-bed reactor). With the end of nuclear technology in Germany, this technology was transferred to other fields of application, among them food technology. One of the early applications was the preparation of encapsulated yeast used to ferment champagne (Hill, 1991). Since then, many applications have been freshly developed, but also a lot of ideas have been newly redeveloped with this technology, as it was not economically possible to produce sufficient amounts with little effort.

Vibrational drip casting is a process working with a laminar flow breakup according to the Rayleigh flow breakup with a vibration of the flow under resonance to give same-sized droplets (see Figure 10.2). In this process, the liquid feeds (one for the core and one for the shell material) are extruded through an annular gap nozzle. The flow is kept laminar so that it disrupts the droplets. By applying a vibration in resonance axial or lateral to the flow—either via the nozzle or the liquid directly—the resulting droplets have a very narrow monomodal size distribution. By adjusting the flow rates for core and shell nozzle separately, the load can be adjusted easily during processing.

These processes can easily be applied for room temperature or heated processes by installing a heating chamber around the nozzle head.

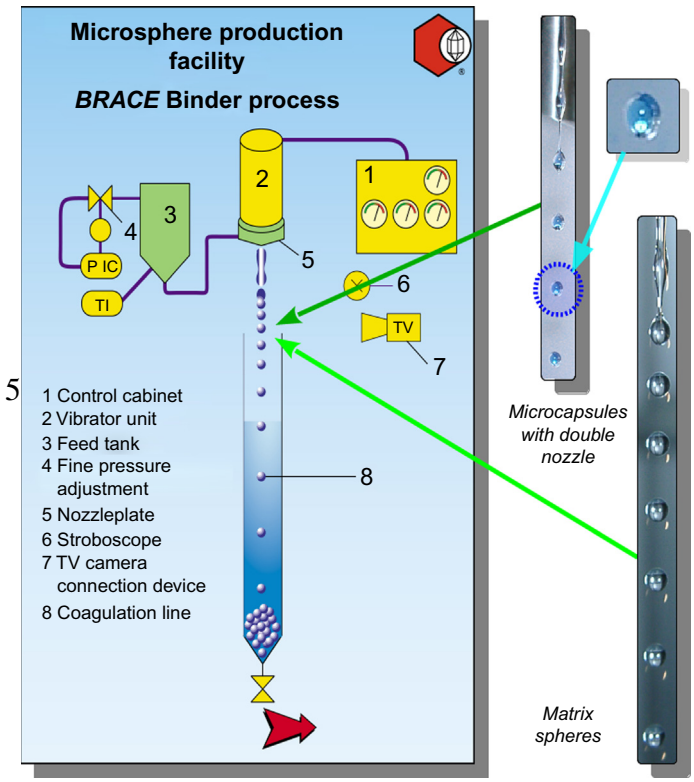

FIGURE 10.2 Schematic principle of annular jet nozzle processes with vibrating nozzles. Shown are the room temperature processes of reactive mixtures (e.g., alginate, pectin, cellulose, etc). Optional heating and cooling towers used in process drying or spray cooling are possible or other reactive processes. On the right side, microspheres (matrix encapsulation) and microcapsules (core–shell encapsulation) are shown during processing. *Courtesy of BRACE GmbH, Germany, www.brace.de.*

Another big advantage of those processes is that it can be scaled easily by increasing the number of nozzles. By using multinozzle plates, capacities of several hundred kilograms per hour can be reached. To further increase the capacity, the number of drip-casting heads can be increased to reach capacities of 20 tons/h and more.

There are different types of nozzles used in the market such as double nozzles (a core and a shell liquid), triple nozzles (a core, an intermediate, and a shell), and even quadruple nozzles. But with double nozzles, the mechanical capabilities even of advanced tooling shops are already limited, so that the use of larger-scale triple and quadruple nozzles is not widespread. As the tooling capabilities are bound to improve in the future, there is a reasonable hope that triple and quadruple nozzle systems will be available for large-scale production in the foreseeable future.

10.2.3 Submerged Nozzle

The differences between submerged nozzle systems and vibrational drip casting systems are subtle, but essential with regard to the products produced. A submerged nozzle system works by introducing the nozzle—inner and outer nozzle—into a transport medium (Suzuki et al., 1999). In most cases this is an oil. This transport medium flows codirectional to the nozzle and separates the droplets due to the external disruption introduced. The resulting droplets are of spherical shape due to the interfacial tension of the droplet shell material (usually gelatine) to the transport medium (usually oil). A vibration in this case is not necessary, but in some cases it is applied. The vibration applied here is in a low frequency range and is typically little varied (around 50 Hz). The resulting capsules range in size of about 2 mm up to 10 mm. The scalability of this process is technically difficult, as the flow has to be applied evenly around each nozzle. Large-scale equipment with this process is not available on the market currently and the prices for small-scale equipment are typically quite high in comparison to vibrational drip casting processes. It is, however, not possible to produce such small particles as it is with vibrational drip casting.

10.2.4 Flow Focusing

A special niche annular jet-based process is the flow focusing technology (Ferrari, 2006). Here, instead of using a liquid shell material, a matrix sphere is produced, but the outer nozzle is operated with an inert medium (usually gas) (see http://en.wikipedia.org/wiki/Flow_focusing). In this case, a monomodal range of droplets can be produced that have a mean diameter that is substantially smaller than the nozzle diameter. The size distribution is less narrow as with, for example, the vibrational drip casting method; however, with this technology, it is possible to produce very small droplets, and even those that could not be processed in other nozzle-based technologies, as the primary particle size in the feed mix is larger than the to-be-used nozzle.

10.2.5 General Principle

The general principle of this process is that two liquids are extruded concentrically through an annular gap nozzle in a laminar flow. With the flow rates of the two liquids, their ratio in the final capsule can be varied through a wide range. The nozzle, or the liquid itself, is moved typically in the direction of the flow. By finding the right resonance frequency, the vibration introduces a disturbance in the flow that leads finally to breakup. The separated parts of the flow form a spherical droplet due to the surface tension of the liquids. Then, the droplet becomes solidified quickly. This can be done in different ways, but always depends on the materials, mostly the shell material. Typically in food applications, the shell consists of hydrocolloids such as gelatine, alginate, agar, or fats; therefore, solidification is performed by cold oil, calcium chloride solution, or cold air.

10.3 EQUIPMENT

Annular jet-based processes are nowadays used in foods frequently to produce highly monodispersed core−shell encapsulated products. Those products, referred to as "microcapsules," are typically used in confectionery such as chewing gums (Marmo and Rocco, 1981), breath refresher, in tobacco products, ready meals, soft and hard drinks, and dairy products. Most of the time they are used as flavor (Kiefer et al., 1997) or additive containment for special effects ("flavor burst"), but also for delivering ingredients such as vitamins, caffeine, probiotics, essential oils, and minerals. Last, but not least, some ingredients in "health food" have poor taste and need to be encapsulated so that the bad taste does not affect the consumption of the foods.

There is a wide range of equipment available that uses different forms of annular jet nozzle systems for food applications. The following subsections describe some examples of different sized equipment from various suppliers around the world. As the processes deployed are different between this equipment, results are usually not fully transferable. This is to be kept in mind when developing with these devices, as only some of the vendors supply pilot equipment and even fewer supply production-scale equipment. Typically available equipment sizes are listed where available at the vendors' site.

10.3.1 Nisco Engineering

Nisco Engineering AG, Zurich, offers a range of small-scale laboratory devices that work with different processes. Besides vibrational drip casting, Nisco offers electrostatically driven breakup and air-driven flow focusing processes. Most of the units can be obtained also in GMP versions.

Typically, the Nisco devices are well suited for small-scale tests and development works on a feasibility test basis or if only very limited quantities are needed. The units are small and simple to operate. The vibrational drip casting or laminar jet breakup devices of the Var-D series are available with single nozzles for matrix beads as well as with annular gap nozzles for core–shell encapsulation. Variations with more than one nozzle or contained systems are currently not available with annular gap nozzles to produce capsules, but only for matrix encapsulation.

For electrostatically driven jet breakup, Nisco offers the Var-V1 series (Figure 10.3). These small units are also available with multinozzle systems to produce larger amounts. However, due to the electrostatic nature of the drip casting, scaling up with multinozzles is very difficult. Here, coagulation can often be seen.

For flow focusing, the Var-J1 (Figure 10.4) devices are available. These units work according to the theory described elsewhere in this book. Here, the flow of a single nozzle is focused by an air beam into a much smaller droplet. The throughput with these devices is pretty small; however, very small droplets can be produced. A triple nozzle version to produce capsules with flow focusing is currently not available.

Lastly, the Var-W1 is available with annular gap nozzles for gravity dripping, meaning that there is no vibration or means to strip out the droplets from the nozzle other than gravity.

FIGURE 10.3 Nisco Var V1, electrostatically driven jet breakup. *Courtesy of Nisco Engineering, Zürich, Switzerland, www.nisco.ch.*

10.3.2 BUCHI

The BUCHI Encapsulators (Figure 10.5) (formerly known as Inotech Encapsulators) are small-scale microsphere and microcapsule generating devices, mainly used for feasibility tests and small-scale processing, for example, in university

FIGURE 10.4 Coaxial air flow droplet generation. *Courtesy of Nisco Engineering, Zürich, Switzerland, www.nisco.ch.*

FIGURE 10.5 BUCHI Encapsulator B-390. *Courtesy of BUCHI Labortechnik AG, Switzerland, www.buchi.com.*

or for early development stage use. As bench-top ready-to-use devices, they are easy to install and use out-of-the-box. With their small process volume, they are predominantly used for very expensive or difficult to generate materials.

The units are based on vibrational drip casting with a focus on electrostatic dispersion. The electrostatic dispersion works by using vibrational drip casting as a source for generating identically sized droplets. These are superficially charged with a high voltage load that repels the pellets from each other (Figure 10.6), so coagulation of the droplets

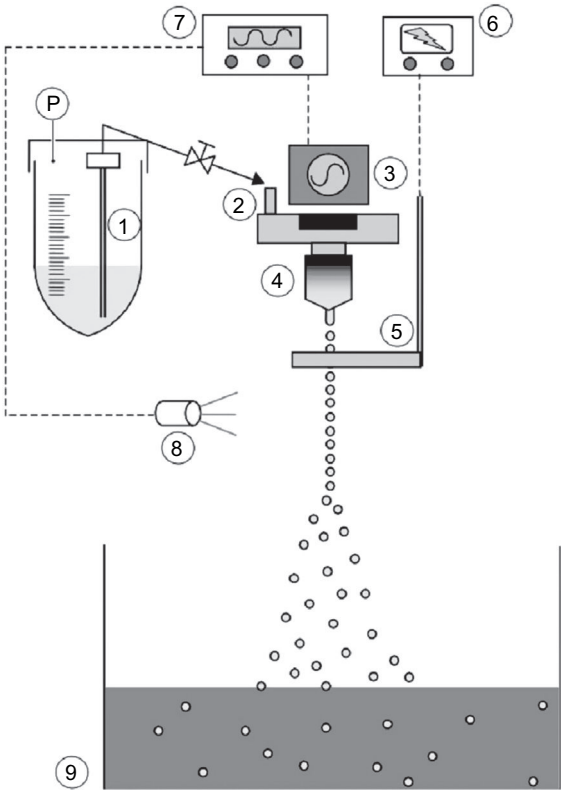

FIGURE 10.6 Process scheme BUCHI Encapsulator. *Courtesy of BUCHI Labortechnik AG, Switzerland, www.buchi.com.*

during the free-fall period is reduced if not eliminated. The drawback of this technology is that it only works well for a single nozzle and the space consumption of the reception bath is increased dramatically. For larger droplets, the electrostatic dispersion is seldom used, as those droplets sink into the reception liquid fast enough to avoid coagulation.

10.3.3 BRACE

BRACE is currently the only manufacturer of machinery that uses laminar flow breakup technology that is able to provide units from small laboratory size equipment up to large-scale production facilities. The focus of the BRACE units is on industrial manufacturing. The Spherisator Series is designed as modular machinery, and pilot and production size equipment is designed for specific products. The Spherisator S and M units are designed for laboratory use, for example, for feasibility tests or small sample productions. They are convenient desktop systems, but both can be equipped with numerous options starting from different tank sizes up to continuous production systems. The Spherisator M unit is commonly used to match production-scale processes in the laboratory to simulate and develop formulations. All results can be transferred 1:1 to any production unit, so a production can be started quickly by using identical parameters and recipes. Scalability was the focus on the design of all BRACE units, and they are clearly targeted for the industrial market. The size range of the BRACE units is from about 10 μm up to about 10 mm and they offer some of the broadest particle sizes in the market. With capacities starting around 1 L/h up to 20,000 L/h, they are the largest machines available for annular gap nozzle equipment. These units are used in all industries, for example, chemical industries processing and producing grinding media, catalysts, catalyst carriers, proppants, waxes, intermediates, metal

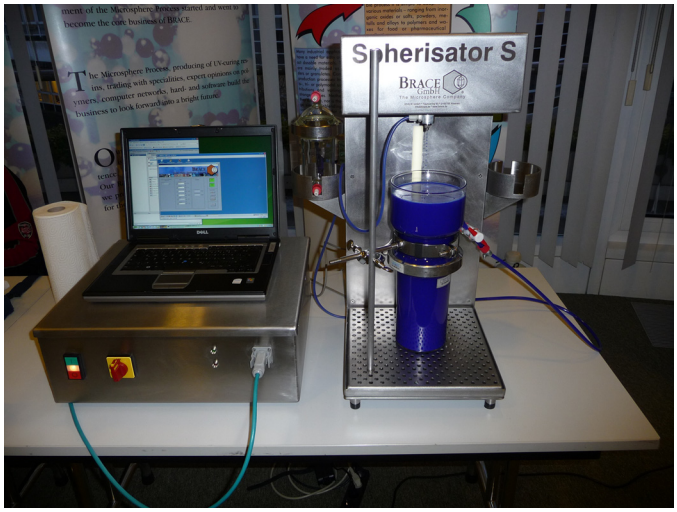

FIGURE 10.7 BRACE Spherisator S. *Courtesy of BRACE GmbH, Germany, www.brace.de.*

FIGURE 10.8 BRACE Spherisator M. *Courtesy of BRACE GmbH, Germany, www.brace.de.*

and metal alloy microspheres, phase-change-material capsules, and various other chemicals; food industries producing matrix and core−shell encapsulates of flavors, pre- and probiotics, yeast, nutritious additives, vitamins, and such; as well as cosmetic and pharmaceutical industries producing cosmetics, exfoliants, pharmaceutical formulations, excipients, and feed products.

A Spherisator S (see Figure 10.7) is a very small desktop unit with a compact control and a PC for displaying and operating the device. In can be equipped with single nozzle heads (i.e., one nozzle) for matrix and core−shell encapsulation. As a compact desktop unit, it is mainly designed for feasibility tests and very small-scale processing. The maximum throughput is about 1 L/h.

The Spherisator M (see Figure 10.8) is the "little bigger brother" of the Spherisator S. Being still a desktop unit, it can be equipped with more than one nozzle, thus making it possible to produce microspheres or microcapsules up to 10 L/h. With a number of options such as feed tanks up to 100 L and continuous production options, this unit is very well suited for producing pilot batches.

Both units can be equipped with heating chamber systems and cooling towers for use with hot materials (e.g., gelatine, agar, waxes, metal alloys, etc.). With their design being compatible with GMP/GLP pharmaceutical, cosmetic, food, and even normal chemical development such as grinding media, catalyst carriers or such are easily possible.

For pilot and production operation, BRACE offers customer-tailored machinery and equipment up to turnkey installations with capacities of 20,000 L/h and more. Those units are specifically made for one or a number of products and are fully automatic for the production. Besides drip casting, BRACE also offers auxiliary equipment for preparation, vessels, washing and separation, drying, sieving, and sorting machinery; in short, it offers the full turnkey with optimized equipment.

10.3.4 Freund Corporation

The Freund Corporation's Spherex is a small-scale unit capable of producing core−shell microcapsules in a diameter range of 1 to 8 mm. It works with a submerged nozzle process, meaning that the nozzles are in a transport medium (typically an oil). The oil flows in the direction of the flow from the nozzles and transports the capsules away from the nozzle. By changing the speed of the flow, the capsule size can be adjusted. Production rates of up to 200 capsules per second place the equipment in the small laboratory size category. Because these machines are usually equipped with only one nozzle, they need to be scaled by a number of machines. As the materials are limited to nonreactive materials such as gelatine or agar, the applications are mostly in the pharmaceutical and food market (see Freund Corporation, www.freund.co.jp).

10.3.5 Other Annular Jet Systems

Apart from the previously described annular jet systems, there are also a number of extrusion systems working with annular jet nozzles. Most of them are used for processing high viscous materials such as cereals or other wheat-based products in food applications. As there is no outstanding vendor but numerous vendors offering basically identical equipment, a detailed list or description is not given here. However, a general search for extruder companies should lead quickly to a desired result.

10.4 MATERIALS

Annular jet processes are fairly generous when it comes to choosing materials. This means that a very broad range of different materials can be used, in general. "In general" means that once again, as is so often in microencapsulation, only trials can decide if one material is usable or not.

As annular jet processes work with two material flows, those two materials should be compatible with each other. This means that they do not react in such way that they solidify (quickly) or dissolve, for example, lemon oil in Eudragit®. It is possible to produce very nice capsules with a lemon oil core and a solid Eudragit shell. However, within a few minutes the shell is dissolved by the lemon oil and all that is left is a squishy mass.

For food technology, not only is the chemical compatibility important, but also the properties of the finished product and, of course, regulation issues. In this chapter, regulation issues are not addressed, as this subject is far too complex and evolving to be covered here.

As each application is different, as well as each material to be encapsulated, a number of questions have to be asked:

- Is the material to be encapsulated hydrophobic or hydrophilic?
- Will it be released and, if yes, how and when?
- Are there constraints for the size?
- What is the purpose of the encapsulation?

These are very basic questions. However, experience shows that most of the time during project start times not even these four very basic questions can be answered clearly. When the answers to those questions are clear, the next steps are to select and understand a group of materials that will be used for the encapsulation—the shell materials. These materials should be different in the hydrophobocity to the core materials. Typically, the higher the difference, the easier the encapsulation process.

Release is another important factor when selecting the shell material. Normally, many possible options that would be suitable after the first question can be eliminated here. The size of the microcapsules will also affect the possible choice. High solid content shell materials such as gelatine or starch usually yield thicker shells than alginates or pectin. Wax shells are much bigger than any of the hydrocolloids and to obtain reasonable loads in the core diameters, less than 1 mm is seldom possible.

Lastly, the purpose of the encapsulation is important when selecting a shell material that either dissolves or breaks. A chewing gum formulation is, of course, different to an enteric formulation that passes the stomach.

10.4.1 Encapsulation of Hydrophobic Materials

For food applications, a hydrophobic material such as a flavor will be the most common form of active to be encapsulated. For those materials, a hydrophilic material such as a shell made of alginate, gelatine, agar, gellan gum, pectin, shellac, (modified) cellulose, starch, or similar hydrocolloid is usually acceptable. If a release during chewing or on mechanical pressure is needed, all shells are suitable. The shell should in any case be thin enough to be either not noticeable by the user or quickly dissolve in water. In case the release has to be triggered by temperature and water or by water alone, the choices are limited to gelatine or agar. There are some materials that are soluble in water but only at elevated temperatures, for example, agar needs a substantial temperature increase to be dissolved. Table 10.1 gives an overview of commonly used shell materials with their respective applications. Values given in brackets mean that the material by itself is not a good choice but needs to be combined, for example, with temperature or with an additive, to be useful under the conditions.

Modifiers that change the dissolution properties of these shell materials are usually sugars. High sugar content modifies pectin or agar to a low temperature melt formulation.

It should, however, always be kept in mind that most of these commonly available materials are standardized (with sugar). This is good for the manufacturer, but bad for encapsulation, as it changes the properties of the resulting microcapsules. In the laboratory, this does not make a big difference, as there are other parameters to investigate first. But for production purposes, it will be necessary to discuss this issue closely with the manufacturer or even better, standardize the materials oneself.

As hydrocolloid shell solutions used during processing consist mostly of water, the shell of such capsules will shrink considerably. A shell thickness of 300 μm molded with an alginate shell ends up—depending on the solid content—after drying as a shell of 30 to 50 μm thickness. This, of course, makes the hydrocolloids highly attractive for this type of encapsulation, when active load is high and shell is so low that the consumer usually does not even notice it.

The biggest applications for such hydrocolloid capsules are chewing gums and tobacco products, where alginate and gelatine shells are used. The use of gelatine is, however, declining, as it cannot be used for all markets. The use of alginate, however, or agar or other nonanimal-derived products, is increasing considerably. With the choice of the right encapsulation materials, production can be dramatically facilitated, which makes encapsulation very economical.

One of the major obstacles to encapsulating flavor is the flavor itself. Some of the components react with the shell materials (e.g., alcohols with alginates), but others do not remain tightly in the capsules. In these cases, both materials have to be optimized. The shell material can be enhanced with additives such as glycerol, sugars, which "close" the hydrocolloid mesh and "tighten" the shell. But also the flavor can be optimized for microencapsulation. Here, it is necessary to talk to the flavorists. Most of the commonly used flavors have been developed for spray-drying applications and it can be difficult to communicate with a flavorist and make him/her aware of the problems with the different

TABLE 10.1 Overview of Commonly Used Shell Materials with Their Respective Applications

	Pressure	Temperature	Water	Enteric
Alginate	+	−	−	+
Agar	+	0	(0)	0
Gelatine	+	+	+	−
Pectin	+	(0)	−	−
Gellan gum	+	−	−	−
Shellac	+	−	(−)	+
CMC	+	(0)	+	−
Starch	+	+	(+)	−

processes. Interfacial tensions play a major role in such annular jet processes and the effects of different carriers can be huge. It is strongly advisable to involve the flavorists in such an encapsulation project right from the start, otherwise it may end up as a short excursion into microencapsulation with limited success.

10.4.2 Encapsulation of Hydrophilic Agents

Hydrophilic actives are usually more challenging to encapsulate than hydrophobic ones. Although hydrocolloids are very helpful for the majority of the actives, they are miscible with these actives, which makes it impossible—or at least very difficult—to get a good phase separation between the core and the shell. Under some circumstances, it is possible to change the physicochemical properties of the shell material in a manner so that the core can still be kept inside, but the resulting microcapsules tend to have a lower quality than with a hydrophobic core. Even if microcapsules can be formed, products typically need to be dried. During drying, the core is in most cases extracted, which leads to high material losses and therefore uneconomical products.

There are a number of applications where this can work. For example, gelatine forms a tight diffusion barrier against alcohols—even ethanol—when the water content of the gelatine is low enough. Under these circumstances, a gelatine capsule can be formed nicely and the losses can be compensated by varying the alcohol component in the core material.

An alternative to the normal route of encapsulation for hydrophilic agents is the so-called inverted solidification. Instead of using the shell material directly in an annular jet nozzle process, the shell material forms the reception bath and a reactive solution is used for the outer liquid. Here, especially, alginates or other reactive hydrocolloids are used. For alginates, for example, a calcium chloride solution is used as the "shell" material and drip casted into an alginate solution. The reaction with the alginate is very fast so capsules are formed ("inverted crosslinking") (Klein, 1985). As the calcium continuously diffuses into the reception bath, the shell thickness can be adjusted by the hardening time. It is evident that even for laboratory tests, such products can only be produced with continuous processing equipment where the residual time in the hardening bath can be closely controlled. Other materials that can be likewise processed are all reactive hydrocolloids such as gellan gum, carageenans, pectins, etc. Even agars can be used as reception baths, as the solidification of agar below 42°C is only reversible at high temperatures.

The addition of a reactive ingredient such as a calcium salt to an active agent can be tolerated under some circumstances, for example, when cell slurries are encapsulated; however, these additives do not come for free. A yeast slurry tolerates quite substantial amounts of calcium chloride and can be encapsulated well in alginate for fermentation processes. A sweet flavor for a chewing gum, however, can end up with an intolerable salt level.

If the actives are quite potent or a lower load is acceptable, it is also possible to form an emulsion for the core. Here, the hydrophilic core is emulsified first with a hydrophobic material (e.g., an oil or fat) and the emulsion is used as core material with a hydrophilic shell. Emulsions are generally difficult to process, but by preparing very fine emulsions, agreeable microcapsules can be obtained. If a fat or a hydrogenated oil is used, the solid core at room temperature even has enhanced features such as a very strong flavor retention and increased stability. There are numerous ways to change the properties of these hydrophilic materials, which can yield a totally new range of products with outstanding properties.

When a hydrophilic shell and a hydrophilic core are essential for the application and the previously-mentioned methods fail, there are still possibilities—a triple nozzle system, for example, an where intermediate substance such as hydrophobic material is introduced as a separating agent. Such "fish-eye capsules" can be an attractive, if expensive, way to solve the hydrophilic−hydrophilic problem for the customer (see www.jintanworld.com/english/capsule/sinka.html).

Even though there are a number of options to encapsulate hydrophilic materials with hydrophilic shells, there will still be unsolved problems, for example, the shell has to be prepared from hydrophobic shells. The basic process here, of course, is the same. However, the disadvantages are numerous: a fat or wax-type shell is not shrinking during drying, the visibility during processing is difficult, and the hardness of wax shells is seldom comparable with, for example, a gelatine capsule. On the positive side, wax capsules are insoluble in water, form a tight barrier for water diffusion in or out, and microbiological contamination is less of a problem than with the hydrocolloids. Lastly, there is no additional drying step as the capsules are directly produced as dry material.

For shell materials, fats or hydrogenated oils are commonly used. The hydrogenated oils have the advantage that their melt points are sharp in comparison with fat, while they form harder capsules. Waxes such as carnauba can be used too; they even produce extraordinary hard capsules with a very good diffusion barrier.

Often, microencapsulation and especially annular jet encapsulation depends dramatically on the properties and behavior of all ingredients. A phase of trial and error is unavoidable. This phase is even more difficult as encapsulation materials and additives behave differently, not only when they are of different specification, but also when a vendor is changed. It happens quite frequently that by changing the vendor of a carrier oil (e.g., medium chain triglycerides), an encapsulation that was giving very bad results ends up giving very good results—and vice versa.

10.5 CONCLUSION

Annular jet nozzle processes are widespread in industry and commonly used to produce core−shell encapsulated products (and many nonencapsulated products, too). With the focus on food technology, a number of technologies have been brought up to scale so that already a good number of products are available. Here, especially, vibrational and nonvibrational laminar flow breakup processes are the predominant technology. They produce monomodal size microcapsules with tight size distribution and can be used for a vast array of materials. The resulting products are better, easier to handle, and when looking at the full application, are usually cheaper than regular spray-dried products. A number of companies use and produce equipment for such processes from laboratory to large-scale production size. However, the process technology is not yet as far advanced as much older technology such as extrusion or spray drying, where basic knowledge about the processes can be assumed with any engineer, but they demand a good evaluation of materials and knowledge for successful implementation. As the processes are more widely used, the basic technology will improve, but microencapsulation with annular jet nozzles is still a system with a lot of parameters. To an outsider, what an experienced operator of a system can achieve by choosing the right materials and combining them might look like magic.

Certainly, the future will bring a great deal of highly advanced products, as knowledge increases and raw material makers start to understand the needs of encapsulation chemists and adopt their products so that a large amount of the current trial and error work is reduced, and equipment makers can improve their machinery in such a way that the chemist can concentrate on the real work: to create a better tomorrow.

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Chapter 11

Monodispersed Microencapsulation Technology

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11.1 INTRODUCTION

Microencapsulation science has led to many applications of great utility in pharmaceuticals, vaccines, cellular biology, agriculture, and food science. A common theme with microencapsulation technologies is the desire to control the release of a drug, protein, organism, flavor, or fragrance in different environments, at specific times, or in a particular fashion. When the microencapsulated form of an active is contained within a uniformly shaped vehicle, such as a spherical particle, the ability to predict release characteristics can be relatively straightforward with mathematical modeling. Because release rate and mechanism is typically governed by particle diameter (or shell thickness), a population of differently sized spherical particles will behave, at best, as an average of all particle sizes within that population. If, however, all particles in the population are of uniform size, prediction and repeatability of release mechanisms, timing, and duration are straightforward. Furthermore, many recent microencapsulation technologies focus on creating particles with a high degree of monodispersity, specifically with sizes in the micrometer range. This chapter covers the salient characteristics of technologies that can allow uniform microsphere production from submicron to millimeter range, and how the field of the food and flavors industry can benefit from such capabilities.

11.2 MONODISPERSE PARTICLE FABRICATION TECHNOLOGIES

A number of technologies for monodisperse particle fabrication have recently emerged. They differ from each other in complexity of the setups, ability to handle the precursor materials (e.g., viscosity of the feed material), capability of producing particles of certain shapes and size ranges, scalability, and application-specific cost effectiveness. In this section, we highlight the prominent features of these technologies in their current form and provide examples, where possible, of their existing or potential use in the food and flavors industry.

11.2.1 Microfluidics

Microfluidics has been widely studied for use in microelectronics and for molecular analysis (Whitesides, 2006), however, more recently it is being used to produce uniform microspheres and microbubbles (Dendukuri and Doyle, 2009). As the field has matured, three general types of microfluidic processes have been adapted to pharmaceutics, nutraceutics, and the food industry: droplet based, lithography based, and colloid based (Dendukuri and Doyle, 2009). The common fundamental feature of these microfluidic processes is the concerted microscale fabrication mechanism, which is capable of extreme complexity.

From an industrial perspective, droplet-based microfluidics is generally regarded as continuous, rather than a batch process. The formation of microspheres is achieved by using two or more immiscible fluid phases flowing either adjacent or perpendicular to one another. Microsphere droplets are formed as a result of shear forces and surface tension between the two intersecting streams (Figure 11.1A). One (or multiple) stream contains a polymer or material solution that the microspheres are made of, and the surrounding fluid acts to break, stabilize, carry, and collect the droplets

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00011-X

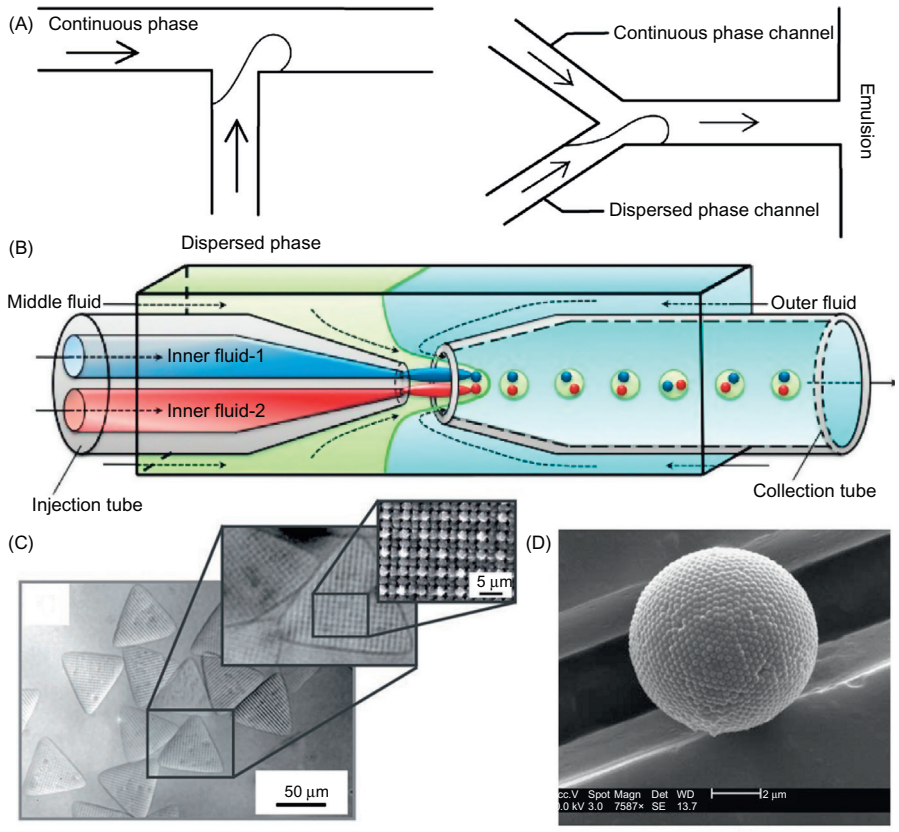

FIGURE 11.1 Microfluidic processes. (A) Schematic of a T-junction and Y-junction used to create uniform droplet breakup in a microchannel. (B) Schematic of coaxial flow of multiple streams leading to binucleated droplets. (C) Triangular-shaped microstructures from lithographic microfluidics. (D) Colloid sphere formed from controlled aggregation of microemulsion droplets. *(A) Reproduced with permission from Maan et al. (2011). © Elsevier 2011. (B) Reproduced with permission from Sun et al. (2010). © American Chemical Society 2010. (C) Reproduced with permission from Jang et al. (2007). © Wiley & Sons 2007. (D) Reproduced with permission from Yi et al. (2003). © Elsevier 2003.*

either individually or en masse. Microsphere size and shape are governed by velocities, orifice diameters, and architecture of the stream interfaces (Figure 11.1B), which can enable more complex geometries such as rods (Dendukuri et al., 2005), capsules (Sun et al., 2010; Abate et al., 2011), and biphasic droplets (Shepherd et al., 2006). Depending on the formulation, before exiting the stabilizing architecture of the microfluidic process, sometimes droplets are consolidated (hardened, stabilized, or crosslinked) by light (Lewis et al., 2010), chemical exposure (Trivedi et al., 2009), or other natural mechanisms (Breslauer et al., 2010; Wassén et al., 2012).

Lithography-based microfluidics uses the same principles as droplet based, but is analogous to a pseudobatch process, which capitalizes on the ability to create highly complex morphologies. The formation of microstructures is achieved by injecting the material stream of interest into a micromold or chamber and arresting the flow just long enough for the hardening or crosslinking step to occur, after which flow resumes, carrying away the stabilized microstructure and making space for the next injection (Figure 11.1C). The lithography-based process is capable of producing unique shapes such as triangles (Jang et al., 2007), gears (Shepherd et al., 2008), and other compound structures (Chung et al., 2008). A cGMP nonwet method for creating small volumes of reproducible structures was recently developed by Liquidia. Liquidia's PRINT™ technology (DeSimone et al., 2007) is designed to control the kinetics of active or biological molecule presentation on the surface of a uniform structure, through the formulation of specialized polymers. Moreover, compared to droplet-based microfluidics, lithography-based processes are better candidates for more detailed microsphere and microstructure formulations.

Lastly, a superset of droplet-based and lithography-based microfluidics utilizes assembly of colloids to form larger structures (Figure 11.1D). Here, microstructures previously formed through the basic fluidic systems, acting as colloids, are combined together to form large-scale three-dimensional structures, such as zipper shapes (Vanapalli et al., 2008) and even larger spheres (Yi et al., 2003). Microsphere arrangement is guided by complex channel orientation, magnetic fields, phase separation techniques, or thermal guidance. Interface bonding between the colloids is performed by methods similar to other microfluidic applications, such as light crosslinking or chemical bonding (Dendukuri and Doyle, 2009).

Depending on the complexity of the microsphere or microstructure, the size range capabilities of microfluidics are generally limited to below 500 μm (Dendukuri and Doyle, 2009). Specifically, simple spheres created with droplet-based or lithography-based techniques can be below 10 μm or up to 200 μm, whereas more complex shapes typically

fall in the range of 50 to 200 μm. Only colloid assembly applications can reach structure sizes near 1.0 mm, by massive assembly of smaller substructures.

While microfluidics excels beyond other monodisperse fabrication technologies in process control, intricacy, efficiency, and reproducibility, there is paucity with regard to the potential for high volume scalability. Major factors that can be considered limiting to the scalability of microfluidics include low flow rates and precision machining of microchannels and arrays. Several examples exist, however, of how microfluidics can be scaled for the aforementioned specialized applications. These assemblies typically multiplex flow channels parallel to one another in a lattice-like structure (Kobayashi et al., 2005) or radially oriented (Nisisako et al., 2006). As such, this approach is a "wet method" for microparticle production, thus further particle purification and/or drying may be needed.

For applications requiring modest quantities of product (e.g., cell carriers (Sugiura et al., 2007) or e-paper applications (Nisisako et al., 2006)), microfluidic processes represent a suitable candidate. For commodity and consumer products (e.g., perishables/food items, lotions/gels, and major pharmaceutical formulations), the reasons for selecting microfluidics as the primary scaled process are less compelling, although there are a handful of food science examples to date (Neves et al., 2008; Chuah et al., 2009; Maan et al., 2011; Wassén et al., 2012). Utilization of microfluidic technology for food applications would more likely focus on impurity detection and subsequent filtration from food products before distribution to the consumer, rather than be integrated within the process to produce the consumable. Thus, using microfluidics for large-scale production of monodisperse microspheres has captured a limited number of, yet important, market niches.

11.2.2 Electrohydrodynamic Spraying

Electrohydrodynamic spraying (EHDS), also referred to as electrostatic spraying, is an atomization process that utilizes an electric field to generate the droplets of a primarily liquid feed stream (for more details, see review in Jaworek, 2008). The feed material consists of a liquid (or a suspension) with a polymer solution, an emulsion (water-in-oil or oil-in-water) or a melted material as the primary matrix, which is passed through a nozzle that usually consists of a hypodermic needle (Lopez-Herrera et al., 2003). The feed material is charged by applying a high voltage between the hypodermic needle (or, in some instances, the high voltage is applied directly to the feed material (Berkland et al., 2004)) and a grounded electrode. Due to the Coulombic interactions of the charges, the liquid is drawn out of the nozzle and takes the shape of a meniscus. At this stage, depending on the process conditions, the feed material can be (1) spun in the form of a fiber, (2) atomized into droplets due to charge repulsion (known as the *dripping mode*) (Figure 11.2A), or (3) in the form of a cone through which a jet of material emanates, breaking up into small droplets at some distance from the cone (known as the *jet mode*) (Jaworek, 2008; Xie et al., 2008; Rezvanpour et al., 2012). The formation of the jet occurs as a result of electrical forces overcoming the surface tension. In specific cases, when the meniscus forms an axisymmetric and stable cone (Taylor cone), the spraying occurs via the *cone-jet mode* or *Taylor cone mode* (Taylor, 1964; Jaworek, 2008; Xie et al., 2008) (Figure 11.2A). There are two sets of EHDS variables that affect the droplet generation and their size, namely, the process parameters and material parameters. Prominent process parameters affecting EHDS include the feed flow rate, applied voltage, and ambient temperature, whereas some of the most important material parameters include the viscosity of the feed, surface tension, and solvent properties (such as dielectric constant, conductivity, and vapor pressure) (Meng et al., 2009). In addition to the formation of matrix-type microspheres, the process is amenable to producing microcapsules with a single core and a multilayer structure where the feed streams are coextruded through a coaxial dual capillary nozzle (Loscertales et al., 2002; Lopez-Herrera et al., 2003) or a tri-needle (Ahmad et al., 2008), respectively (Figure 11.2B).

Microspheres produced using EHDS are not always uniform. In fact, it is extremely common to have bimodal and multimodal distributions of the particles, where primary droplets (on the order of jet diameter), satellite droplets (formed due to instabilities), and offspring droplets (formed due to Coulomb fission of the primary droplets when the Rayleigh limit is reached) may form (Tang and Gomez, 1996; Hartman et al., 2000; Meng et al., 2009; Rezvanpour et al., 2012). However, spatial expansion of the spray due to electrostatic inertia can be exploited during the collection to obtain highly monodispersed particles (Gomez and Tang, 1994; Tang and Gomez, 1996; Hong et al., 2008; Meng et al., 2009) (Figure 11.2C). The efficiency of the monodisperse particle collection could therefore be increased by adjusting the process and material parameters in a manner that minimizes the offspring and satellite droplet formation, for which there is typically a very narrow window (Tang and Gomez, 1996; Rezvanpour et al., 2012). With respect to the size of the microspheres, EHDS can produce a wide range of microsphere sizes. The dripping mode usually produces larger particle sizes (in the order of mm) (Poncelet et al., 1994; Moghadam et al., 2008), whereas the cone-jet mode can produce much smaller microparticles or microcapsules (in the range of 1−100 μm or lower) with a high

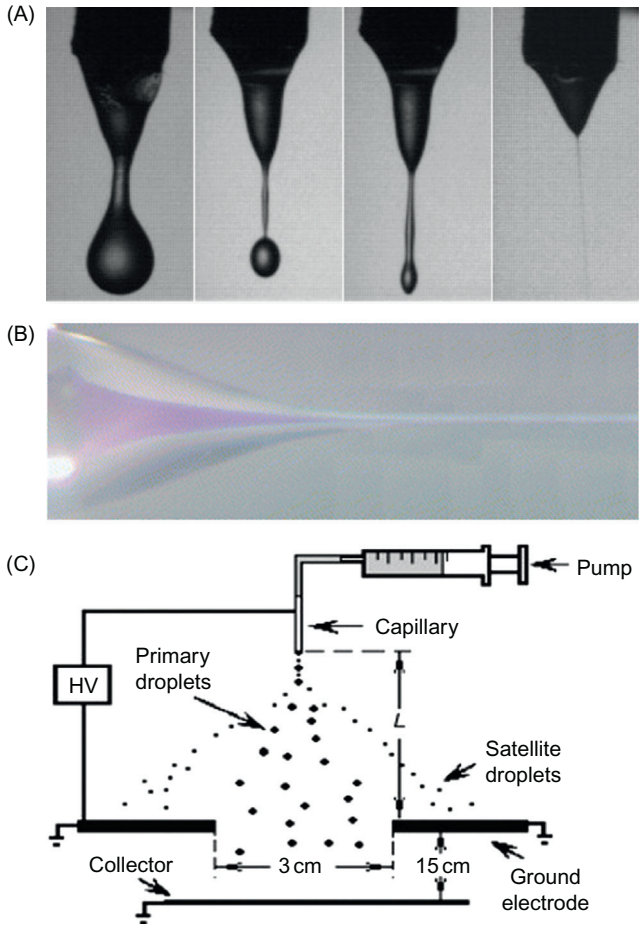

FIGURE 11.2 Electrohydrodynamic spraying (EHDS). (A) A jet of material emerging from a nozzle under various modes: dripping mode without applying voltage difference, dripping mode under a low voltage, dripping mode under a higher voltage, and Taylor cone-jet mode (from left to right). (B) A close-up view of coextruded feed streams through a coaxial dual capillary nozzle. (C) A schematic representing an EHDS setup with a modified collection method, where spatial expansion of the spray due to electrostatic inertia was exploited for the elimination of lighter satellite droplets. *(A) Reproduced with permission from Xie et al. (2008). © Elsevier 2008. (B) Reproduced with permission from Lopez-Herrera et al. (2003). © Elsevier 2003. (C) Reproduced with permission from Hong et al. (2008). © Elsevier 2008.*

degree of monodispersity (Loscertales et al., 2002; Lopez-Herrera et al., 2003; Bocanegra et al., 2005a; Hong et al., 2008; Bohr et al., 2011). To predict the particle size in the cone-jet mode, Fernandez de La Mora and Loscertales proposed a scaling law for droplet size determination, as described in Eq. (11.1):

$$d = \alpha\left(\frac{Q\varepsilon_0\varepsilon_r}{K}\right)^{1/3} \tag{11.1}$$

where d is the diameter of the droplet, α is a constant, Q is the feed flow rate, ε_0 is the permittivity of the free space, ε_r is the relative permittivity of the liquid, and K is the liquid conductivity (Fernandez de La Mora and Loscertales, 1994).

EHDS has been widely explored for use in the pharmaceutical area. Comparatively, there are limited examples for its use in food and flavors applications. In one example, EHDS was applied to encapsulate curcumin, a naturally occurring nutraceutical and also a natural food dye, in matrix-type zein nanoparticles in the size range of 175 to 900 nm (Gomez-Estaca et al., 2012). In another example, cocoa butter was used as a shell material to encapsulate sugar solutions and emulsions in the core, forming monodisperse capsules with mean diameters in the range of 10 to 20 μm (Bocanegra et al., 2005a). In one study, a melt of α-eicosene wax was subjected to EHDS for the purpose of generating uniform disc-shaped wax particles with reported mean droplet diameters of as low as ≈1 μm (Mejia et al., 2009). There is also another example where EHDS was applied to encapsulate invertase (an enzyme) in sodium alginate (Watanabe et al., 2001), but the particle preparations were not reported to be monodisperse.

One of the biggest challenges associated with EHDS for its application in the food industry is the issue of low throughput, which becomes more challenging if the required size of the microspheres is fairly low (in the order of 1−10 μm). As a single nozzle cannot process liters of feed materials in an hour, multinozzle and linear-slit nozzle designs have been shown to scale the systems for relatively higher production rates (Poncelet et al., 1994; Almekinders and Jones, 1999). Another point of concern is the safety aspect. Application of high voltages, especially when fine particles are to be produced, accompanies major safety concerns (personal injury or fire hazard due to arching) (Yeo et al., 2005).

11.2.3 Jet Cutting

The jet cutting technique, developed by Vorlop and Breford, utilizes mechanical breakup of a liquid jet by means of a rotating cutting tool consisting of several wires (Figure 11.3A) (Prüße et al., 1998b,c; Vorlop et al., 2002). The feed material is passed through a nozzle and the emanated jet is cut into uniform cylinders using the rotating wire brush. As

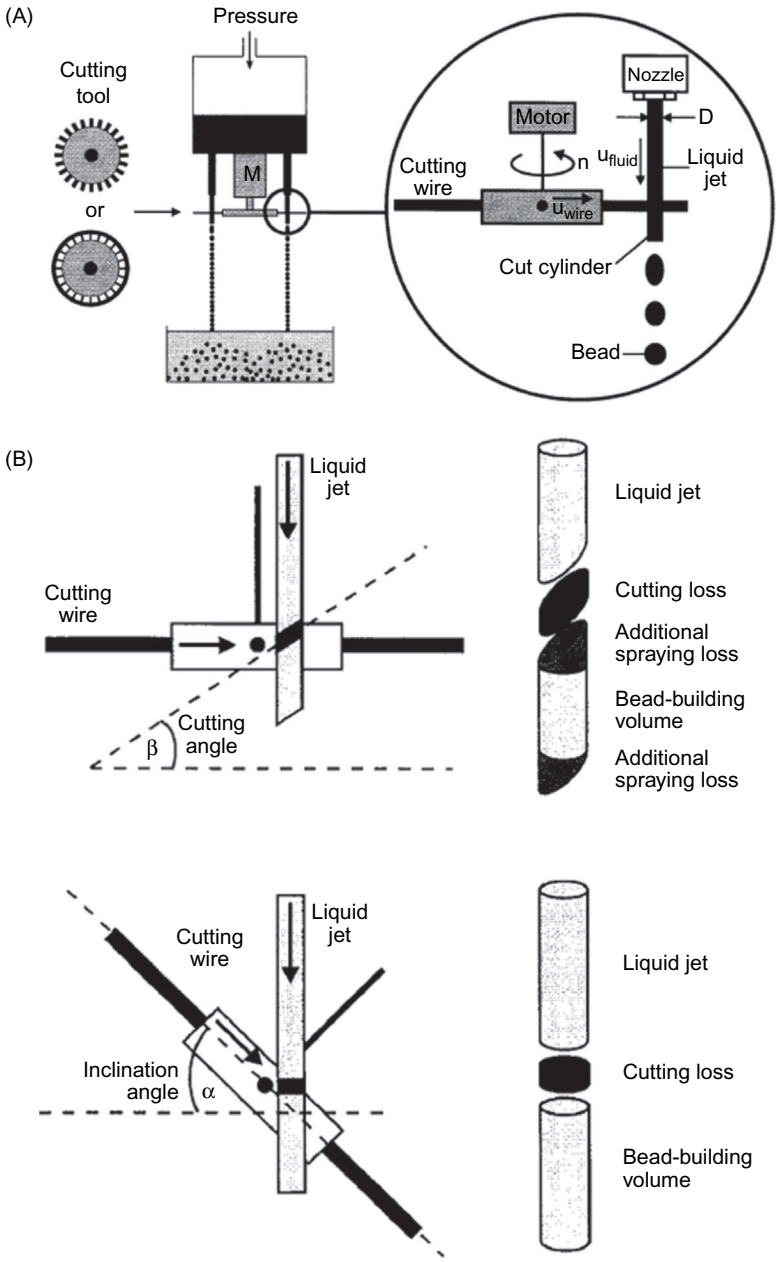

FIGURE 11.3 Jet cutting process. (A) A schematic representation of the jet cutting process. (B) The angle of the cutting tool with respect to the feed stream affects the cutting loss, where a diagonal angle of cutting can reduce the loss. *(A) Reproduced with permission from Prüße et al. (1998b). © Springer 1998. (B) Reproduced with permission from Prüße et al. (1998a). © John Wiley and Sons 1999.*

a result of surface tension, the jet fragments take the form of spheres and transform into particles after consolidation (Prüße et al., 1998b). Because the cutting wire removes some materials during the fragmentation of the jet, there is a *cutting* or *spray loss* associated with the process, which is a function of wire diameter and nozzle diameter, as well as the pitch of the wire relative to the jet axis (Figure 11.3B) (Prüße et al., 1998a, 2002; Vorlop et al., 2002). Variables that affect the size of the droplets include rotating frequency of the cutting tool, number of wires in the cutting tool, diameter of the wires, material feed rate, and nozzle design (Prüße et al., 1998b,c). Using a simplified model with the assumption of a diagonal cutting and a perfectly cylindrical cutting loss, the droplet diameter could be predicted with Eq. (11.2):

$$d = \sqrt[3]{\frac{3}{2}D^2\left(\frac{V_{fluid}}{n.z} - d_{wire}\right)} \tag{11.2}$$

where d is the droplet diameter, D is the diameter of the nozzle, v_{fluid} is the velocity of the fluid, n is the number of rotations of the cutting tool, z is the number of cutting wires, and d_{wire} is the diameter of the wire (Prüße et al., 2002).

Droplets produced using the jet cutting method are fairly monodisperse, as long as the jet flow is laminar. It is difficult, however, to produce particles in the 1 to 100 μm range using the jet cutting method alone. Reported particle sizes range from 150 μm to several millimeters in diameter with a relatively narrow standard deviation in size (Prüße et al., 1998b). Another disadvantage with the process pertains to the aforementioned cutting losses, which reduce the production yield. Cutting losses, however, can be minimized by recycling and adjusting the cutting angle (Prüße et al., 2002). The major advantage of jet cutting over other methods is related to the viscosities of the feed material that this technique can handle, which allows processing of highly viscous materials (Prüße et al., 1998c). This process also results in a high throughput, where high fluid velocities (up to 30 m/s)—a prerequisite to produce a continuous jet—lead to high production rates (Prüße et al., 2002). Multinozzle designs and increased jet cutting frequency are suggested as two possible routes to scale the system up (Prüße et al., 2002). A number of materials commonly used in the food industry, such as alginate, chitosan, gelatin, cellulose derivatives, waxes, etc., have been found to be suitable for use with this technique of microencapsulation (Prüße et al., 2002).

11.2.4 Rotary Disc Atomization

Rotary disc atomization or spinning disc atomization is a process where centrifugal forces are used to generate uniform droplets. Another variation of this process is a rotating nozzle atomizer (Prüße et al., 2002). In rotary disc atomization, a feed stream is dropped directly onto a rotating disc. Centrifugal forces discharge the feed stream into the surrounding area in the form of droplets. In certain optimal rotating conditions with respect to the viscosity of the feed stream, centrifugal forces stretch the jet in the form of ligaments or threads outside of the periphery of the disc, and the droplets thus produced are relatively uniform in nature (Figure 11.4) (Prüße et al., 2002; Teunou and Poncelet, 2005). Both viscosity of the feed stream and rotating speed affect the droplet size (Mackaplow et al., 2006). Advantages of this process, similar to the jet cutting process, are the high throughput and higher viscosities of the feed material that the process can handle and simplicity of the setup. However, the particles produced usually have a much wider size range than most other technologies discussed in this chapter (Mackaplow et al., 2006). Improved collection schemes can be implemented to segregate particles of different sizes during the collection (Figure 11.4) (Teunou and Poncelet, 2005).

11.2.5 Vibratory Process

Lord Rayleigh first described the phenomenon of creating spherical droplets from a jet through a series of publications (Rayleigh, 1878, 1879a,b, 1885). Rayleigh noted that although droplet formation would occur naturally as a result of surface tension, there existed a range of wavelengths (if imposed on a fluid stream) that would result in uniform droplet breakup. Likewise, droplet formation by jet instability provided a scientific foundation for the most established method of producing uniform microspheres, and has generated considerable momentum in progressing the field of precision microencapsulation and vibration technology.

The components of the vibrating nozzle, at minimum, typically consist of a nozzle with defined orifice diameter, a waveform generator attached to a frequency transducer, and a pump for feeding one or more fluid streams into the nozzle (Whelehan and Marison, 2011). The material streams are flowable either by means of solvent addition or heat

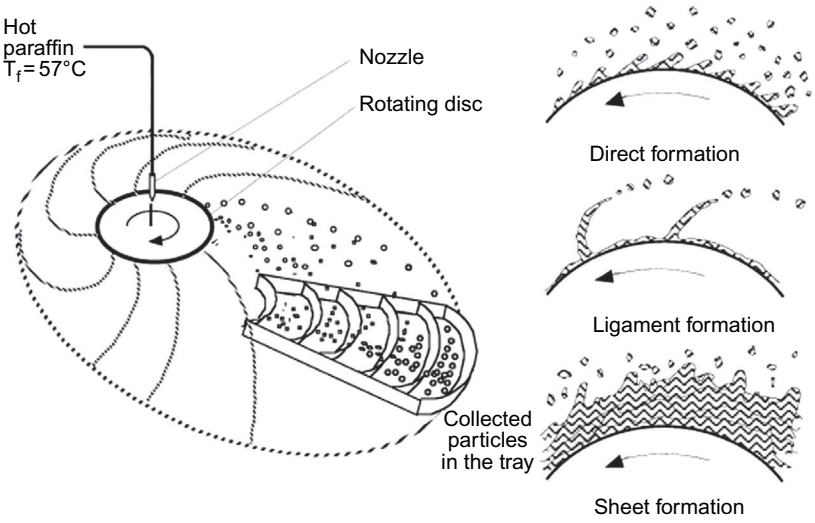

FIGURE 11.4 A schematic representation of the rotating disc process. Under different speeds of rotations, droplets may form through different mechanisms, including direct droplet formation straight from the disc, controlled droplet generation through ligaments projected from the disc, or random droplet generation from a sheet formed on the disc. Shown on the left, an improved collection process was adapted to segregate particles based on their sizes. *Reproduced with permission from Teunou and Poncelet (2005). © Elsevier 2005.*

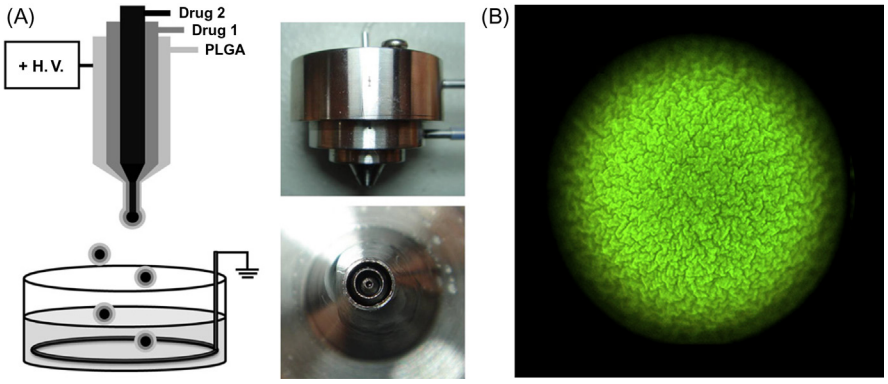

FIGURE 11.5 Vibratory instrumentation and beads. (A) Vibrating nozzle head with concentric compartments for producing layered beads. (B) Confocal image of fluorescently labeled whey protein bead produced with vibratory technology. *(A) Reproduced with permission from Lee et al. (2011). © Elsevier 2011. (B) Reproduced with permission from Doherty et al. (2011). © Elsevier 2011.*

application. Once the fluid reaches the nozzle tip, a combination of jet velocity and vibratory excitation breaks the stream into uniform segments (Figure 11.5A). The diameter of the resulting droplets can be predicted by Eq. (11.3):

$$r = \left(\frac{3r_j^2 v_j}{4f}\right)^{1/3} \tag{11.3}$$

where r is the droplet radius, r_j is the radius of the undisturbed jet, v_j is the linear velocity of the jet, and f is the frequency of vibration (Rayleigh, 1878). Higher frequencies specified by the user result in shorter wavelengths, and hence smaller droplets, assuming the wave amplitude is sufficient to disrupt the jet. Conversely, low frequencies produce larger droplets. In some instances, the amplitude of vibration can be adjusted to aid in breaking more viscous solutions and/or larger diameter jets. Vibration technology sets itself apart from flow focusing and more complex systems by the fact that droplet formation occurs only due to surface tension and vibration of the material stream itself. In some applications, droplet breakup and dispersion is promoted by downstream charge induction right before collection (Kim and Kim, 2010; Lee et al., 2011).

Nozzle architectures can be simple, or more complex, having two or three concentric compartments (Whelehan and Marison, 2011). Multiple compartments can be used to isolate streams of different materials (Figure 11.5B) such as polymers, oils, and liquefied actives, which can produce capsules, multinucleated microspheres, or beads that are carriers for biologics such as cells (Heinzen et al., 2002). A few important considerations for the nozzle design include material of construction (considering operating temperature and/or contacting fluids), simple assembly, and accessible contact surfaces for inspection and ease of cleaning.

For applications where solvents need to be removed (as seen in biodegradable polymer formulations), the droplets require solvent evaporation and hardening (Lee et al., 2011). Conversely, if microsphere hardening takes place immediately, either thermally (wax (Brandau, 2002), chitosan (Liu et al., 2011)) or chemically (acrylate (Kim and Kim, 2010), alginate (Seifert and Phillips, 1997; Brandenberger and Widmer, 1998)). Thus, postprocessing or drying steps could be minimized and usage or storage can take place immediately.

Unlike other uniform microsphere fabrication processes, vibration technology is restricted to primarily larger sizes (Whelehan and Marison, 2011). Matrix (noncapsule) particles under 150 μm are rarely produced, while the minimum size typically reported for capsules is just slightly larger. A common rule of thumb is that the diameter of the jet produced from a vibrating nozzle is approximately half the smallest achievable droplet diameter. The upper size limit, however, is essentially undefined; higher flow rates and larger nozzle orifices lead to larger droplets. Thus, common diameters for vibration-based droplets range up to several millimeters.

The simplicity of vibration-based microsphere technology, combined with large particle sizes (which translates to high volume throughput), makes it the very best candidate for scalability. In fact, several companies have taken this technology beyond experimental units to pilot-scale production (Buchi Encapsulator B 390/395, Nisco VAR E/C) and large-scale processing (BRACE GmbH). With these options, production rates can reach up to 1000 liters per hour or more.

Currently, the usage of vibration technology for microencapsulation is only excluded from instances requiring microsphere sizes below 150 μm. These small particle sizes, however, require small orifices to produce a jet (roughly half the microsphere diameter). Decreasing orifice size increases the probability of nozzle plugging and limits the use of viscous encapsulation materials. If particles larger than 200 μm can be utilized, products in pharmaceutics, taste masking, cell transplantation, cosmetics, pigments, agriculture, and food science can benefit. Specific examples relating to the food industry include formulation of whey protein microcapsules (Doherty et al., 2011) or encapsulation of vanilla flavoring (Manojlovic et al., 2008). While vibration technology may be the least complex and customizable method for creating monodisperse droplets, it compensates with breadth of application, scalability, and robustness.

11.2.6 Flow Focusing

Traditionally, droplet breakup in a jet can be induced by gravity or produced in a uniform manner with vibratory disturbance (Rayleigh, 1878, 1879a,b, 1885). Without vibration, however, uniform droplet size can be achieved by a method called flow focusing (Martín-Banderas et al., 2005). Flow focusing does not rely on an external disturbance (such as vibration) of a laminar jet of fluid. Here, shear caused by a sheath fluid leads to concentric stretching of the stream in the direction of flow. Tapering of the fluid jet imparts a downward shear force greater than that of gravity alone, which forces jet breakup at a much smaller diameter than seen with vibratory processes.

Flow-focusing instrumentation is extremely similar to that of its vibratory and microfluidics process counterparts, in that a nozzle with a specified orifice diameter is used. The additional criterion for flow focusing is that a concentric outer stream of either air or liquid, typically flowing at a much higher rate, is used to taper the production stream (Figure 11.6A). This focusing stream does not become part of the droplet and no vibration is needed. Like all fluid

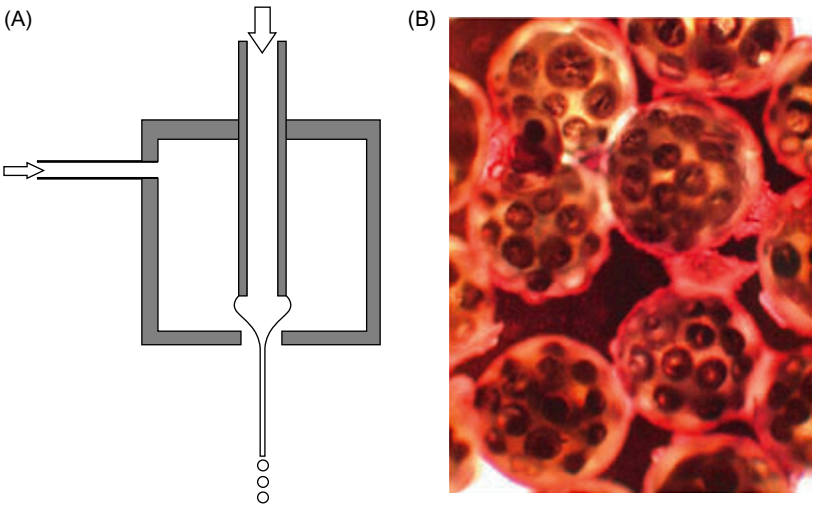

FIGURE 11.6 Flow-focusing technology. (A) Schematic of flow-focusing device. Material stream (top arrow) tapered by air jet (side arrow) breaks up droplets. (B) UV-cured capsules produced after a flow-focused stream was tuned to include multiple cores. *Reproduced with permission from Martín-Banderas et al. (2005). © Wiley & Sons 2005.*

mechanics processes, limits of operation to achieve uniform breakup exist, and such limits depend on fluid pressure, velocity, diameter, and surface tension. As derived by Martín-Banderas et al. (2011), the diameter of the droplet could be predicted by Eq. (11.4):

$$d = \left(\frac{3\pi C}{2k\rho_P}\right)^{1/3}\left(\frac{Q_i}{Q_o}\right)^{1/2} D \tag{11.4}$$

where d is the droplet diameter, C is the polymer concentration (wt/vol), ρ_P is the density of the polymer solution, k is the wavenumber of the fastest growing perturbation on the jet, Q_i is the inner fluid flow rate, Q_o is the outer fluid flow rate, and D is the inner diameter of the nozzle orifice (Martín-Banderas et al., 2011). Like other processes, flow focusing can also utilize an inner stream to microencapsulate a core phase. The number of cores can be adjusted by varying the velocity of the focusing stream, such that breakup of the core droplets occurs in singles or multiples (Figure 11.6B).

Unlike vibratory and microfluidics processes, flow focusing can produce particles in the 2 to 50 μm range, and the droplet size is not limited by the orifice diameter. The flow-focusing process is also scalable and has low associated costs. Most published applications utilize flow focusing with biopolymers such as PLGA (Martín-Banderas et al., 2005), PCL (Cheng et al., 2010), PS (Martín-Banderas et al., 2006), PEG (Talu et al., 2006), PDMS (Umbanhowar et al., 2000), or silica (Liu et al., 2012), but the technique could be used in the food space as easily as the vibratory and microfluidic processes. Collection, drying, and product preservation can take place in existing industry facilities that handle traditional emulsion or spray drying (nonmonodisperse) microsphere production.

11.2.7 Vibratory Process Combined With a Carrier Stream

This chapter has discussed myriad technologies for producing uniform microspheres and microcapsules: electrohydrodynamic spraying, flow-based processes, spinning discs, jet cutting, and simple vibration-based methods. A formulation scientist can define which characteristics are most important for the end use (materials, actives, size, scalability, morphological characteristics) and select an appropriate method for their product. Recently, the emergence of a technology combining flow to reduce jet diameter with vibration to assist jet breakup has been developed, and is referred to as precision particle fabrication, or PPF (Berkland et al., 2001). This particular adaptation of creating monodisperse microspheres has the scalability of vibration-based processes, maintains the complexity of most microfluidic approaches (porosity, coating thickness, surface texture), and has a particle size range that spans all of the previously existing technologies.

PPF technology can create precise particle sizes using almost any solution because the immiscible, coaxial fluid/fluid or fluid/gas flows reduce the diameter of viscous jets of melts or dissolved materials (Figure 11.7A). Increasing the annular "carrier" stream flow rate or decreasing the polymer stream flow rate reduces the diameter of the emitted polymer jet without having to reduce the nozzle diameter. In this manner, previous limitations of vibrating nozzle technology (e.g., clogging or material viscosity) are obviated. Once the desired jet diameter has been set, the emitted coaxial jet is periodically disrupted by a controlled acoustic wave that is tuned to produce the desired droplet size. Here, the jet breakup is caused primarily by vibration. High viscosity polymer solutions, water−oil emulsions, suspensions, and melts have been successfully sprayed as uniform droplets that quickly harden after cooling, drying, solvent extraction, polymerization, coacervation, or crosslinking (Figure 11.7B).

In advanced demonstrations of the technology, core−shell microcapsule morphologies have been produced from a wide variety of materials. For example, microcapsules have been formed with a polymer shell filled with a second polymer, oil, or even water (Berkland et al., 2007). These particles can provide a delayed burst of active ingredients depending on the degradation characteristics of the shell material. In addition, unique shell materials (e.g., polyesters, celluloses, etc.) and discrete control over shell thickness facilitate novel applications that demand capsule rupture in response to an environmental cue (e.g., pH, shear, etc.). The PPF setup changes only slightly for these core−shell formulations, by adding an additional concentric nozzle to the traditional solid particle nozzle.

Using PPF technology, a wide range of particle sizes is achievable ($\approx$2 μm to $>$1 mm) with precise control over particle size and shell thickness. The flexibility of PPF technology allows for a single-step process that can easily integrate into existing industrial fabrication and collection schemes, specifically those used for spray drying and spray chilling. Size and scalability characteristics position PPF as a technology with few compromises (Figure 11.8).

PPF is patent protected (US and PCT) and exclusively licensed to Orbis Biosciences (Kim et al., 2001a,b). Although there have been variants using acoustic waves from a loudspeaker to propagate the frequency disturbance (Bocanegra et al., 2005b), only the PPF process can achieve the lower spectrum of particle sizes ($<$50 μm) with a core−shell

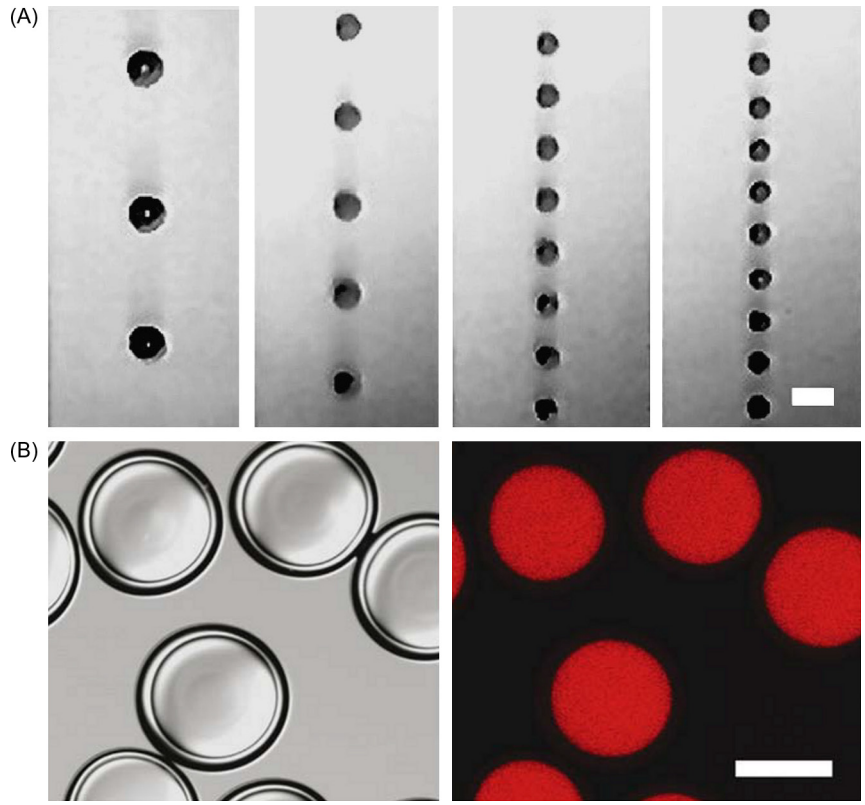

FIGURE 11.7 Precision particle fabrication combining vibratory disturbance and cocurrent flow. (A) Increasing frequency of vibration decreases droplet size. Scalebar = 100 μm. (B) Optical (left) and confocal (right) microscopy of uniform capsules filled with sulforhodamine B-labeled protein. *(A) Reproduced with permission from Berkland et al. (2001). © Elsevier 2001. (B) Reproduced with permission from Berkland et al. (2007). © Springer 2007.*

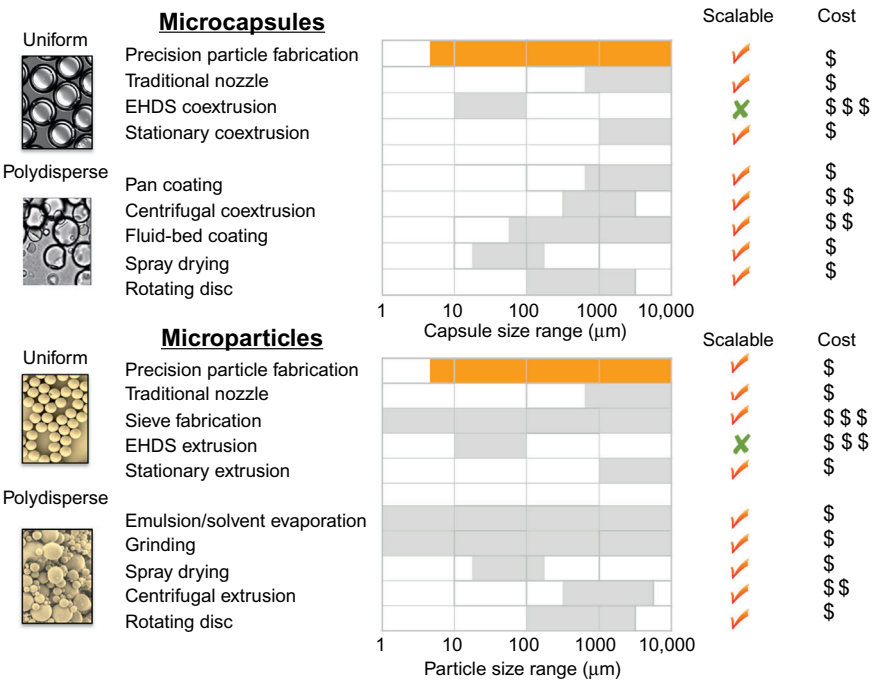

FIGURE 11.8 A comparison of some common microparticle and microcapsule production technologies showing their capabilities, including uniformity, possible size range, scalability, and associated cost.

morphology. The technology has been applied to pharmaceutics, vaccines, dermal applications, nutraceuticals, agriculture, food flavoring, and taste-masking applications.

11.3 CONCLUSION

In this chapter, a review of prominent existing monodisperse microparticle fabrication techniques was provided. Some of these technologies came into existence to fulfill needs in the field of pharmaceuticals, particularly the drug delivery area, and have not been extensively explored for food and flavor applications. Many challenges still remain, including scalability and cost effectiveness, which limit the use of some of these technologies for the food and flavors industry. Continuous progress is being made in the field of microencapsulation to address the needs of evolving food and flavors applications. The integration of advances in microencapsulation sciences combined with high-throughput cost-effective ways to generate monodisperse microparticles would promote the use of such technologies for differentiated food and flavors products in the future.

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Chapter 12

Coacervation Processes

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12.1 INTRODUCTION

Coacervation is the separation of a colloidal system into two liquid phases, as defined by the International Union of Pure and Applied Chemistry (IUPAC, 1997). The coacervation process can be categorized into two types: simple or complex coacervation, depending on the number of polymers involved. Complex coacervation occurs mainly through electrostatic interactions between two or more oppositely charged polymer solutions, and results in two immiscible liquid phases: one is the polymer-poor continuous phase; the other is the polymer-rich dense phase, also called coacervate phase, which is used to coat a variety of active core ingredients. Normally, coacervates generated by complex coacervation possess improved functional properties compared to each of their individual constituting polymers (Schmitt and Turgeon, 2011; Zhang et al., 2012; Souza et al., 2013). While simple coacervation only involves one polymer, it has been hardly used in food industry. Therefore, this chapter focuses on complex coacervation.

Complex coacervation is mainly controlled by the charge density of the biopolymers, which is primarily influenced by pH and ionic strength of the dispersion (Souza et al., 2013). Dilution promotes complex coacervation, which is a unique property distinguishing it from other polymer phase-separation phenomena (Thies, 2007).

Although the first phenomenon of phase separation of oppositely charged hydrocolloids was reported a century ago (Lv et al., 2013), Bungenberg de Jong and Kruyt were the pioneers who investigated the phenomenon systematically based on the gelatin−gum arabic system (Bungenberg de Jong and Kruyt, 1929; De Kruif et al., 2004). Since then, complex coacervation has been studied extensively and applied in various consumer products, such as carbonless copy paper, scent strips, fragrance samplers, lipophilic drugs, flavor ingredients, and functional foods (Zhang et al., 2012). Carbonless copy paper was the first commercial application of coacervation by the National Cash Register Company in the late 1950s (Green and Schleicher, 1956, 1957).

As one of the microencapsulation technologies in the food industry, the complex coacervation process has been used for encapsulating reactive, sensitive, or volatile additives or nutrients, such as flavors, pro-/prebiotics, antioxidants, vitamins, minerals, phytosterols, peptides, lutein, lycopene, polyunsaturated fatty acids, and so on to increase their shelf-life under different storage conditions (light, oxygen, moisture, and temperature), to allow alternative food processing (heat, shear, and redox potential), and to mask the taste or to control release of encapsulated components (Yan et al., 2008; Fang and Bhandari, 2010; Qv et al., 2011; Zhang et al., 2012). Protein and polysaccharide are the most widely used wall materials, as they both are major macronutrients for a balanced diet, present together in many food systems, and contribute to the structure, texture, and stability of foods (Doublier et al., 2000). Polysaccharides are usually used as polyanions, which include gum arabic (GA), pectin, alginate, carrageenan, agar, gellan, chitosan, guar gum, and glucan, along with polyphosphate and carboxymethyl cellulose, as they are generally recognized as safe (GRAS). Polycations are mainly proteins, such as gelatin, soy proteins, casein (caseinate), pea proteins, whey proteins, β-lactoglobulin, bovine serum albumin, and egg albumin (Gouin, 2004; Fang and Bhandari, 2010; Nedovic et al., 2011).

The complex coacervation process principally consists of three basic steps: emulsification, coacervation, and shell formation and/or hardening (Zhang et al., 2012). It requires two extra steps (coacervation and hardening) as an encapsulation method compared to the spray drying method, which is the current dominant encapsulation technology in the food industry (Thies, 2007; Taneja and Singh, 2012). Thus, it is considered a rather long processing, complex, and expensive technology (Gouin, 2004; Fang and Bhandari, 2010; Taneja and Singh, 2012). However, coacervation

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00012-1

technology offers numerous advantages over spray drying for protecting high-value and labile functional ingredients, such as mild preparation conditions (neither organic solvent nor drastic temperatures required), high core loading level (up to 99%), high shell integrity, high encapsulation efficiency, and excellent controlled-release characteristics, because the resulting microcapsules are water immiscible (Gouin, 2004; Jin et al., 2007; Beindorff and Zuidam, 2010; Kralovec et al., 2012; Taneja and Singh, 2012; Zhang et al., 2012). The coacervation process is affected by multiple factors, including the nature of shell materials and core ingredients (molecular weight, conformation, and charge density), their composition, total solid percentage, as well as the aqueous conditions, such as pressure, shearing, temperature, pH, ionic strength, etc. (Schmitt and Turgeon, 2011; Souza et al., 2013).

Many original research articles, along with industrial patents on complex coacervation for food applications, have been published. The thermodynamic, physicochemical, structural, and functional aspects of various complex coacervation systems based on food-grade polymers have been reviewed recently in several excellent review papers and book chapters (Barrow et al., 2007; Jin et al., 2007; Drusch and Mannino, 2009; Schmitt and Turgeon, 2011; Thies, 2007; Veis, 2011), which provide a guide for developing coacervation systems in the food industry. This chapter reviews the most recent progresses in both academic and industrial fields, with a focus on the practical side of coacervation based on commercial examples. It is divided into five sections. The first section describes shell material selection. The second section illustrates coacervation processes. The third section discusses important parameters influencing coacervation. The fourth section outlines coacervation evaluation methodologies, and the last section discusses their stability, controlled release, and bioavailability.

12.2 SELECTION OF WALL MATERIALS

Many wall materials are suitable for production of microcapsules. However, compared to the pharmaceutical, agrochemical, or cosmetic industries, selection of wall materials in the food industry to encapsulate functional ingredients via coacervation is often challenging, due to tight regulations and low price margin. As a rule of thumb, a price increase of $0.15 per serving for a new food product is acceptable by customers, and the loading level of functional ingredients in foodstuffs is low (1−5%), so a maximum cost for a microencapsulation process in the food industry can be roughly estimated at $0.15/kg (Gouin, 2004). Meanwhile, wall material particularly affects the capsules' stability, the process efficiency, and the degree of protection of the active core ingredients (Nesterenko et al., 2013). Therefore, wall material selection is one of the major concerns for applying coacervation technology in the food industry (Kralovec et al., 2012). Proteins and polysaccharides are the most widely used wall materials in the food industry, because they are natural products, relatively inexpensive, and it is easy to obtain food regulatory approval. Furthermore, their coacervates demonstrate superior new functional properties by combining advantages of both protein and polysaccharide (Souza et al., 2013).

12.2.1 Proteins

Proteins are biological polymers comprised of amino acids with a variety of structures, such as fibrous and globular, depending on their amino acid sequence, prevailing environmental conditions, and history, exposure to different temperatures, pressures, solvents, pH values, and ionic compositions (Matalanis et al., 2011). Other than cost, their physicochemical characteristics must be considered when selecting proteins for coacervation technology, particularly their electrical characteristics, because electrostatic interactions are the main driving forces for complex coacervation. Their electrical characteristics can be described by their ζ-potential change profile with pH, where the electrical charge on proteins changes from positive below their isoelectric point (pI), to neutral at the pI, and to negative above the pI. Even though a protein is neutral at its pI, the protein still has both positive and negative charged groups on its surface. Thus, it can be involved in attractive and/or repulsive electrostatic interactions. Proteins can vary widely in their pIs depending on their biological origin and extracting process. For example, Type A gelatin, which is produced by acid hydrolysis of collagen, has a pI typically around pH 8 to 9, while the pI of Type B gelatin is around 4 to 5, due to hydrolysis of pendant amide groups under alkaline conditions (Thies, 2007; Subramaniam and Reilly, 2009). Other important characteristics include their emulsifying ability, viscosity, surface tension with the core ingredient, thermal denaturation temperatures (for globular proteins), helix−coil transition temperatures (for gelatin or collagen), pI, and sensitivities to specific monovalent or multivalent ions, or susceptibility to specific enzyme or chemical crosslinking or degradation reactions (Matalanis et al., 2011). It is also important to assess their properties in their coacervates, such as morphology, charge, stability to pH, ionic strength, temperature, and enzymes. These factors will determine how their coacervates may impact the stability and functional characteristics in their final food applications (LaClair and Etzel, 2010; Matalanis et al., 2011).

Proteins extracted from both animal and plant sources have been used for encapsulating active core ingredients through coacervation, such as gelatin, whey proteins, and casein from animals, and soy proteins, pea proteins, and cereal proteins from vegetables (Nesterenko et al., 2013). Gelatin has been the most commonly used protein in complex coacervation due to its excellent emulsifying capacity, gelling ability, and high crosslinking activity through its primary amino group (Thies, 2007). Gelatin can be paired up with a series of polysaccharides, such as GA, agar, pectin, gellan, and glucan, as well as polyphosphate. Commercial gelatins are of varying purity, and usually blended to achieve some particular goal, such as viscosity, gel strength, or adhesiveness. The actual molecular weight distributions within a gelatin preparation are neither predictable nor reproducible (Thies, 2007). At the same time, gelatin is relatively expensive, and can be problematic in monitoring for vegetarian and kosher versions. A number of other proteins, particularly globular proteins, such as egg white, soybean, and whey proteins that demonstrate good emulsifying, gelling properties, and industrial significance, have attracted much attention in the past a few years. Their gelation is traditionally achieved through heat treatment, and gels are produced by the unfolding of polypeptide chains with concomitant exposure of initially buried hydrophobic amino acid residues and subsequent self-aggregation of protein molecules into a three-dimensional network (Chen et al., 2006). These proteins have been studied to obtain a variety of special capsules via complex coacervation followed by gelation process for different food applications.

Among vegetable proteins, soy protein isolate, pea protein isolate, and cereal proteins have been used as wall materials in microencapsulation via coacervation. For the majority of vegetable proteins in aqueous solution, the isoelectric point is located in a pH range of 3 to 5 (Nesterenko et al., 2013). To obtain good solubility, they have to be dispersed in alkaline solution first. Soybean proteins possess good emulsification capacity, gelation, and film-forming properties. Meanwhile, they are good substrates for crosslinking, particularly when using transglutaminase as the crosslinking agent, thus they are the most commonly used vegetable proteins for encapsulation via coacervation. Cereal proteins, such as oat, barley, wheat, and corn, are more advantageous from the nutritional standpoint, and they have attracted research and commercial attention for this reason (Nesterenko et al., 2013).

The use of vegetable proteins as wall materials reflects the present "green" trend in the food industries, as they are known as less allergenic compared to animal-derived proteins (Nesterenko et al., 2013). The protein ingredient industry has been turning toward plant sources in the last decade, and vegetarian diets are becoming more popular as public health awareness increases. Currently, the majority industry uses animal proteins for encapsulation via coacervation, with very limited use of plant proteins. This tendency is going to be reversed in the near future (Yan et al., 2008; Nesterenko et al., 2013).

12.2.2 Polysaccharides

Polysaccharides are often classified as either homo-polysaccharides, which consist of only one type of monosaccharide, or hetero-polysaccharides, which are made of different types of monosaccharide, depending on their monomer sequence, the prevailing environmental conditions, and their history (Matalanis et al., 2011). Polysaccharides differ from one another chemically in terms of the type, number, sequence, and bonding of the repeating unit within the polymer chain. These chemical differences lead to differences in molecular properties, such as molecular weight, degree of branching, structure, flexibility, and electrical charge. In turn, these molecular differences lead to differences in functional properties, such as solubility, thickening, gelation, water holding capacity, surface activity, emulsification, and digestibility (Matalanis et al., 2011). Like proteins, when selecting polysaccharides for encapsulation via coacervation, besides their price, their physicochemical properties must be considered, especially their electrical characteristics (ζ-potential versus pH). The electrical charge on polysaccharides depends on the nature of the ionic groups along the chain background, as well as solution conditions (Kizilay et al., 2011; Li and McClements, 2011). Some polysaccharides are anionic, such as alginate, carrageenan, xanthan, and GA. Some are cationic, for example, chitosan. The magnitude of the electrical charge on ionic polysaccharides depends on the pH relative to the pK_a of the charge groups. Anionic polysaccharides tend to be neutral at pH values sufficiently below their pK_a value but negative above, whereas cationic polysaccharides tend to be neutral at pH values sufficiently above their pK_a value but positive below. The most commonly charged groups on polysaccharides are carboxyl groups, such as pectin, alginate, xanthan, and carboxymethylcellulose; sulfate groups (carrageenan); and amino groups (chitosan): $-CO_2H \rightleftharpoons -CO_2^-$ (p$K_a \approx 3.5$); $-SO_4H \rightleftharpoons -SO_4^-$ (p$K_a \approx 2$); $-NH_3^+ \rightleftharpoons -NH_2$ (p$K_a \approx 6.5$). The electrical charge on polysaccharides may also be altered by interactions with other ionic species in their environment. These interactions typically involve monovalent or multivalent ions such as sodium or calcium that bind to oppositely charged groups on the biopolymer chain, altering overall charge characteristics. Other important characteristics include their emulsifying ability, viscosity, helix−coil transition temperatures (for carrageenan, alginate, pectin), sensitivity to specific monovalent or multivalent ions, or susceptibility to enzyme or chemical reactions (Matalanis et al., 2011). At the

same time, the type of environmental and solution conditions present within a particular food is also important. For example, pectin can start to depolymerize when exposed to neutral or alkaline conditions as a result of a base-catalyzed β-elimination reaction that breaks down its sugar chain. Over time, this breakdown can result in a decline in viscosity and loss of texture (Matalanis et al., 2011).

GA, one of the mostly widely used industrial polysaccharides, due to its good solubility, low viscosity, and high surface activity, is mainly produced in regions of Africa, and obtained from trees of the *Acacia* species. It is a hetero-polysaccharide containing about 2% protein, and chemically consists of a 1,3-linked β-D-galactopyranose backbone with branches of 1,6-linked galactopyranose units. Both the main and the side chains contain units of α-l-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl-β-D-glucuronopyranosyl. About 20% of the sugar units contain a carboxyl group. It is a weak polyelectrolyte that carries a net negative charge above pH 2.2 due to its carboxyl groups (Thies, 2007; Yang et al., 2012).

Alginate is a natural polysaccharide, commonly obtained from brown seaweed. Alginate molecules are linear polymer chains with a carboxyl group on every sugar unit (Thies, 2007). Pectin is a polysaccharide associated to the cell and intercellular wall of plants and fruits. It is an anionic commercial polysaccharide with acid D-galacturonic unities obtained from sterification of high metoxilation pectin. Its pK_a is around 3.6 and its main monomer is glucoronate (Sila et al., 2009; Zimet and Livney, 2009; Al-Hakkak and Al-Hakkak, 2010; Souza et al., 2013). Carboxymethylcellulose is also from plants, and obtained from treatment of cellulose via Williamson reaction, which results in partial replacement of hydroxyl groups from glucose by the group $-CH_2-COOH$. The treatment attributes to interesting functional properties such as high solubility and viscosity in cold or hot water; its pK_a is around 2.0 and its main monomer is methylated glucose (Kita et al., 2007; Souza et al., 2013). Xanthan gum is a hetero-polysaccharide produced from a microorganism (*Xanthomonas campestris*) through extracellular aerobic fermentation with a linear/helical structure. Its pK_a is around 2.8 and its main monomer is β-D-glucose (Ye, 2008; Souza et al., 2013). Carrageenan is a polymer with a linear/helical structure, obtained from several species of marine algae, and located in the cell wall and in algae intercellular matrix tissue. Different species and sources of algae produce different kinds: kappa (κ), iota (ι), and lambda (λ). Their pK_a is around 4.3, and their main monomers are sulfated galactan (Souza et al., 2013). Gellan is a linear anionic extracellular bacterial polysaccharide discovered in 1978, with a tetrasaccharide repeating sequence that consists of two residues of β-D-glucose, one of β-D-glucuronate and one of α-L-rhamnose. Acyl groups present in the native polymer are removed by alkaline hydrolysis in normal commercial production, giving the charged tetrasaccharide repeating sequence for coacervation. It is the most recent addition to the range of gelling agents available commercially for use in food (Morris et al., 2012). Sodium polyphosphate, an inorganic compound, as a polyanion, has phosphate groups distributed along its chain. Only freshly prepared polyphosphate solution is used for coacervation, due to its quick hydrolysis (Thies, 2007).

12.3 COACERVATION ENCAPSULATION PROCESSES

The pioneering work of Bungenberg de Jong on the complex coacervation of gelatin and GA provides the basic procedure of coacervation encapsulation (Green and Schleicher, 1956, 1957). The first step is emulsification, where the core material is added to a warm (around 50°C) gelatin solution under agitation, at a gelatin concentration of 8 to 11 w/w % to obtain a desired emulsion droplet size. The second step is coacervation, where polysaccharides are added along with dilution water to lower the total polymer solids content. Then the pH is adjusted to initiate electrostatic interactions and bring emulsion droplets together to form agglomerates. The third step is out-shell formation and/or hardening, where the aqueous system is cooled below the gelling point of gelatin, so the wall materials deposit on the agglomerates and provide an outer layer (Green and Schleicher, 1956, 1957; Thies, 2007).

Emulsion can be produced by several methods: high pressure homogenization, mechanical agitation, or phase inversion. High pressure homogenization is high energy consuming, and produces the smallest (in the nanometer range) and the most stable droplets/capsules (Gonnet et al., 2010). Emulsion stability can be improved by soluble complexes formed between the protein and polysaccharide at a suitable pH range (Elmer et al., 2010).

Coacervation can be initiated by changes in environmental conditions, which are able to affect polymer solubility in the aqueous phase, such as lowering pH, addition of water or an opposite charged polymer, heating up, or cooling down (Thies, 2007; Jones and McClements, 2010; Nakagawa and Nagao, 2012). The coacervation process is affected by numerous parameters, discussed in detail in the following section. The process can be easily adapted to industrial scale, and usually produces capsules ranging from five to several hundreds of μm (Gonnet et al., 2010).

The microcapsule walls formed by coacervates are usually unstable and low in mechanical strength, due to the ionic nature of interaction between the biopolymer layers. Gelatin-based coacervate capsule shells are extremely swollen and melt if reheated, and so release the core ingredients, which can be desirable in certain food applications, but also poses

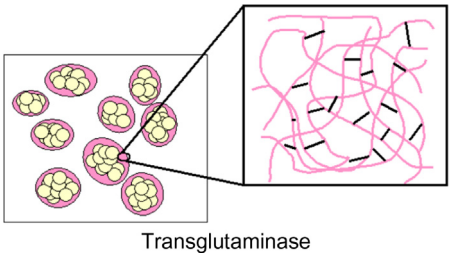

FIGURE 12.1 Crosslinking to harden the shell.

issues during isolation, drying, and application in foods (Thies, 2007). Therefore, crosslinking is necessary to stabilize and reinforce them before being dewatered and dried, as illustrated in Figure 12.1.

Previously, chemical crosslinking was used to stabilize and modify the structure and properties of complex coacervates for specific utilizations, such as refraining or promoting release of active ingredients. Chemical crosslinking agents, such as aldehydes, have been used through linking hydroxyl residues on polysaccharides and/or amine residues on proteins (Thies, 2007; Farris et al., 2010). Tannic acid also was used as a chemical crosslinker by rapidly and dramatically shrinking the shells, and reducing its water content. But it is prone to discoloration and has a characteristic taste, which limits its applicability to food systems (Thies, 2007; Zhang et al., 2011). Overall, genotoxic effects on the use of such chemical crosslinking agents in food applications is of great concern (Speit et al., 2008; Chen et al., 2012).

In the past decade, it has been demonstrated that the strength of polysaccharide/protein complex can be improved through crosslinking by heat treatment and different enzymes, such as transglutaminase (TG), genipin, laccase, and ribose (Gan et al., 2008; Selinheimo et al., 2008; Jones and McClements, 2010; Chen et al., 2012; Kralovec et al., 2012; Zhang et al., 2012).

For systems with globular proteins, heat treatment above their thermal denaturation temperature is commonly used to promote crosslinking through protein self-association via hydrophobic attraction and disulfide formation (Matalanis et al., 2011; Chen et al., 2012; Kralovec et al., 2012).

TG, a naturally occurring enzyme, catalyzes the formation of isopeptide bonds between the ε-amino group of polypeptide-bound lysines and the γ-carboxamide group of polypeptide-bound glutamines. It is used as an edible crosslinker and has been widely applied in cheese making, meat processing, and edible film production. It has been successfully used as a hardening agent for a variety of encapsulation systems with gelatin and soy proteins (Jin et al., 2008; Yan et al., 2004; Zhang et al., 2012). For example, TG was used to crosslink gelatin/GA coacervates with microalgal oil encapsulated in, and the optimum parameters were: hardening for 6 h at 15°C and pH 6.0 with a TG concentration of 15 U/g gelatin. The oil released from the microcapsules could be regulated by changing the crosslinking parameters: hardening time, pH, temperature, and TG concentration (Zhang et al., 2012). Ribose is known for its high reactivity with protein via the Maillard reaction and capability to crosslink proteins, which can be formed if a protein is heated in the presence of a reducing sugar that undergoes the Maillard reaction. Ribose crosslinked soy protein isolate-based fish oil microcapsules showed much lower release rate and longer shelf-life during storage than those crosslinked by TG (Gan et al., 2008). Genipin has been used to crosslink gelatin/GA for encapsulating the model flavors D-limonene and 2-phenylethanol (Buldur and Kok, 2011). Laccase has been applied to stabilize and reinforce whey protein isolate (WPI)/beet pectin capsules through ferulic acid in beet pectin. The crosslinking had a remarkable influence on the physical properties of WPI−beet pectin complex coacervates and resulted in fine networking structures (Selinheimo et al., 2008; Chen et al., 2012).

In the current food industry, gelatin is still the first choice for microencapsulation via coacervation, for example, Ocean Nutrition Canada's commercial products: Powder-loc™ powders are mainly based on gelatin. Ocean Nutrition Canada (ONC) patented its technologies of producing multicore and multishell microcapsules, in which an agglomeration of primary microcapsules is encapsulated by an outer shell based on gelatin and other proteins, particularly vegetarian proteins (Yan et al., 2004; Jin et al., 2008; Yan et al., 2008). The detailed procedure for commercial production is shown in Figure 12.2.

Because proteins and polysaccharides are of biological origin, it is difficult to isolate and characterize them for purity, homogeneity, and molecular weight, and particularly charge density and backbone charge distributions (Veis, 2011). Each coacervation system operates under a unique set of conditions and has a unique set of properties once formed. Thus, in a commercial scale, minimizing lot-to-lot variation to ensure the quality consistency of coacervates is the marriage of science and art (Wieland and Soper, 2006; Thies, 2007).

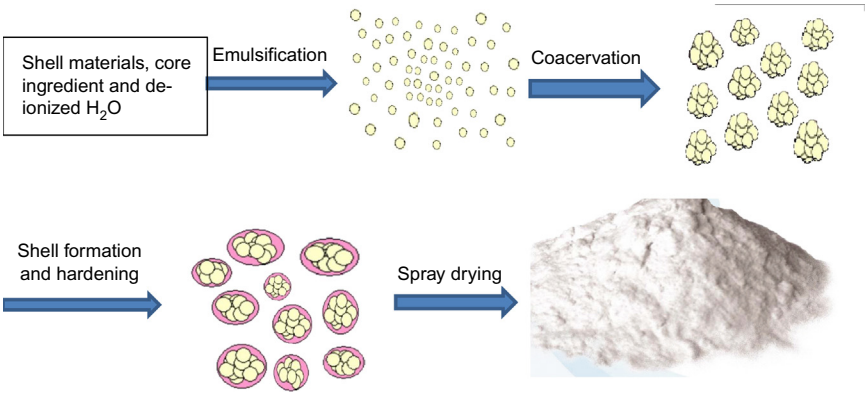

FIGURE 12.2 Coacervation process based on ONC commercial production of microcapsules.

12.4 PARAMETERS INFLUENCING THE FORMATION OF COACERVATES

Coacervation is highly sensitive to many parameters, such as the nature of proteins, polysaccharides, and core ingredients (their molecular weight, charge density, and conformation), and their ratio, total solid content, and aqueous conditions, such as pressure, shearing, temperature, pH, and ionic strength (Jones and McClements, 2010; Schmitt and Turgeon, 2011; Nakagawa and Nagao, 2012; Lv et al., 2013), as shown in Figure 12.3.

To obtain the optimal reaction conditions, all these parameters are individually optimized depending on the morphology, size, and entrapping efficiency of microcapsules, which can be a tedious and time-consuming task. In addition, the conditions attained from the laboratory are always confronted with problems for the practical application due to the quality fluctuations of raw materials (Zhang et al., 2012).

Generally, turbidity titration accompanied by other analysis, such as ζ-potential and electrophoretic mobility measurements, light scattering, size and distribution analysis, gravimetric and thermal gravimetric analysis, and spectroscopy visualization and analysis, is the most commonly applied technology for disclosing the course of coacervate formation (Nakagawa and Nagao, 2012). However, in terms of mechanism elucidation for coacervates, the information collected already is far from satisfactory. Currently, few studies have been reported regarding the interactions among proteins and polysaccharides at the molecular level (Lv et al., 2013).

Complex coacervation is mainly controlled by the charge density of the biopolymers, which is primarily influenced by pH and ionic strength of the aqueous system. pH is vital for protein−polysaccharide coacervate formation. Complex formation generally occurs between the pK_a of the polysaccharide and the pI of the protein (Schmitt and Turgeon, 2011; Klemmer et al., 2012). For example, α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) are important whey proteins with isoelectric points of pH 4.80 and 5.34, respectively. Chitosan (Ch) exhibits a cationic property under pH 6.5. α-La, β-Lg, and Ch precipitated at pH values of approximately 5.0, 5.0, and 7.0, respectively. The mixtures of α-La and Ch as well as β-Lg and Ch coacervated at a pH range of 6.0−6.5 (Lee and Hong, 2009).

In general, complex coacervation involves two main pH-induced structure-forming events associated with the formation of soluble and insoluble complexes. Soluble complexes form at a pH corresponding to the first experimentally

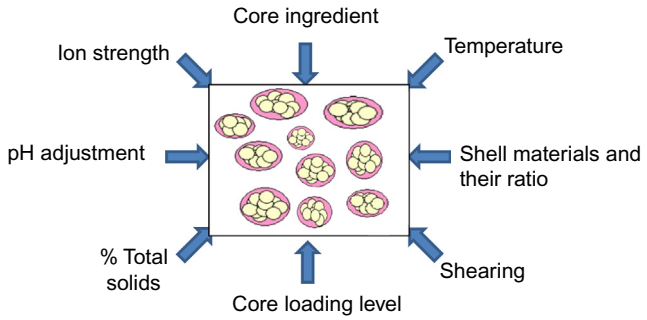

FIGURE 12.3 Parameters influencing the formation of coacervates.

TABLE 12.1 Critical pH Values of the Most Recently Reported Coacervation Systems

Coacervation System	pH_c	$pH_{\phi 1}$	pH_{opt}	References
Pea protein isolate (PPI)/gum arabic (GA) at 2:1	4.23	3.77	3.60	Elmer et al. (2010)
PPI/alginate (AL) at 1:1	5.00	2.98	2.10	Klemmer et al. (2012)
Lentil protein isolates (LPI)/GA at 1:1	5.87	3.62	3.50	Aryee and Nickerson (2012)

detectable increase in turbidity during a pH titration, which is denoted as pH_c. The formation of insoluble complexes appears at a pH with a large rise in turbidity, which is stated as $pH_{\phi 1}$. Optimal coacervate formation occurs at a pH where both biopolymers reach their electrical equivalence (denoted as pH_{opt}), which is followed by precipitation of the complexes at lower pH ($pH_{\phi 2}$), due to protonation of reactive groups on the polysaccharide backbone (Weinbreck et al., 2004; Schmitt and Turgeon, 2011). For example, soybean protein isolates (SPI) and/or α-gliadin/GA systems, pH_c 4.2 for SPI at 3:1, and pH_c 3.0 for α-gliadin at a mixing ratio 1:1 (Chen et al., 2012). For the SPI/Ch pair, the optimum coacervation pH was 6.0 to 6.5 (Huang et al., 2012). Among the most recent reported coacervation systems for food applications, the majority of them are vegetable protein based, which confirms that vegetable proteins are becoming more and more popular. Their critical pH values are listed in Table 12.1.

pH may also induce structural transitions of protein and polysaccharide. The effect of proteins and polysaccharides ratio on coacervation becomes pH dependent. For instance, soluble and insoluble fish gelatin and GA complexes were formed even in a pH region where both biopolymers were net-negatively charged (Yang et al., 2012). Meanwhile, ionic strength may change the pH value that triggers their structure transition (Souza et al., 2013).

Normally high ionic strength suppresses coacervate formation or dissociates coacervates, as evidenced by a variety of coacervation systems, such as β-Lg/low-methylated (LM) pectin, bovine serum albumin (BSA)/LM, whey protein isolate (WPI)/GA, and SPI/GA. For instance, 70 mM NaCl suppressed coacervation formation of WPI/GA, while 20 mM dissociated BSA/LM coacervates (Schmitt and Turgeon, 2011; Xiao et al., 2011). NaCl also inhibited the coacervation between SPI and Ch (Huang et al., 2012).

The proteins and polysaccharides mixing ratio is critical for controlling the charge balance, the intensity of interactions, and the degree of self-aggregation during coacervation. For example, with an LPI/GA system, the addition of GA pushed the pI of LPI from pH 4.70 to 3.17, and as the LPI/GA ratio increased, critical pH values shifted upwards until reaching the 1:1 mixing ratio, afterwards becoming relatively ratio independent (Aryee and Nickerson, 2012). With the PPI/AL system, as mixing ratios increase from 4:1 and 8:1, critical pH values shifted upwards too (Klemmer et al., 2012). The same trend was observed with the PPI/Ch system, as the PPI−Ch mixing ratio increased from 1:1 to 12.5:1, critical pH values shifted upwards, and progressively behaved similar to those for PPI alone. At PPI/Ch ratios >15:1, mixed systems resembled that of PPI alone (Elmer et al., 2011).

The charge density of the polysaccharide is also vital for coacervate formation, as demonstrated by comparing WPI coacervation with λ-carrageenan and a lactic bacteria exopolysaccharide (EPS B40). Complex formation of WPI with λ-carrageenan (sulfated polysaccharide) led to precipitates due to the high charge density of λ-carrageenan. The phosphorylated EPS B40 falls in-between GA (carboxylated) and λ-carrageenan. The effect of the charge density on the formation of electrostatic complexes was also confirmed on a milk protein/polysaccharide model (skimmed milk powder), which showed that the more sulfated polysaccharides led to complex formation at a higher critical pH_c compared to polysaccharides with a lower charge density (carboxylated). Ninety-six percent of β-Lg could form complexes with LM pectin at pH 4.5 and a mixing ratio of 4:1, whereas only 78% were complexed when high-methylated (HM) pectin was used, showing again the importance of polysaccharide charge density on complex formation (Schmitt and Turgeon, 2011).

The effect of total polymer concentration on complex coacervation was also reported. For example, with the WPI/GA system, complex formation could be observed up to 15 wt% total biopolymer concentration at pH 4.0, but the optimum conditions for coacervation were found for a total biopolymer concentration of 2 wt% and 2:1 of WPI/GA. For the gelatin/pectin system, a total biopolymer concentration of about 17 wt% led to self-suppression of complex formation (Schmitt and Turgeon, 2011).

The effect of temperature and shear was studied on the β-Lg/GA, gelatin/GA, and egg yolk lipoprotein/polysaccharides systems (Schmitt and Turgeon, 2011; Nakagawa and Nagao, 2012; Souza et al., 2013). Combinations of both parameters could either lead to a stable system (low temperature/complex shear) or to a very unstable one (high temperature/

complex shear), with a marked phase separation due to the flocculation and coalescence of coacervates (Schmitt and Turgeon, 2011). Recently, a freezing process has been reported to control the kinetics of gelatin/GA complex coacervation (Nakagawa and Nagao, 2012).

The ability to undergo solvent exchange is a unique property of microcapsules made by coacervation, which means that a water-immiscible liquid originally encapsulated inside a capsule can be exchanged with a more water-miscible liquid by diffusion. This ability provides an alternative method to encapsulate liquids and flavors that cannot be handled at the time of capsule formation (Thies, 2007).

12.5 EVALUATION OF COACERVATES

The performance of microcapsules via coacervation within a food product depends on their structure, size, stability, and release characteristics. Therefore, it is useful to highlight their most important characteristics (Matalanis et al., 2011).

12.5.1 Structure

The structure of coacervate microcapsules is significantly influenced by homogenization pressure during the emulsification process. Normally, single core microcapsules are prepared with a lower homogenization pressure than multicore microcapsules (Nesterenko et al., 2013). Single or multicore microcapsules with multiple shells prepared by complex coacervation have been patented by ONC. Inside these microcapsules, the core ingredient, fish oil, is covered by a first shell through emulsification, and then these monoshell emulsion droplets are optionally agglomerated and wrapped with a second shell through coacervation, gelation, and crosslinking. A third shell can be applied through a number of coating technologies such as fluidized bed, prilling, or spinning spray (Yan et al., 2004, 2008; Jin et al., 2008). Their structure is illustrated in Figure 12.4 along with a light microscopic picture and a transmission electron microscopy (TEM) picture of one microcapsule in Figure 12.5. These commercial products have served as a benchmark in the food industry in recent years (Drusch and Mannino, 2009).

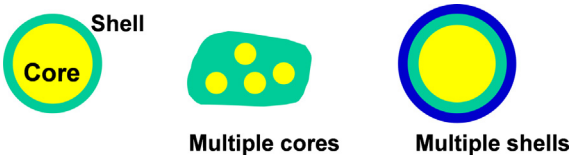

FIGURE 12.4 Structure of coacervates.

12.5.2 Size and Size Distribution of Capsules

The size and size distribution of capsules affect the texture and sensory properties of foods. It is desirable that the addition of encapsulated ingredients should not affect the texture and sensory properties, color, or flavor of food products and the food industry usually aims to prevent this. As particle size affects texture, large capsules are undesirable in most cases, explaining why microencapsulation becomes crucial in the food sector (Gan et al., 2008). The diameter of coacervates can vary from nanometers to a few hundred micrometers (Schmitt and Turgeon, 2011). For example, PepsiCo patented the size of gelatin/GA coacervates in the range of 0.1 to 5.0 μm (Kohane et al., 2009). For ONC commercial fish oil powder capsules, the fish oil emulsion droplet typically is less than 5 μm, as shown in Figure 12.6, and the size of end coacervates is normally in the range of 30 to 100 μm as shown in Figure 12.7 (Yan et al., 2004, 2008; Wieland and Soper, 2006; Jin et al., 2008; Kralovec et al., 2012).

The protein concentration (as wall material) during the emulsification step is strongly related to the stability and size of coacervates. Due to protein surfactant properties, increasing protein concentration would result in a decrease of emulsion droplet size, improving encapsulation efficiency and the droplets' coalescence resistance (Thies, 2007; Nesterenko et al., 2013).

The size of the capsule and its characteristics can be varied by changing the pH, the ion strength, the type of matrix, concentration and ratio, the type of core ingredient, and loading level (Vos et al., 2010; Klassen and Nickerson, 2012; Lv et al., 2013).

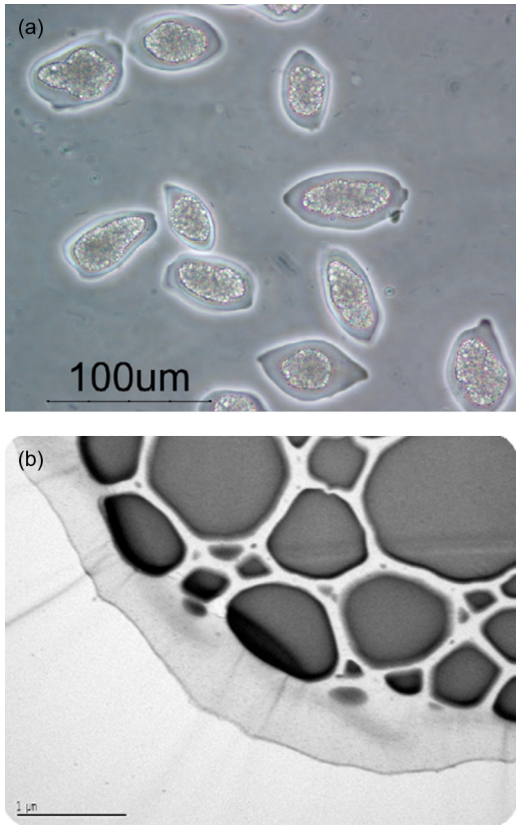

FIGURE 12.5 Multishelled ONC fish oil coacervates. (a) Light microscopy image; (b) TEM image.

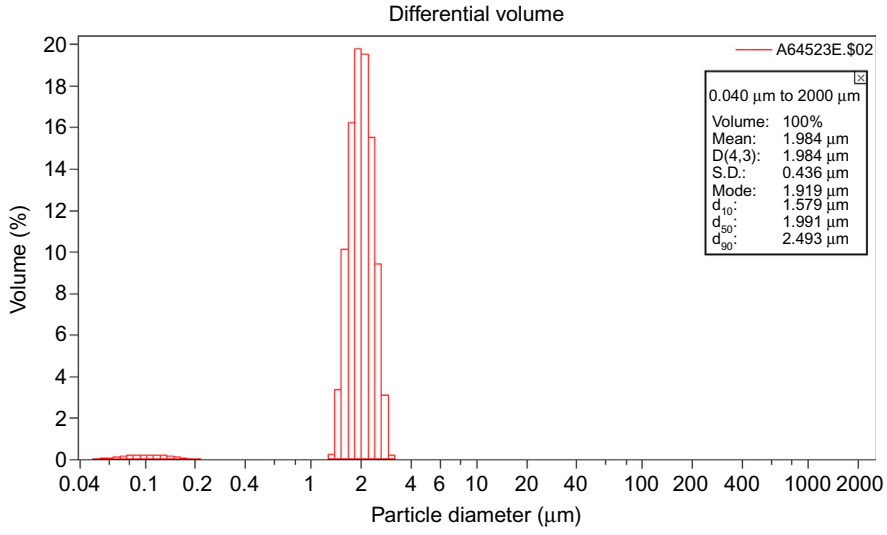

FIGURE 12.6 Size and distribution of ONC fish oil emulsion droplets.

12.5.3 Encapsulation Efficiency

Encapsulation efficiency is a significant indicator to appraise the quality of the encapsulated products, which is defined as (Qv et al., 2011):

$$\mathrm{E}\,(\%) = (w_1/w_2) \times 100\%$$

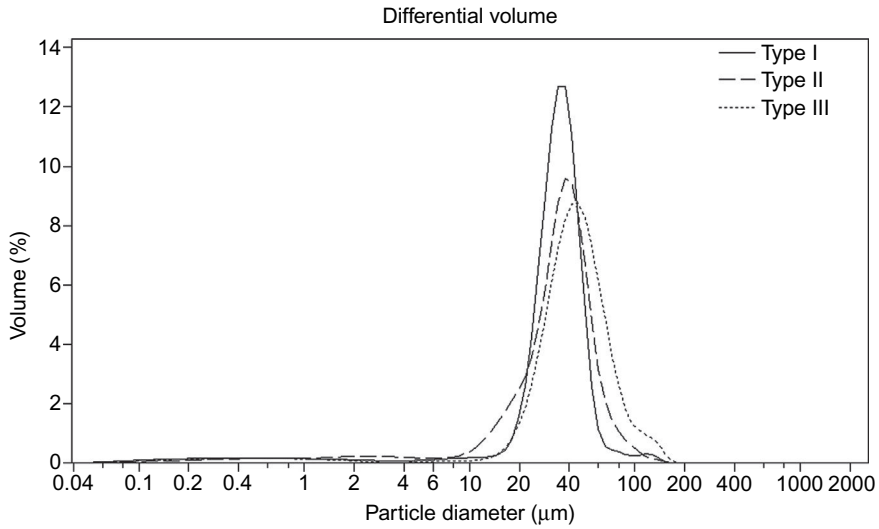

FIGURE 12.7 Size and distribution of ONC Powder-loc™ capsules.

where E is the encapsulation efficiency, w_1 represents the weight of core ingredient loaded inside the capsules, and w_2 stands for the total feeding amount of the core ingredient.

E is determined by the loading level of core ingredient, nature of wall materials, their concentration and ratios, temperature, and pH of the media. When the loading level of active core ingredients exceeds 50% (w/w), a decrease in E is generally observed (Mendanha et al., 2009; Xiao et al., 2011). For example, when using the gelatin/GA system to microencapsulate lutein, E changed with the concentration of wall material, core loading level, and pH. E increased from 77 to 84% as pH increased from 4.4 to 4.7 (Qv et al., 2011). While the wall/core ratio of SPI/pectin decreased from 1/1 to 1/3, E increased from 78 to 83%, and further increased to 93% with sucrose addition at sucrose/SPI 1:1 (Xiao et al., 2011).

A more common way to describe encapsulation efficiency of microencapsulated essential oils in the food industry is the so-called nonencapsulated oil or extractable oil %, which is a key parameter to evaluate the quality of the capsules, because nonencapsulated oil or extractable oil is prone to oxidation and limits the shelf-life by rancidity development (Jin et al., 2008; Kralovec et al., 2012). Capsules made by coacervation exhibit much lower nonencapsulated oil % and a higher loading level than those made by spray drying (Jin et al., 2008; Kralovec et al., 2012). High encapsulation efficiency (up to 99.9%) is one of the major advantages over other encapsulation technologies.

12.6 STABILITY, CONTROLLED RELEASE, AND BIOAVAILABILITY

Like other encapsulation technologies, the stability of microcapsules obtained by coacervation technology is governed by a few parameters, such as storage temperature, water activity, oxygen concentration, the shell permeability and thickness, the quality of core ingredients, the presence of trace amount of minerals, and food matrix (Drusch et al., 2007; Serfert et al., 2009).

Microcapsules obtained by coacervation technology exhibit excellent controlled-release characteristics in food systems, as most food systems are aqueous and water immiscible. The control release property of coacervate microcapsules is determined by the structure, size, loading level, crosslinking degree, shell material, mechanical treatment, pH variations (acidic conditions in the stomach, neutral in the intestine), and enzymatic action to specific gastrointestinal targets (Thies, 2007; Schmitt and Turgeon, 2011).

The unique gelling−melting profile of gelatin generates interesting properties for microencapsulation. Viscous coacervates can be prepared at 50 to 60°C, a temperature higher than the gel point of gelatin, while on cooling, interface rigidity increases resulting in a stable gelled shell around the microcapsule. During consumption, the shell will be disrupted as gelatin is readily melted in the mouth. The diffusion release rate depends on the ease and rate at which the encapsulated material can migrate through the coacervate layer (Schmitt and Turgeon, 2011). Flavor delivery is the most promising application of coacervates, and gelatin/GA coacervates were used to encapsulate flavors to be released during cooking in baked goods (Thies, 2007; Schmitt and Turgeon, 2011).

Smaller microcapsules (5−40 μm) possess bigger surface area and thinner membrane, therefore the core material is easier to diffuse into the release medium through the microcapsule membrane. Comparably with small oil-containing microcapsules, the medium size microcapsules (50−100 μm) are more suitable for controlled core release applications. The increase of loading decreases the protective effectiveness of microcapsules, improving the release rate of the core material. Microcapsules sufficiently hardened possess excellent heat-resistant and moisture-resistant properties, so as to tolerate high-temperature and high-moisture dispersing medium. The experiments have proved that the allium oil microcapsules by coacervation obtained exceptional applicable effectiveness in baking (Dong et al., 2011).

Process parameters as environmental conditions (pH, ionic strength, macromolecular ratio) and mechanical treatments (spray drying, homogenization) also allow modulation of the coacervate layer properties, such as thickness, swelling rate, and so on (Nori et al., 2011). The release rate of gelatin/GA encapsulated microalgal oil exhibited a higher rate at the beginning, while the release profile became constant indicating some sustained release after the first hour (Zhang et al., 2012). For gelatin/GA encapsulated peppermint oil, the release separately followed the first order release kinetics model in hot water and zero order release kinetics model in a high-temperature oven. The release of microcapsules in hot water exhibited initial rapid release phase and following slow release phase, where effect of core/wall ratio on the release rate was different due to the increase of loading and particle size with the core/wall ratio. Only 7% of peppermint oil released during storage in cold water for 40 days and exhibited good storage stability (Dong et al., 2011).

The bioavailability study of ONC's commercially microencapsulated fish oil Powder-loc™ powders through gelatin/polysaccharide coacervation technology was conducted and compared with the standard fish-oil soft-gel capsules. It was found that phospholipid levels of long-chain omega-3 fatty acids increased equivalently in both subject groups, which indicates that omega-3 fatty acids have equivalent bioavailability when delivered as microencapsulated complex coacervates or as soft-gel capsules (Barrow et al., 2007).

12.7 CONCLUSION

As one of the microencapsulation technologies in the food industry, the complex coacervation process has been used for encapsulating, protecting, and delivering functional ingredients, such as flavors and polyunsaturated fatty acids, with the purposes of increasing their shelf-life under different storage conditions, allowing alternative food processing, masking the taste, or controlling release of encapsulated core ingredients. The complex coacervation process consists of three basic steps: emulsification, coacervation, and shell formation and/or hardening, which can be affected by multiple factors. It is a rather long processing, complex, and expensive technology with numerous advantages for protecting high-value and labile functional ingredients. Selection of wall materials in the food industry to encapsulate functional ingredients via coacervation is often challenging, due to tight regulations and low price margins. Proteins and polysaccharides are the most widely used wall materials to pair up for complex coacervation because they are natural products, relatively inexpensive, and it is easy to obtain food regulatory approval.

Encapsulation via coacervation technology has been commercially used for more than half a century, and will continue to serve various commercial applications. However, properties of the final capsules are very sensitive to small changes in many parameters, such as the structure, molecular weight, and charge density of proteins and polysaccharides, core ingredients, their ratio, total solid content, and aqueous conditions such as pressure, shearing, temperature, pH, and ionic strength. Proteins and polysaccharides are of biological origin, and there are variations among different batches. Thus, in a commercial scale, consistent quality control is very challenging. Therefore, products made by coacervation commercially available in the food market are very limited. ONC's microencapsulated fish oil Powder-loc™ powders through gelatin/polysaccharide coacervation technology have served as a benchmark in the food industry in recent years.

Nevertheless, this technology continues to diversify and expand. Significant progress has been achieved on protein−polysaccharide coacervates as delivery systems in the food industry during the past decade, particularly vegetable protein-based systems, which reflects the present "green" trend in the food industries.

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Chapter 13

Application of Liposomes in the Food Industry

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13.1 INTRODUCTION

There are many common microencapsulating processes that are used in the food and nutraceutical industry, such as spray drying, fluid coating, spray chilling or cooling, melt injection, melt extrusion, emulsification, emulsion with multilayers, coacervation, preparation of microspheres via extrusion or dropping, preparation of microspheres via emulsification, coextrusion, inclusion complexation, liposome entrapment, freeze or vacuum drying, or encapsulation of rapid expansion of supercritical fluids.

Most encapsulating processes are based on first making droplets of the active agent, which are then subsequently surrounded by a membrane via the different processes listed above. Exceptions to this process include melt extrudates, inclusion complexation technologies, liposomes, and the use of natural encapsulates such as yeast.

There are two main types of encapsulates, the reservoir type and the matrix type. The reservoir type has a single membrane around the active agent, and is commonly referred to as capsule, single-core, mono-core, or core−shell type. The contents of the capsule can be released upon pressure of the membrane wall. Poly- or multiple-core-type encapsulates also exist. The active agent in the matrix type tends to be more dispersed over the membrane; the active reagent can be in the form of small droplets or more homogeneously distributed over the encapsulate. In the matrix type, the active agent can also be present on the surface of the membrane; in contrast, this is not seen in the reservoir type (Zuidam and Nedovic, 2010).

Liposomes can form both reservoir- and matrix-type microencapsulation. The definition of a liposome, an explanation of how it is able to form both types, and its uses will be explored further in this chapter.

13.2 WHAT ARE LIPOSOMES?

The liposome can be thought of as a hollow sphere whose size can range from a few nanometers to a few microns. Liposomes are generally made from phospholipid molecules, which are the same molecules that comprise the cell membrane. Phospholipids are made up of a glycerol backbone, polar head group, and fatty acid chain. Phospholipids are known as amphipathic, that is, their polar head group is water soluble (hydrophilic or water loving) and the fatty acid chain is oil-like (hydrophobic or water fearing or fat soluble). Therefore, when added to water, the water-soluble part of the phospholipid interacts with the water and the oil-like part of the molecule avoids the water (see Figure 13.1).

In order to accomplish this, the phospholipids align themselves side by side with their oil-like portions orienting themselves towards each other. This structure is known as a phospholipid bilayer. This bilayer extends itself in water to form a sheet, which then curls into a liposome. The interior of liposomes is filled with water and therefore molecules that are soluble in water can be encapsulated in the interior of the liposome. A less recognized but very important property of liposomes is that molecules that are not water soluble, or oil-like, can be entrapped in the oil-like portion of the phospholipid bilayer. Consequently, liposomes can serve as carriers for all types of molecules including both water-soluble and water-insoluble compounds. In fact, a single liposome can carry both types of molecules or combinations of each type of molecule.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00013-3

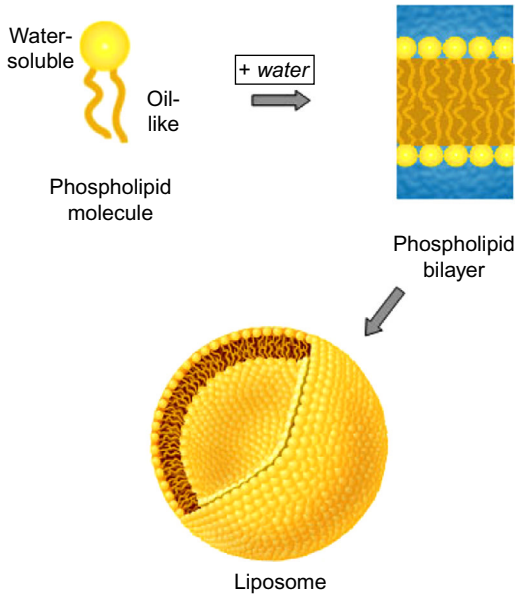

FIGURE 13.1 Phospholipid molecule, lipid bilayer, and liposome structure.

FIGURE 13.2 Unilamellar liposome containing one lipid bilayer.

FIGURE 13.3 Onion shape multilamellar liposome containing concentric phospholipid spheres.

Liposomes smaller than about 200 nanometers usually consist of one bilayer (reservoir type), and are known as unilamellar liposomes, as shown in Figure 13.2. Larger liposomes often are comprised of several unilamellar vesicles that form one inside the other in diminishing size, creating a multilamellar structure of concentric phospholipid spheres separated by layers of water (see Figure 13.3). Multilamellar liposomes (matrix type) have been described as looking like an onion. Finally, a multilamellar liposome that does not contain concentric phospholipid spheres is called a multi-

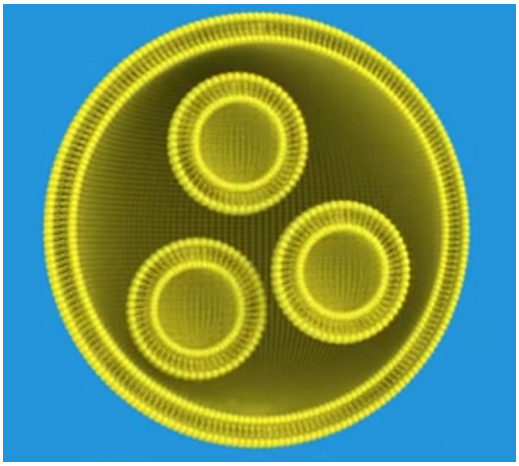

FIGURE 13.4 Multi-vesicular liposome containing non-concentric phospholipid spheres.

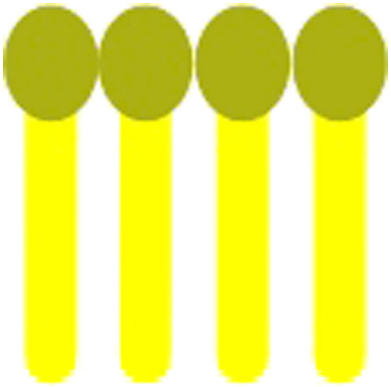

FIGURE 13.5 Saturated lipids forming tightly packed membrane.

vesicular liposome, as shown in Figure 13.4. These liposomes are made up of several small liposomes that are formed and encapsulated inside a larger liposome (matrix type).

It is the liposomes' natural tendency to form into the closed microencapsulation vesicles. Techniques such as sonication, extrusion, and microfluidization are not used to form the liposomes, which spontaneously form in an aqueous environment, but rather to size them to a desired dimension.

Liposomes can be made of a single type of phospholipid or by mixtures of different phospholipids. By varying the type of phospholipid used to make liposomes and/or by attaching certain molecules to the surface of liposomes, they can be engineered to have many useful properties. Non-phospholipid components such as cholesterol, fatty acids, and other lipid soluble molecules such as tocopherol are commonly added to liposomes to change their useful properties.

Fatty acids are considered saturated when their carbon atoms are bound covalently by a single bond and each carbon has the maximal number of hydrogen atoms bound to it. Unsaturated fatty acids have at least one double carbon bond causing the chain to kink. Saturated lipids, shown in Figure 13.5, have straight chains that form a tightly packed liposomal bilayer; on the other hand, the kink of the unsaturated fatty acid causes the liposome to be loosely packed. During incorporation of molecules such as vitamin A, tocopherol, or cholesterol, this kink becomes important as the molecule is able to be incorporated in the liposome in the space provided by the double bond kink, as shown in Figure 13.6.

13.3 LIPOSOME STABILITY

The difficulty in using liposomes in a food or nutraceutical formulation centers on the stability of the phospholipids at room temperature. Chemical degradation such as hydrolysis and/or oxidation, aggregation, and fusion often occur over time.

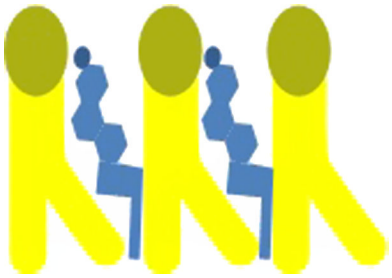

FIGURE 13.6 Unsaturated lipids form loosely packed liposomes and lipid soluble molecules such as cholesterol can sit in the space between the lipids.

13.3.1 Hydrolysis of Liposomes

Hydrolysis is a natural process. Both saturated and unsaturated lipids will hydrolyze over time. The hydrolysis of a single molecule of phosphatidylcholine provides the following five hydrolysis products: 1-acyl-lyso-PC, 2-acyl-lyso-PC, free fatty acids, and glycerophosphorylcholine. In Figure 13.7, the hydrolysis of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) as the most natural occurring lipid is shown.

The formation of lyso-PC is one of the main reasons for chemical and physical instability of liposomes. Lyso-PC has very strong detergent properties. Lyso-PC got its name due to its ability to lyse red blood cells. Lyso-PC can exchange into and out of liposomal membrane and it can destabilize bilayers resulting in leakage of encapsulated aqueous contents. Lyso-PC can also stabilize non-bilayer forming lipid into bilayers and induce aggregation and/or fusion in liposome formulations.

As shown in Figure 13.8, over an 8-day span the concentration of hydrogenated soy PC disappears as the products of hydrolysis increase. Keep in mind that in this study extreme conditions such as high temperature and low pH are used (Grit and Crommelin, 1993).

There are several ways to decrease the rate of hydrolysis and formation of lyso-PC and therefore to increase the shelf-life of the liposomes. The rate of hydrolysis is temperature dependent. As shown in Figure 13.9, over 40% of the

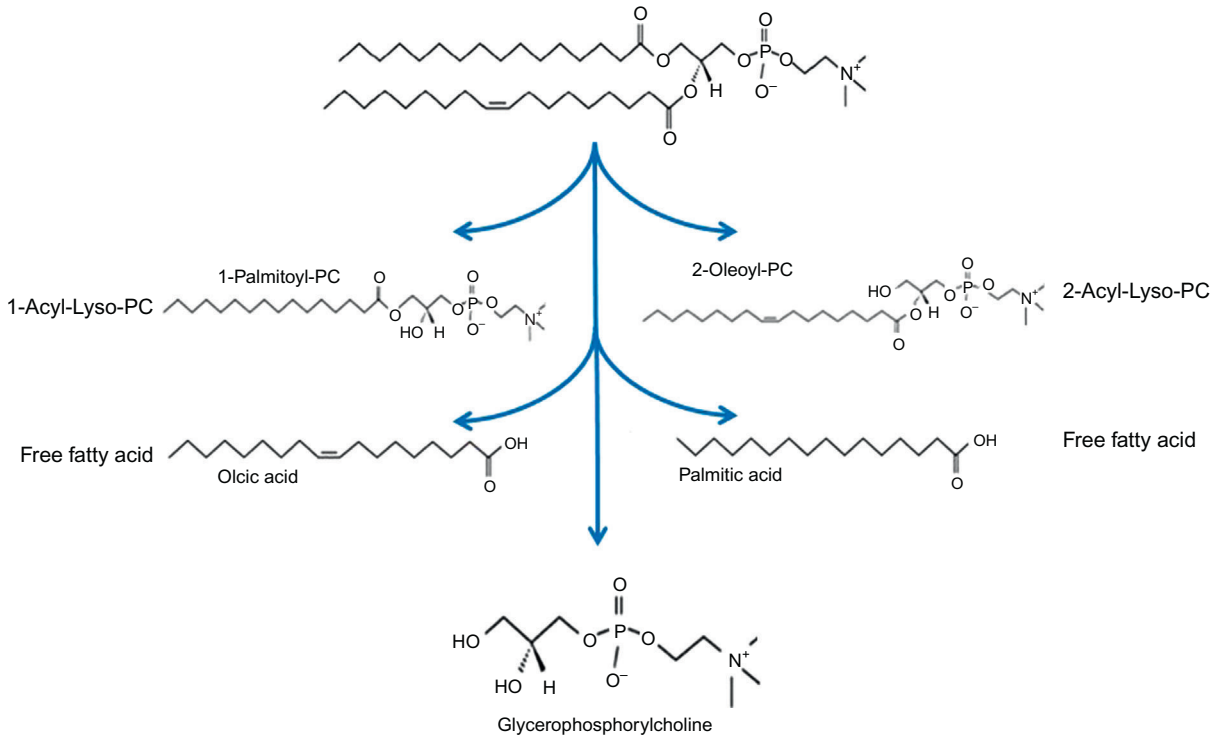

FIGURE 13.7 Hydrolysis of POPC (the most naturally occurring phospholipid) and formation of five molecules.

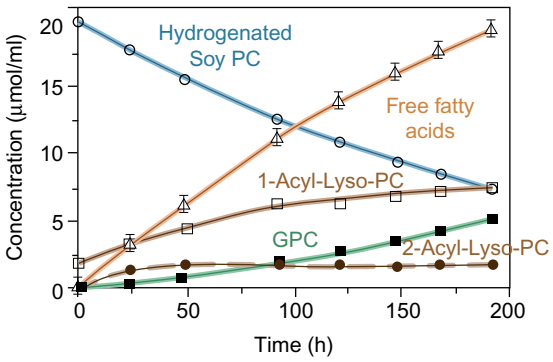

FIGURE 13.8 Chemical degradation of phospholipids and the formation of products of phospholipid hydrolysis. Disappearance of HSPC and appearance of hydrolysis products monitored for 8 days. HSPC liposomes incubated at 70°C in 0.05 M acetate at pH 4.0.

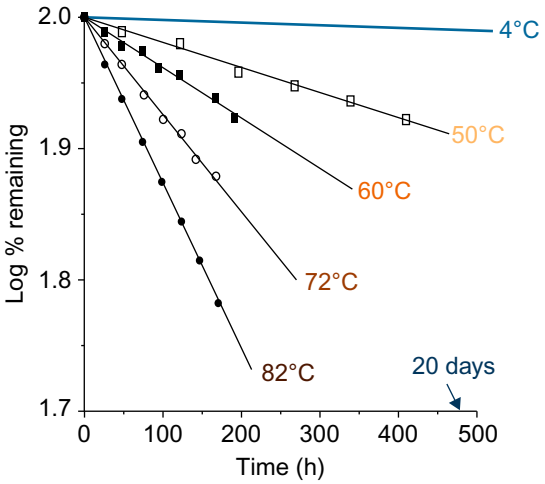

FIGURE 13.9 Rate of hydrolysis of soy PC in citrate buffer ($\mu = 0.06$) at pH 6.5.

lipid degrades after 8 days, when the liposome solution is kept at 82°C. There is almost no lipid degradation when the same liposome solution is kept at 4°C in a refrigerator (Grit et al., 1989).

13.3.2 Effect of Buffer and pH

Hydrolysis reaction produces free protons (H^+). Phospholipid acid hydrolysis is autocatalytic. Without buffer, hydrolysis accelerates and therefore the liposome formulation must be buffered. In Figure 13.10, first-order rate constant k'_{obs} at each pH value is calculated from lipid degradation data at 72°C in various citrate buffer concentrations and then extrapolated to zero concentration of citrate. The experiment shows that in order to maximize the shelf-life of the liposomes, a high concentration of buffer should be avoided and a minimum effective concentration of buffer should be used. Figure 13.11 shows that at pH 6.5 lipids have the lowest rate of hydrolysis regardless of the temperature of their storage (Grit et al., 1993).

13.3.3 Oxidation of Unsaturated Phospholipids

The oxidation of the double bond in unsaturated phospholipid can be an issue. However, it turns out not be a major issue if the formulation is composed of monosaturated phospholipids of high purity. Oxidation of lipids only becomes problematic when the formulation is composed of polyunsaturated phospholipids. Oxidation of phospholipids can be significantly decreased when the buffers are degassed and purged with nitrogen or argon.

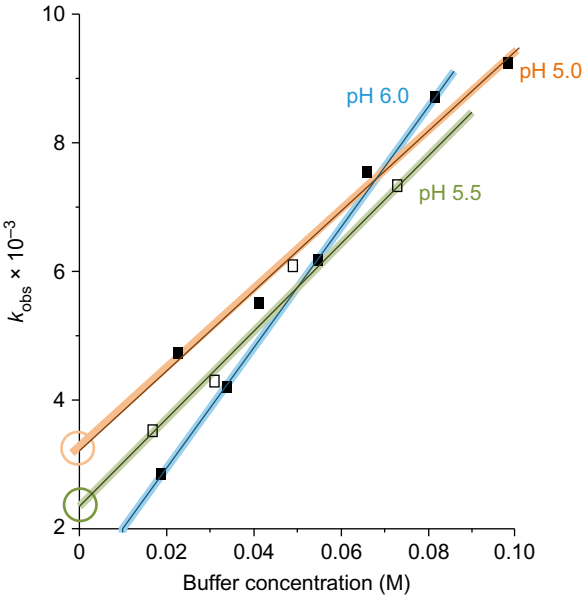

FIGURE 13.10 Rate of hydrolysis of soy PC in citrate buffer at 72°C.

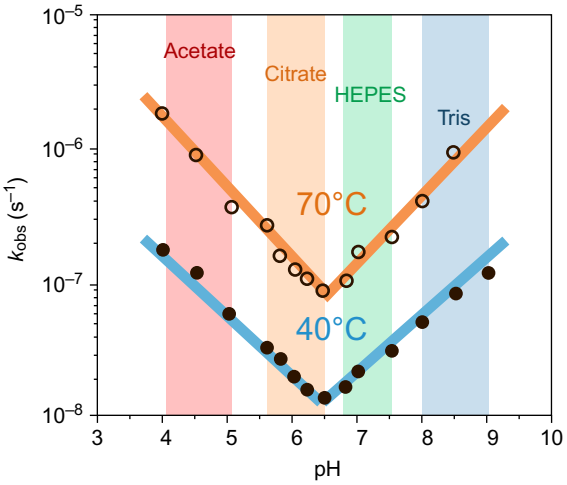

FIGURE 13.11 Rate of hydrolysis of HSPC in 0.05 M buffer.

13.3.4 Saturated Ether Lipids

As discussed above, there are two reasons for instability of liposome formulation: hydrolysis and to a lesser extent oxidation. It is possible to use phospholipids that can neither be hydrolyzed nor oxidized. This class of phospholipids is called saturated ether lipids (see Figure 13.12). These lipids are synthetic and very expensive and have limited research application at this time. Due to the high cost of the lipids, they cannot be used in food and nutraceutical industries (Mangold, 1983).

13.3.5 Application of Liposome as a Solubility Tool

One of the main applications of liposomes in the food and nutraceutical industries is their property to dissolve water-soluble and lipid-soluble molecules at the same time. This is important when various molecules should be delivered together in order to have a synergistic effect. As an example, ascorbic acid and tocopherol—both powerful antioxidants—have synergistic function. Ascorbic acid is a water-soluble molecule and tocopherol is a fat-soluble molecule. Ascorbic

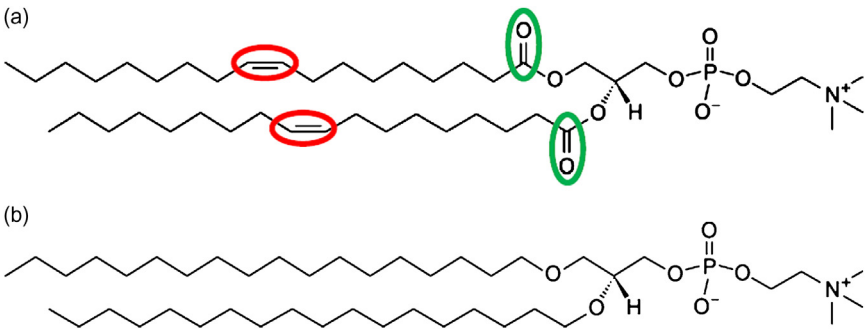

FIGURE 13.12 (a) A typical unsaturated phospholipid molecule that is prone to hydrolysis and oxidation. (b) A saturated lipid molecule. It is not prone to hydrolysis and oxidation as it does not have any carbonyl groups or double bonds.

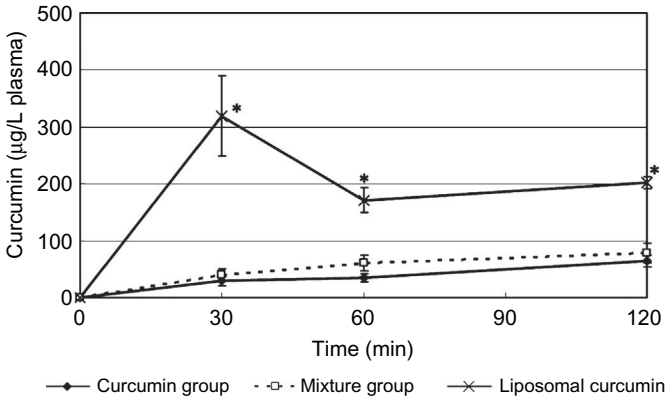

FIGURE 13.13 Concentration of curcumin in rat plasma after a single oral administration of: curcumin, a mixture of curcumin and lecithin, and liposomal curcumin (100 mg curcumin/kg body weight). The asterisks represent a significant difference at $P < 0.01$ (vs. curcumin group).

acid can be encapsulated inside the aqueous core of the liposomes and tocopherol is incorporated into the lipid bilayers of the liposomes. One liposome contains both molecules and can deliver both at the same time to the site of action.

Curcumin is an important functional food. Unfortunately, due to its poor solubility, the molecule has very low bioavailability. Curcumin is a fat-soluble molecule, so it can easily be incorporated into the lipid bilayer of the liposomes. Liposomal formulation of curcumin has significantly better solubility and bioavailability compared to unencapsulated curcumin. However, the cell lining of the human intestine contains several types of enzymes that convert curcumin into relatively inactive substances. These enzymes are UGT (UDP-glucuronosyltransferase) enzymes, sulfotransferase enzymes, alcohol dehydrogenase, and P450 enzymes. The same enzymes are also found in the liver and other tissues and can rapidly destroy most of the curcumin thus restricting its bioavailability. There are two substances that inhibit these enzymes:

1. Piperine (extracted from black pepper) inhibits UGT enzymes and P450 enzymes.
2. Quercetin (extracted from various plants) inhibits sulfotransferase enzymes.

Piperine and quercetin are both fat-soluble molecules. These two molecules can be incorporated into the lipid bilayers of the liposomes along with curcumin. Liposomes not only increase the solubility and bioavailability of all three molecules but also deliver all three molecules to the gastrointestinal tract at the same time (Kaur and Chintamaneni, 2011).

The bioavailability of liposomal curcumin has been extensively studied by various groups. A group in Japan has studied the bioavailability and antioxidant properties of liposomal curcumin in rats. Three forms of curcumin formulation, unencapsulated curcumin, a mixture of curcumin and lecithin, and liposomal curcumin, were administered orally to Sprague-Dawley rats at a dose of 100 mg curcumin/kg body weight. The pharmacokinetic parameters following curcumin administration were determined in each form. Pharmacokinetic parameters after oral administration of liposomal curcumin were compared to those of curcumin and a mixture of curcumin and lecithin (see Figure 13.13). High bioavailability of curcumin was evident in the case of oral liposomal curcumin; a faster rate and better absorption of curcumin were observed as compared to the other forms. Oral liposomal curcumin gave higher C_{max} (maximum concentration (peak) of the drug that can be achieved in a specific area) and shorter T_{max} (the time at which C_{max}

is observed) as well as a higher value for the area under the blood concentration−time curve, at all time-points. These results indicated that curcumin enhanced the gastrointestinal absorption by liposomes encapsulation. Interestingly, the plasma antioxidant activity following oral liposomal curcumin was significantly higher than that of the other treatments (Takahashi et al., 2009).

13.3.6 Application of Liposomes in the Food and Beverage Industry

To date, liposomes have not been widely used as ingredients in liquid beverages, in large part due to stability issues and difficult and expensive manufacturing processes which involve the use of organic solvents. In order to avoid oxidation, saturated lipids can be used; the problem is that saturated lipids in comparison to unsaturated lipids are very expensive. In addition, during the manufacturing process the liposomal formulation has to be heated to above the liquid-to-gel phase transition temperature of the saturated lipids, often nullifying the commonly used food-grade temperature-sensitive active agents. Both unsaturated and saturated lipids can be hydrolyzed; to slow down the process, a buffer with a pH around 6.5 can be used. The majority of commercialized beverages have acidic pH (below 3.0). In addition, beverages go through thermal processing such as sterilization or pasteurization. The majority of commercialized beverages are kept at room temperature. Beverages that require refrigeration are expensive and have limited distribution. In order to use liposomes in fortified beverages, a non-phospholipid-based lipid should be used. Many of these amphiphilic liposome-forming molecules have not been commoditized and they are not available in large scale and commodity price.

Furthermore, another issue crippling the production of liposomal preparations in beverages is due to the fact that addition of a fat-soluble molecule such as vitamin A, tocopherol, and CoQ10 causes the liposomal solution to be turbid. In order to make the beverage solution transparent, the size of the liposome has to be below 50 nanometers. Sizing the liposomes to this dimension is an expensive and time-consuming process and is not applicable to the large production scale required for the food industry.

Even though liposomes in general are not good candidates for production in beverages, there are many research papers in which they have been used to encapsulate nutrients such as liposomal encapsulation of ferrous sulfate in order to fortify the aqueous apple cider beverage, and liposomal encapsulation of calcium ions to prevent protein coagulation in soy milk (Arnaud, 1995; Sue et al., 1996).

While liposomes may not be suitable for the beverage industry, the nutraceutical industry has widely used liposome formulations of active ingredients. While liposomes in nutritional supplements do not increase the bioavailability of many active ingredients despite the marketing claims, there are some studies that suggest that bioavailability of fat-soluble molecules such as curcumin can be increased when the liposome formulation is used. In addition, mucoadhesive liposome formulation can be extremely beneficial. Such studies suggest that the mucoadhesive formulation of curcumin liposomes stays in the intestine longer than free curcumin and therefore curcumin will be in contact with colon cancer tumors/pre-cancer cells for a longer period of time.

Due to the instability of phospholipids at high temperature and low pH, liquid formulation of liposomes with a long shelf-life at room temperature cannot be developed. All the liquid liposome formulations that are developed in the pharmaceutical industry are kept in refrigerators. One way to get around this problem is to use an innovative packaging solution.

One technique to package liposomes for commercial use is by formulating freeze-dried proliposomes and placing the contents into powder release caps. When the caps are placed on water bottles, their contents are released and dispersed in water and liposomes are instantly formed. By packaging liposomes in this way, the shelf-life of the product is instantly increased. Furthermore, because the liposomal formulation remains as a dried powder until the consumer is ready to use the product, lipid degradation and destabilization is no longer an issue. The freeze-dried liposomes can be kept at room temperature without the need to use overnight shipping on cool packs. Figure 13.14 shows Curcusome® formulation packaged in a powder released cap. Figure 13.15 shows the difference in solubility of curcumin and liposomal curcumin in water.

13.3.7 Application of Liposomes in Protecting Small Molecules and Enzymes

Many studies have used liposomes as a tool to protect the active encapsulated molecule from degradation, hydrolysis, and/or premature reaction with other molecules in the environment. As an example, ascorbic acid (vitamin C) has been encapsulated inside the liposomes; the liposomes protect vitamin C from reaction and oxidation for a short period of time (after a few months) (Kirby et al., 1991). Liposomes cannot tolerate low pH for a long period of time and, as

FIGURE 13.14 The left-hand side picture shows a regular bottled water and a universal powder release cap containing freeze-dried liposomal formulation of curcumin/quercetin/piperine, known by the brand name Curcusome®, which can be put on the top of any bottle. The right-hand side picture shows liposomal formulation of curcumin that was instantly formed upon the release of powder into the water.

FIGURE 13.15 The left-hand side bottle contains liposome-encapsulated curcumin and the bottle on the right-hand side contains the same amount of curcumin in free and unencapsulated form.

shown before, low pH will cause rapid hydrolysis of lipids and formulation of lyso-PC and destabilization of liposomes. Upon destabilization of liposomes, the active ingredient leaks out of the liposomes.

Another example of using liposomes as a protective tool is the encapsulation of β-galactosidase in liposomes. β-Galactosidase is added to milk for people who are lactose intolerant. Supplementing the milk with free enzymes can cause a flavor problem. In order to solve this flavor problem, the enzyme is encapsulated into the liposomes. The encapsulated enzyme stays active for 20 days inside the milk (Rao et al., 1994).

Liposomes can also incorporate many fat-soluble flavors into the lipid bilayer. Flavors such as vanilla, citral, and menthol are lipid soluble. Due to the instability of phospholipids, many liposomologists have tried to use other types of chemically stable amphiphile molecules for liposome formation. Novasome vesicles are a class of liposomes that are made from non-phospholipid-based molecules. Fat-soluble vanilla flavor has been incorporated into Novasome vesicles. These liposomes retained the flavor without having the stability issues of traditional phospholipids (Mathur and Capasso, 1995).

13.3.8 Liposome Encapsulation of Antimicrobials

Nisin is a cationic polypeptide composed of 34 amino acids produced by *Lactococcus lactis* strains. Nisin has received much attention because of its broad inhibitory spectrum against a variety of Gram-positive bacteria, including food pathogens and contaminants such as *Listeria monocytogenes.* However, the use of nisin in its free form (unencapsulated) is associated with loss of activity due to degradation or deactivation and emergence of nisin-resistant bacterial strains. Liposome encapsulation will protect nisin from inhibitors and undesired interaction, and will reduce the affinity of nisin to non-target components. Liposomal nisin acts as a long-term preservative with controlled release properties. Liposome-encapsulated nisin has enhanced antimicrobial activity and has been used to control the growth of bacteria during cheese storage. It was shown that the liposomal formulation could be used for the control of food-borne pathogens in cheese (Colas et al., 2007).

Despite the extended uses of nisin, addition of nisin to raw meat products has been largely unsuccessful, unless used at very high concentrations or post-treatment. Nisin is an expensive antimicrobial agent and cannot be used in high quantities. Several studies have shown that the inactivation of nisin in meat is mainly due to its reaction with meat components, such as the presence of proteases and reaction of nisin with constituents such as glutathione and poor solubility of nisin at the pH of meat. The inactivation of nisin in a raw meat system has been extensively studied and as a result has been the cause of a major setback, prohibiting its wider application as an antimicrobial agent in raw meat. Other studies have shown that nisin remains active when it is added to the cooked meat. The temperature increase most likely causes denaturation of glutathione and other meat constituents involved in nisin inactivation.

A heat-triggered release liposomal nisin formulation has been developed by a group in Canada. Nisin is encapsulated inside liposomes composed of 100% DPPC. DPPC is a saturated lipid with a lipid-to-gel phase transition temperature of 41°C. DPPC lipid is in gel form below 41°C and in liquid form above 41°C. In these studies, liposomes leaked out nisin when they reached ≥ 37°C. Nisin was tested at 3200 AU/ml of meat. Unencapsulated nisin was added to the raw beef and mixed at 4°C, and the meat was tested at 0, 15, 30, and 60 minutes after the initial mixing. Free nisin incubated with meat did not produce inhibition zones no matter the length of the exposure. It was completely deactivated due to the direct contact with raw meat. Encapsulated nisin was mixed at room temperature and the meat was tested at 0, 15, 30, and 60 minutes after the initial mixing. Encapsulated nisin incubated with meat did not produce inhibition zones no matter the length of the exposure. This is due to the stability of liposomal nisin. At room temperature, nisin will not leak out of the liposomes. Encapsulated nisin was mixed at room temperature and the meat was tested at 0, 15, 30, and 60 minutes after the initial mixing and heating to above ≥ 37°C. A zone of inhibition was produced due to heating, which indicated that nisin leaked out of the liposomes. These properties of liposomal nisin make an ideal antibacterial formulation for meat that is exposed to cooking temperatures (Boualem et al., 2013).

13.3.9 Application of Liposomes in the Accelerated Ripening of Cheese

Cheese ripening, also known as cheese maturation, is one of the components of the process of cheese making. It is responsible for the development of the distinct flavor of cheese. Duration is dependent on the type of cheese and the desired quality. Ripening can range anywhere from 3 weeks to a couple of years; most cheeses usually require 2 or more years of ripening. Acceleration of cheese ripening has been proposed as a way to produce a fast ripening curd for processed cheese and to reduce the cost of cheese manufacturing. Various methods have been developed for accelerating the cheese manufacturing process.

One of the methods that is widely used is the addition of exogenous enzymes; however, significant amounts of enzyme are lost to the whey and can have adverse effects on the whey quality. Liposome encapsulation of the enzyme has evolved as a method to combat losses to whey and improve enzyme retention in the curd. The application of liposome-encapsulated enzymes on the acceleration of cheese ripening has been studied for years. For example, Alkhalaf et al. studied encapsulation efficiency and retention time of the proteolytic enzymes as a function of the liposomal charge (neutral, positively charged, or negatively charged). In their studies, the authors encapsulated proteolytic enzyme (neutrase) in neutral, positively charged, or negatively charged liposomes. The liposomes-encapsulated enzyme was then added to Saint-Paulin cheese milk. Charged liposomes (cationic and anionic) encapsulated more enzyme than did neutral liposomes. The percentage of retention of the liposomes in the cheese was highest for positively charged, then negatively charged, and then neutral liposomes. Liposome stability in milk was lower at acidic pH and also decreased with increasing temperature and salt concentration. Neutral and positive liposomes were more stable than negative liposomes (Alkhalaf et al., 1988, 1989).

Lariviere et al. focused on the method of preparation and manufacturing of enzyme-loaded liposomes during cheese ripening. In these studies, they prepared and characterized multilamellar (MLV), multilamellar-microfluidized (MLV-MF), microfluidized (MF), and dehydrated-rehydrated (DRV) liposomes. They concluded that microfluidization can be used as a successful method for scaling up production. Laloy et al. studied proteolytic release from liposomes in the cheese matrix as a function of time. In these studies, changes in proteolysis and in residual enzymatic activity as a function of time were compared in model cheeses, made with either free enzymes or liposomes containing enzymes and in control model cheeses. Cheeses were ripened under different conditions of pH, fat content, and temperature. The release of enzymes from liposomes was significantly stimulated by increasing the fat content from 0 to 20% and the pH from 4.9 to 5.5. Ripening temperature (6 or 13°C) did not affect the rate of enzyme release from liposomes. Ripening is usually done in a cool environment and therefore the liposomes cannot be exposed to high temperature. Although enzyme release started as early as the first day after manufacture, after 2 months of ripening, proteolysis was 30% lower in liposome- than in free enzyme-treated cheeses, indicating a possible inhibition of released enzymes (Laloy et al., 1998).

Kheadr et al. demonstrated an improvement in the mature texture and flavor intensity of cheese after the addition of liposome-encapsulated enzymes such as lipase, bacterial protease, fungal protease, and Flavourzyme®. In these studies, cheddar cheese proteolysis and lipolysis were accelerated using liposome-encapsulated enzymatic cocktails. Flavourzyme, neutral bacterial protease, acid fungal protease, and lipase (Palatase M) were individually encapsulated in liposomes and added to cheese milk prior to renneting. Flavourzyme was tested alone at three concentrations (Z1, Z2, and Z3 cheeses). Enzyme cocktails consisted of lipase and bacterial protease (BP cheeses), lipase and fungal protease (FP cheeses), or lipase and Flavourzyme (ZP cheeses). The resulting cheeses were chemically, rheologically, and organoleptically evaluated during 3 months of ripening at 8°C. Levels of free fatty acids and appearance of bitter and astringent peptides were measured. Certain enzyme treatments (BP and ZP) resulted in cheeses with more mature texture and higher flavor intensity in a shorter time compared with control cheeses. No bitterness defect was detected except in 90-day-old FP cheese. A full-aged Cheddar flavor was developed in Z3 and ZP cheeses, while BP treatment led to strong typical Cheddar flavor by the second month and no off-flavor was exhibited when ripening was extended for a further month (Haynes et al., 1992; Kheadr et al., 2002, 2003).

13.3.10 Encapsulation of Maillard Browning Reagent in Liposomes

The Maillard reaction or browning reaction can be defined generally as the action of amino acids and proteins on sugars. The carbohydrate must be a reducing sugar, because a free carbonyl group is necessary for such a chemical reaction. The reaction proceeds with the eventual formation of melanoidins, which are brown-colored polymers. The rate and extent of the browning reaction is influenced by a number of factors such as the particular amino acid or protein, the carbohydrate, and the presence of lipids. Different foods react at different rates and do not brown to the same extent.

Microwave cooking is one of the most desirable methods for cooking. One of the disadvantages of microwave cooking unlike oven cooking is the inability to produce the highly desirable brown color on the surface of the food. The brown color is particularly desirable on meats, breads, and pastries. Microwave cooking does not raise the surface temperature of the food to a high enough temperature for a period of time long enough to brown the food.

Temperature-sensitive liposomes can easily leak their content upon exposure to heat and this trigger release property of liposomes was used to develop a microwave-activated browning composition. A basic amino acid such as lysine is encapsulated inside the liposomes. A reducing sugar containing a free carbonyl group is outside the liposomes. Liposomes release their content upon exposure to heat/microwave. Lysine reacts with sugar and provides Maillard browning products.

In the Maillard reaction, the basic amino group is consumed, and so the initial pH of the system has an important effect on the rate of the reaction. The browning reaction slows down as the environment becomes more acidic and the pH decreases, and therefore the reaction tends to be self-inhibitory as it proceeds. To maintain proper pH, a buffer can be added to the system. The pH of the food is dependent in part on the concentration of the amino acid and the amount of moisture in the food. When a large amount of water is present, most of the browning occurs by caramelization of sugars. At lower water levels and at pH levels greater than about 6, the Maillard reaction is the predominant cause of browning (Haynes et al., 1992).

13.4 CONCLUSION

As shown in this chapter, the research prospects of liposomes in the food industry are boundless. They can be employed to solve any number of issues such as taste (i.e., encapsulation of flavor), texture, protection of sensitive active agents

(i.e., nisin, vitamin C), as a way to improve solubility in the ripening process, and as a tool to deliver protective agents such as antimicrobials.

However, even though the number of uses for liposomes is vast, and they have proven to work within the research laboratories, to date, commercialization and the complications that they cause have crippled the evolution of the use of liposomes in the food and neutraceutical industries. A wide range of problems need to be addressed in order for liposomes to be utilized fully within these industries, such as stability and chemical degradation in the form of hydrolysis, oxidation, aggregation, and fusion. Furthermore, solutions to increase shelf-life fallbacks such as temperature, buffer pH, size, and color all need to be addressed.

Looking to the future, liposomologists will have to concentrate on developing strategies to overcome the commercial barriers that have plagued the food, beverage, and neutraceutical industries, such as time, cost, chemical degradation, packaging, and shelf-life. Once these barriers are crossed the true extent and the degree of utilization of liposomes in these industries will be limitless. Therefore, future research has to focus on the production of the lipid vesicles through safe, reliable, scalable, and cost-effective methods.

The newly commercialized Curcusome® attempts to avoid these pitfalls by forming a freeze-dried proliposome that is packaged into powder release caps; only time will tell if this method will prove to be a stepping stone for the introduction of liposomes in the large-scale manufacturing needed to be successful in the food and beverage industries.

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Chapter 14

Nanoencapsulation in the Food Industry: Technology of the Future

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14.1 INTRODUCTION

The term nanoencapsulation describes the application of encapsulation on the nanometer scale with films, layers, coverings, or simply microdispersion. The encapsulation layer is clearly of nanometer scale forming a protective layer on the food or flavor molecules/ingredients. Often the active ingredient is in the molecule or nanoscale state. The major benefit is the homogeneity it imparts, leading to better encapsulation efficiency as well as physical and chemical properties. For the purposes of definition in this chapter, the scale considered for nanoencapsulation ranges from 0.1 nanometers to 1 micron (1000 nanometers). For the purposes of reference, a molecular dispersion of a true solution has a scale of 1 to 10 angstroms (i.e., 0.01 to 0.1 nanometers).

14.2 TECHNOLOGY ADVANTAGES

The major benefits of nanotechnology for food ingredient microencapsulation are (Gouin, 2004; Madene et al., 2006; Weiss et al., 2006):

- An increase in surface area may lead to improvement in bioavailability of flavors and food ingredients: This is especially important for flavor ingredients that have low solubility and/or low flavor and odor detection thresholds.
- Improvement in solubility of poorly water soluble ingredients: For example, omega-3 fish oil solubilization using a micelle-based system.
- Optically transparent (important in beverage application): Nanoemulsions and microemulsions that have oil droplet sizes of less than 100 nm are optically transparent.
- Higher ingredient retention during processing (volatile organic carbon (VOC) reduction) during spray drying.
- Closer to true molecular solution (homogeneity in system properties such as density): For example, molecular inclusion complexes based on amylose and cyclodextrins.
- Higher activity levels of encapsulated ingredient, e.g., antimicrobials in nanoemulsion/microemulsion forms.

There are numerous examples of nanoencapsulates in nature such as casein micelles in milk (<100 nm), mitochondria (500−10,000 nm), and viruses (10−300 nm). Nanotechnology in the food industry, including nanoencapsulation-related review papers, has been extensively published in recent years (Neethirajan and Jayas, 2011; Cushena et al., 2012; Nazzaro et al., 2012). In this review, the current status of nanoencapsulation as applied to the food industry will be presented with an emphasis on practical applications that have been in the commercial arena.

14.3 CLASSIFICATION OF NANOENCAPSULATED SYSTEMS

The nanoencapsulated systems are divided into liquid−liquid, solid−liquid, and solid−solid systems. Nanoparticles can be formed by two mechanisms. The first method is by size reduction from larger micron size particles to nanoparticles

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00014-5

(top-down approach), e.g., micronization, high-pressure homogenization, or by some other means with high energy density input. The second method is formation of nanoparticles by particle formation through some form of precipitation or coacervation processes (bottom-up approach). By proper control and understanding of these processes, appropriate nanoparticle-based encapsulates can be prepared.

14.4 LIQUID–LIQUID SYSTEMS

In the liquid–liquid system, which is mostly applicable to food beverages and drinks, intimate mixing of oil and water phases is critical. For example, in food ingredients such as flavors, omega-3 oils, and other essential lipophilic media, where the ingredient has very low water solubility, i.e., solubility in water is less than 0.1 to 1 mg/ml, food scientists use nanoemulsion processes to create an intimate emulsion. The basic properties food scientists need to consider before choosing a proper system for solubilization are saturation solubility of active ingredient in water and oil phases, partition coefficient, flavor/food taste threshold, flavor/food aroma/odor threshold, and appropriate dose loading requirements for nutraceuticals such as omega-3 fish oil. The definitions for these properties are as follows.

Partition coefficient is the ratio of saturation solubility of the unionized form of the flavor or food active molecule in the oil phase and the water phase. The saturation solubility in the oil phase is measured with octanol as a model solvent representing the oil phase. The partition coefficient is also called the octanol–water partition coefficient and is a measure of lipophilicity.

$$\log P_{\text{octanol/water}} = \log\left(\frac{\text{Saturation Solubility of Solute in Octanol}}{\text{Saturation Solubility in Water (un-ionized form)}}\right)$$

Flavor/food taste threshold is the minimum concentration at which taste sensitivity to a particular flavor and food ingredient can be perceived by a human. It is imperative to choose the appropriate solubilization system, such that the solubilized concentration of the flavor and food ingredient is above the flavor and taste threshold for product likeability.

Flavor, aroma or odor threshold is the lowest concentration of the molecule that is detected and perceived by the human nose. The solubilized concentration of flavor or food ingredient must be above the aroma or odor threshold to be effective.

The main liquid–liquid systems that are commonly used for solubilization are oil–water emulsion-based systems. These are illustrated in Figures 14.1 and 14.2 (Chen et al., 2006).

Table 14.1 describes the salient features of these common nanoparticulate liquid–liquid systems. For these oil–water-based two-phase systems to be stable, a surfactant needs to be added. Surfactant reduces the interfacial tension between the oil and the water phase, thereby stabilizing the oil droplets and preventing them from agglomerating. Box 14.1 describes the list of possible flavor and food ingredients with low water solubility which may benefit by using emulsion-based nanoparticulate liquid–liquid systems in food product formulations.

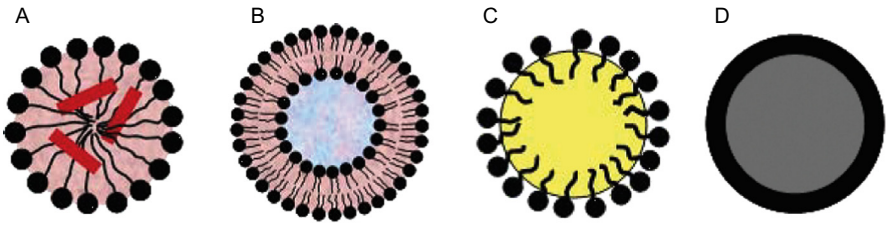

FIGURE 14.1 Typical nanoparticulate liquid–liquid systems. A: Microemulsion; B: Liposome; C: Nanoemulsion; D: Biopolymeric nanoparticle (with shell core structure).

14.5 MICROEMULSIONS

Microemulsions consist of oil, water, and an amphiphile (surfactant). Water is a continuous phase. The surfactant typically forms micelles above its critical micelle concentration (CMC). The inside of the micelle is a lipophilic/hydrophobic region where typically oil-soluble flavor/food ingredient can be solubilized. Typical micelle size is of the order of 5–50 nm. Micelles are optically transparent and have a limited capacity for solubilizing flavor/food ingredients. They are thermodynamically stable (Gaonkar and Bagwe, 2003).

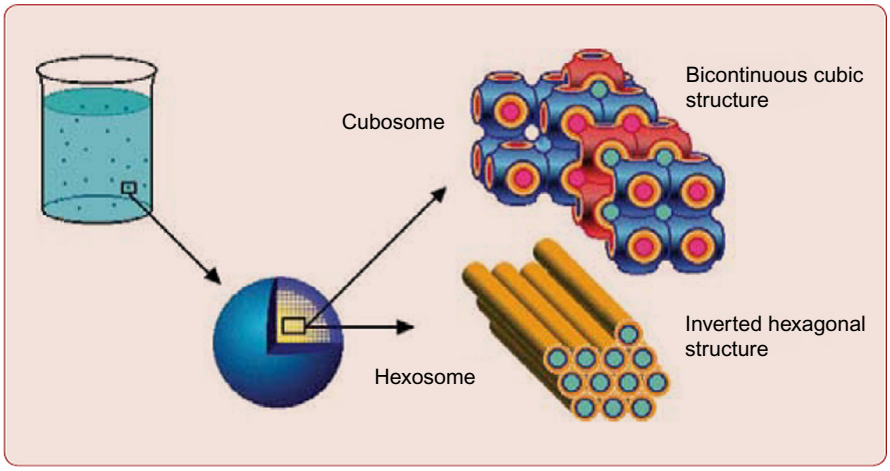

FIGURE 14.2 Other nanoparticulate structures: cubosomes and hexosomes.

TABLE 14.1 Typical Emulsion-Based Nanoparticulate Liquid–Liquid Systems

Name	Particle Diameter	Thermodynamic Stability	Appearance	Surfactant to Oil Ratio	Example
Emulsion	0.1–100 microns	No	Opaque	<1:10	Milk
Liposome	100 nm–0.1 microns	Yes	Clear/cloudy	N/A	Liposomal CoQ10 enzyme
Microemulsion	5–50 nm	Yes	Clear/cloudy	>1:1	NutraLease Technology Products
Nanoemulsion	10–100 nm	No	Clear/cloudy	≈1:1	High-pressure homogenized emulsions

Box14.1 List of Flavor and Food Ingredients with Low Water Solubility Which May Benefit by Using Emulsion-Based Nanoparticulate Liquid–Liquid Systems for New Products

- Alpha-lipoic acid
- Alpha-tocopherol (Vitamin E)
- Ascorbic acid
- Astaxanthin
- Benzoic acid
- Beta-carotene
- Citric acid
- Coenzyme Q10
- Flavor oils
- Flaxseed oil
- Gamma-tocopherol (Vitamin E)
- Isofavone
- Lutein
- Lycopene
- Omega-3 fish oil from fish and algae
- Orange
- Mint
- Phytosterols
- Rice bran oil
- Rosemary extract
- Sorbic acid
- Vitamin A acetate and palmitate
- Vitamin B
- Vitamin D
- Vitamin D3
- Vitamin E
- Vitamin K
- Vitamin A

In beverage applications, AQUANOVA AG has launched polysorbate (Tween) surfactant-based micelle-based solubilization technology for food and nutraceutical applications (Behnam, 2004). Zymes LLC (http://zymesllc.com/science/omega-3/) has developed a polyoxyethanyl-α-tocopheryl sebacate (PTS)-based micelle-driven solubilization system for omega-3 fish oil. Vitamin E TPGS NF, d-α-tocopheryl polyethylene glycol 1000 succinate, from Eastman Chemicals, can also be used as a solubilizer of flavors and nutraceuticals for beverage applications (Cook, 2004).

In the area of self-emulsifying systems, NutraLease Ltd. offers a self-emulsifying composition for solubilization of flavors and nutraceuticals (Garti et al., 2007). This system is micelle based and self-emulsifies when ingredients are mixed. It is useful for solubilizing lipophilic flavors/food ingredients such as omega-3 fish oil, vitamins, nutraceuticals, phytosterols, flavors, and others.

Micelle-based systems are relatively easy to produce. Initially, surfactant is dissolved in water with a concentration just above its CMC. Once the surfactant dissolution is complete, food ingredient/flavor is added to the solution for solubilization. Research will need to find the optimum time required for complete dissolution by using design of experiments. After the dissolution is completed, the solution is filtered to remove any undissolved food ingredient/flavor. Some examples of vitamin E-TPGS-based solubilization are given in an Eastman patent application by Cook, 2004.

14.6 NANOEMULSIONS

Nanoemulsions are oil–water emulsions with water as a continuous phase. The oil droplet sizes are typically less than 100 nm. The droplets are kinetically stable and need high energy input to obtain nanoemulsion. They do not form spontaneously. Major benefits of a nanoemulsion-based system are (Mason et al., 2006):

- Higher surface area: much larger surface area to volume ratio. Useful for improving taste perception for poorly water-soluble flavors/ingredients (improvement in bioavailability).
- Optically clear: applicable in beverages.
- Reduction in viscosity.
- Reduced amount of surfactant required.

Nanoemulsions are made by the use of high-pressure homogenization and/or high-shear mixing. The oil/water/surfactant/ingredient slurry is forced through a micron size piston-gap under high pressure to create cavitation and turbulence leading to particle size reduction. For nanoemulsions, high pressures of the order of 10,000 to 20,000 psi are used.

Huang et al. (2010) recently reported use of nanoemulsions for solubilization of phytochemicals such as curcumin, resveratrol, and carotenoids such as lycopene, β-carotene, lutein, and zeaxanthin. They also reported that these formulations may help in improving bioavailability. Weiss et al. (2009) reported use of nanoemulsions for the delivery of antimicrobials. Due to small oil droplet size (high area to volume ratio), antimicrobials such as lysozyme, nisin, and lauric arginate will have a better activity.

14.7 LIPOSOMES

Liposomes are spherical particles with a core sphere containing water and the sphere is coated with a bilayer of lipids. The lipid bilayer acts as a carrier for lipophilic substances. Liposome particles are good carriers for both hydrophilic and lipophilic food actives. Liposomes are difficult and expensive to manufacture (Weiss et al., 2009). A detailed account of liposomes as an encapsulating system is covered in Chapter 13 of this book.

14.8 SOLID–LIPID NANOPARTICLES

There are other liquid–liquid systems such as solid–lipid nanoparticles, double-layer nanocapsules based on layer-by-layer (LbL) electrostatic deposition (polyelectrolyte complexes), multi-assembled nanocapsule aggregates, and others (Weiss et al., 2009).

14.9 SOLID–SOLID SYSTEMS

For solid–solid systems containing nanoencapsulates, typically the formulation starts with making a nanoemulsion or a homogeneous dispersion of the active ingredient with the carrier shell material in a liquid state. Using atomization and/or

unique nozzle systems, the small droplets or fibers are created, followed by a precipitation step to generate nanoencapsulates (Jaworek, 2008). The major benefits of this approach are:

- High surface to volume ratio, leading to very high surface area, and improvement in bioavailability of ingredient.
- Controlled release of flavor including stimuli responsive, fast dissolve or burst effect.
- Unique surface morphology may prove beneficial in improving shelf-life.
- Near room temperature process may work well for encapsulation of thermally labile ingredients.

14.10 NANOFIBERS

Electrospun nanofibers are made by dissolving the polymer carrier in an organic solvent/water mixture with the flavor/functional ingredient to form a suspension/solution. Fibers are made by electrospinning the fiber using a very high DC voltage of up to 30 kV (Fernandez et al., 2009). Electronspun nanofibers of zein polymer have been made to encapsulate beta-carotene as a protection against light.

Amylose, beta-cyclodextrins, and pea starch can be used for encapsulation of hydrophobic food actives based on molecular inclusion (Conde-Petit et al., 2006; Boursier, 2010). A smaller guest (ingredient) molecule fits into and is surrounded by the host molecule. Examples of host molecules are amylose starch and cyclodextrins. Wacker Chemie (http://www.wacker.com/cms/en/products-markets/products/product.jsp?product = 12975) sells beta-cyclodextrin-based encapsulates including curcumin.

14.11 CONCLUSION

Nanoencapsulation-based systems are relatively few in the food industry due to cost as well as complexity. The nanoparticulate formulations for food and flavor ingredient encapsulation offer distinct benefits such as increase in solubility leading to higher bioavailability; improved shelf stability; controlled release of active ingredients; and others. Nanoencapsulation is well established in the beverage segment of the food industry especially with emulsions.

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Chapter 15

Aqueous Two-Phase Systems for Microencapsulation in Food Applications

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15.1 INTRODUCTION

Biopolymers are very common components in various food systems, and a large group of materials are suitable and regulatorily accepted for applications in foods. Biopolymers provide many of the functional properties that are desired in foods, such as thickening, gelation, film formation, and replacement of fat with maintained mouthfeel. By controlling the behavior of biopolymers and their mixtures and interactions, the food technologist can design the properties of a food product.

When biopolymers are mixed in water, the biopolymers can phase separate and an aqueous two-phase system (ATPS) is formed, where water is the solvent in both phases and the phases differ in their composition. Depending on the properties of the (bio)polymers, different kinds of phase-separated systems can be obtained (e.g., Tolstoguzov, 2000; de Kruif and Tuinier, 2001):

- *Co-solubility* occurs at low concentration for mixtures of polymers. The compatibility is due to mixing entropy and excluded volume effects. Co-solubility increases with decreasing excluded volume.
- *Associative phase separation (coacervation)* occurs when two oppositely charged polymers, e.g., a protein and a polysaccharide, are mixed and the polysaccharide can adsorb onto the protein. If the polysaccharide concentration is low, one polysaccharide molecule may bridge two protein molecules to create aggregates, a process called complex coacervation. These aggregates are surface active and tend to interact with and deposit on hydrophobic surfaces, such as emulsion droplets. This is used in a classic application of complex coacervation, namely, carbon-free copying paper, where ink is encapsulated in complex coacervates composed of gelatin and gum arabic.
- *Segregative phase separation (thermodynamic incompatibility)* is a process that is driven by the low entropy of mixing for two polymers in a common solvent, due to the large molecular size and the rigidity of the polymers. The excluded volume effect also promotes the phase separation process, by generating an attraction between like particles or polymers due to overlapping depletion zones that do not allow interpenetration by a different polymer (e.g., Tolstoguzov, 2000; de Kruif and Tuinier, 2001). This process is described by the Flory−Huggins theory. The thermodynamics of phase separating systems in foods were thoroughly reviewed by Tolstoguzov (2000, 2003) and de Kruif and Tuinier (2001). Phase separation can occur for pairs of charged (e.g., a protein and a polysaccharide), uncharged (e.g., dextran and modified cellulose), or one charged and one neutral polymer (e.g., protein and maltodextrin), depending on the pH and ionic strength. Phase separation is not complete; one phase in the ATPS will be enriched in one of the components while the other phase is enriched in the other component.

The phase separating system can be described in a phase diagram, see Figure 15.1. By convention, the polymer dominating the lower phase is on the *x*-axis and the polymer dominating the upper phase is on the *y*-axis. In the area above the phase separation line (the binodal), the system will phase separate to create two phases in equilibrium with the compositions that are found at either end of the tie line (B−C in Figure 15.1). The relative phase volumes can be calculated from the distance from the mixing point (A) to the points describing the two different phases (B and C) using

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00015-7

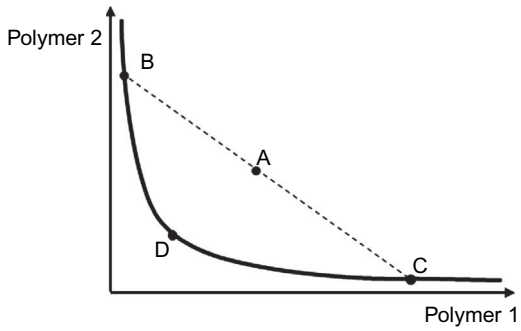

FIGURE 15.1 Schematic phase diagram for a phase separating system composed of polymers 1 and 2. The solid line is the binodal, and point A represents a mixture that phase separates into two phases of different composition, B and C. The dashed line BAC is called a tie line. Point D is the critical point.

the inverse lever rule. The locations of the binodal and the tie lines for a given pair of polymers are affected by the temperature, pH (for charged polymers), the ionic strength, and the molecular weight of the polymers.

An ATPS can also be formed by a salt and a polymer, usually sodium phosphate and polyethylene glycol (PEG), as first described by Albertsson (1960). This type of system has been used, for example, for purification of organelles in biotechnology applications.

The interfacial tension in an ATPS is very low, thus having a minimal tendency to denature proteins, and water is the solvent in both phases, hence providing very mild conditions. Due to low interfacial tension (between 0.0001 and 0.1 mN/m compared with 1−20 mN/m for conventional water−organic interfaces) between the polymer phases, adsorption of surface active materials (e.g., protein) at the liquid−liquid interfaces is less likely to occur (Forciniti et al., 1990). The low interfacial tension further implies that only a low level of energy is required to obtain and maintain an emulsion structure in the ATPS.

Low-molecular-weight solutes have a tendency to distribute evenly between the phases, while macromolecules, cells, cell organelles, etc. often partition preferentially to one of the two phases. The partitioning is directed by the surface properties of the partitioning material (Zaslavsky, 1995). Molecules are partitioned in an ATPS according to their size and surface properties. Thus, for relatively large molecules such as proteins, the partitioning is governed mainly by the surface properties, i.e., hydrophobicity and charge. The partitioning of a solute is described by the partitioning coefficient K:

$$K = \frac{C_{top}}{C_{bottom}}$$

where C_{top} is the concentration of the solute in the top phase and C_{bottom} is the concentration of the solute in the bottom phase.

ATPSs have been used for a variety of different applications, such as in analytical applications (e.g., Zaslavsky, 1995), bioseparation (e.g., Rosa et al., 2010; Asenjo and Andrews, 2011), extractive biotransformation processes (e.g., Banik et al., 2003), and structuring in foods (e.g., Wolf et al., 2000). In food applications, it is also of interest to encapsulate flavors, fragrances, nutraceuticals, and supply taste masking, and encapsulate bioactive peptides, proteins, and probiotics. All these different active ingredients have different properties and demand different encapsulation systems to give the desired performance. The encapsulation capacity is as important as the control of the release profile and release conditions. This chapter will describe the use of the ATPS for microencapsulation in food systems in different ways.

15.2 ENCAPSULATION IN FILMS, GELS, AND DISPERSED GEL PARTICLES

Microencapsulation in different films and gels has been studied by several authors. Films are used in, for example, food packaging, where there may be an interest in controlling the release of preservatives, etc.

Multiple emulsions have attracted interest as delivery systems and a means to reduce fat in foods without sacrificing the organoleptic properties. Water-in-oil-in-water (W/O/W) emulsions have been explored for this purpose, and the difficulty of stabilizing the water-in-oil (W/O) emulsion is a bottleneck in the development of these systems in food applications. When a biopolymer-stabilized emulsion is mixed with a phase separating mixture of biopolymers, an oil-in-water-in-water (O/W/W) multiple emulsion can be formed due to the thermodynamics of the system. Kim et al. (2006)

studied a system composed of whey protein-stabilized vegetable oil emulsion that was mixed with heat-denatured whey protein and pectin at such concentration that a phase separating system was created. The phase diagram is quite skewed, so that the pectin concentration is substantially lower that the protein concentration to achieve phase separation. The heat-denatured whey protein isolate (HD-WPI) is present as protein aggregates, i.e., it appears as larger molecules than in the native whey protein, and hence phase separation occurs at lower protein concentration. In order to prepare O/W/W emulsions of vegetable oil, an ATPS of HD-WPI and pectin was prepared, the phases separated, and then mixed back with an WPI-stabilized vegetable oil emulsion. The resulting O/W/W emulsions were structured so that the emulsion resided in the protein-rich phase, which could be dispersed in the pectin-rich phase. The system tends to macroscopic phase separation over a period of 24 h, and thus the authors pointed out the need for a method to stabilize the HD-WPI droplets with the included oil phase in order to find applications for this type of system.

In a later study from the same group, Matalanis et al. (2012) refined this system using pectin and caseinate to encapsulate fish oil with the purpose to improve the oxidation stability of the emulsified fish oil. The microcapsules were produced by first preparing an ATPS of caseinate and pectin and separating the two phases. The pectin-rich phase (90% by volume) was then mixed with the caseinate-rich phase (5%) and fish oil emulsion stabilized by caseinate (5%), so that the final oil concentration was 1%. By stirring and reducing the pH from 7 to 5, pectin was adsorbed onto casein and hydrogel particles were formed, in effect an O/W/W emulsion. The particles were then stabilized by adding transglutaminase to crosslink casein covalently, and the final size of the microcapsules was around 8 μm in diameter. The oxidation stability was substantially enhanced compared to fish oil in conventional emulsions stabilized by Tween, and had similar stability to a caseinate-stabilized emulsion. The authors claim that the main stabilizing effect arises from the antioxidative properties of food proteins. There may, however, be benefits of pectin in the system for the purpose of controlling release in the mouth, stomach, or intestine, although this was not explored in this paper. An issue in practical applications may be the low oil content in the final system (1% oil). The same group has refined the production method and reduced the number of processing steps. Matalanis and McClements (2013) investigated effects of the order of mixing and increased the oil content in the microparticles. It was found that the emulsion should be prepared prior to making up the ATPS in order to encapsulate the oil efficiently, and that the oil content could be increased substantially. Interestingly, it was possible to density match the hydrogel particles to water, so that sedimentation of the hydrogel particles was very slow.

15.3 ENCAPSULATION IN PARTICULATE SYSTEMS

15.3.1 Spray-Dried Particles

In the introduction, it was mentioned that a W/W emulsion is easily formed in an ATPS due to the low interfacial tension. It can be expected that the phase separation occurring in the ATPS in the liquid state is preserved during rapid drying, such as spray drying, by kinetically arresting the phase separation that ultimately tends to macroscopic phase separation. In spray drying, it may be anticipated that encapsulation of polymer droplets containing the active ingredient will reduce the level of interaction with the air−liquid interface and the contact with the external particle surface in the dried powder. For application of the ATPS in spray drying, some constraints exist: (1) the polymers must form an ATPS at reasonably low polymer concentrations so that the viscosity of the ATPS can be kept sufficiently low to allow spray drying, (2) polymers that exhibit extensional viscosity may cause practical difficulties with respect to powder production and powder structure, (3) the glass transition temperature (T_g) of the polymers must not be too low, and (4) the active ingredient should preferentially partition to one of the phases.

15.3.1.1 Spray-Dried ATPS for Enzyme Encapsulation

The purpose of using an ATPS as carrier in spray drying is to achieve encapsulation of the protein in the powder for controlled release and protect it from interaction with the spray-droplet surface during the drying process, which may cause structural damage to the protein, and thus activity loss in the case of bioactive proteins. It has been shown that proteins accumulate at the powder surface, and this can cause loss of activity (Millqvist-Fureby et al., 1999). The surface denaturation of the protein can be reduced by adding surfactants (Millqvist-Fureby et al., 1999) or surface active polymers (Elversson and Millqvist-Fureby, 2006). However, these techniques do not allow for control of the release of the protein, but this may be accomplished using ATPS formulations. A prerequisite for successful encapsulation of the protein in the dispersed phase of the ATPS is that the protein partitions strongly to this phase. The polymer pair must be chosen to suit the protein that is to be encapsulated in terms of protein stability and partitioning behavior. The partitioning can be improved in several ways (pH changes, salt additions, covalent modifications of the target protein,

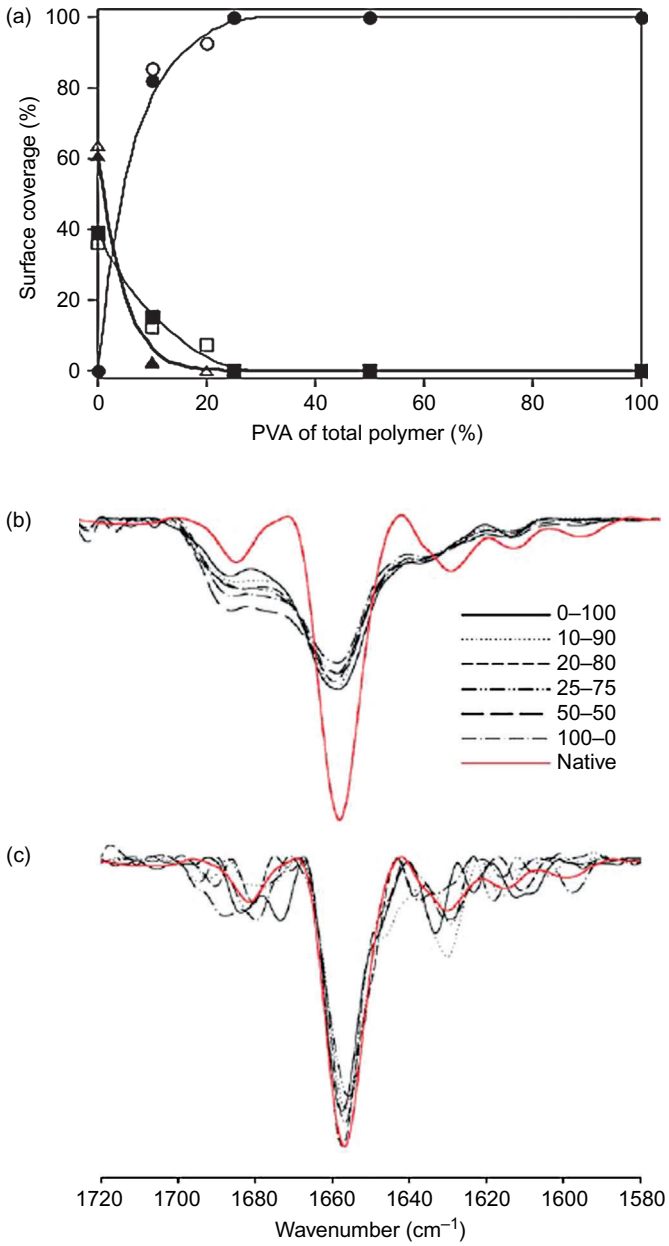

FIGURE 15.2 (a) Surface composition and (b) secondary structure of BSA in spray-dried PVA−dextran ATPS, and (c) secondary structure of BSA in reconstituted ATPS.

polymer choice, etc.), but the efficiency of these additives/changes in the phase system varies (e.g., Johansson et al., 1996; Delgado et al., 1997). When choosing the phase-forming polymers, attention must also be paid to the suitability of the polymers for spray drying.

As model protein for microencapsulation in spray-dried ATPSs, bovine serum albumin (BSA) was used in a polyvinyl alcohol (PVA)−dextran system, where the protein preferentially partitions to the dextran-rich phase (Elversson and Millqvist-Fureby, 2005). If the protein concentration is too high (above a few weight percent), the ATPS is altered since protein is also a macromolecule in the system, and the partitioning of BSA becomes less favorable for the dextran-rich phase. This is due to BSA being a charged polymer, which then favors more equal distribution between the two phases. This effect could possibly have been reduced by increasing the ionic strength of the system. When the PVA−dextran system with BSA was spray dried and the particles were analyzed for surface composition, it was found that the surface tended to be dominated by PVA, and also when the dextran-rich phase was the continuous phase (Figure 15.2a). This is

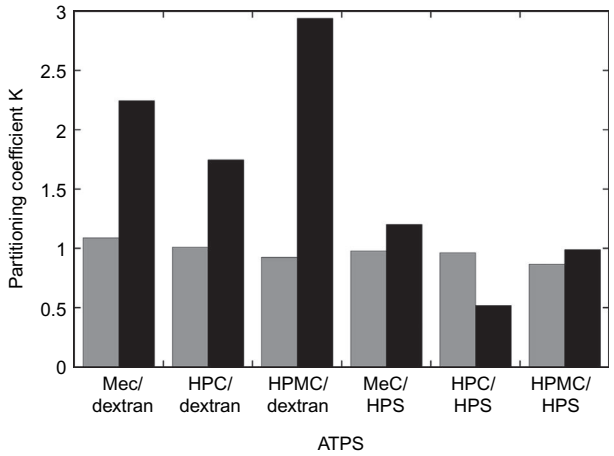

FIGURE 15.3 Partitioning of lysozyme and trypsin in ATPS with different polymers. All systems contain 100 mM $NaClO_4$. The systems consisted of 3% of each polymer, and 5% of protein in solids (i.e., 0.3% in solution). Light gray bars: trypsin; black bars: lysozyme.

due to the presence of surface active PVA in the dextran-rich phase, and already at low concentrations PVA is adsorbed at the air−water interface of the drying droplets. The protein coverage of the powder surface is very low, and surface-induced denaturation is avoided. The protein secondary structure was analyzed using Fourier transform infrared spectroscopy and the second derivative of the spectrum (Figure 15.2b). The dried samples had clearly partly lost the α-helix structure, while it was recovered after reconstitution (Figure 15.2c).

Lysozyme is a much smaller protein than BSA, is compact, and has a higher isoelectric point, pI = 11.3, compared to BSA, pI = 4.7, and therefore lysozyme is an interesting candidate for comparison to BSA. In addition, lysozyme is an enzyme and the preservation of its structure after drying and reconstitution can be measured as the retention of its initial enzymatic activity. The partitioning of lysozyme in PVA−dextran was poor, and therefore three hydrophobically modified celluloses (hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), and methyl cellulose (MeC)) and a modified starch (hydroxypropyl starch, HPS) were used in addition to dextran. The modified celluloses may be considered more hydrophobic than PVA, and may thus influence the partitioning of lysozyme. The surface charge of lysozyme can be altered by a shift in pH as well as affected by addition of salt, especially chaotropic salts. Furthermore, hydrophobic patches at the protein interface may interact with hydrophobic ions such as triethylammonium phosphate (TEAF) such that a positive charge is "added" to the protein, which then could be experienced as being more hydrophilic. It is known that hydrophobic anions (e.g., ClO_4^-) have a tendency to move a positively charged protein to the more hydrophobic phase (Lu et al., 1995; Johansson et al., 1996), while hydrophilic anions, such as TEAF, act in the opposite way.

Sodium perchlorate was explored for its ability to enhance the partition of lysozyme in six different polymer systems as shown in Figure 15.3. For lysozyme, there are two systems that exhibit a fairly good partitioning of the protein, HPMC/dextran and HPC/HPS.

Two ATPS were chosen as the matrix for spray drying of lysozyme, HPMC/dextran, and HPC/HPS. For comparison, lysozyme was spray dried in lactose at the same solids content. The residual activity after spray drying in the different matrices was high for all powders, including the one with lactose (Table 15.1). There may also be a connection to the low surface coverage of protein in the ATPS-based powders (Table 15.1). In this sense, there is a dramatic difference between the powder based on ATPS, which has a protein surface coverage of 5%, and the lactose-based powder with about 52% protein on the surface. Calculations indicate that the surface is dominated by the most surface active (and hydrophobic) polymer, irrespective of the continuous phase, as in the case of PVA, polyvinylpyrrolidone (PVP), and HPMC in other applications (Millqvist-Fureby et al., 2000; Elversson and Millqvist-Fureby, 2005, 2006).

15.3.1.2 Encapsulation of Probiotics in ATPS

It is well known that both cells and organelles can partition strongly in an ATPS (e.g., Albertsson 1960; Hatti-Kaul, 2001), and the possibility was investigated to utilize the partitioning of cells in spray drying of bacteria with the objective of increasing the stability of the cells. When dry forms of bacteria are created, freeze drying is the most commonly used technique, although spray drying is used in some cases. Probiotic bacteria and strains used in the dairy industry

TABLE 15.1 The Residual Activity of Lysozyme after Spray Drying in Different ATPS or Lactose as Compared to Untreated Lysozyme. Polymers were used in 50/50 Ratio

Matrix	Residual Activity (%)	Protein Coverage (%)
HPMC/dextran	97	5
HPC/HPS	89	5
Lactose	82	52

are of interest to supply in dried form, if the shelf-life can be increased, and if the drying process is more economical than freeze drying. Availability of dried bacteria also enables the creation of new products such as probiotic capsules and tablets, follow-on formula with added probiotics, as well as foods and snacks with added probiotic bacteria in dry or semidry products with long shelf-life. Microencapsulation of probiotics is covered in Chapter 36, and here only microencapsulation of probiotics in spray-dried ATPS is reviewed.

15.3.1.3 PVP–Dextran Conceptual Study

The concept of an ATPS for stabilizing bacteria in spray drying was first investigated using an established ATPS that, however, was not made up of food-grade polymers. The system consisted of PVP and dextran, which form an ATPS at low polymer concentrations if the molecular weight of the polymers is large (Millqvist-Fureby et al., 1999). The bacterium studied was *Enterococcus faecium* M74, a lactococcus with probiotic properties. The bacteria were found partitioned completely to the dextran-rich phase; it would then be anticipated that the bacteria can achieve a double encapsulation after spray drying, provided that the dextran-rich phase is dispersed in the PVP-rich phase. *E. faecium* was formulated in a series of PVP–dextran ratios and spray dried, and the survival rate was determined immediately after drying and after storage at room temperature under dry conditions, see Figure 15.4. The survival rate was highest at 40–60 PVP–dextran ratio (w/w), indicating that the best conditions were close to the phase inversion region (around 40–45% PVP in the system at 8% total polymer content).

15.3.1.4 Polysaccharide-Based Systems

In order to improve the system for applications, the non-food system used with *E. faecium* was replaced by protein–polysaccharide systems, and two different lactobacilli strains (*E. faecium* and *Lactobacillus plantarum*) were used (Elofsson and Millqvist-Fureby, 2001). At first, different neutral biopolymers were tested for their suitability as encapsulation systems, and methyl cellulose–dextran was chosen based on the partitioning of the bacteria. The cells partitioned to the dextran-rich phase in this system. Both strains were spray dried with different ratios of methyl cellulose–dextran, and it was found that the viability and stability was influenced by the composition. In the case of *E. faecium*, it appears that a methyl cellulose continuous system favored storage stability (Figure 15.5), while *L. plantarum* exhibited highest stability when dextran was the continuous phase, but still with some methyl cellulose present coating the particles. Surface chemical analysis of the powders showed that methyl cellulose was the dominating polymer at the powder surface already when the content of this polymer was at least 25%. This indicates that an additional coating can be positive for the survival rate of the bacteria. The lack of improvement of the cell stability for *L. plantarum* in the double encapsulated system may be due to a very high cell density in the dextran-rich phase.

Disaccharides have been found to be particularly advantageous for stabilization of liposomes (Suzuki et al., 1996) and proteins (Prestrelski et al., 1993; Mazzobre et al., 1997) during freeze drying. Similar results have also been found for freeze drying of bacteria (Leslie et al., 1995). This effect has been attributed to the disaccharides acting as "water replacers" in hydrogen bonds with the liposomes or proteins in the dried state (Carpenter and Crowe, 1989). Small molecules can come in closer contact with the material to be protected than can the polymer components, and thus are more efficient in this sense. Here, three different disaccharides, lactose, sucrose, and trehalose, as well as sodium caseinate, were used as additives in the MeC–dextran system. Figure 15.6 shows that the addition of disaccharides further improved the survival rate for both bacteria. There was no notable difference in the effect on survival during the drying step dependent on which disaccharide, sucrose, lactose, or trehalose was used. However, addition of trehalose gave significantly better storage properties of *L. plantarum* dried in MeC–dextran. Sodium caseinate, on the other

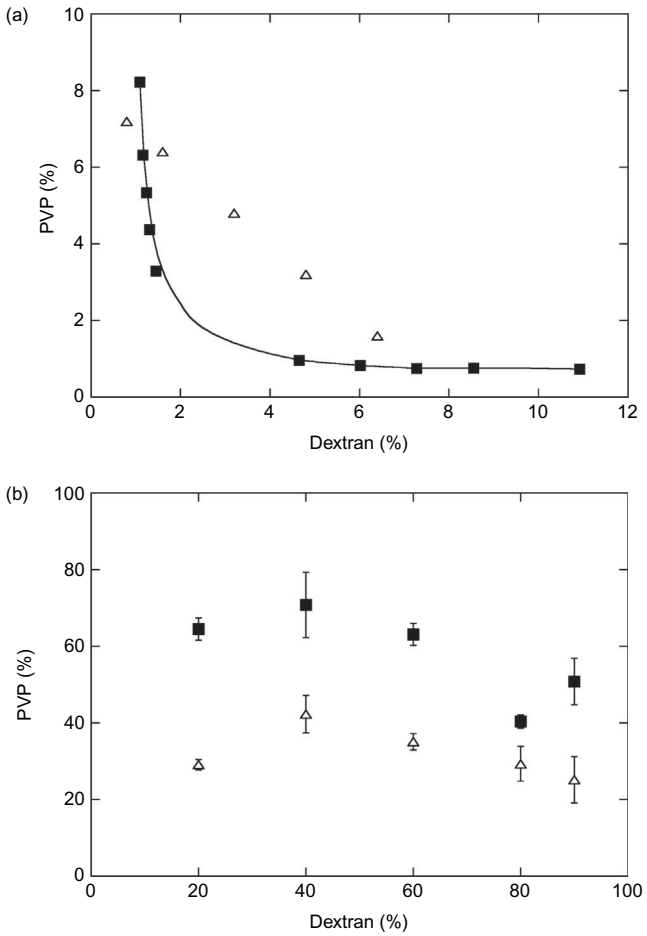

FIGURE 15.4 (a) Location of the points investigated in the phase diagram. (b) Survival rate of *E. faecium* after spray drying (△) and after storage at room temperature for 4 weeks (■), both compared to viability before drying.

hand, was less efficient than the disaccharides in stabilizing the cells during drying. According to the reasoning above, sodium caseinate consisting of relatively large polypeptides (19−23 kDa) would not have the ability to replace water in the same manner as the disaccharides may do, while all the disaccharides improved the stability of the bacteria.

15.3.1.5 Protein−Polysaccharide Systems

Pectin forms an aqueous two-phase system when mixed with reconstituted skim milk powder at neutral pH, due to segregative phase separation caused by the interaction between different polymers being more repulsive than the attractive interaction between like polymers (Syrbe et al., 1998). A phase diagram for milk protein and pectin is illustrated in Figure 15.7 (Tolstoguzov, 1986). As can be seen from the phase diagram, only a small amount of pectin is needed to provide a two-phase system. At lower pH but still above the pI of the caseins, phase separation may still occur, but the mechanism is different: pectin adsorbs to the surface of the casein micelles, causing bridging flocculation (Syrbe et al., 1998). If the pH is still lower (between the pI of the caseins and the pI of pectin), complex coacervation occurs. The polymer complexes will form a dense phase in equilibrium with a larger phase with low polymer content. Here we used pH 7, and thus segregative phase separation occurred.

In this system, *E. faecium* and *L. plantarum* partition to the concentrated protein-rich phase. Two concentrations of pectin were used, 2% and 3%, whereas the skim milk powder (SMP) concentration was 7.5% as dry substance (corresponding to 2.7% milk protein, the remainder being mainly lactose). This results in a pectin-rich phase with $\approx$2% (3%) pectin and a protein-rich phase with $\approx$28% (33%) milk protein, according to the phase diagram in Figure 15.7. The solutions also contained 0.9% NaCl and approximately 0.8% *E. faecium* or 0.1% *L. plantarum*. Compared to the dextran-containing systems previously discussed, the survival rate is increased significantly in this system for both

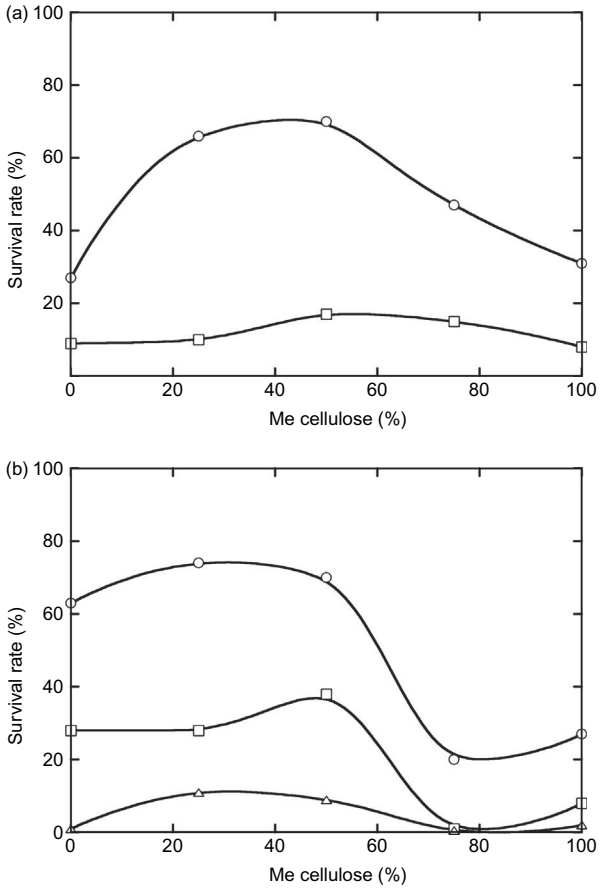

FIGURE 15.5 Survival rate of (a) *E. faecium* and (b) *L. plantarum* in MeC−dextran after spray drying. (○) Fresh powder, (□) powder stored 5 weeks at 4°C, and (△) powder stored 5 weeks at room temperature.

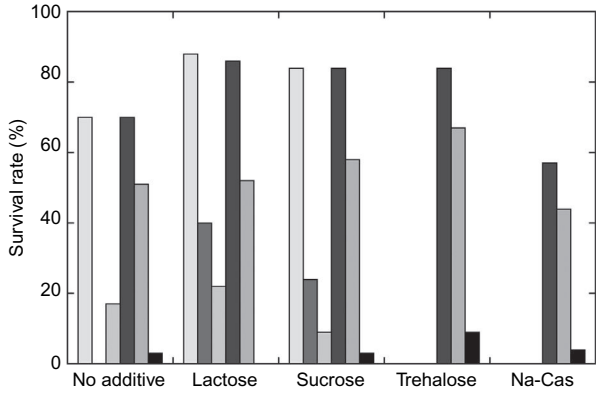

FIGURE 15.6 The effect of additives on the survival rate of *E. faecium* and *L. plantarum* in spray-dried powders.

bacteria (Figure 15.8). In particular, the viability after storage is exceptionally high, both at 4°C and at room temperature. The two-phase system did not bring any benefits compared to the simple SMP system.

The dissolution time of the SMP−pectin systems were, as expected, dependent on the pH of the fluid (Table 15.2). Similarly, the dissolution of a control system without pectin was slow at low pH, due to the aggregation of the milk proteins at this pH. This shows that the presence of milk protein is sufficient to provide some gastric protection of the probiotic bacteria.

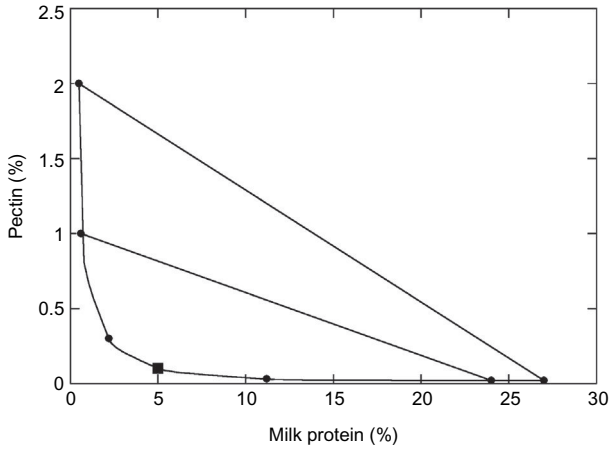

FIGURE 15.7 Phase diagram for milk protein and pectin at pH 6.5 and 20°C. Data redrawn from Tolstoguzov (1986). (■) Critical point, (●) compositions of equilibrium phases, connected by tie-lines.

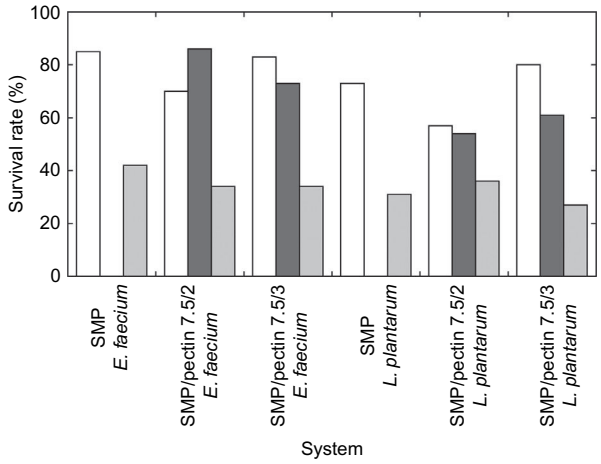

FIGURE 15.8 The survival rate of *E. faecium* and *L. plantarum* in an SMP−pectin two-phase system. White bars: freshly dried powder; dark gray bars: after storage at 4°C for 5 weeks; and gray bars: after storage at room temperature for 5 weeks.

TABLE 15.2 Dissolution Times for MP/Pectin Powders in Different Fluids

Dissolution Medium	*E. faecium*			*L. plantarum*		
	SMP/pectin (min)	WPC/pectin (min)	SMP (min)	SMP/pectin (min)	WPC/pectin (min)	SMP (min)
GI fluid*	>330	40	>300	>420	102	>300
0.1 mM HCl	22	21		36, 14, 30	16, 24	
Milli-Q	21	27	12, 25	38, 36	22, 36	13

**2 g NaCl and 7 ml 37% HCl in 1000 ml, pH 1.1.*

15.3.2 Core−Shell Particles

Core−shell microcapsules are a well-known concept for microencapsulation of oils in a hydrocolloid shell (e.g., gelatin). The technique is also well developed in the pharmaceutical field. There is equipment available for the preparation of such microcapsules, and microencapsulated products are available on the market.

The particles can be formed using different technologies, such as concentric nozzles, where the shell material encloses the core material in a concentric flow and where droplets are formed typically by vibrations of the nozzle, induced by, for example, an ultrasonic field. During the fall from the nozzle into a receiving vessel containing a hardening bath, the liquid segment will form a core–shell structure spontaneously. Depending on the material, different types of hardening baths or gelling baths can be used; in the most common case, alginate is gelled with calcium ions or other divalent ions. For research purposes, microfluidic preparation can also be employed, where concentric tubes allow for controlled formation of core–shell particles (e.g., Liu et al., 2013). The receiving fluid can be a gelation fluid as for concentric nozzles or an antisolvent such as an oil. Both techniques mentioned here result in particles with narrow size distribution. It is also possible to create core–shell microparticles using a self-assembly approach based on ATPS. This technology is still in the research phase and is being explored by several groups.

Ziemecka et al. (2011) have developed a research system based on microfluidics for preparation of water-in-water-in-water droplets of PEG–dextran–PEG phases. The device contains three different mixing junctions, where first dextran and PEG solutions are mixed, followed by sheathing in a dextran solution, and finally ejection into a PEG solution. This system is thus completely free of surfactants, oils, and organic solvents. The authors' intentions are to use this system for studies of biological systems such as cells. However, the stability of the core–shell capsules is very low; within 20 minutes, the PEG core has merged with the continuous PEG phase. In order to use this technology for the manufacture of core–shell capsules for controlled release, it is expected that the coating layer will need to be gelled or crosslinked to create a stable shell.

A different approach to formation of core–shell microcapsules based on ATPS was taken by Wassén et al. (Fransson et al., 2009; Wassen et al., 2012, 2013). Their systems are based on a premixed polymer solution composed of two polymers in water and where the polymers will be phase separated. Further, at least one of the polymers has the ability to gel under different conditions. Both gelatin–maltodextrin (temperature-induced gelation) and gellan gum–whey protein isolate (pH-induced gelation of preheated WPI) were used in the studies (Fransson et al., 2009; Wassén et al., 2013). The microcapsules were created using different dispersion technologies: emulsification by mixing the premixed polymer solution with a vegetable oil containing polyglycerol ricinoleate (PGPR), and microfluidic droplet generation with the same oil/PGPR combination as the receiving fluid. The droplet size depended on the manufacturing technique, so that emulsification resulted in droplets in the range 4–300 μm (Fransson et al., 2009), while microfluidic manufacturing produced monodispersed droplets in the size range 115–160 μm (Wassén et al., 2012). The phase separation inside the aqueous droplets was followed in a confocal laser scanning microscope (CLSM), and a dye was added to color one of the phases. It was observed that the phase separation process was affected by the size of the droplets, so that in larger droplets (>20 μm) one of the phases coated the aqueous droplet while smaller droplets of this phase were dispersed in the other aqueous phase. In small droplets (<20 μm), phase separation leading to a core–shell structure was formed, but other phase-separated structures, such as hemispheres of each polymer phase (janus-like particles), were also observed. In most particles (except the janus particles), one of the phases is coating the droplet, regardless of droplet size and internal structure, and this is the phase that preferentially wets the interphase between the aqueous phase and the oil phase. In the case of gelatin–maltodextrin, maltodextrin coats the particles, and in the case of WPI–gellan gum, the droplets are coated by WPI.

The effect of quenching of the phase-separated structure by gelation of the polymer was also investigated as a means to control the microcapsule morphology. The holding time at a temperature above the gelation temperature strongly affected the internal structure in the droplets, so that immediate cooling to 20°C did not allow for as extensive a phase separation as a longer holding time (5–20 minutes) before cooling to 20°C (Fransson et al., 2009). It was shown for the larger core–shell particles produced in a microfluidic device that the polymer type and cooling profile together determined the internal phase-separated structure in the microcapsules. In this size range, no purely core–shell structure particles were found; instead different internal structures were observed (large phase-separated regions, bicontinuous structures, small phase-separated regions) (Wassén et al., 2012).

15.3.3 Microspheres Produced in ATPS

The ATPS can easily form water-in-water (W/W) emulsions, and if one of the phases is coagulated in some way, microcapsules can in principle be formed. This approach has been used to make microcapsules for different purposes within the field of controlled release. The essential step in this approach is the crosslinking or solidification of the dispersed phase, and the subsequent washing steps and potential drying step. The integrity of the microspheres may be difficult to maintain during these processes. Chemical crosslinking has been a predominant approach of solidification (Yu and Liu, 1994; Dziechciarek et al., 2002; Li et al., 2009), but non-covalent approaches relying on recrystallization

(Reslow et al., 2002) have also been used. In most cases, microparticles are formed by an emulsification method using a W/O emulsion, where the oil may be hexane, toluene, vegetable oil, or other organic solvents. The particles are recovered once the solidification is completed, and the solvent is usually removed by a washing procedure. The use of organic solvents makes these processes less attractive for food application, although such processes are used for manufacture of pharmaceutical products.

A similar approach was used by Anand and Ambarish (2011), where ATPS was formed from octenyl succinic acid starch (OSA-starch) and PEG. The OSA-starch was crosslinked using trisodium trimetaphosphate (TSTP) as the crosslinker. The OSA-starch solution was mixed with TSTP and NaOH to obtain sufficiently high pH. The solution was added dropwise to the PEG solution, and particles were obtained after incubation for 8 hours at 30°C; absolute ethanol was added and the particles were collected. After washing with deionized water and absolute ethanol, the particles were dried under vacuum. The particle size was in the range 10−40 μm, and the particles were smooth and spherical. No loading or release experiments have been presented for these microparticles.

A very similar methodology was used by Li et al. (2012), when their technique to prepare starch microspheres crosslinked using TSTP in a W/O system (Li et al., 2009) was converted to a W/W system where starch and PEG are the phase-forming polymers (Li et al., 2012). Particles produced in the two systems were compared and found to be similar, albeit with some differences in yield and crosslinking degree. The crosslinking degree was related to the amount of TSTP as well as to the incubation time. The preparation process for starch microspheres in the W/W system involved preparation of a stock solution of soluble starch (16%), containing 2% NaOH and 6% TSTP, and a stock solution of PEG (28−44%, molecular weight 10,000 or 20,000). The stock solutions were mixed in ratios 4:7−4:11 at 30°C, and the mixture was stirred for 6 minutes and then incubated without stirring for at least 30 minutes. The ATPS was diluted by adding an equal volume of deionized water, and the particles were collected by centrifugation and then washed with distilled water and absolute ethanol prior to vacuum drying. The particles thus obtained were in the approximate size range 2−15 μm, highly spherical, and with a smooth surface. The particles are claimed to be non-porous, based on the external appearance in scanning electron microscopy. No loading or release experiments have been presented for these microparticles.

Reslow et al. (2002) described the preparation and properties of starch microspheres in an ATPS composed of starch and PEG and encapsulating human growth hormone, where the starch was crosslinked by means of non-covalent interactions due to recrystallization of starch. In order to provide sustained release of the peptide, the microspheres were coated by poly(DL-lactide-co-glycolide) (PLG). This microsphere system (Biosphere™) was developed for delivery of peptides and proteins. However, the concept of starch microspheres for controlled delivery could also be extended to food systems. The production conditions and their effects on the quality and properties of the microspheres have been studied in detail by Elfstrand et al. (2007a,b). Later, the system was developed further and was used without the PLG coating for release of BSA and insulin (Elfstrand et al., 2009). The general procedure of preparing these starch microspheres involves preparation of a concentrated starch solution (30% starch by weight) using an acid-hydrolyzed and mechanically treated starch quality, and preparation of a PEG stock solution (38% by weight). PEG solution is added to the starch solution, and the mixture is stirred slowly, and then followed by an incubation program with different incubation times at 6 and 37°C. Finally, the starch microspheres are collected by centrifugation, washed with buffer and again collected by centrifugation, and finally freeze dried. The typical particle size is 10−100 μm. The microspheres obtained are porous but swell in water. Some of the crystallinity and order in the starch microspheres is lost during freeze drying. The release of insulin and BSA in buffer was studied by Elfstrand et al. (2009), and the time to release 75% of the load was about 1 hour. The presence of amylase increased the release rate for BSA but had no effect on the release rate of insulin, indicating that the starch microspheres have large pores that do not hinder the diffusion of insulin. BSA may have been entrapped to some extent, explaining the higher released fraction in the presence of amylase. The encapsulation efficiency was low for insulin (up to 49%) and higher for BSA. The lower encapsulation efficiency for insulin may be due to losses during the manufacturing process. This indicates that starch microspheres may be less suitable for controlled release of smaller molecules, unless a coating is applied.

An alternative to starch particles is presented by crosslinked gelatin particles produced in ATPS, as described by Plichta et al. (1994) and Kong et al. (2011). The procedures are similar in these papers, although Plichta et al. use formaldehyde to crosslink gelatin, while Kong et al. use glutaraldehyde. Gelatin solution was mixed with PVP or PEG solution to form an ATPS at 60°C. An emulsion was formed by vigorous stirring and the gelatin droplets were gelled by rapid cooling to 5°C. After washing in chilled water and collecting the particles by centrifugation at low temperature, they were dehydrated in isopropyl alcohol, followed by vacuum drying (noncrosslinked particles) or crosslinked by glutaraldehyde prior to drying. In both cases, spherical particles were obtained in the size range 10−40 μm. No data were shown for loading or release from these particles.

15.4 CONCLUSION

Aqueous two-phase systems are interesting from a food science perspective, because phase-separated systems can be obtained using only water as the solvent and various food-grade polymers. The properties of the ATPS can be used to aid in the regulation of the rheology of a food material, and also to provide controlled release properties. Several methods for obtaining microencapsulation using ATPS have been described in this chapter, but so far none of these has reached the market in full-scale applications. An issue may be that biopolymer hydrogels tend to be permeable to small molecules, making encapsulation of flavors and nutrients difficult. However, macromolecules such as enzymes, and indeed probiotics, can be microencapsulated in biopolymer hydrogels to provide controlled release properties. In addition, coating of the microcapsules may further improve the possibilities of controlling and directing the release of the encapsulated material.

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Part IV

Materials Used in Microencapsulation

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Chapter 16

Selection of Materials for Microencapsulation

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16.1 INTRODUCTION

Formulation of food ingredients such as flavors, aromas, vitamins, minerals, spices, carotenoids, essential oils such as omega-3 and -6 oils, salts, phytochemicals, nutritional supplements, functional enzymes, and coenzymes are microencapsulated for various reasons. One key reason is to protect the sensitive active ingredient against oxygen, pressure, heat, light, and water. Furthermore, protection against oxygen is critical to prevent rancidity or off-tastes that may develop during oxidation. In different instances, the physical state of the food ingredient needs to be protected. By physical state one means that the stability of the colloidal or emulsion, or insoluble active, particle dispersion in an aqueous media needs to be protected. There are also occasions where actives need to be protected by extreme physical changes in heat, pressure, or pH during the processing of the product.

This chapter focuses on the selection of the matrix material for microencapsulation. For example, using shelf stability as a criterion, materials are classified by the governing physical property to provide protection against oxygen, pressure, heat, pH, light, heavy metals, and water. The governing properties in turn can be effectively used to impact taste masking, aroma masking, color masking, color alteration, dissolution, controlled or triggered release, delayed release, product stability, component incompatibility, and enhanced processing.

To achieve the design goals, a combination of materials is used to create a suitable balance of the properties. Figure 16.1 demonstrates the interconnected relationship between the different properties that must be balanced for a successful product development program.

The development of a suitable recipe for the microencapsulated active agent requires several factors including formulation design, material selection, and process selection. In this chapter, we will integrate the formulation design with material selection for the different microencapsulation processes. The summary of the different processes is described in Chapter 4, while Part 3—Chapters 4 through 15—detail the different processes.

16.2 MORPHOLOGICAL DESIGN

The microcapsule morphology is the first step to assess whether a specific application is suitable for a defined structure by virtue of its design attributes. In Figure 16.2, different morphologies are shown.

One must first consider the morphological design since the structure in which the active ingredient is configured into a microcapsule or microsphere can significantly impact stability and release of the active ingredient. Table 16.1 summarizes the comparison of the different morphologies in terms of ability to protect against oxygen, pressure, heat, light, and water.

16.3 MATERIAL SELECTION

Although any food-grade coating material can be conceptually used as a candidate for the microcapsule shell material, most commercial microcapsules produced to date utilize a relatively small number of different shell materials. This is

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00016-9

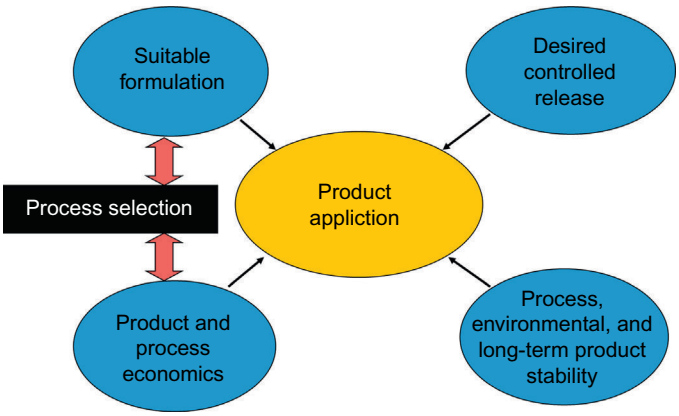

FIGURE 16.1 Rational design map for microencapsulation-based product development.

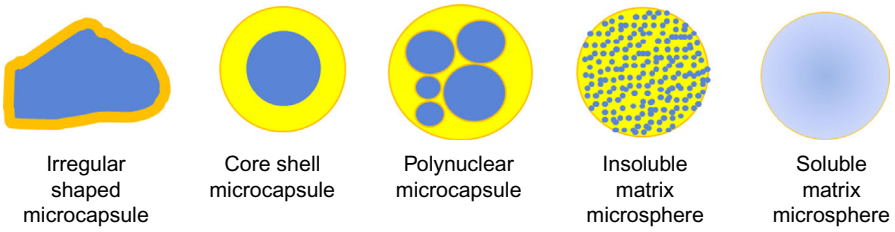

FIGURE 16.2 Microencapsulation morphologies.

TABLE 16.1 Matrix Material-Dependent Impact of Morphology to Provide Enhanced Stability

Protection Against	Irregular-Shaped Microcapsule	Core–Shell Microcapsule	Multi-Nuclear Microcapsules	Insoluble Matrix Microsphere	Insoluble Matrix Microsphere
Oxygen	Fair	Good	Excellent	Fair	Poor
Water	Fair	Good	Excellent	Fair	Poor
Pressure	Excellent	Poor	Fair	Good	Excellent
Heat	Good	Fair	Good	Poor	Poor
Light	Excellent	Good	Good	Fair	Poor

because the shell materials for food products are limited to materials that are approved by the US Food and Drug Administration (FDA). The database is maintained by the Center for Food Safety and Applied Nutrition (CFSAN) in the FDA under *Everything Added in Foods in the United States* (EAFUS, January 1998). The EAFUS list of substances contains ingredients added directly to food that the FDA has either approved as food additives or listed or affirmed as generally recognized as safe (GRAS).

The more than 3000 total substances together comprise an inventory in EAFUS. This list can be found at http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt = eafuslisting. The database is continuously updated under an ongoing program known as the Priority-based Assessment of Food Additives (PAFA). PAFA contains administrative, chemical, and toxicological information on over 2000 substances directly added to food, including substances regulated by the FDA as direct, "secondary" direct, color additives, and GRAS.

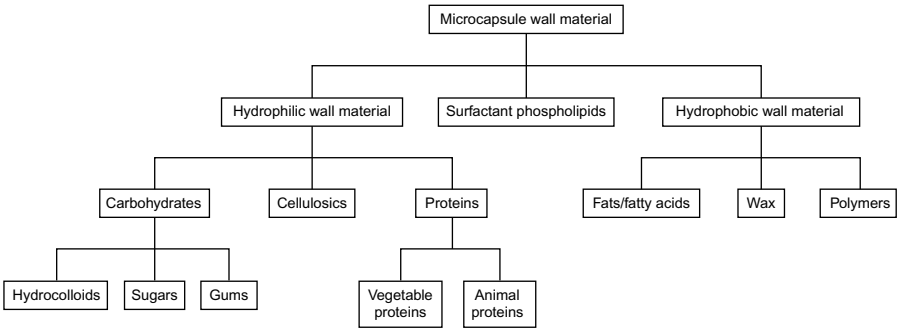

FIGURE 16.3 Microencapsulation material genealogy based on barrier properties.

TABLE 16.2 Representative Examples of Matrices with Typical Applications

Shell Materials Used to Produce Commercially Significant Microcapsules

Shell Material	Regulatory Status	Chemical Class	Encapsulation Process	Applications
Gum arabic	Edible	Polysaccharide	Spray drying	Food flavors
Gelatin	Edible	Protein	Spray drying	Vitamins
Gelatin–gumarabic[a]	Nonedible[b]	Protein–polysaccharide complex	Complex coacervation	Carbonless paper
Ethylcellulose	Edible	Cellulose either	Wurster process or polymer–polymer incompatibility	Oral pharmaceuticals
Polyurea or polyamide	Nonedible	Cross-linked polymer	Interfacial polymerization *in situ* polymerization	Agrochemicals and carbonless paper
Aminoplasts	Nonedible	Cross-linked polymer		Carbonless paper, fragrances, and adhesives
Maltodextrins	Edible	Low molecular weight carbohydrate	Spray drying and desolvation	Food flavors
Hydrogenated vegetable oils	Edible	Glycerides	Fluidized bed	Assorted food ingredients

[a]*Treated with glutaraldehyde.*
[b]*For intended application, i.e., carbonless paper.*

The choice of material suitable for the microencapsulation of the food ingredient is dependent on the physical and chemical properties of the active agent as described in Chapter 2. However, under most circumstances, it is essential that the selection of the materials must show three important characteristics:

- Excellent barrier properties
- Excellent film-forming properties, that is, excellent wettability
- Phase transition point at which the matrix undergoes phase transformation.

A schematic approach to material selection in microencapsulation formulation can be defined based on the three above properties. Based on barrier properties, matrix materials used in microcapsulation can be grouped by hydrophilic, surfactant phospholipid, and hydrophobic class of wall matrix materials, as shown in Figure 16.3.

To construct an optimal shell material for a given application can be complex, especially since many interacting parameters determine the success of a given capsule shell material. Microencapsulation of food ingredients such as flavors, vitamins, minerals, essential oils, carotenes, salts, or enzymes requires stabilization in the environment in which they are placed. Furthermore, some of the material class is linked to the process selection and cannot be used in other processes. Table 16.2 lists representative examples of these materials along with typical applications.

Considering the number and variety of competing factors, the selection of materials can be a daunting task. The best way to approach this challenge is by asking ourselves a sequence of questions that will narrow our search for the most suitable material. Table 16.3 describes the fact list that defines the targeted product profile, shelf-life, barrier properties required, and processing challenges that need to be overcome.

Multiple factors must be considered to help narrow the list of matrix polymers, including core material, particle morphology, particle size, size distribution, production quantities, budget, and process availability. In many cases, the original microencapsulation development objectives and expectations may need to be adjusted to accommodate a material limitation towards stability or microencapsulation processing. For example, microspheres with a large amount of modified starches may be appropriate to use via the spray drying process and this may be commercially justifiable when the microencapsulated essential oil along with the citrus powdered drink is stored in a vacuum sealed bag. In such a case, the modified starch simply serves as a taste-masking matrix component in a microencapsulated form. In contrast, gelatin-based core−shell microcapsules offer a high core loading manufactured by a complex coacervation process.

The encapsulating matrix or shell material is generally applied as a liquid to permit enrobing of the core material. The interfacial tension between the core and shell material must favor the wetting of the former by the latter. The shell material may be a molten coating material, a solution of dissolved shell material, a suspension of shell material and

TABLE 16.3 Assessment of Target Product Profile

Product	Property	Example
Target profile	Description of the formulation objectives	Protect of omega-3 oils in a concentrated powder mix of orange juice
Product shelf-life	In years	2 years in finished product at 25 ± 5°C
Microcapsule size	100 nm to 1 mm	<50 μm with a non-critical normal distribution
Release type	Immediate release, controlled release, triggered release, sustained release	Controlled or triggered past the stomach
Physical state of the active	Solid, liquid, emulsion (water-in-oil or oil-in-water), dispersion	Liquid oil
Thermodynamic profile of the active ingredient	Heat, pressure, and light sensitive?	Heat, light sensitive
Critical barrier property	Oxygen, water	Oxygen
Single versus multicomponent active	Is the core material one unique molecule or does it include several components?	Including components of essential oils (omega-3, omega-6)
Critical processability attribute and processing limitations	Temperature limits? Salt sensitivity? pH limitation? Mixing time limits? Nitrogen blanket? Enclosed system or yellow light?	Minimum thermal energy, i.e., min dT/dt, with nitrogen blanket. Mixing time during process up to 24 h incorporated with an inert gas blanket. Microcapsules will be placed at a pH of 4−5 with presence of salts when reconstituted
Material type	Hydrophilic, hydrophobic, surfactant class	Hydrophilic
Critical performance attribute of the polymer?	Gel point > processing temperature > 30 + 5°C	
Which class of hydrophilic polymers is most suitable?	Carbohydrates, proteins, or celluloses?	Carbohydrates and proteins
Options	List specific polymers	Gelatin, carrageenan, modified starches, cyclodextrins in combinations

binder, or a composition of multiple dissolved and suspended components. Viscosity must be low enough for the shell material to flow around the core material. Furthermore, the miscibility of the core and shell material must be minimized to prevent blending of the two phases prior to solidification of the shell material. While some miscibility between a shell material solvent and core material is acceptable, the encapsulated product stability and shell integrity will be degraded if the solidified shell material has some solubility in a liquid core.

Once the core material is surrounded by the shell material, the liquid shell material must be solidified to form the microcapsule. Solidification mechanisms include solvent evaporation, gelation, freezing, or chemical reactions. Solvent evaporation is one of the most common shell solidification mechanisms, especially when using water as the solvent. Shell material is dispersed or dissolved into an aqueous solution, followed by the application of heat to remove the water once the solution has encapsulated the core material. The resulting precipitation of dissolved solids results in the formation of a solid shell. Common examples include starch or gum acacia. Other volatile solvents can be used when appropriate.

Hydrophobic and hydrophilic materials can be distinguished by their solubility in water. It is obvious that hydrophilic wall materials for microencapsulation are most suitable for hydrophobic active ingredients. Depending on type and modification of the hydrophilic polymer matrix materials, the rate of dissolution, ability to taste mask, oxygen heat, and pressure stability would change.

16.4 HYDROPHILIC MATERIALS

All hydrophilic colloidal particles in suspension exhibit a surface charge. Hydrophilic coating materials can be distinguished between proteins, gums, and carbohydrates by virtue of their surface charge and can be captured by a measurement called *zeta potential* (Hunter, 1981). This measurement is critical for predicting formulation stability and interactions, and also as a simple method of quality control. The size and concentration of particles are the key parameters that determine the zeta potential. In solution, the presence of a net charge on a particle affects the distribution of ions surrounding it, resulting in an increase in the concentration of counter-ions. The region over which this influence extends is called the *electrical double layer*. This is illustrated in Figure 16.4.

As shown in Figure 16.4, the inner region of strongly bound ions is known as the *Stern layer*. The outer layer of loosely associated ions is called the *diffuse layer*. At some distance from the particle, there is a boundary beyond which ions do not move with the particle. The boundary is often termed the *slipping plane*, and exists somewhere within the diffuse layer. It is the potential that exists at the slipping plane that is defined as the *zeta potential*.

Zeta potential is crucial in determining the stability of a colloidal suspension. If the zeta potential is low, the tendency for flocculation is increased. When all the particles have a large negative or large positive charge, they will repel each other, and so the suspension will be stable. In colloidal chemistry, pH often alters the chemical potential of the solution. Therefore, zeta potential is often discussed with respect to pH. In Figure 16.4, a plot of zeta potential versus pH exhibits an *isoelectric point*, a particular value of solution pH where the net charge on the particles is *zero*. At this point the suspension is highly unstable, and flocculation is at its most likely. The mobility is used in predicting zeta potential, colloidal stability, solubility differences, and incompatibilities at different pH conditions. When the value of μ is zero, it means that there is no mobility of the colloid under an electric field. In fact, Noerenburg plotted the electrophoretic mobility, μ, which is defined as the velocity at which a colloid is moving in a 1 V/cm electric field, with units of μ in μm/s/V/cm as a function of [H +] concentration, a measure of pH to develop the analytical method to assess zeta potential.

This property is used to assess the net charge of the hydrocolloid. If the net charge of the molecule is negative, that is, when the molecule is anionic, the μ value will be negative. Hydrocolloids, such as gums, unmodified starches, cellulose, pectin, alginates, and carrageenans are anionic hydrophilic materials. Similarly, when the net charge of the molecule is positive, that is, when the molecule is cationic, the μ value will be positive. Under specific pH conditions, i.e., pH lower than the isoelectric point, protein becomes cationic. Because of the dual nature of a protein molecule, which means that it exhibits both positive and negative charge at different pH conditions, such polymers are called amphoteric polymers.

16.4.1 Proteins

All proteins are amphoteric in nature. They contain amine (-NH_2) and a carboxylic acid (-COOH) group within the molecule. Under strong acidic conditions, the -NH_2 protonates to form NH_3^+ while the COO^- picks up a proton to form -COOH. In contrast, when the pH is alkaline, the protein is negatively charged, because amino groups are present as -NH_2 while -COOH deprotonates to form -COO.

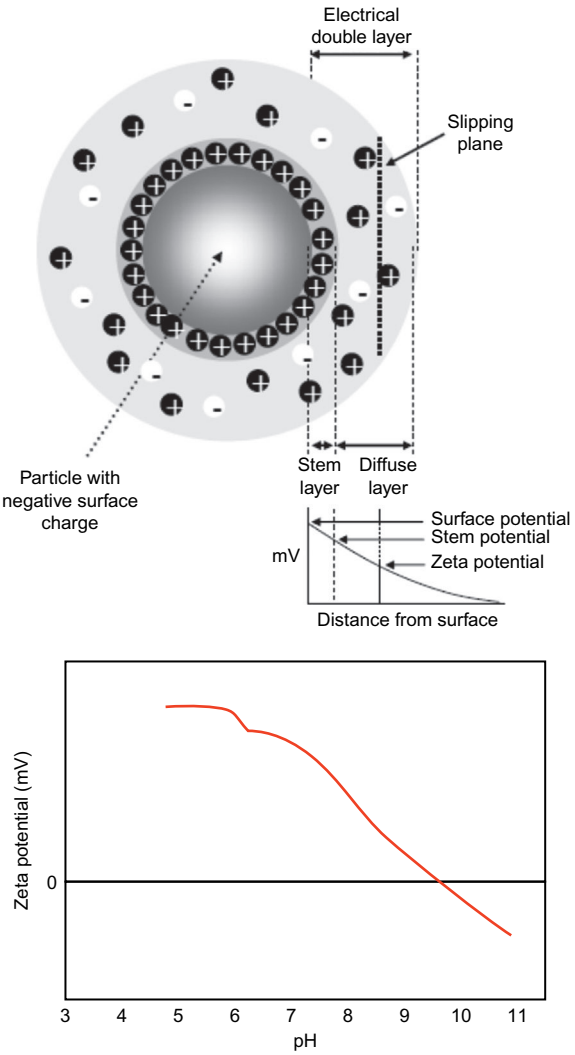

FIGURE 16.4 Concept of zeta potential, ζ.

Proteins may be animal or vegetable derived and depending on its derivation, the physical and chemical properties for microencapsulation are exploited. Examples of vegetable proteins include soy proteins, wheat gluten, zein or corn protein, and rice-bran protein. Animal-derived proteins include gelatin, fish gelatin, casein, whey, and egg albumin. Gelatin is one of the most commonly used proteins in the food industry.

The key properties of gelatin, which is exploited for the purposes of microencapsulation, are:

- Gel point
- Zeta potential, ζ, or electrophoretic mobility, μ
- Bloom strength.

Typically, at a temperature less than 35°C, gelatin exhibits gel properties. This is important because the *gel point* of gelatin is slightly lower than the body temperature in the mouth, which is an important property that can be exploited. The *electrophoretic mobility*, μ, is a critical property that maintains electrostatic stability of the colloidal gelatin droplets in suspension. A greater μ value allows for greater stability in suspension. This property is exploited to disperse gelatin over prolonged periods of time. For example, gelatin can be dispersed in an acid medium, and such dispersions are stable for 10−15 days with little or no chemical changes and are suitable for microencapsulation coating purposes or to maintain colloidal stability of microencapsulated product. Figure 16.5 shows significant differences in the isoelectric points between type A and type B gelatin, and differences in which the μ value remains asymptotic beyond a pH. By definition, type A gelatin is a porcine skin protein processed in acid, while type B gelatin is bovine bone gelatin

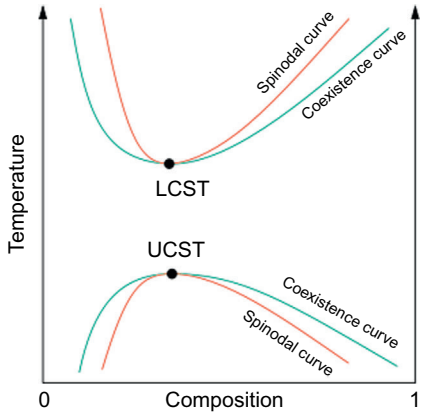

FIGURE 16.5 A plot of polymer binary solution phase behavior including both a low solution critical temperature (LCST) and a upper critical solution temperature (UCST).

processed in an alkaline medium. As depicted, type B gelatin shows an isoelectric point of 5 and at a pH of 6 or greater the electrophoretic mobility remains unaltered. In contrast, type A gelatin exhibits an isoelectric point of 9. This property is exploited to develop the process of complex coacervation. In addition, Figure 16.5 can be used to define an optimum pH range of product stability when gelatin-based microencapsulation systems are considered.

Bloom strength is a measure of the mechanical properties of gelatin or other gels. The test was originally developed and patented in 1925 by O.T. Bloom. The test determines the weight (in grams) needed by a probe (normally with a diameter of 0.5 inches) to deflect the surface of the gel 4 mm without breaking it. The result is expressed in the range of between 30 and 300 bloom. The higher the Bloom strength the stronger the gel will be. This property is exploited in microencapsulated systems that warrant hammer milling or pressure-based processing stability.

16.4.2 Carbohydrates

Among the class of hydrophilic matrices used in the field of microencapsulation, carbohydrates are the most commonly used material. Carbohydrates are classified into four categories: simple sugars or *monosaccharides*, such as glucose and fructose, *disaccharides*, such as sucrose and lactose, *oligosaccharides*, such as maltodextrin and dextrins, and *polysaccharides*, such as starches and cellulose. While all carbohydrate types can be used as fillers and additives, the longer chained saccharides are considered suitable as a wall matrix. Polysaccharides are generally considered in this class of materials. Polysaccharides also include modified starches, where the polysaccharide is structurally and compositionally modified to offer unique solubility, partitioning, and barrier properties to the active food ingredient.

Monosaccharides and disaccharides offer both low viscosity in solution and an effective means of flavor microencapsulation. However, they do not offer ability to emulsify the flavor oils. As a result, smaller amounts of stabilizing colloids are used in synergy (Huang, 2012). By nature of the molecular size, mono- and disaccharides are smaller and readily fit into the interstitial space to prevent formation of a crystallization or crystalline grain boundary within a polysaccharide, which allows for greater stability of the microencapsulated flavor. It is well established that entrapment of flavor oils in an amorphous state offers greater stability than matrices with crystallinity. Therefore, low-molecular-weight mono- and disaccharides are often used with polymer material that inherently exhibits crystalline characteristics. The key properties of carbohydrates that are exploited for the purposes of microencapsulation are:

1. Gel point
2. Lower or upper critical solution temperature.

Note that common carbohydrates that exhibit *gel points* include agar, agarose, carrageenan, pectin, guar gum, and Konjac®, all of which are considered as an alternative option to gelatin. Critical solution temperature is a property of polymer solution where miscibility is defined. Figure 16.5 depicts the characteristics of critical solution temperatures. The *lower critical solution temperature (LCST)* is the *critical temperature* below which the components of a mixture are completely miscible for all compositions. At temperatures below LCST, the system is completely miscible in all proportions, whereas above LCST partial liquid miscibility occurs. Similarly, the *upper critical solution temperature (UCST)* is the temperature above which complete miscibility occurs. Certain celluloses such as hydroxypropyl cellulose show LCST characteristics. At temperatures greater than 40°C, the hydroxypropyl cellulose phase separates in solution.

This property is used both for release and creation of microcapsules. Similarly, methyl cellulose has an LCST between 40 and 50°C. At temperatures below the LCST, it is readily soluble in water; above the LCST, it is not soluble, which has a paradoxical effect whereby heating a saturated solution of methyl cellulose will turn it solid, because the methyl cellulose will precipitate out.

16.5 HYDROPHOBIC MATERIALS

Hydrophobic coating materials can be distinguished from their hydrophilic counterpart by virtue of water immiscibility and affinity for oleophilic components such as fats, fatty acid, fatty alcohols, and waxes. A common term for hydrophobic materials is lipids. Dependent on the structure and composition, lipids may be either solid or liquid at room temperature. The term oils is usually used to refer to fats that are liquids at normal room temperature, while fats are used for solids at room temperature. Examples of edible animal fats are lard, fish oil, butter, and whale blubber. These fats are obtained from milk, meat, or skin. Examples of edible plant fats include peanut, soya bean, sunflower, sesame, coconut, corn, and olive oil.

From the standpoint of chemical composition, fats are derivatives of fatty acids and glycerol. The properties of any specific fat molecule depend on the particular fatty acids that constitute it. Different fatty acids are composed of different numbers of carbon and hydrogen atoms. Fatty acids with long chains are more susceptible to intermolecular forces of attraction that raise their melting point. Therefore, a saturated fatty acid with a greater molecular weight has a higher melting point than a saturated fatty acid with a lower molecular weight. A saturated fatty acid has a higher melting point than unsaturated fatty acid. Because fats exhibit melting point as a critical property, this property in lipids is most commonly used for microencapsulation resulting in thermal solidification.

Similarly to thermal solidification induced at temperatures lower than the gel point, microencapsulation in fats is induced by congealing of the fat at temperatures below their melt point. Common examples include fats, waxes, or lipids where the melt temperature is above 50°C for processing. Lower melting coating materials can be used, but may require climate controlled storage after formation of the encapsulated products.

16.6 CONCLUSIONS

Several review chapters pertaining to choice of materials for microencapsulation of food ingredients can be found. Starting from Balassa and Fanger in 1971, the art of microencapsulation has not changed much over the decades. From Dziezak (1988), Risch and Reineccius (1995), to Garti (2008) only a handful of new materials have been added to the user list.

Traditionally, the formation of a microencapsulation requires that there be incompatibility between the shell and the active so that a coating will exist at the surface of the active ingredient. In most practical cases, the active is somewhat soluble in matrix. This said, our desire to microencapsulate hydrophobic actives is best achieved by hydrophilic material, and, similarly, where a hydrophilic active requires encapsulation, a hydrophobic material should be used as a matrix or coating material. A wide variety of polysaccharides, proteins, and polymers have been used for wall materials for microencapsulation, while hydrophobic materials include lipids, waxes, and polymers.

Tzhere are no all-encompassing matrix materials that can apply to the microencapsulation of food ingredients. Each situation demands a tailor-made solution because of the wide variation in food, storage, the need for different release triggers, and consumption requirements. Regulatory compliance often narrows the selection.

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Chapter 17

Cellulose Polymers in Microencapsulation of Food Additives

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17.1 INTRODUCTION

Derivatives of cellulose polymers have been known and used for many decades. Cellulose ethers, for instance, have been commercially available and applied for over 75 years, and have been employed in food microencapsulation applications for decades. As a class, these polymers provide a variety of useful and unique functional attributes in food products. They find valuable use in the formulation of, for instance, salad dressings, dietetic foods, fried foods, bakery products, nondairy whipped toppings, heated convenience foods, extruded and shaped foods, frozen desserts, beverages, and confectionery products. For example, many cellulose ether products possess unique properties that are particularly useful to the food product formulators and processors. Thermal gelation of cellulosic ethers, for instance, generates a hydrophilic, water-containing gel, which, when applied as a food coating or batter, limits penetration of oil during frying of items such as potatoes. This property is also useful in strengthening the structure of baked goods prepared with low-gluten flours. A useful overview of the application of cellulose ethers in food formulation can be found in Coffey et al. (1995).

This review will focus on applications of cellulose derivatives in microencapsulation of food additives. For this reason, cellulosic polymers with minor roles in food encapsulation (i.e., hydroxyethyl cellulose, HEC; or hydroxyethyl methylcellulose, HEMC) are not covered in detail here. As well, chitin (N-acetyl-2-aminocelluloses), chitosan, and α-1,4-glucosyl polymers (starches, dextrins, dextrans) are not discussed. This important food polymer class, starch, and its derivatives will be discussed in greater detail in subsequent chapters.

17.2 PROPERTIES OF CELLULOSIC POLYMERS

17.2.1 General Properties

The production of cellulosic derivatives begins from natural wood or cotton cellulose. Cellulose is a naturally derived, linear polymer of anhydroglucose with the β-*O*-glucopyranosyl structure (see Figure 17.1).

For preparation of the cellulosics most useful in microencapsulation applications, cellulose is usually derivatized by substitution of chemical moieties on available hydroxyl groups, resulting in functionalized polymers. The properties of a specific cellulose derivative depend on the type, distribution, and uniformity of the substituent group. For each β-*O*-glucopyranosyl ring, there are three hydroxyl groups available for nucleophilic substitution reaction. The term *degree of substitution* (DS) is used to identify the average number of sites reacted per ring. The maximum value is 3, corresponding to the number of hydroxyls available for reaction. When side-chain formation is possible, in cases resulting in hydroxylethyl or hydroxypropyl derivatives (i.e., HEC; hydroxypropyl cellulose, HPC; hydroxypropyl methylcellulose, HPMC), for instance, further reaction of the added side-chain hydroxyl moiety is possible. The term *molar substitution* (MS) is used and the value can exceed 3 (Greminger and Krummel, 1980).

Key examples of the structure of cellulose derivatives include methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) as shown in Figures 17.2 and 17.3.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00017-0

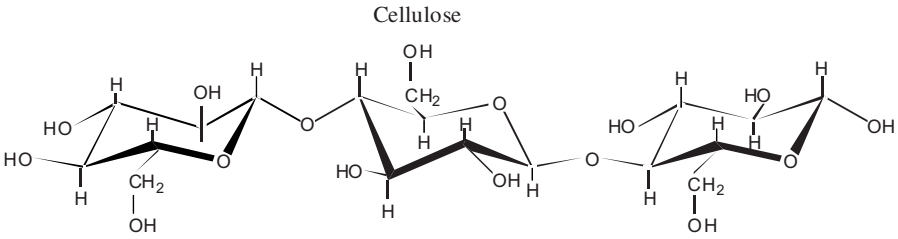

FIGURE 17.1 Cellulose is a naturally derived, linear polymer of anhydroglucose with the β-*O*-glucopyranosyl structure.

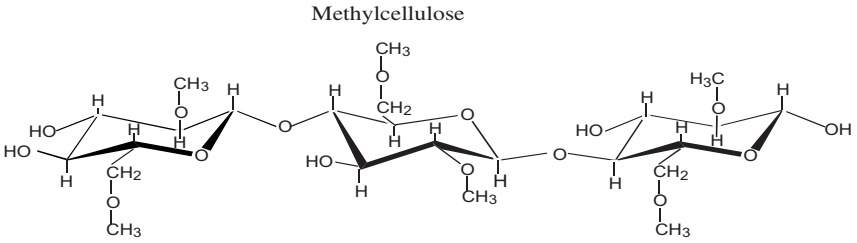

FIGURE 17.2 Structure of cellulose derivative methylcellulose (MC).

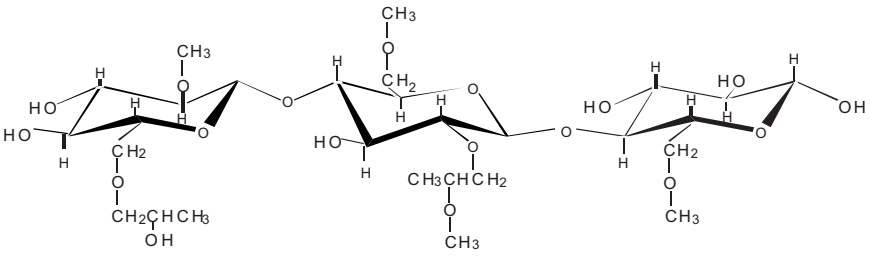

FIGURE 17.3 Structure of cellulose derivative hydroxypropyl methylcellulose (HPMC).

MC and HPMC are two examples of this versatile class of water-soluble hydrocolloid derived from the etherification of cellulose. MC and HPMC cellulose ethers are polymers having the useful properties of thickening, thermal gelation, surface activity, film formation, and adhesion. Additionally, to tailor a product for a specific end use, the properties of MC and HPMC may be modified by changing the polymer molecular weight or the relative amounts of etherifying reagents.

Another cellulose ether, carboxymethylcellulose (CMC, or its sodium salt, sodium carboxymethylcellulose, CMC-Na) is a water-soluble, anionic, linear polymer, broadly known as cellulose gum in the food industry. It is prepared by substitution reaction on alkali cellulose, resulting in carboxymethyl substituent groups on the cellulose polymer backbone. Commercial CMC products have DS values from about 0.4 to about 1.4, with a DS of 0.7 to 0.8 as the most common grade available. Such a grade has about the same acid strength as acetic acid, with a pK_a of about 4.4 (for DS 0.8 product) (Stelzer and Klug, 1980). Therefore, at pH 7, such a CMC material would be a predominantly deprotonated, ionic carboxylate anion form, containing very little of the free acid form. Given the pK_a value, useful ratios of deprotonated, anionically charged form to protonated, uncharged, free acid form can be estimated at any formulation pH value.

Cellulose esters are prepared by esterification of cellulose (i.e., cellulose acetate, CA), cellulose ethers (i.e., hydroxypropyl methylcellulose phthalate, HPMCP), or multiple esterifications of cellulose (cellulose acetate phthalate, CAP; or hydroxypropyl methyl cellulose acetylsuccinate, HPMCAS). Key properties of cellulose esters, and cellulose ethers as well, are described in the following subsections.

17.2.2 Solubility

The substitution type and level of a particular nonionic cellulose derivative determines to a large extent its aqueous solubility. Cellulose, though a highly hydroxylated polysaccharide, is swellable but not soluble in water. This is ascribed to high levels of intramolecular and, to a lesser extent, intermolecular hydrogen bonding between hydroxyl groups in and between glucopyranosyl rings within a polysaccharide chain and between adjacent polysaccharide chains in bundled fiber structures of cellulose.

Many commercially available cellulose ethers, such as MC, HPMC, and CMC, are water-soluble polymers. Commercial MC products have an average DS ranging from 1.5 to 2.0; commercial HPMC products have an average DS for methyl group substitution of 0.9 to 1.8 and the MS of hydroxypropyl groups ranges from 0.1 to 1.0. Altering the amounts of methyl and hydroxypropyl substitution also affects the solubility properties of MC and HPMC cellulose ethers. Decreasing the level of substituent groups below a DS of 1.4 gives products whose solubility in water decreases. Increasing the substitution above an MS of 2.0 improves solubility in polar organic solvents.

MC and HPMC possess the rather unusual property of solubility in cold water and insolubility in hot water, so that when a solution is heated, a three-dimensional gel structure is formed (Sarkar, 1995; Sarkar and Walker, 1995). By modifying production techniques and by altering the ratios of methyl and hydroxypropyl substitutions, it is possible to produce products whose thermal gelation temperature ranges from 50 to 90°C (122−194°F) and whose gel texture ranges from firm to rather mushy. Hydroxypropyl cellulose (HPC) also exhibits cold water solubility and thermal gelation. It becomes insoluble above about 42°C and is soluble in a broad range of polar organic liquids (Desmarais and Wint, 1975).

Aqueous solubility of ethylcellulose (EC) depends on its degree of ethylation (DS) and also on the distribution of ethyl substituent groups on the glucopyranosyl rings. EC with DS 0.7 to 1.7 is water soluble and exhibits thermal gelation. Selective synthesis of 3-mono-*O*-ethylcellulose has been reported (Koschella et al., 2006), resulting in a positional isomer not readily found in EC prepared by conventional reaction of ethyl chloride with alkali cellulose. EC from conventional preparation processes typically possesses about equal proportions of 2- and 6-positional isomers with low partial DS at position 3 (Koschella et al., 2006). 3-Mono-*O*-ethylcellulose is soluble in both aprotic-dipolar organic media and in water, and exhibits thermoreversible gelation at 58.5°C, about 30°C higher than the cloud point temperature of conventional water-soluble EC.

Ethylcellulose, once described in pharmaceutical formulation as "probably the most widely used water-insoluble polymer in film coating due to its good film forming properties that enable tough, flexible coatings to be produced" (Narisawa et al., 1994), is beginning to find application in microencapsulation applications in food. Commercially representative ethylcellulose, of DS >2.0, dissolves in polar organic solvents such as ethanol or ethyl acetate, and in aqueous-solvent systems (i.e., 85% ethanol-water or higher). Theoretical values for ethylcellulose−solvent interaction parameters have been calculated for 112 solvents, and such parameters can be used to identify useful solvents or solvent−nonsolvent systems for preparation of microcapsules using coacervation (Robinson, 1989). Commercial EC (DS >2.0) is thermoplastic, softens at 130°C, flows at 140 to 160°C, and can be used for preparation of films by melt extrusion (Koschella et al., 2006). Careful manipulation of EC solubility properties in various solvents has been used to develop coacervation coating systems. In particular, non solvent addition, emulsion-solvent evaporation, and temperature reduction-poor solvent methods have been employed to achieve very effective EC coatings in microencapsulation systems (Nixon, 1985; Chemtob et al., 1986; Nixon and Wong, 1990; Sveinsson and Kristmundsdottir, 1992; Amperiadou and Georgarakis, 1995; Jones and Pearce, 1995; Song et al., 2005; Choudhury and Kar, 2009). Select examples of these coating methods will be indicated described in Section 17.3. Finally, aqueous dispersions of ethylcellulose are sold into the food industry and are used widely in the pharmaceutical industry, but have not generally been adopted by food formulators for microencapsulation coating applications.

Cellulose esters exhibit quite different solubility profiles than do cellulose ethers. For example, because the phthalate substituent of cellulose acetate phthalate (CAP) is ionizable in solution above its pK_a value, this cellulose derivative is insoluble in acid media at pH 5 or below, but is soluble at pH higher than 6. This profile is generally representative of the class of cellulose esters, though particular pK_a values for select esters may vary. Such solubility behavior provides useful cellulose ester coatings for pH-activated release of microencapsulated active ingredients, such as nutrients and vitamins, in the gastrointestinal (GI) tract.

17.2.3 Thermal Gelation

Cellulose ethers are known to reversibly gel at elevated temperatures, a property that is used to advantage in many cooking applications. Methylcellulose is commercially available with a methyl substitution (DS) of about 1.6 to 1.9. Although nonionic, the addition of the methyl ether group to the cellulose backbone gives this gum some unique properties, such as cold water solubility and thermal gelation. The most common grade of methylcellulose gels between 48°C and 64°C (118°F and 147°F). Methylcellulose solutions gel and maintain a solid structure as the product temperature increases. This gelling forces the heat transfer from convection to conduction, which subsequently limits the movement of the product and prevents boiling out of the filling during baking. The thermal gel structure also reduces moisture loss during heating (Nieto and Akins, 2011). This gelling behavior is reversible and reproducible. After cooling, the gel reverts to the original liquefied form, providing suitable product texture and mouthfeel.

Characterization of the thermal gelation properties, including gel strength, kinetics of gelation, and gel point, has been investigated for both methylcellulose and hydroxypropyl methylcellulose (Sarkar, 1995; Sarkar and Walker, 1995). Kinetic expressions detailing the influence of concentration, temperature, polymer molecular weight, and substitution patterns (DS for MC and HPMC, and MS, also, for HPMC) on the rate of gelation and on the gel modulus for both are reported (Sarkar, 1995). The rate of gelation for methylcellulose was found to be strongly dependent on concentration, temperature, and methoxyl content, and only marginally dependent on polymer molecular weight. Gel strength was found to be strongly dependent on concentration, very strongly dependent on methoxyl content, and only marginally dependent on temperature (above the cloud point) and molecular weight. The rate of gelation of HPMC was found to be highly dependent (a second power relationship) on the relative degree of methoxyl substitution (DS) compared to the molar substitution (MS) of the hydroxypropyl groups. As with MC, the rate of gelation for HPMC was highly dependent (a third power relationship) on polymer concentration in solution. Gel strengths of HPMC solutions increased with an increase in methoxyl DS and a decrease in MS.

Gelation of ethylcellulose in organic solvents (nonsolvents) has been described in detail (Robinson, 1989). The impact of DS and of ethyl-substituent positional isomer distribution on both solubility and on thermal gelation has been described (Koschella et al., 2006). Recent work has studied in much more detail the self-association of EC polymer derivatives necessary for occurrence of thermal gelation (Sun et al., 2009).

17.2.4 Surface Activity

The surface activity of cellulose derivatives (Table 17.1) contributes to key applications, such as emulsion stabilization, dispersant activity, and film coating integrity. Surface activity modification works in concert with the solution thickening or viscosity building ability of cellulosics to stabilize aqueous emulsions and dispersed particle solutions.

Surface active water-soluble polymers have long been used as emulsifiers to stabilize oil−water emulsions in many food applications. Such polymers have an amphiphilic character, by which they adsorb to and from hydrophilic layers of the surface of dispersed oil droplets. Studies with surface active HPMC of varying molecular weight (polymer chain length) were conducted to examine the effect of emulsifier structure on stabilization of silicone oil droplets (about 20−45 μm diameter) in aqueous solution (Hayakawa et al., 1997). The rate of change of droplet size and size distribution of oil droplets over time was used as a measure of emulsion stability. It was found that the size of oil droplets increased with increasing molecular weight of HPMC at fixed HPMC concentration, whereas droplet size decreased with increasing HPMC concentration at constant HPMC molecular weight. Adsorbance of HPMC of varying molecular weights on silicone oil droplets was found to be constant. Further work (Yonekura et al., 1998) indicated that brief mixing times (10 s) were required to achieve stable emulsions of silicone oil in aqueous solutions of HPMC, indicating efficient formation of stabilized emulsions; increased mixing times reduced the size and the size distribution (narrower distribution) of the dispersed oil droplets. Overall, though, development of steady-state interfacial tension was found to be time dependent. Results reported using METHOCEL™* F50 (weight average MW 70,000 g/mol) and F4M

TABLE 17.1 Surface Activity of 1% Aqueous Solutions

Gum Type	Surface Tension (mN/m)
Water (control)	72
Xanthan	69
CMC	68
Na alginate	62
PG alginate	58
MC	53−59
HPMC and HPC	45−55

Source: Wallick (2012).

* METHOCEL is a trademark of The Dow Chemical Company.

(weight average MW 800,000 g/mol) show that the higher molecular weight sample appeared to be slower in attaining a steady-state value than did the lower molecular weight sample (Sarkar, 1984). Diffusion of polymer molecules to the interface is believed to control the rate of decrease in interfacial tension, with the larger molecules expected to diffuse more slowly than the smaller molecules. High internal potential energy barriers for long polymer molecules must be overcome as the polymer adopts its optimal solution conformation at the oil−water interface, leading to attainment of the optimal interfacial tension. Further examination of the physicochemical process of stabilization of emulsion interfacial layers—diffusion of polymer from the bulk to the interface, followed by polymer adsorption at the interface—can be found (Wollenweber et al., 2000; Camino et al., 2009).

Several other factors have been shown to affect the interfacial tension achieved by MC and HPMC (Sarkar, 1984). Interfacial tension was found to decrease with increase in the total degree of methyl and hydroxypropyl substitution. Interfacial tension was found to decrease first with increasing temperature, then increase at higher temperature for samples that gelled or precipitated. Interfacial tension was found to increase slightly with increasing polymer molecular weight. Solution pH was also found to affect the particle size and the stability of emulsions formed using sunflower oil and HPMC (Camino et al., 2011; Camino and Pilosof, 2011). The lowest interfacial tensions were found for polymers possessing "blockiness," or nonuniformity, of substitution along the backbone. Interfacial tension depends not only on the number of hydrophilic and lipophilic sites in the polymer, but also on their distribution. If the polymer molecules were a perfect block polymer, with the hydrophilic and lipophilic block lengths large enough to allow the polymer to orient at the interface such that the blocks would preferentially be in the oil and water phases, the attractive interactions would be maximized, the entropy state of the polymer would be low, and the interfacial tension would be extremely low (Sarkar, 1984).

Stabilization of emulsions can also be undertaken using blends of cellulose ethers with other additives, such as β-casein or β-lactoglobulin protein-MC/HPMC stabilizing systems (Arboleya and Wilde, 2005) and Emultop™ lecithin surfactant-HPC systems (Mezdour et al., 2008). Use, then, of cellulose ethers as emulsion stabilization aids may be modified (competitive adsorption or synergy, for instance) by other formulation additives and should be explored during development of microemulsion or spray-dried micro- and nanoencapsulant formulations. Further description can be found in Section 17.3.

The literature reveals growing exploration of novel emulsion stabilization systems based on Pickering emulsions. Finely divided colloidal particles can be used as emulsifying agents, whereby they partition between oil and water phases to very effectively reduce interfacial tension. Water-insoluble ethylcellulose particles have been reported (Melzer et al., 2003) to exhibit such behavior, and may be considered yet another means of using cellulosic polymers for emulsion stabilization in microencapsulation systems.

Cellulosics have also been used as stabilizers for dispersed solid particle systems, in analogy to the need to maintain dispersed microencapsulant beads and avoiding agglomeration. Adsorption of nonionic water-soluble cellulose polymers (HEC, HPC, HPMC) onto a variety of solid substrates was investigated (Law and Kayes, 1983), demonstrating that adsorbed cellulosic layers at the solid−solution interfaces produced a steric stabilization effect. The magnitude of this effect was dependent on both the composition (e.g., hydrophobic interaction, between polymer and substrate) and thickness of the adsorbed surface layer. It is likely that the functional groups of both the anhydroglucose and substitution units on the polymer contribute to the degree of stabilization.

The surface free energy parameters of aqueous-based cellulose ether films (MC, HEC, HPC, HPMC) were determined using the Lifshitz−van der Waals/acid−base approach using data from contact angle measurements of sessile drops of apolar and polar liquids on cellulose ether cast films (Luner and Oh, 2001). Cellulose ether surfaces were found to be predominantly electron donating and had acid−base surface energy components that accounted for about 5 to 10% of the total surface energy. Rationalization of the results was found to be reasonable on the basis of cellulosic structure and explained both aqueous solubility and hydrophilicity of cellulose ethers satisfactorily. Surface chemical characteristics of the cellulosic polymers were found to be consistent with measurements made with cellulose and EC polymers. Of particular importance to microencapsulation applications, including surface adsorption, coating technology, agglomeration, bioadhesion, and wetting effects, are calculations of the work of adhesion of the polymers to a variety of surfaces demonstrating that the acid−base contributions to surface free energy may enhance adhesion of cellulose ethers.

17.2.5 Stability

The cellulose polymer backbone imparts a broad range of chemical and physical stability to cellulosic polymers. For instance, methylcellulose, as representative of the cellulose ether class, is stable over the pH range 3 to 11 (Nieto and

Akins, 2011). Under more acidic conditions, though, acid-catalyzed hydrolysis on the cellulose backbone results in polymer chain scission and molecular weight degradation. In the presence of molecular oxygen, base-catalyzed oxidation takes place at high pH values and also leads to a decrease in polymer molecular weight (Glover, 1975). Acid-stable cellulose esters, on the other hand, de-esterify and dissolve under neutral physiologic conditions, allowing their use as enteric coatings for delayed release of encapsulated additives in the GI tract. This application will be described in more detail in the next section.

Cellulose derivatives, and in particular cellulose ethers, are stable in terms of compatibility with a wide range of active ingredients. There are exceptions, as already mentioned and otherwise, but over decades of application of cellulosics in food, in pharmaceuticals, and in other specialty areas, these materials exhibit good stability and very low reactivity with other chemicals.

Cellulose derivatives are also physiologically stable during ingestion and do not degrade in the GI tract. This behavior is in stark contrast to many other polysaccharide polymers, such as starches, which undergo ready digestion and contribute caloric value to the foods containing them. Cellulose ethers, in particular, are nondigestible and do not contribute caloric value to foods containing them. For this reason, MC finds use in, for example, the formulation of dietetic foods (Glover, 1975). Cellulose gum (CMC) is also physiologically inert and has no caloric value (Stelzer and Klug, 1980).

17.3 APPLICATIONS OF CELLULOSIC POLYMERS IN MICROENCAPSULATION

The manufacture of foods demands the addition of functional ingredients to better engineer flavor, color, texture, preservation, and nutritive properties. The inclusion of healthy, but inherently unstable additives, such as antioxidants and probiotics, in our food is increasing. The preparation of functional foods requires the stabilization of bioactive compounds during processing, storage, and ingestion in the human body. With new food additives, such as nutrients and beneficial microorganisms, enhancing the bioavailability at the site of nutrient absorption or the site of bacteria physiologic activity is also demanded. Microencapsulation of such additives is a leading means of providing the requisite control of stability and delivery. Cellulose derivatives play significant roles in the formulation of microencapsulated food systems. Key applications of cellulose derivatives, as emulsion and dispersant stabilizers, as formulation binders, and as film and barrier coatings, will be highlighted with liberal reference to the literature for those desiring more information.

While the experience base of cellulose polymer encapsulation in food applications is useful but modest, much can be extrapolated from the rich base in pharmaceutical sciences. Cellulosic polymers find considerable encapsulation application in, for instance, controlling drug release from dosage forms. The realms of food and pharmaceutical applications of microencapsulation overlap, especially in the area of nutraceuticals and dietary supplements, so this section cites some pharmaceutical literature sources that will lead the reader to a better appreciation of application with food additives. Those desiring more information about the application of cellulose ether polymers for microencapsulation of drug actives are directed to recent literature reviews (Rogers and Wallick, 2012a–c).

17.3.1 Emulsion Stabilizers and Dispersants

A variety of fish oil microencapsulation techniques have been performed using cellulose derivatives to stabilize oil–water emulsions. Nanocapsules of less than 200 nm size were prepared by emulsification of MC stabilizer, sodium caseinate, sucrose, and poly(vinyl alcohol) with fish oil or plant oil extracts (Tanaka et al., 2006). Alternately, an emulsion formed from 4% HPMC and 30% fish oil was blended with 20% maltodextrin or 20% acacia, then spray dried to yield microencapsulants with improved oxidative stability (Wu et al., 2005). Further, an emulsion containing up to 40% of long chain omega-3 polyunsaturated fatty acids (PUFA), sourced from fish oil, was stabilized with MC and spray dried to improve the stability of the oil for dietary applications (Kolanowski et al., 2004). Microencapsulated fish oil of favorable encapsulation efficiency and good particle morphology, prepared by spray drying the oil in MC, was incorporated into bread products with no significant modification of sensory characteristics (Davidov-Pardo et al., 2008).

Analogously, other food oils have been encapsulated using cellulose ethers as emulsion stabilizers. In one example, lemon oil (18 g) was combined with 5% aqueous MC to form an oil–water emulsion, then microencapsulated with 70 g of 25% aqueous hydrolyzed starch, osmotically dewatered with 25% aqueous sodium chloride, recovered, and rinsed to give microcapsules capable of rapid swelling and dissolution in water (Takahashi et al., 1989).

A food fortification additive comprising a core–shell microcapsule core of calcium carbonate, calcium hydrogen phosphate, iron pyrophosphate, and iron orthophosphate, with CMC dispersant, and a shell of sucrose ester or tripolyglycerol ester, offered high dispersion stability and no flavor contribution to food products (Liu et al., 2009).

17.3.2 Formulation Binders

CMC has been used at 0.5 to 1.2 w/w % levels as a binder in the formulation of high loading capsules (Wu et al., 2009). The invention was reported to impart heat resistance and low activity loss to encapsulated phytase. Alternately, CMC has been used in the formulation of microencapsulated zinc oxide granules as a feed additive for the treatment of baby pig diarrhea (Li et al., 2011). A formulation containing, by weight percent, starch 5 to 20, CMC-Na 5 to 10, zinc oxide powder 50 to 80, stearic acid 5 to 15, and an unspecified resin 3 to 5, was found to protect the high levels of zinc oxide from gastric acid and provide sustained release in the pig intestinal tract.

17.3.3 Film and Barrier Coatings

Edible films and coatings containing cellulose derivatives provide functional attributes such as barrier properties; protection against thermal, acid, and enzymic inactivation of sensitive active ingredients; and structural or morphological benefits in microencapsulation applications. Such coatings also can be used to moderate the delivery of beneficial additives, such as nutrients, antioxidants, and biocides. Examples include antioxidant-containing, HPMC-based film coatings on almonds (not explicitly a microencapsulation application, but illustrative of additional opportunities). Ascorbic acid, citric acid, or ginger essential oil antioxidants were applied to toasted almonds as HPMC-based dispersions and examined for their effectiveness in protecting against development of rancidity (Atares et al., 2011). Incorporation of these into HPMC polymer coatings revealed that the two acid compounds exhibited crosslinking, serving to tighten the porosity of the coatings and reduce permeability of oxygen, increasing the oxidative stability of the almonds. The hydrophobic nature of ginger essential oil was thought to reduce water vapor permeability at low temperatures.

A spray-dried HPMC matrix was used to provide protection for acidic colistin sulfate (Zhou and Ye, 2010). The powder was then coated with starch to further isolate colistin sulfate from deleterious reaction with sensitive vitamins and trace elements in animal feed.

Rose essential oil was encapsulated, in part, in an emulsion containing HPC and HPMC capsule wall polymers. The resultant microcapsules exhibited stability, little irritation, improved storage and handling, and slow release (Li and Liang, 2007).

Incorporation of vanillin, an inhibitor of microorganisms, in chitosan/MC was conducted to explore its diffusion into fruit juices (Sangsuwan et al., 2009). Migration of vanillin was affected by vanillin concentration, temperature, and pH, with higher temperature and lower vanillin content giving higher diffusion coefficient values, and lower juice pH resulting in higher migration rates.

Coacervates containing blends of HPMC, NaCMC, and surfactants (sodium dodecylsulfate, SDS) were used to microencapsulate sunflower oil (Katona et al., 2010). Coacervate deposition at the oil−water interface of sunflower emulsions provided useful powders after spray drying. It was found that HPMC−SDS complexes could form, depending on SDS concentration, and these complexes were thermodynamically incompatible with NaCMC. The amount of recoverable oil was found to depend on the viscoelastic properties of the coacervate shell in the microencapsulants.

CAP and similar cellulose ester coatings, which are stable at acidic pHs but dissolve in neutral or basic pHs, find some use as enteric coatings for food and nutritive additives, and much broader use for enteric coatings of drugs (Nixon, 1985). Such coatings protect sensitive active ingredients from low pH environments in and near the stomach, but then dissolve at higher pH in the GI tract downstream from the acidic stomach area, providing delayed release of the actives. These enteric coatings are used to provide targeted or site-specific delivery of encapsulated additives or nutrients to their physiological absorption/uptake sites in the small or large intestines.

Some examples of the application of cellulose ester enteric systems are provided: dietary supplementation of microencapsulated arginine has been demonstrated in juvenile *Penaeus monodon* shrimp, which cannot effectively use crystalline amino acids in their diets. Arginine encapsulated in a CAP matrix exhibited favorable weight gain and feed conversion during 8-week feed studies in shrimp (Chen et al., 1992). Encapsulation of *Ampelopsis grossedentata* flavonoid extracts in cellulose derivatives—EC, CMC, MC, HPMC, cellulose acetate butyrate (CAB), and others—was shown to provide increases in animal health and growth rates when provided as nutritive feed additives (Shi et al., 2006).

Nanoencapsulants of lutein in HPMCP were prepared using supercritical anti-solvent precipitation techniques. Lutein loadings up to 15.8%, in spherical particles of 160 to 220 nm, in high yield (95%), and encapsulation efficiency (88.4%) were achieved (Jin et al., 2009).

Enzymes have been encapsulated using cellulose derivatives. For example, lactase was microencapsulated in protective particles using shellac/HPMC or gum acacia/HPMC matrices (Solomon, 2008). Further, microencapsulation of

probiotic bacteria with cellulose ester enteric coatings has been used to achieve targeted delivery in the gastrointestinal tract while protecting the sensitive strains from inactivation during transit through the stomach's acidic environment. A useful review (Anal and Singh, 2007) cites examples of *B. pseudolongum* encapsulated in CAP using an emulsion technique, enabling survival of bacteria at high levels (10^9 CFU/mL) in an acidic environment for 1 hour (compared to total loss of viability for unencapsulated strain samples), as well as encapsulation of *B. lactis* and *L. acidophilus* in CAP using spray-dry methods, also enabling protection to the encapsulated samples in an acidic environment while affording complete dissolution of the polymer wall material and release of bacteria in a pH 7 bile solution.

CMC has been used in select microencapsulation applications as an alternative to gum arabic in traditional gum arabic−gelatin shell coating materials via complex coacervation. Due to batch-to-batch inhomogeneity of stability, regional sourcing difficulties, and higher prices, utilization of gum arabic is becoming limited. As an anionic long-chain polysaccharide, CMC has been compared to gum arabic in such coacervation applications. It possesses a much higher anionic charge density, which can often lead to precipitation of coacervates rather than the preferred liquid coacervate system. A new combination of wall materials—CMC-gelatin—has been reported (Lv et al., 2012), which required careful mixing levels to maintain liquid coacervate. Zeta-potential was used to select key blend ratios, and then turbimetric titration was conducted to get approximate blend solution pH and mixing ratios. This technique allowed formation of uniform, spherical, oil-containing microcapsules of about 45 μm size.

Lipid-soluble vitamins, such as vitamin E acetate, have been encapsulated at 30 to 90% levels in a primary polymer coating (75% METHOCEL E 15LV (HPMC)), followed optionally by additional encapsulation in secondary (2%) or tertiary (10%) polymer coatings, resulting in a free-flowing particulate material suitable for use in food, animal feed, or pharmaceutical applications (Boyle and Chang, 1996).

HPMC (METHOCEL E6) was employed for extrusion preparation of microencapsulated ferrous fumarate and iodine double fortified salt for dietary micronutrient fortification applications. HPMC barrier film overcoating of ferrous fumarate particles prevented diffusion of deleterious or reactive small molecules, such as water or iodate, into the ferrous fumarate core. Therefore, barrier film coated iron premix particles were produced in this fashion (Li et al., 2010).

Microparticles containing antioxidant, α-tocopherol, were produced by spray drying using pea protein, CMC, and mixtures of these, with maltodextrin wall material. Spherical particles with mean sizes below 7 μm were used as ingestible food additives (Pierucci et al., 2007). Similarly, ascorbic acid was microencapsulated by spray drying using CMC-maltodextrin as a matrix material, resulting in regular and smooth particles with mean diameter less than 8 μm (Pierucci et al., 2006).

Taste masking of unfavorable food tastes is an important role for encapsulation in food formulation. Cellulose derivatives are used to advantage in such applications. Fish oil, for instance, has been successfully microencapsulated using cellulose ethers or cellulose esters (Cantor et al., 1989). An emulsion of 20 parts of fish oil (omega-3 fatty acids) and 80 parts of 25% EC in ammonium hydroxide solution was atomized under nitrogen into 5% acetic acid. The resultant microcapsules were filtered, washed, and dried. The capsules were found to have good storage stability and the aftertaste of the fish oil was eliminated. Additionally, in another instance, encapsulation of edible oils, such as omega-3 oils, was conducted using a formulation comprising, by weight percent, water 3.5 to 4.2, whey protein 40.3 to 47.8, CMC 8.0 to 9.5, oil 5 to 20, and citric acid 28.2−33.5 (Slater, 2007).

EC microcapsules containing folic acid have been reported (Prasertmanakit et al., 2009), which serve to shield the nutrient from degradation on exposure to the acid gastric fluids in the stomach, while allowing its release until the intestinal tract and its site of physiologic absorption. These delayed release forms of folic acid in EC were prepared by oil-in-oil emulsion solvent evaporation using a dispersed phase of 9:1 acetone:methanol in a continuous phase of light liquid paraffin. Span™ 80 surfactant was used as an emulsion stabilizer and sucrose was added to the shell polymer formulation as a pore former. Release kinetics of folic acid depends on the concentrations of EC, surfactant, sucrose, and folic acid active. The average diameter of the microcapsules increased from 300 to 448 μm, while the folic acid release rate decreased from 52 to 40%, as the ethyl cellulose concentration was increased from 2.5 to 7.5% (w/v). Increasing the Span 80 concentration from 1 to 4% (v/v) decreased the average diameter of microcapsules from 300 to 141 μm and increased the folic acid release rate from 52 to 79%. The addition of 2.5 to 7.5% (w/v) of sucrose improved the folic acid release from the microcapsules. The entrapment efficiency was improved from 64 to 88% when the initial folic acid concentration was increased from 1 to 3 mg/mL. Optimization of formulation component amounts provided many dimensions of improvement for folic acid release rates, microencapsulant particle morphology, and processing efficiencies. A further example of the utility of cellulose ether coatings (though not at microencapsulation scale), employing MC, HPMC, CMC, and EC for folic acid fortification of rice, may be useful (Shrestha et al., 2003).

Polyphenolic extracts from bayberry have been microencapsulated using a phase separation method and EC coating material (Zheng et al., 2011). Both the storage stability and the gastrointestinal stability and delivery rates of the

bayberry polyphenols could be controlled and improved. Analogously, EC microencapsulation of blueberry anthocyanins afforded light stability to the nutrients (Li et al., 2009a). EC coating has also been used to microencapsulate vitamin D2-chitosan spray-dried powders (Shi and Tan, 2002). Encapsulated preservatives have also been demonstrated using EC as a barrier coating. Sodium metabisulfite was encapsulated in EC, using elevated temperature/poor solvent coating methodology (see Section 17.2.2), to afford a dispersion suitable for coating on food-contact paper (Li et al., 2009b).

A microencapsulation process was demonstrated (D'Onofrio et al., 1979), which allowed an oil slurry of microcapsules to be filled into soft gelatin capsules, a potential delivery option for feeding of nutraceuticals, probiotics, or other dietary additives. EC solutions in ethyl acetate were desolvated by the addition of the antisolvent light liquid paraffin so that the EC shell coat was deposited onto a core of active ingredients. The use of light liquid paraffin enabled the resulting slurry to be filled directly into soft gelatin capsules without the usual filtering, drying, and redispersion steps. Release characteristics for the microencapsulated actives could be controlled by varying the ratio of EC polymer to actives during encapsulation. Using aspirin (acetylsalicylic acid) active compound as a model, release in simulated gastric fluid was achieved consistent with first-order kinetics for up to 12 hours.

Coatings of EC have also shown utility in the bacteriostat-mediated stabilization of microencapsulated probiotic bacteria for use in high moisture foods and beverages at ambient temperatures (Hong et al., 2012). Core−shell microspheres containing, for example, 61.92% *Lactobacillus rhamnosus*, 29.28% EC, and 8.79% lauric acid (bacteriostat) were shown to stabilize the viability of the probiotic bacteria for up to 12 weeks in aqueous beverages at ambient temperature. Other citations demonstrate the application of EC as an enteric barrier coating to protect encapsulated probiotic bacteria from stomach acid during ingestion (Chen, 2007). Such probiotics were positioned for application in livestock feed.

Edible microcapsules containing food additives were protected from high temperatures during food processing operations by incorporation of shell material that exhibited thermal gelation in the temperature range of about 90 to about 160°F (Lew, 2000). Preferred shell materials were MC, HPC, HPMC, and mixtures thereof, at a shell thickness of 3 to 50 μm. Additives could be added to the shell materials to moderate the gelation temperatures and optimize the thermal stability of the encapsulated flavorings. Sorbitol and glycerine, at 20 wt % levels, depressed the gelation temperature of MC (METHOCEL A15C) or of HPMC (METHOCEL F15C or METHOCEL K4M) by about 10 to about 36°F, while ethanol or propylene glycol, at 20 wt % levels, increased the gelation temperature of these cellulosics by about 45°F. This technology was targeted at additives in liquid-based foods such as soups, stews, and sauces.

The protection concept afforded by use of thermoreversible gelling polymers has been employed for stabilization of encapsulated probiotic bacteria in liquid-based food products (Penhasi, 2012). In this case, HPC and Pluronic™† ethylene glycol-propylene glycol-ethylene glycol triblock thermogel-forming polymers were preferably used as thermoprotective shell materials, with MC or HPMC also suitable as long as their higher gel temperature characteristics could be accommodated. Such materials underwent a sharp increase in viscosity associated with gelation at suitable processing temperatures, thereby preventing water penetration into the encapsulant, minimizing solubilization of the encapsulated additive, and limiting effect of higher bulk temperatures at the inner core. HPMC (METHOCEL E3 and E5) was employed as binder and subcoating layer materials, respectively. Improvement in the viability retention of probiotics through heat processing operations during food manufacture had been identified as a major challenge confronting broader acceptance of probiotics in foods (Anal and Singh, 2007).

Further, the use of temperature-responsive gels (polymeric hydrogels exhibiting a large temperature-dependent change in volume) for flavor retention at cooking temperatures has been discussed (Heitfeld, 2006; Heitfeld et al., 2008). Specifically, gels with a lower critical solution temperature (LCST) swell at low temperatures and collapse at high temperatures. In the collapsed state, the polymer acted as a transport barrier, keeping the volatile flavors inside.

Derivatized HPC gels that exhibited this volume change have been prepared containing an oil phase inside the gel. The flavor-loaded, encapsulated oil exhibited an increased release time when compared to similar gelatin capsules.

Examples such as these, which describe the employment of functional attributes of polymer materials in formulated systems for increased utility, such as enhanced thermal stability, show the exciting potential and value of a materials engineering dimension for improving product and process development in microencapsulated systems.

Furthering the concept of engineered solutions, blends of cellulosic polymers have been used to modulate the release rate of encapsulated additives. While the following example illustrates the controlled release of the water-soluble drug theophylline, extrapolation of the example to water-soluble food additives should be clear. Three microencapsulation coating systems—EC-cellulose triacetate (CTA) composite microcapsules, EC-CTA dual-walled microcapsules, and EC microcapsules containing CTA matrices—were prepared using nonsolvent addition phase separation methods. The

† Pluronic is a trademark of The Dow Chemical Company.

effects of cellulose triacetate on the release of theophylline were slower than those obtained from comparable EC microcapsules alone. The EC microcapsules containing CTA matrices exhibited longer release half-times and smaller particle size (smaller release surface area) than did the other capsule preparations (Wu et al., 1994). Additional work indicated that blends of EC with poly(α-methylstyrene) could also be used as a cowall material to achieve reduced rates of theophylline release versus that achieved with EC matrices, though only to about one-third the magnitude of release rate reduction achieved with the earlier EC-CTA matrix system (Tsai et al., 2000).

Blends of alginates with cellulose derivatives have been used to provide controlled additive release properties (Borgogna et al., 2010). HPMC was used to moderate controlled release behavior in microencapsulants consisting of alginate three-dimensional network hydrogels afforded by calcium crosslinking. HPMC also provided moderation of the rate of water evaporation from the microcapsules during drying (i.e., spray drying operations).

17.4 PROCESS CONSIDERATIONS USING CELLULOSIC POLYMERS

Functional cellulosic polymers also find advantage in microencapsulation process operations. A novel technique being developed for the rapid and low-cost production of microcapsules required core and coat materials of different dielectric constants. CMC was cited as offering the highest coating efficiency when treated at 1200 W microwave irradiation intensity for 400 seconds (Abbasi and Rahimi, 2008).

Fluid-bed coating processes are affected by both the processing parameters and the properties of the coating materials. Coating with three water-soluble cellulose derivatives—MC, CMC, and HPC—was investigated (Nienaltowska et al., 2009), with particular attention to how coating solution viscosities affected the coating quality. In this study, the best coating quality was achieved using HPC coatings, which were not influenced by solution viscosity and provided low variations in quality over a variety of fluid-bed coating runs.

Spray-dry processing of a variety of cellulosic polymers—HPMCAS, HPMC, MC, and NaCMC—was examined (Wan et al., 1992a). HPMC and MC produced coated products with similar dissolution profiles and particle flow properties.

While careful selection of various cellulose ester polymers can be made to control release of actives, the microencapsulation processes themselves can also be selected to moderate active compound delivery. A comparison of paracetamol drug release rates from three emulsion-based microencapsulation systems illustrated this control mechanism (Sprockel and Prapaitrakul, 1990). Emulsion-solvent evaporation (ESE), modified emulsion-solvent evaporation (MESE), and emulsion nonsolvent addition (ENSA) processes employing CAB microencapsulation of the active were compared. Drug release from microspheres prepared by ESE was significantly slower compared to MESE microspheres or ENSA microspheres, but the drug loading of ESE microspheres was significantly lower than those of MESE or ENSA microspheres. Preparation times for the various microencapsulation methods must also be considered when assessing these techniques.

Manipulation of the permeability of coatings and films has been shown to moderate the release profile of encapsulated additives. The effect of plasticizers—triethylcitrate, polyethylene glycol, propylene glycol, glycerin, and citric acid—on microencapsulated particles processed using spray drying of HPMC-containing formulations has been investigated (Wan et al., 1992b). Plasticizers exhibited effects on coating porosity by modification of film hydrophilicity−lipophilicity or cohesiveness, or by formation of pores in coatings as water-soluble plasticizers dissolve in aqueous matrices, or by changes in matrix pH (i.e., citric acid); all effects have contributed to changing dissolution rates of microencapsulated additives. In other cases, the encapsulated core active compounds themselves have been shown to plasticize shell coating materials. An example is α-tocopherol (vitamin E) plasticization of EC (Kangarlou et al., 2008). This points to another phenomenon worth considering when designing microencapsulated core−shell systems: are core components compatible with and will they mobilize into shell materials, possibly modifying desired barrier coating properties? Plasticization, then, provides another means to moderate the release and delivery of encapsulated food additives.

Additional means of using formulation modifiers, the use of pore formers or pore inducers to enhance the permeability or porosity of barrier coatings, was already mentioned (Prasertmanakit et al., 2009). The use of sucrose in that example illustrated the ability of water-soluble pore formers to increase release rates of hydrophilic additives from encapsulants, especially those using barrier coatings such as EC. Pore formers typically dissolve from hydrophobic coatings after exposure to aqueous fluids, leaving water-filled pores or voids in the shell coating and effectively reducing the path length for diffusion or dissolution through the coating shell. This formulation technique has been used to expedite release kinetics of actives from microencapsulants. Additional examples of the use of pore formers can be found in the literature (sucrose—Song et al., 2005; sodium chloride—Tikkonen and Paronen, 1992; HPMC—Sansukcharearnpon et al., 2010; HPC—Marucci et al., 2009).

Processing conditions can also be used to moderate film-coating permeability. The porosity of ethylcellulose films can be controlled by selection of the appropriate blend ratio of ethanol−water solvent in the spray drying process operation (Narisawa et al., 1994). Film porosity was found to increase considerably with decreasing ethanolic concentration, while the polymer concentration in the spray solution and the polymer molecular weight had only slight effects. Temperature and relative humidity also exhibited some effects on resultant film porosity. The relationship between film porosity and permeability (described by a power function) suggested that permeation of actives from the microencapsulants occurred through water-filled microscopic pores in the film coating, and therefore depended to a large degree on the film structure.

Therefore, both the choice of coating polymer materials and the processes and processing conditions used to perform microencapsulation should be evaluated to optimize loading levels of active ingredients, delivery release rates, and other important delivery criteria.

In summary, cellulosic derivatives find varied and useful application in the microencapsulation of food additives. Their functional properties, such as solubility, thermal gelation, and surface activity, can be used to engineer stabilization of emulsions or dispersants, to act as formulation binders, or to provide barrier protection, stabilization, and controlled delivery of active nutritional compounds during food formulation, processing, or ingestion in humans and animals.

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Chapter 18

The Use of Starch-Based Materials for Microencapsulation

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18.1 INTRODUCTION

Starch is one of the most abundant, economical, and renewable biopolymers (BeMiller and Huber, 2012). Starch by nature is an energy storage biopolymer for plant regeneration and it is commonly consumed as a major carbohydrate source for human nutrition. In 2006, world starch production was about 60 million metric tons mostly from the harvest of corn/maize (73%), cassava/tapioca (14%), wheat (8.1%), and potato (37%) (Fachverband der Starke-Industrie, 2006).

Over the years, the native and modified starches have been widely used as functional ingredients for food and beverage, industrial, cosmetic, and pharmaceutical applications (Singh et al., 2010). Starches typically provide texture, stability, and film forming to food products, or aid manufacturing processes. Starches are also widely used in non-food industrial applications, especially in the production of paper, paperboard, cosmetics, pharmaceutical products, renewable packaging, etc. (Thomas and Atwell, 1999; Bertolini, 2010).

Octenyl succinic anhydride modified starch (OSA starch), i.e., sodium starch octenyl succinate (SSOS) as a surface active food additive, is widely used in microencapsulations of oil-based flavors, nutrients, fragrances, agri-chemicals, and pharmaceutical actives, among other biopolymers such as gelatin, protein, and gum arabic (Wandrey et al., 2010). With designed structures and product consistency, OSA starches are preferred for many microencapsulation processes due to their high functionality, versatility, and efficiency. In spray drying microencapsulation, modified starches offer high oil retention, long shelf-life, and high manufacturing efficiency.

18.2 STARCH AND STARCH MODIFICATIONS

18.2.1 The Nature of Starches

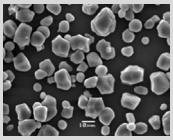
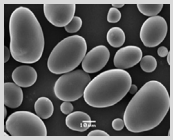
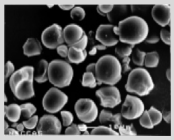
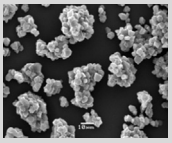
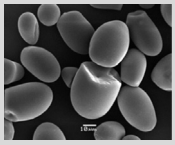
Starch occurs in a granular form in most cereal seeds and tuber roots. Starch granules vary in their size, shape, and crystallinity in different crops and different hybrids, as shown in Table 18.1.

The granule structure and its response to processing are very important for starch modifications and starch applications. Starch granule structure allows economical chemical modifications. Compared to water-soluble hydrocolloids, granules are easier to recover from the slurries in which they are modified. In most applications, starch undergoes gelatinization in order to become functional. Gelatinization is a transition of starch from crystalline/granule forms to a disassociated molecular form, typically taking place at elevated temperatures in the presence of high moisture. The gelatinization temperature of a starch depends on its granule morphology, crystallinity, molecular structure, and chemical modification. Pre-gelatinized starches are referred to as cold water-soluble starches (CWS) or pregel starches.

Very fine granules, such as rice, with particle size about 3 μm, may be used as substrates for delivery systems. Certain enzymes can create microporous structures on starch granules that help absorb active oil molecules (Zhao et al., 1996). Such active containing granules can be further coated by fluidized bed techniques to create different active release profiles. Fine granules may also be used for Pickering emulsions (Binks and Fletcher, 2001). Pickering

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00018-2

TABLE 18.1 A List of Different Starch Sources, Their Granule Sizes, Granule Morphologies, and Approximate Amylose Contents

Source of Starch	Corn	Potato	Tapioca	Rice	Sago
Granule size	14 μm	41 μm	17 μm	3 μm	33 μm
Amylose content	25%	20%	17%	19%	28%
Morphology (SEM)					

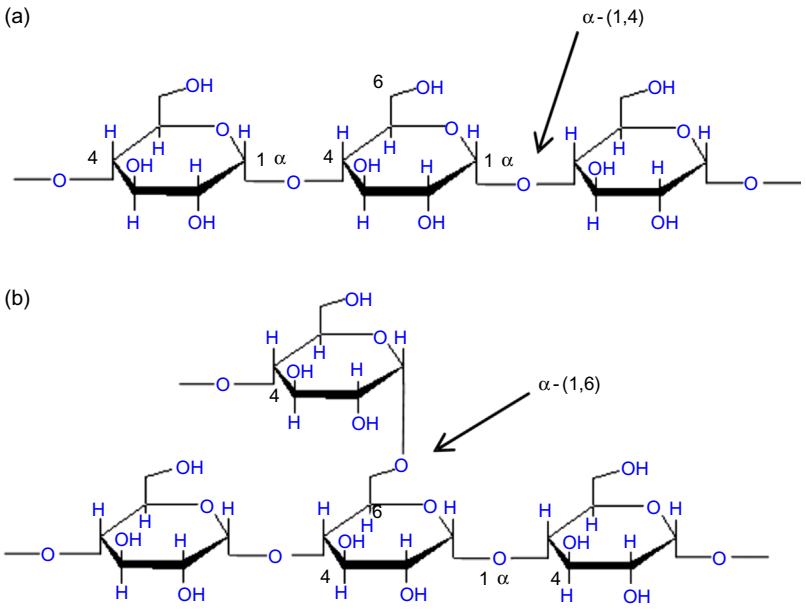


FIGURE 18.1 Drawings of molecular structures of (a) amylose (mostly with α-(1,4) linkage) and (b) amylopectin (containing significant α-(1,6) linkage).

emulsions are stabilized by fine granules other than amphiphilic surface active agents. Spray drying Pickering emulsion may potentially offer an approach for clean label encapsulation.

Starch [9005-25-8] chemically refers to poly-D-glucose linked by glycosidic bonds (Whistler and Paschall, 1967). There are two general types of starch molecules, amylose and amylopectin, as shown in Figure 18.1. Amylose [9005-82-7] has a linear molecular structure through predominantly α-(1,4) linkages, although some amylose molecules are slightly branched. Amylopectin [9037-22-3] has a branched molecular structure consisting of short chain amylose, having α-(1,6) linkage at the branch points. Regular starches contain 70−75% amylopectin by weight. Special hybrids may have extremes of amylose or amylopectin content (Table 18.1). For example, a waxy corn hybrid contains about 99% amylopectin, and Hylon VII (a product of Ingredion) contains about 70% amylose.

Amylose and amylopectin, due to their difference in molecular structures, are expected to have distinct behaviors (Frazier and Donald, 1997), as summarized in Table 18.2. Linear amylose molecules have strong intramolecular and intermolecular interactions. They form double-helical structures, which tend to crystallize. Such crystalline structure is utilized for improved digestion resistance (Ebihara, 2005) and for the spheronization process in drug delivery (Dukic et al., 2006). Strong molecular interactions allow amylose to form mechanically strong films (Krogars et al., 2003). For the same reason, amylose typically requires a very high temperature cook-up, and upon cooling, it tends to reassociate, i.e., retrograde, observable by gel formation, increasing viscosities, or precipitation, as shown in Figure 18.2A.

Amylopectin has weaker intermolecular interactions due to steric hindrance by the branched structure. It therefore has better solution stability, and is suitable for high moisture applications. Amylopectin also has much higher molecular

TABLE 18.2 Comparison of Amylose and Amylopectin

	Linkage	Structure	MW	Properties	Applications
Amylose	α-(1,4)	Linear	10^{5-6}	Retrogradation Moisture resistance High T_g	Film forming Gelling Controlled digestion Lipid complexation
Amylopectin	α-(1,6) α-(1,4)	Branched	10^{7-8}	Solution stable High viscosity	Texturizing Stabilization Encapsulation Emulsification

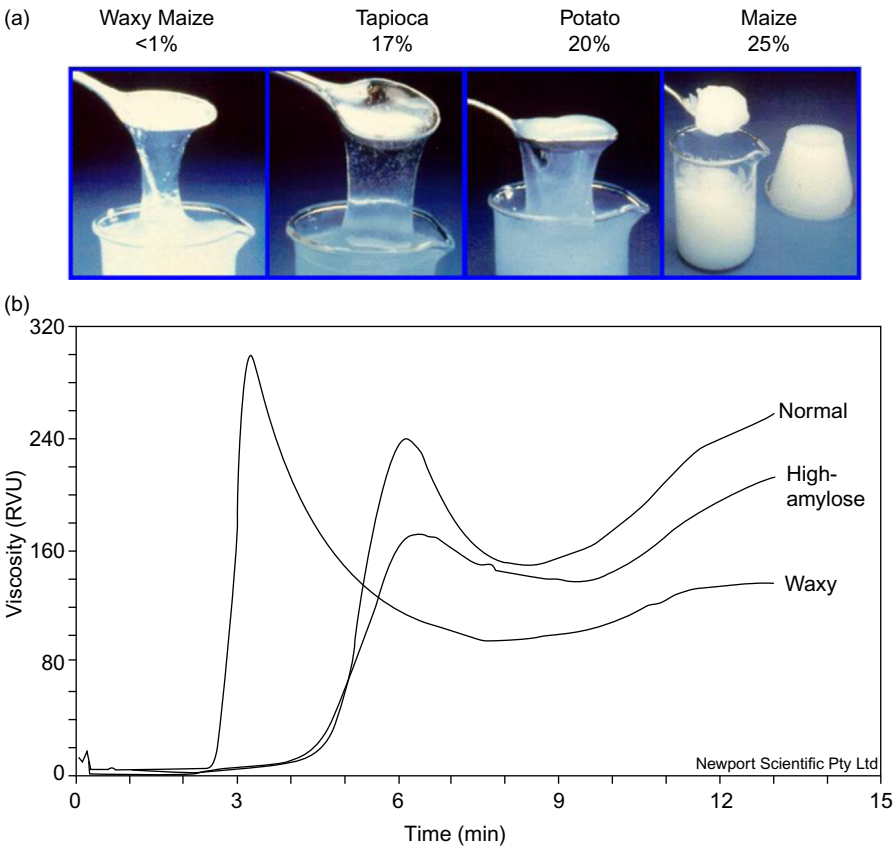


FIGURE 18.2 Spoon texture of 6% water solution of different starches with increasing amylose content from <1 to 25%.

weight, up to 50 million daltons (Millard et al., 1999), providing higher viscosifying power compared to amylose. Its gelatinization temperature is lower, and it is typically easier to use than amylose.

Figure 18.2B shows amylose impact on gelation and peak viscosity of rice starches using Rapid Visco Analyzer RAV-4 (Newport Scientific, Warriewood, Australia) according to the AACC 76-21 method (American Association of Cereal Chemists, 2000). The onset of viscosity, the peak viscosity in RA unit showed that waxy or amylopectin has lower gelatinization temperature and higher viscosifying power (Blazek and Copeland, 2008).

18.2.2 Food Starch Modifications

Modified starches are used in almost every food and beverage application due to the benefits of sensory enhancement, storage stability improvement, nutrition enrichment, and acting as a processing aid. General chemistries and processes for starch modifications are well documented (Wurzburg, 1986; BeMiller and Huber, 2012). Important modifications

via hydrolysis, chemical modification, physical treatment, and enzymatic, hydrophobic modification relevant to microencapsulation are highlighted below.

Starches, especially in waxy varieties, are high-molecular-weight polymers (Table 18.2). Their molecular weights are often reduced by hydrolysis to achieve lower viscosities and enhanced functionalities. Such viscosities can be characterized by modern viscometry (e.g., Brabender/RVA) and traditionally by water fluidity (WF), which measures the reciprocal of viscosity.

Hydrolysis naturally occurs in the human digestion process. It is not a surprise that acids and enzymes are widely used to degrade starch. Hydrolysis of starch can also be achieved by oxidation using peroxides, or mechanical scissoring via extrusion.

Heating starches at high temperature under acidic conditions is a unique type of hydrolysis process, in which hydrolyzed sugary fractions repolymerize to generate additional branching. This process is called dextrinization and the resulted products are called dextrins (Stephen et al., 2006). Dextrins are highly water soluble and stable, even at low temperatures, due to low molecular weight and branched structures.

Enzyme hydrolysis is widely used to produce hydrolysates of very low molecular weight or low degree of polymerization (DP), including maltodextrins (MD), oligosaccharides, syrups, and sugars. Dextrose equivalent (DE) is a term commonly used to describe the degree of hydrolysis, based on the fact that each molecule has one reducing end (or reducing sugar) (Campbell and Farrell, 2012). In short, DE of dextrose is 100, i.e., 100% dextrose units are reducing sugars. DE of a low-molecular-weight starch derivative is defined as 100/DP. For instance, a di-sugar such as maltose or sucrose has a DP of 2; therefore a DE of 50, i.e., 50% dextrose units are reducing sugars. A maltodextrin having a DP of 5 has a DE of 20, etc. DE of common products based on popular conventions (Nakakuki, 2002) are summarized below:

- Sugars: DE 50−100
- Syrups DE 20−45
- Oligosaccharides: DE 10−50
- Maltodextrins: DE 5−20.

18.2.2.1 Chemical Modifications

In food manufacturing, products may undergo high shear, high temperature, and severe pH and salinity processes in order to meet product sensory, safety, and stability requirements. Two major types of chemical modifications are widely used to improve the viscosifying power, process tolerance, and product stability:

(1) Stabilization, meaning linking small molecule moieties such as propylene oxide, ethylene oxide, and acidic anhydride onto starch backbones, which reduces the tendency of retrogradation and therefore improves cold temperature or freeze−thaw stability. Such modifications also reduce the gelatinization temperature of starch granules.

(2) Crosslinking, typically combined with stabilization, enhances the viscosity profile in food processes and applications, and improves process tolerance. Such chemical modifications also influence starch interactions with other ingredients.

Both treatments hinder digestion of starch but only crosslinking is utilized in controlled release, especially for pharmaceutical applications (Dumoulin et al., 1999; Mulhbacher et al., 2001; Simi, 2007; Mundargi et al., 2008).

18.2.2.2 Physical Treatments

Physical treatment typically involves temperature, moisture, and shear to alter the product form for improved functionality and process convenience. One major treatment is to pre-gelatinize starches using jet cooking/spray drying, drum drying, extrusions, and compaction processes. Such pregel starches may also undergo agglomeration for enhanced water dispersion. Pregelled or partially pregelled starches are often used as pharmaceutical excipients. Thermally treated starches, as a clean label alternative, may provide similar functionality to chemically treated starches. Physical treatment may also impact the crystallinity/polymorphism of starches and therefore their digestion profile.

18.2.2.3 Enzymatic Treatment

Besides α-amylase, which is widely used for random hydrolysis of starches, there is an increasing list of specialty enzymes emerging in the market. Specialty enzymes can produce unique molecular structures and unique performances, otherwise not achievable by chemical modifications. For instance, β-amylase and glucoamylase hydrolyze α-(1,4)

linkages producing maltose and glucose, respectively. Isoamlyase, however, hydrolyzes starches at α-(1,6) linkages. Transferase enzymes may extend the linear chain length, or generate branched structures.

18.2.2.4 Hydrophobic Modification

For encapsulation and emulsification applications, starches are often modified by octenyl succinic anhydride, which is an eight hydrocarbon hydrophobe with one double bond. The reaction opens up the anhydride ring and forms a sugar ester likely at positions 2 and 3 (Bai and Shi, 2011), and at the same time forms a carboxyl group, which renders OSA starch slightly negatively charged. The hydrophobic treatment, typically in combination with hydrolysis, makes a starch very surface active.

FDA allows a maximum of 3% OSA treatment on a dry-weight basis for food additives. Other regulations are similar but with variations on residual OSA level and its testing method. Table 18.3 summarizes key regulatory limits for OSA starches in food.

Theoretically, 3% OSA treatment is equivalent to a degree of substitution (DS) around 0.02, which means on average that for every 50 glucose units there is only one octenyl hydrophobe. This suggests that an OSA starch is still very hydrophilic, or has a high hydrophilic−lipophilic balance (HLB) (Griffin, 1949) number.

The method for OSA treatment was described by O.B. Wurzburg, P. Trubiano, and N. Lacourse, and later reviewed by Singh (Liu et al., 2008; Singh et al., 2010). Figure 18.3 describes a general process of OSA treatment, which is often combined with hydrolysis for better surface activity and process efficiency. By choosing a proper starch base, method and degree of hydrolysis, physical treatment, and a combination of them, one can tailor-make optimized molecular structures that best suit specific encapsulation applications.

TABLE 18.3 Regulatory Limits for Starch Sodium Octenyl Succinate. The Testing Methods for Octenyl Succinic Groups are Defined Independently for Each Regulation/Region

	Japan	JECFA	US/FCC	EU
OSA treatment limitation	–	–	≦3.0%	–
Octenyl succinic groups	≦3.0%	≦3.0%	–	≦3.0%
Residual octenyl succinic acid	≦0.8%	≦0.3%	–	≦0.3%

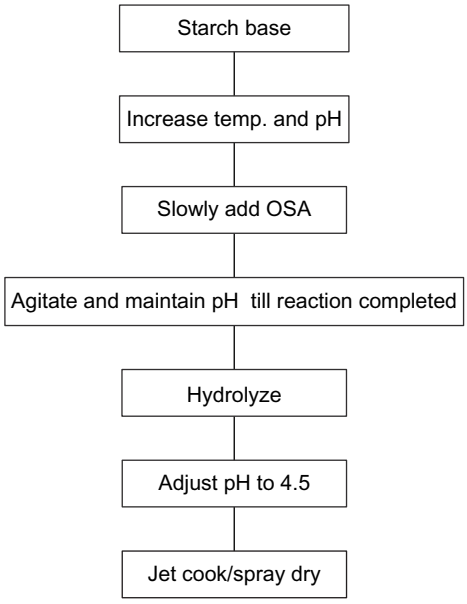


FIGURE 18.3 A typical process of octenyl succinate anhydride treatment.

18.3 CHARACTERISTICS OF OSA STARCHES

OSA starch is highly surface active and its emulsification properties are less sensitive to salinity and pH conditions in comparison to gum arabic and proteins (Qian et al., 2011). Figure 18.4 shows that a well-designed OSA starch is very effective in reducing surface/interfacial tensions. It is worth mentioning that OSA starch is not salt sensitive, due to the fact that it has a very low linear charge density (Qian et al., 2011). Typically, with addition of 1% NaCl, the surface tension may decrease by about 5%.

Figure 18.5 shows that the surface tension of OSA starches increases with increasing pH. This may be due to increased charge density at high pH, which in principle further increases the HLB value, resulting in higher surface/interfacial tension.

OSA starches typically have low viscosities, which is extremely important for spray drying, spray congealing, extrusion, and other encapsulation processes. The low viscosity allows high process efficiency as well as high performance, which will be discussed in detail in Section 18.4.1.

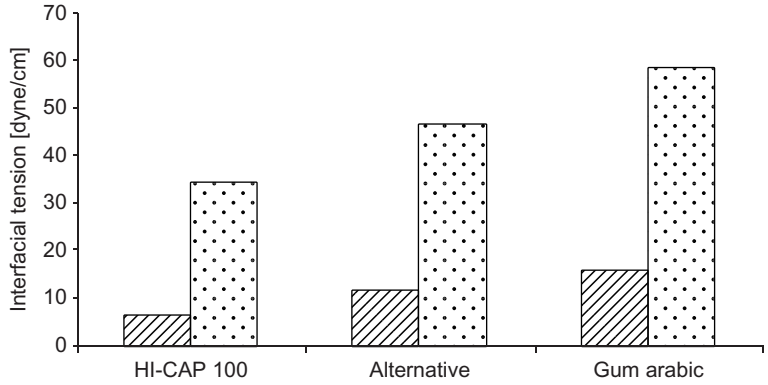


FIGURE 18.4 D-limonene–water interfacial tension (lines), and surface tension (dots) of HI-CAP® 100, an alternative OSA starch, and gum arabic 10% aqueous solutions at pH 3.

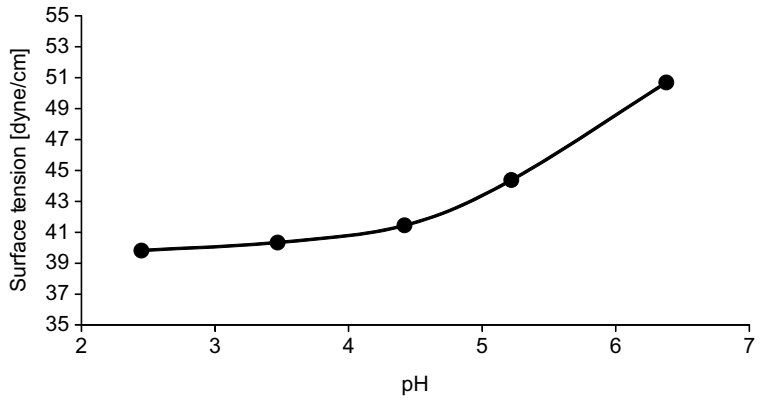


FIGURE 18.5 Surface tension of CAPSUL® as a function of pH, using 10% water solutions.

18.4 USING MODIFIED STARCHES FOR MICROENCAPSULATION

Replacing gum arabic using modified starches dates back to the 1870s for adhesive applications (Wurzburg, 1986). In the last several decades, hydrophobically modified starches have been widely used to replace gum arabic in microencapsulation and in beverage emulsion applications.

Naturally harvested gum arabic varies in its protein content and protein conjugation with arabinogalactan, depending on the growth and harvest conditions (Al-Assaf et al., 2007), resulting in inconsistent emulsification and encapsulation performances. Starch modifications follow tight control in the manufacturing process and final product specifications, and therefore OSA starches offer high consistency from batch to batch. Their basic properties and advantages in encapsulation are summarized in Table 18.4.

TABLE 18.4 Summary of Advantages of using Modified Starch for Spray Drying Microencapsulation

Features	Performance	Benefits
High surface activity	Fine oil droplet size	Low surface oil
	High specific surface coverage	High oil load
Low viscosity	High solids	Production efficiency
		High flavor retention
Film forming	Dense matrix formation	Active protection

18.4.1 Typical Spray Drying Practices Using OSA Starches

Table 18.5 lists examples of emulsion formulations at 40% solids, targeting 20% and 40% oil (flavor) load. In industrial practices, the flavor is typically compounded with multiple components, and so is the matrix. The preparation of spray drying ready emulsions is rather straightforward. Cold water-soluble starches are typically easy to hydrate. However, slow adding, gentle agitating, and optionally heating the solution to around 43°C helps faster dissolution and avoids foaming. Should any foam form, allow enough time for it to settle, and then slowly add flavor oil under high shear to form an emulsion. High energy homogenization may be needed to achieve the desired emulsion droplet size of about 1 μm (measured by Beckman Coulter) in case of heavy oils such as omega-3 and vitamin E. A two-stage APV homogenizer is often used, and the typical first stage and second stage pressures are 4500 PSI and 500 PSI respectively.

During the entire spray drying process, the emulsion should be kept agitated in the feeding tank and the emulsion droplet size should not change significantly. Figure 18.6 shows emulsion droplet size and its pot-life stability, where emulsions were made using a matrix material containing CAPSUL® (OSA starch of Ingredion) and maltodextrin at

TABLE 18.5 Examples of 40% Solids Flavor Emulsion Formulations for Spray Drying with Oil Load of 20% and 40%

	20% Oil Load	40% Oil Load
Flavor	8%	16%
Matrix material	32%	24%
Water	60%	60%

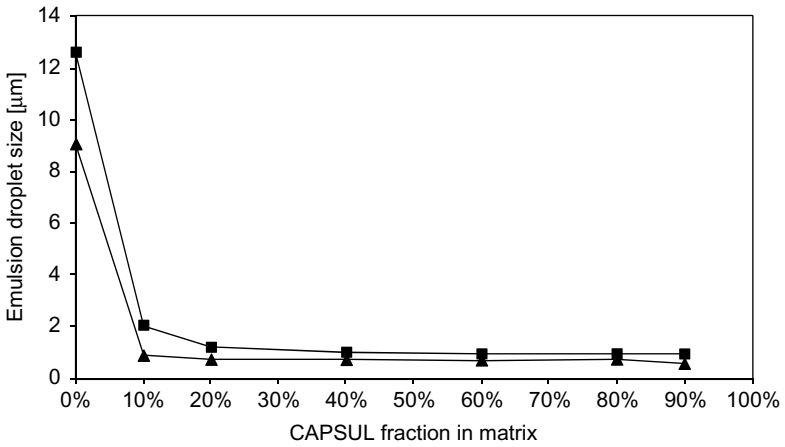


FIGURE 18.6 Flavor emulsion droplet size of fresh emulsions (triangles) and aged emulsions (squares) stored at room temperature for 24 hours, as a function of CAPSUL percentage in the matrix. The emulsion contains 8% one-fold orange flavor, and 32% matrix, which contains CAPSUL and maltodextrin (DE10, a product of Ingredion). Emulsions were produced using 7000 rpm for 3 minutes using a ROSS HSM-100 LCI high shear mixer.

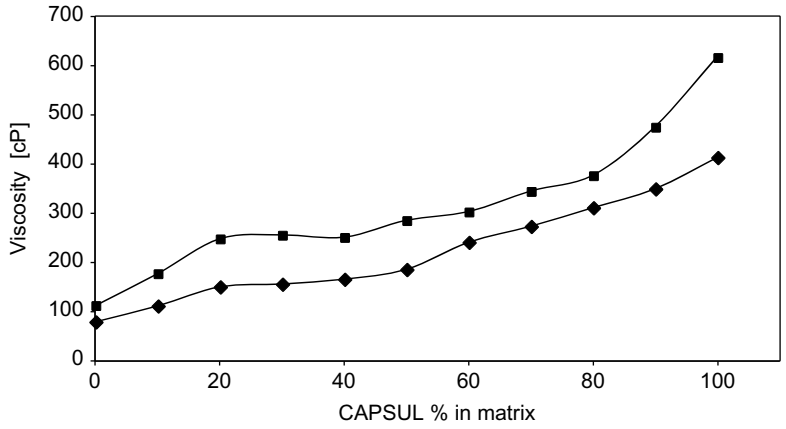


FIGURE 18.7 Brookfield viscosity as a function of CAPSUL fraction in the matrix, where the matrix contains CAPSUL and maltodextrin DE10. Diamonds represent 40% matrix material in water, and squares indicate emulsions containing 10% one-fold orange flavor and 40% matrix material.

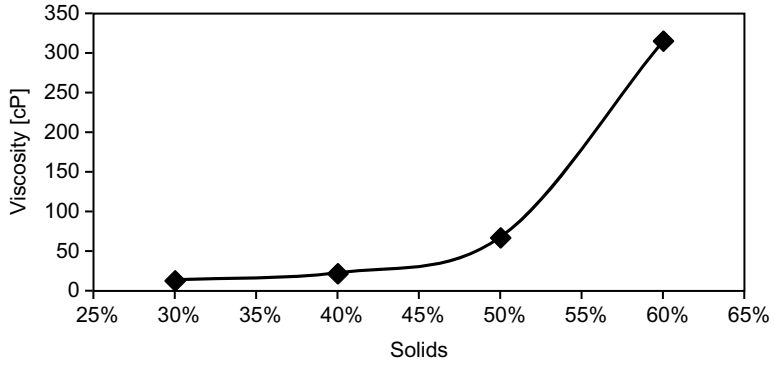


FIGURE 18.8 Brookfield viscosity of emulsions with different solids levels, where in the solids, 20% is one-fold orange flavor, 16% is CAPSUL, and 64% is maltodextrin DE18.

different ratios. It is evidenced that CAPSUL has sufficiently high emulsifying power that even a small amount of CAPSUL can produce fine emulsion droplets, which are stable during 24 hours' storage under ambient conditions. However, as the amount of CAPSUL decreases to about 20% in the matrix, or a CAPSUL:flavor ratio of about 1:1, both emulsion droplet size and stability deteriorate.

Figure 18.7 shows the viscosity of solutions and emulsions of encapsulation matrices containing CAPSUL and maltodextrin at different ratios. Figure 18.8 shows the viscosity of emulsions as a function of solids. Figures 18.7 and 18.8 provide guidance for formulating the emulsions with the right viscosities. Overall CAPSUL offers low viscosity for a large range of solids and compositions, including at 50% solids and using low DE maltodextrin.

Table 18.6 shows a summary of typical spray drying conditions using modified starches. Many of these parameters are interdependent. For instance, the outlet temperature is influenced by the inlet temperature and the solids level, as well as the feed rate. The powder particle size is influenced by the atomization, the viscosity, and the feed rate. Practically, the outlet temperature is a good starting point to set the process conditions. Too low an outlet temperature may lead to high moisture in the powder, causing low yield or caking.

Once the microcapsules are formed, they are analyzed for oil retention, surface oil, morphology, flowability, etc. The microcapsules are typically aged at 50°C for 14 days and then tested for the oxidation. Sometimes the reconstitution of microcapsules into water is also observed. Table 18.7 lists some recommended analytical methods for emulsions and spray-dried microcapsule powders. Surface oil is typically extracted by a non-destructive solvent, such as hexane or pentane. The total oil is extracted by a destructive solvent such as acetone or an alcohol−water mixture, and in some cases with the aid of enzyme hydrolysis. The extracted oils are quantified by HPLC/GCMS (high performance liquid chromatography/gas chromatography−mass spectroscopy). The total oil can also be analyzed by distillation and gravimetry. To measure the oxidation in case of orange flavor, limonene oxide/carviol/carvone (Thomas and Bessière, 1989) are used to account for the total oxidates using head space GC. The aged samples need to be handled carefully to avoid loss of volatiles. An elevated temperature is typically used during measurement to ensure that all volatiles are sampled.

TABLE 18.6 Typical Flavor Spray Drying Conditions using OSA Starches

Parameters	Conditions
Outlet temperature	80–95°C
Inlet temperature	160–200°C
Flavor load	20–40%
Solids in emulsion	30–50%
Emulsion viscosity	<200 cP
Emulsion droplet size	≈1 μm
Centrifual atomizer speed	20,000–25,000 rpm
Power particle size	30–70 μm
Feed rate	Depending on dryer capacity
Powder moisture	3–6%

TABLE 18.7 Summary of Analytical Methods Useful for Flavor Microencapsulation

	Equipment	Method	Typical Values
Emulsion viscosity	Brookfield	Spindle LV62/60 rpm	50–300 cP
Oil droplet size	Beckman Coulter	Static light scattering	1–2 μm
Powder particle size			30–70 μm
Surface oil[a]	HPLC	Hexane extraction	<1%
Oil retention[b]	HPLC/Gravimetry	Alcohol extraction	80–90%
Oxidation level[c]	Headspace GC/FID	Aging @ 50°C × 14 days	<1000 ppm
Morphology	SEM	Microtome Gold vapor deposition	500–5000 × magnification

[a]Surface oil is defined as the weight of hexane extracted oil divided by the weight of microcapsule powder.
[b]Retention is defined as the amount of retained flavor divided by the amount of flavor formulated in the emulsion.
[c]Oxidation level is defined as the total weight of oxidates divided by the weight of retained flavor oil.

18.4.2 A Dynamic Model and its Relevance to Matrix Materials

Figure 18.9 shows morphological evolution of a droplet in a highly dynamic spray drying process; an atomized droplet, typically on the order of 40–90 μm to begin with, evaporates and dries within a timeframe of minutes:

1. The atomized droplet contains 50% water, 20% flavor, and 30% matrix material (as an example).
2. On exposure to high temperature, the droplet first dries from the outer surface, a crust so forming.
3. Water vapor permeates from inside through the crust, and at the same time, the solids continuously deposit on the inner surface of the crust, thickening the shell. In the end, all water evaporates a void is left in the center.

Figure 18.10 shows scanning electron microcopy (SEM) images of spray-dried microcapsule morphology and the cross-section of a microcapsule. The cross-section of a microtome microcapsule clearly shows the void in the center and the shell matrix with oil droplets embedded. The thickness of the shell and the size of the void may be influenced by many factors, such as the solids content, the drying temperature, and the relaxation of matrix molecules. In general, the higher the emulsion solids/the lower the spray drying temperature/the faster the matrix relaxation, the smaller the void.

During step 3, the process is rather complex. Water evaporation and matrix material deposition are simultaneous, and at the same time volatile flavor molecules may also evaporate. Flavor molecules, however, permeate slower due to

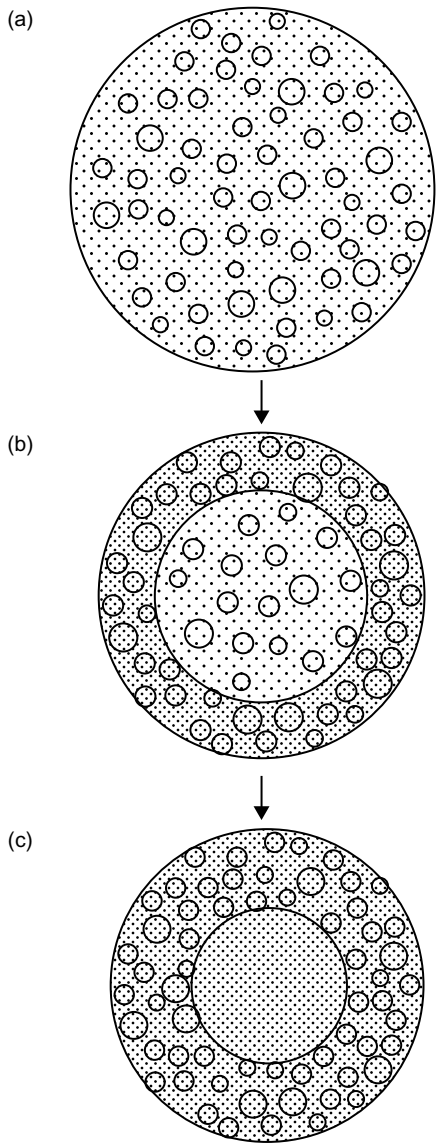


FIGURE 18.9 Schematic illustration of spray drying dynamics. The big circles represent atomized water droplets, small circles represent oil droplets (1−2 μm on average), and dots represent soluble carbohydrate molecules, where a) is a freshly atomized water droplet, typically on order of 40−90 μm, b) crust formed by initial drying from the outer surface, and the core remains having similar composition to a), c) final microcapsule with a void after drying all water.

their reduced vapor pressure by the interfacial layer, lower solubility in a hydrophilic matrix, and higher molecular weight (Section 18.2.2). A small amount of flavor, especially those high volatile or relatively polar fractions, may still permeate through the crust as the primary factor for flavor loss. Air molecules also permeate through the same matrix to fill the void. In case the air molecules permeate slower than water evaporation, the void likely ends up as a partial vacuum. In certain conditions, the shell may fold inward like a deflated basketball, as shown in Figure 18.10.

Such a dynamic model for spray drying encapsulation suggests that high solids level improves the retention of flavors. First of all, higher solids mean shorter drying time. Second, flavor loss has to go through a denser and thicker wall during the process, as shown in Figure 18.9b. It was reported that higher solids significantly improve the retention of flavors, as shown in Table 18.8 (Charve and Reineccius, 2009). Figure 18.11 also shows that higher solids improve the flavor retention as well as flavor protection.

Furthermore, the dynamic model suggests that the preferred matrix materials should have a fast relaxation time to form a dense matrix during the fast drying process. Sugary materials are therefore often used to plasticize

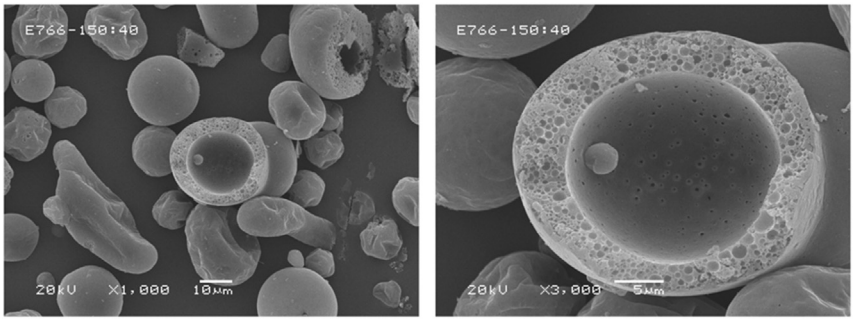


FIGURE 18.10 SEM (scanning electron microcopy) images of spray-dried microcapsules and the cross-section of a microcapsule.

TABLE 18.8 The Retention of Each Compound in a Model Flavor after Spray Drying using 10% and 40% Modified Starch (CAPSUL, a product of Ingredion) in the Emulsions

Flavor Compounds	Retention 10% Modified Starch	Retention 40% Modified Starch
(E)-2-hexenal	3%	63.7%
(R)-(+)-limonene	51.5%	76.1%
Citral	60.6%	97.7%
(E)-cinnamaldehyde	48.9%	85.1%

The model flavor was compounded with (E)-2-hexenal (5%), (R)-(+)-limonene (85%), citral (5%), and (E)-cinnamaldehyde (5%). The flavor to modified starch ratio was 1:4.
Source: Courtesy of G.A. Reineccius.

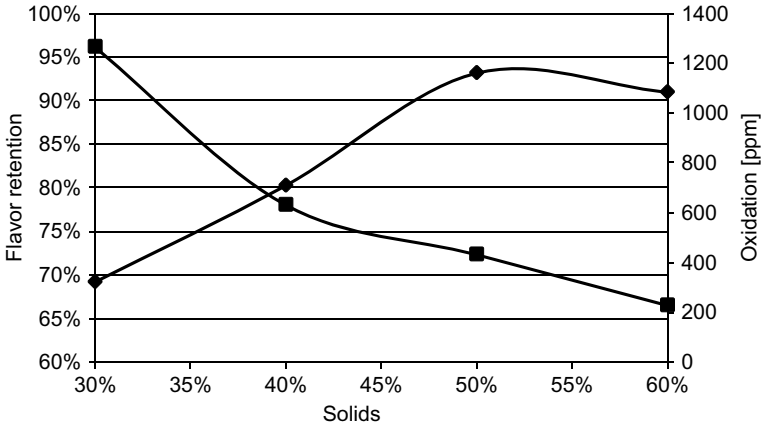


FIGURE 18.11 Impact of emulsion solids on flavor retention (diamonds) and oxidation level (squares). In the solids, 40% is one-fold flavor, 16% is CAPSUL, and 64% is maltodextrin (DE18).

high-molecular-weight films and fill molecular voids. A denser matrix, or glassy matrix, slows down oxygen permeation through the matrix and protects active from oxidation to a greater extent (Ubbink and Schoonman, 2005). However, too much of plasticizers will significantly reduce the T_g of the encapsulation matrix, causing low production yield and higher moisture sensitivity. Empirically, the glass transition temperature of finished microcapsules (similar to that of matrix materials) should be at least 30°C higher than the ambient condition.

A denser matrix, on the other hand, also reduces the permeation of volatile flavor through the matrix during the storage (Krishnan et al., 2005). The vaporized flavor is another mechanism for flavor oxidation, but its significance compared to oxidation in the matrix has not been thoroughly studied.

There have been various attempts to use amylose for spray drying encapsulation due to its film-forming property. Many have reported that amylose films exhibit significantly lower oxygen permeation rate compared to amylopectin films (Forssell et al., 2002).

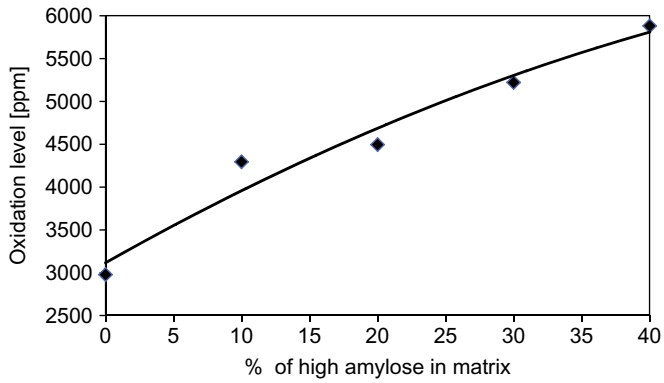


FIGURE 18.12 The oxidation level as a function of amylose content in the matrix after microcapsules were aged at 50°C for 14 days. The spray-dried microcapsules contained 30% one-fold orange flavor, and 30% CAPSUL, and 40% additional carrier that contains high amylose starch and amylopectin starch where both starches were converted to about 80WF.

However, these films are often solution cast where amylose molecules have sufficient time to relax, which does not resemble the rapid film formation during the spray drying process.

Figure 18.12 shows that a high amylose starch may not necessarily provide better oxidation resistance. This is contradictory to the film permeation studies but consistent with the dynamic model. One may have to carefully design an amylose structure in order to take advantage of its benefits.

18.4.3 Case Studies

18.4.3.1 Case 1—Flavor Encapsulation in the Spray Drying Process

Flavors are typically volatile and are prone to oxidation. The challenges for spray drying flavors are to prevent flavor loss during the drying process and flavor oxidation during storage, in order to achieve desirable encapsulation economy and flavor sensory profile.

Figure 18.13 shows the results of spray drying one-fold orange flavor using 40% solids emulsions with different matrix materials. It is observed that the viscosity of these emulsions with a constant oil load depends monotonically on the solution viscosity of the matrix material. CAPSUL has the lowest viscosity and gum arabic typically has much

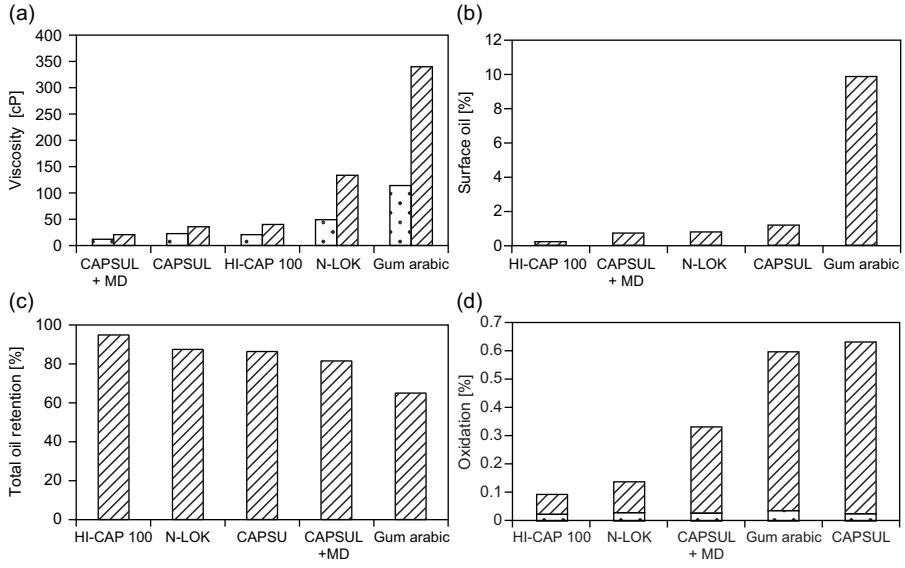


FIGURE 18.13 Results for spray drying flavor encapsulation, using emulsions containing 16% one-fold orange flavor, 24% matrix, and 60% water. (a) Viscosities of emulsions (lines), and viscosity of matrix solutions (dots) before adding the flavor; (b) surface oil; (c) retention of flavor; (d) flavor oxidation after aging at 50°C for 14 days. Dots are for fresh samples, and lines are additional oxidation during aging.

higher viscosity than OSA starches. HI-CAP®100 (OSA starch of Ingredion) shows the best performance on surface oil, flavor retention, and oxidation resistance. N-LOK® (OSA starch of Ingredion) showed high resistance to oxidation.

Hydrolyzed OSA starches, as shown in Figure 18.13, demonstrated advantages in 40% flavor loading encapsulation, and differences in emulsion viscosity, surface oil, flavor retention, and flavor oxidation. One should choose the best starch or combination based on what is most important for a specific application. Lower oil loadings, 20% for example, are relatively less technically, but associated with a high cost of raw material and manufacturing economy. Owing to concern regarding the explosive potential of powder, it is unusual for flavors with an oil load in excess of 40% to be spray dried.

Starch has a bland flavor and is known not to interact with flavors strongly, compared to other ingredients, yet it retains certain flavor compounds to a greater extent than others (Tietz et al., 2007; Charve and Reineccius, 2009). Starch offers fast flavor release due to high digestability in the presence of saliva enzymes when consumed.

18.4.3.2 Case 2—Vitamin Encapsulation

Oil-soluble vitamins, especially vitamin E, are widely used for food fortification and dietary supplements. Vitamin E oil can be emulsified and spray dried similarly to flavor oils using modified starches, with the following exceptions:

- Due to high viscosity of vitamin E oil, both oil and starch solutions are typically heated up prior to emulsification, and high pressure homogenization is employed to achieve a fine emulsion droplet size.
- Vitamin E is less volatile compared to flavors, and therefore vitamin E can be spray dried at an oil load of 50% or higher with no oil retention issue.
- Oxidation is not a crucial factor as vitamin E is often esterified to avoid oxidation during shelf-life.

Surface oil thus becomes one of the most important performance indicators. Figure 18.14 shows that surface oil heavily depends on the emulsion droplet size. Assuming oil droplets uniformly deposit across the matrix (in the microcapsule shell), the probability for a droplet to locate at the air−matrix interface is proportional to d/t, where d is the emulsion droplet size and t is the shell thickness. In principle, the oil droplet size needs to be much smaller than the shell thickness. However, if the oil droplet size is too small, then the oil−matrix interface increases, which may increase the chance of higher oxidation (Soottitantawat et al., 2005).

Modified starches in vitamin spray drying typically provide fine particle size (Figure 18.14), low surface oil (Figure 18.15), and low viscosity for production efficiency. It was reported that modified starches can be used for nanoencapsulation of vitamin E (Chen and Wagner, 2004). In such applications, the vitamin E oil was emulsified into droplets on the order of 100 nm, which enables microcapsules to reconstitute and form clear beverages.

Suppliers of encapsulated vitamins are required to provide active stability over shelf-life. It was found in certain cases that extraction of vitamin E becomes difficult when starch-based microcapsules are stored over a long period of

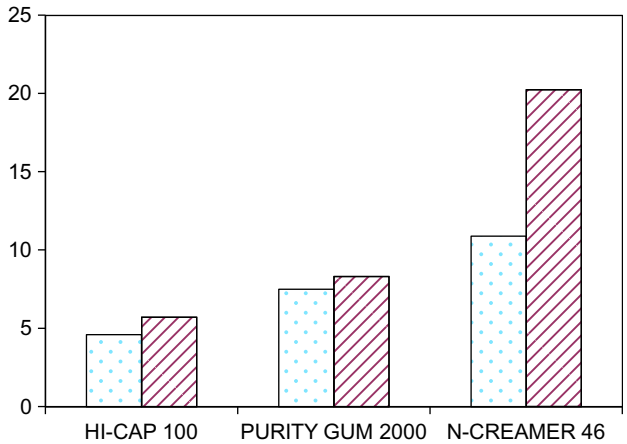


FIGURE 18.14 Emulsion droplet size (dots) in units of μ/10 and surface oil (lines) in percentage for spray drying 50% vitamin E acetate. Emulsions contain 23% of vitamin E acetate, 23% OSA-starch, and 54% water.

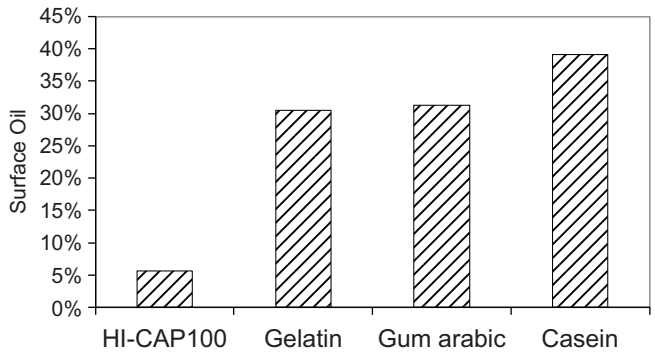


FIGURE 18.15 Surface oil of spray-dried powder of 50% vitamin E acetate using different ingredients. The spray drying emulsion contains 23% vitamin E acetate, 23% matrix material, and 54% water.

time, potentially due to retrogradation or realignment of amylose and amylopectin. Using α-amylase to digest starch prior to oil extraction was demonstrated to be an effective remedy.

18.4.3.3 Case 3—Fat Encapsulation

Fats are often encapsulated to deliver flavor to coffee, savory, and other food applications. Traditionally, casein has been used due to its high emulsifying power and the pleasant dairy note. Modified starches have also been attempted for fat encapsulation due to their cost effectiveness. It has been demonstrated lately that modified starches may partially or totally replace casein, and provide similar performance and sensory. Modified starch therefore became increasingly interesting for food applications, especially at a low pH under which proteins tend to be precipitate.

18.4.3.4 Case 4—Gelatin Replacement in Spray Congealing

Spray congealing has been well established in recent years based on the unique characteristics of gelatin. As discussed in Chapter 20, gelatin provides emulsification and gelling functionality to enable the spray congealing process. Spray congealed products, beadlets, are typically in the range of a few hundred microns providing excellent active protection and superior process tolerance in tableting or pelletizing.

There have been multiple drivers for replacing gelatin in both human and animal nutrition applications. As a response, modified starch-based technologies are emerging in the market. It was disclosed (Chen et al., 2011) that modified starches were successfully used in vitamin A encapsulation to produce beadlets for animal feed, where starch-based beadlets provide high performance in both active retention over time and feed process tolerance.

18.4.3.5 Case 5—Extrusion

Extrusion encapsulation has established its position as a low moisture or nearly anhydrous process, and for its excellent long-term stability. The research on extrusion encapsulation has been overwhelmingly active, as seen in the patent literature. Despite the attractiveness of extrusion, high oil load extrusion remains challenging. OSA starches are reported to be highly effective in this application (Zasypkin and Porzio, 2004) and CAPSUL is a preferred choice due to its low viscosity.

18.4.3.6 Case 6—Plating

Porous starches can be used for plating, which is very attractive from a cost point of view (Bolton and Reineccius, 1991). Simply blending flavor or other oils into a porous starch allows oil to be absorbed. Such powder may be coated by a secondary process to achieve enhanced protection and release profiles.

Special processing can convert a starch granule into an extremely low density and porous structure. Figure 18.16 shows an SEM image of N-ZORBIT® M (a product of Ingredion) that has a bulk density around 0.1 g/cm^3. The porous structure allows the absorption of flavors, oils, glycerine, etc., to as much as two times its own weight, yet the powder remains flowable. The oil can be released upon hydration of the starch.

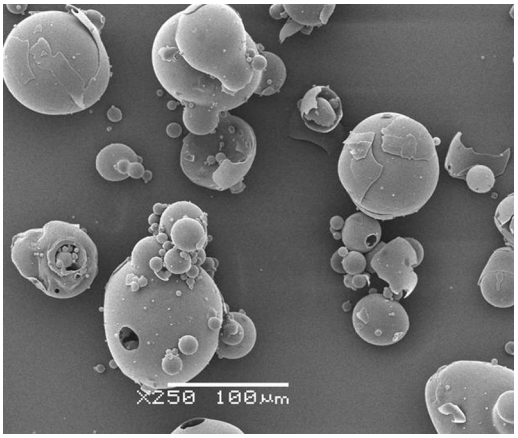


FIGURE 18.16 SEM microscopy of N-ZORBIT® M, a porous starch for oil plating.

18.5 CONCLUSION

Starch is one of many functional biopolymers used in microencapsulation. What makes it different is that starch has many naturally occurring varieties for choosing a preferred structure. Well-established enzymatic, chemical, and physical modification technologies allow tailor-made molecular structures for optimized performance.

Hydrophobically modified starches, acting as surface active agents, are highly effective, consistent, versatile, and economical in microencapsulation of oil-based flavors, micronutrients, fragrances, agri-chemicals, and pharmaceutical actives. In spray drying microencapsulation, modified starches offer high oil load, high volatile retention, long shelf-life, and high manufacturing efficiency. Specifically:

- High surface activity enables high oil load, which lowers both raw material and production cost. It also enables fine emulsions and therefore low surface oil.
- Low viscosity enables high solids and therefore high oil retention and high manufacturing throughput.
- Film forming and fast dynamics allow forming dense matrix providing active protection.

ACKNOWLEDGMENTS

This chapter is sponsored by Ingredion. The author acknowledges the support and contribution from its expert team, especially Yangyang Jin, Amir Malaki Nik, Bob O'Mara, Christopher Lane, Christopher Gregson, and Scott Grare.

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Chapter 19

Use of Milk Proteins for Encapsulation of Food Ingredients

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19.1 INTRODUCTION

The demand for encapsulated food ingredients continues to grow as the food industry seeks to maximize the intended benefits of additives in foods and bioactive components delivered through foods. Encapsulation in the food industry may be used to (1) protect sensitive food additives and bioactives that are not stable in their neat form (e.g., omega-3 oils, probiotics, delicate flavors) against degradation when exposed to various environments (e.g., high temperature, oxygen, extreme pH), (2) isolate food additives and bioactives from undesirable interactions with other food components when incorporated into manufactured foods during processing and storage of the finished food product (e.g., minimizing the interaction of Fe salts with unsaturated oils which lead to oxidation), (3) mask undesirable flavors of functional food ingredients that are added for a health benefit (e.g., peptides, polyphenols), (4) control the release of the food ingredient at the desired time and site, and (5) increase the bioavailability of bioactives (Risch and Reineccius, 1995; Desai and Park, 2005; Anon, 2006, 2007; Augustin and Sanguansri, 2008; Augustin and Hemar, 2009; de Vos et al., 2010; Burgain et al., 2011). Examples of food ingredients that may benefit from encapsulation are given in Table 19.1.

Formulating encapsulated food ingredients is challenging as the food industry is limited to the use of food grade and generally regarded as safe (GRAS) materials as encapsulants. Classes of food materials and examples of these used by the food industry as encapsulating matrices, either alone or in combination with others, are given in Table 19.2. Many of the encapsulating materials such as synthetic polymers (e.g., Eudagrit®) with controlled release properties that are allowed in the pharmaceutical industry are not permitted in foods. When designing encapsulated ingredients for the food industry, it is also essential to consider the cost for producing the encapsulated ingredient as the food industry cannot tolerate the high margins of the pharmaceutical industry. This limits the choice of technology for producing encapsulated food ingredients that are competitive in the marketplace. Gouin (2004) has provided a food industry perspective on the use of various encapsulation technologies and the relative merits of the use of various technologies for encapsulating food ingredients. The material and process used for encapsulation determines the properties of the encapsulated active ingredient. Spray drying is a common technology used in the food industry due to wide availability of the equipment, its versatility, and its low cost compared to other technologies (Gouin, 2004; Gharasallaoui et al., 2007).

A range of dairy ingredients, including proteins, lactose, milk phospholipids, and milkfat can function as encapsulating matrices. Dairy ingredients are effective encapsulating matrices because they possess functional properties that make them amenable to be formulated with a range of food actives and processed into encapsulated food ingredients with the desired functionality. Of the dairy ingredients available, milk proteins are highly sought after as encapsulant materials. This chapter covers the properties of dairy proteins that make them amenable to be used as encapsulating materials. Examples of the use of dairy proteins to deliver a range of hydrophilic and hydrophobic components and probiotics are discussed to demonstrate the applicability of dairy ingredients in the formulation and development of encapsulated food ingredients.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00019-4

TABLE 19.1 Food Ingredients That Benefit from Encapsulation

Ingredient Class	Examples
Flavoring agents	Sweeteners, seasonings, spices
Acids, bases, and buffers	Citric acid, lactic acid, sodium bicarbonate
Lipids	Omega-3 oils (fish and algal oils), conjugated linoleic acid
Enzymes and microorganisms	Proteases, probiotic bacteria
Artificial sweeteners	Aspartame, saccharin
Antioxidants	Flavanols, polyphenols
Preservatives	Sorbic acid, calcium propionate
Essential oils	Orange oil, rosemary oil
Minerals	Calcium, iron, zinc
Amino acids and peptides	Milk bioactive peptides
Vitamins and provitamins	Vitamin A, carotene, vitamin K, vitamin C
Phytochemicals	Lutein, coenzyme Q10, curcumin

TABLE 19.2 Materials Used for Encapsulation in the Food Industry

Class	Examples
Proteins	Albumin, caseinates, gelatin, gluten, peptides, soy protein, vegetable proteins, whey proteins, zein
Simple sugars	Fructose, galactose, glucose, maltose, sucrose
Carbohydrates/ gums	Chitosan, corn syrup solids, cyclodextrin, dextrins, dried glucose syrup, maltodextrins, modified starches, starches Agar, alginates, carrageenan, gum acacia, gum arabic, pectins
Cellulose material	Sodium, carboxymethyl cellulose, cellulose acetate butylate phthalate, cellulose acetate phthalate, ethyl cellulose, methyl cellulose
Lipids	Edible fats and oils, fractionated fats, hardened fats, beeswax
Emulsifiers	Monoglycerides, diglycerides, lecithin, liposomes, food-grade surfactants

19.2 MILK PROTEINS AND THEIR FUNCTION IN ENCAPSULATION

19.2.1 Milk Proteins

Milk contains about 3.5% protein. The major milk proteins are casein and whey protein, with the caseins accounting for $\approx$80% of the total protein in milk. The major caseins are α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein. The major whey proteins are β-lactoglobulin, α-lactalbumin, immunoglobulin, and serum albumin. Genetic variants for caseins and whey proteins exist and this adds to the heterogeneity of the milk proteins. Some inherent properties of milk proteins are given in Table 19.3. Milk proteins can be isolated from milk in a variety of forms or further processed to alter their functional properties to expand their use as ingredients in a variety of applications. Milk proteins in native and processed forms may be used as encapsulants. The diverse range of milk proteins available as encapsulating and/or carrier materials include (1) milk protein products containing both casein and whey proteins (e.g., proteins in skim milk powder (SMP), milk protein concentrate (MPC), milk protein isolate (MPI)), (2) casein products (e.g., micellar casein, casein/caseinates, rennet casein), (3) whey protein products (e.g., whey protein concentrate (WPC), whey protein isolate

TABLE 19.3 Properties of Milk Proteins[a]

Protein	Isoelectric Point	Molecular Weight (daltons)	Amino Acid Residues/ Molecule	Hydrophobicity (kJ/residue)
Caseins				
α_{s1}-Casein-B	5.0	23,614	199	4.9
α_{s2}-Casein-A	5.2–5.4[b]	25,230	207	4.7
β-Casein-A[2]	5.2	23,983	209	5.6
κ-Casein-B	5.6	19,023	169	5.1
Whey Proteins				
β-Lactoglobulin-B	5.3	14,176	123	4.7
α-Lactalbumin-B	4.8	18,363	162	5.1
Serum albumin	4.7	66,267	582	4.3

[a]*Adapted from Walstra and Jenness (1984) and Swaisgood (1982).*
[b]*The isoelectric point is dependent on the degree of phosphorylation of the casein, as a result of post-translational modifications.*

(WPI), individual whey proteins (β-lactoglobulin, α-lactalbumin)), and (4) milk protein hydrolysates (e.g., hydrolyzed casein, hydrolyzed whey protein). A detailed discussion of the properties of the proteins in milk and processes used for the isolation and manufacture of various milk protein products is beyond the scope of this chapter. There are many reviews and book chapters that deal with the processing and functionality of milk protein ingredients (Fox, 2003; Fox and Kelly, 2004; Smithers, 2008; Dalgleish and Corredig, 2012).

19.2.2 Function of Milk Proteins in Encapsulation

Milk proteins are effective encapsulating materials. This is because milk proteins have good solubility, emulsifying, viscosity building and gelling, and film-forming properties. Furthermore, the functional properties of milk proteins may be modified or improved by the application of appropriate processing techniques (Foegeding et al., 2002; Augustin and Udabage, 2007), which expands their use in a variety of applications, including as encapsulation matrices. Milk proteins are versatile encapsulating materials that can be used on their own or in combination with other food-grade materials in the design of microencapsulated food ingredients.

The film-forming and emulsifying properties of milk proteins (e.g., whey proteins, caseins, milk protein isolates, hydrolyzed milk proteins) are employed to stabilize emulsion-based encapsulation systems. The ability of the milk proteins to assemble at an interface and to build viscosity of the bulk phase further stabilizes the emulsions. The proteins also form the matrix that supports and protects the encapsulated component when the emulsion is spray dried. In hydrogel-based encapsulated systems, the ability of the milk proteins to form a gel phase is a useful property that can be capitalized on for embedding food components. In coacervate-based encapsulation systems, proteins interact with oppositely charged biopolymers to form a separate phase that encapsulates components (Augustin and Hemar, 2009). They can also act as carriers of materials due to their specific interactions with various bioactive molecules (Livney, 2010). The ease by which they can be transformed into the dried state using a variety of drying techniques is an added advantage that milk proteins have over some encapsulating matrices.

19.2.3 Encapsulation Technologies Used When Formulating with Milk Proteins

All the common technologies that are used for processing of encapsulated ingredients can be employed for formulations containing milk proteins. Some of the more commonly used methods for preparing encapsulated ingredients formulated with milk proteins include a range of drying techniques (i.e., freeze drying, spray drying, fluid-bed drying), extrusion, methods for production of emulsions and gels, complexation, and coacervation. The choice of method is governed in part by the properties of the active being encapsulated, the composition of the formulation, and the final product application. Encapsulated food ingredients in powder, gel, and emulsion format are most commonly used in the food industry.

19.3 ENCAPSULATION SYSTEMS USING CASEINS AND WHEY PROTEINS

Milk proteins can be used to encapsulate hydrophobic, hydrophilic, and water-dispersible components. The examples given in Table 19.4 demonstrate the interest in the use of milk proteins as encapsulating materials including the various techniques used for the formulations examined.

19.3.1 Milk Proteins and Processes for Encapsulating Hydrophobic Components

The ability of milk proteins to stabilize an oil-in-water emulsion makes them suitable for carrying oils and actives that are soluble in oil. For example, anhydrous milkfat (AMF) loadings of up to 75% have been successfully microencapsulated using whey protein isolate (WPI) or whey protein concentrate (WPC) (Young et al., 1993). This allows the milkfat to be conveniently supplied in a powder format. Compared with nondairy-based polymeric matrices, WPC used on its own was superior to gum arabic alone and a WPC−maltodextrin blend to encapsulate conjugated linoleic acid (CLA) (Jimenez et al., 2006). Furthermore, WPI- and WPC-based wall formulations provide excellent protection against lipid oxidation (Moreau and Rosenberg, 1996; Jimenez et al., 2004).

Sodium caseinate is a superior encapsulant for spray drying of dairy-based oil-in-water emulsions compared to whey protein, having a range of 0.25 to 5 oil-to-protein ratios (Vega and Roos, 2006). This is attributed to the excellent surface-active properties of the caseins and their increased resistance to heat denaturation. For example, sodium caseinate has been used in the formulation of oil-in-water emulsions to produce a range of spray-dried powders containing up to 75% soy oil (Hogan et al., 2001a). However, powders with higher fat contents had low microencapsulation efficiency, as reflected in the higher levels of surface fat due to decreasing protein coverage of the fat globules prior to drying (Hogan et al., 2001a). Sodium caseinate is also an effective wall material for retention of orange oil microencapsulated by spray drying (Kim and Morr, 1996).

β-Lactoglobulin (β-LG) is the major whey protein of bovine milk and is a natural nanocarrier for hydrophobic molecules. Retinoic acid, cholesterol, vitamin D, various aroma compounds (e.g., aldehydes, ketones), and fatty acids (e.g., CLA, palmitate, oleate), for example, bind within its hydrophobic calyx (Kontopidis et al., 2004). The casein micelle can also act as a delivery vehicle of hydrophobic molecules, such as curcumin, a natural bioactive with antitumor activities (Sahu et al., 2008). The encapsulation properties of WPC as coating materials for essential oils of oregano and aroma extracts of citronella and majoram were investigated by spray drying the protein-stabilized oil-in-water emulsions (Baranauskiene et al., 2006). In another example, the excellent gelling and emulsifying properties of whey proteins were used to protect the fat-soluble bioactive retinol via a process of emulsification and cold gelation prior to drying (Beaulieu et al., 2002). The WPI matrix protected retinol from gastric fluid *in vitro*, releasing retinol in the intestinal fluid environment.

19.3.2 Milk Proteins and Processes for Encapsulating Hydrophilic Components

There has been considerable interest in using dairy constituents for encapsulation of various water-soluble components (e.g., food acids, water-soluble flavors and aromas, proteins, proteases) and water-soluble bioactives (e.g., immunoglobulins, caffeine, riboflavin, lactoferrin) (Table 19.4). For instance, the excellent encapsulation properties of WPC to stabilize the water-soluble aroma compound 3-methylbutyraldehyde has been illustrated (Brückner et al., 2007). In that study, encapsulation was achieved by spray drying an aqueous solution containing 3-methylbutyraldehyde within a double emulsion (water-in-oil-in-water, W/O/W). Further, the ability of whey powder to act as an effective carrier for acidic flavors has been illustrated (Bayram et al., 2008). Of the three carrier materials tested—whey powder, SMP, and gum arabic—only the whey powder solution was appropriate for entrapping sumac berry flavor using spray drying.

Microencapsulation consisting of double emulsification and/or gelation of whey proteins has been used successfully to protect proteins against adverse environmental conditions (e.g., heat, pH, metal ions) (Cho et al., 2005; Al-Nabulsi et al., 2006) and for controlled release of water-soluble cores such as caffeine (Gunasekaran et al., 2006; Gan et al., 2009). In all cases, the resultant gels or microcapsules have been conveniently freeze dried or air dried without adversely affecting functionality of the encapsulant system.

Casein has been used to encapsulate citric acid using a novel microwave-assisted technique (Abbasi and Rahimi, 2008). Various biopolymers were evaluated for their encapsulation efficiency and ability to produce high-quality citric acid microcapsules. Casein had superior film-forming properties, producing fine powders containing smooth, uniform-coated crystals with 100% encapsulation efficiency. However, sensory evaluation of the casein-entrapped citric acid in chewing gum showed it was inferior to inulin-coated microcapsules (Abbasi et al., 2009). Controlled proteolysis of the

TABLE 19.4 Examples of the Use of Dairy Proteins as Encapsulants in Powder Delivery Formats

Core	Dairy Protein	Encapsulation Technique	Benefit(s) of Encapsulation	Reference
Hydrophobic Component				
Orange oil	WPI	Spray drying	Protection against oxidation	Kim and Morr (1996)
Carotene	WPC	Electrospraying	Protection against oxidation	Lopez-Rubio and Lagaron (2012)
Soy oil	Sodium caseinate	Spray drying	High encapsulation efficiency (89%)	Hogan et al. (2001a)
CLA	WPC	Spray drying	Protection against oxidation	Jimenez et al. (2004, 2006)
Flaxseed oil	WPI	Spray drying	Protection against oxidation	Partanen et al. (2008)
AMF	WPI	Spray drying	Protection against oxidation during storage	Moreau and Rosenberg (1996)
AMF	WPI, WPC-50, WPC-75	Spray drying	High encapsulation efficiency (> 90%)	Young et al. (1993)
Retinol	WPI	Emulsification/ cold gelation/air drying	Gastroresistance and protection against oxidation	Beaulieu et al. (2002)
Oregano, citronella and marjoram flavors	SMP or WPC	Spray drying	Improved retention of flavors during spray drying	Baranauskiene et al. (2006)
Hydrophilic Component				
3-Methylbutyr-aldehyde	WPC and sodium caseinate or SMP as secondary emulsifier	Double emulsification/ spray drying	Improved retention of aldehyde during storage	Brückner et al. (2007)
Sumac concentrate	Whey powder or SMP	Spray drying	Improved retention of flavor during spray drying	Bayram et al. (2008)
Citric acid	Casein	Cocrystallization	Development of a novel, efficient, and cost-effective microwave encapsulation technique that provided high encapsulation efficiency (100%)	Abbasi and Rahimi (2008)
IgY	WPC as secondary emulsifier	Double emulsion/ gelation/air drying	Protect IgY from highly acidic conditions and heat treatment processes	Cho et al. (2005)
Caffeine	WPC	Hydrogels/air drying	Controlled release of caffeine	Gunasekaran et al. (2006)
Probiotic				
Bifidobacterium sp.	Milkfat and/or denatured WPI	Emulsification/ spray drying	Improved cell viability in yogurt and after exposure to simulated gastrointestinal fluids	Picot and Lacroix (2003, 2004)
Bifidobacterium and *Lactobacillus* sp.	WPI	Freeze drying	Improved cell viability during storage and in yogurt	Kailasapathy and Sureeta (2004)
Lactobacillus sp.	WPI	Freeze drying	Improved cell viability in simulated gastrointestinal fluids	Reid et al. (2005)
Lactobacillus sp.	WPI	Freeze drying	Improved cell viability during the production and storage of biscuits, and improved pH stability	Reid et al. (2007)

whey protein α-lactalbumin (α-LA) results in the formation of self-assembled α-LA nanotubes ($\approx 110 \times 20$ nm) via association of the protein hydrolysates in the presence of Ca ions (Graveland-Bikker and de Kruif, 2006; Ipsen and Otte, 2007). The inner cavity of the tubes (8 nm diameter) is a putative site for encapsulation of molecules such as vitamins or enzymes (Graveland-Bikker and de Kruif, 2006; Ipsen and Otte, 2007).

19.3.3 Milk Proteins and Processes for Encapsulating Probiotics

Probiotic bacteria, when administered in sufficient quantity, confer several physiological benefits to their host. The bacteria, however, are sensitive to moisture, temperature, oxygen, and acid environments and therefore require protection against the detrimental conditions encountered during food processing, storage, and *in vivo* until their release at the desired site in the body. Protein gels offer protection to probiotics during processing and storage (Kailasapathy and Sureeta, 2004; Reid et al., 2005) and extend the food applications of probiotics (Reid et al., 2007). Additionally, proteins have a nutritional advantage over polysaccharide-based systems. Milk proteins, in particular, are well known for their nutritional and bioactive properties (Tomé and Debabbi, 1998; Madureira et al., 2007).

Some examples of encapsulated probiotics formulated with dairy proteins and processes used for their preparation are given in Table 19.4. WPI gels have the potential to improve cell viability of selected microorganisms during food processing and storage (Kailasapathy and Sureeta, 2004; Reid et al., 2005) as well as protect the microorganisms against adverse gastrointestinal environments (Reid et al., 2005). The incorporation of WPI-immobilized probiotics into both dairy and non dairy foods has been illustrated (Reid et al., 2007). The gelled microcapsules can be produced as convenient spray-dried (Picot and Lacroix, 2004) or freeze-dried (Kailasapathy and Sureeta, 2004; Reid et al., 2005, 2007) powders. Although the whey protein gelation process requires further optimization to improve cell viability, these studies demonstrate the feasibility of this approach for delivery of probiotics in food systems.

19.4 MILK PROTEINS IN COMBINATION WITH OTHER MATERIALS AS THE ENCAPSULATING MATRIX

Milk proteins are often used in combination with other food-grade materials in the design of encapsulated ingredients as this widens their application field and allows more control over their release properties (Table 19.5).

19.4.1 Milk Proteins in Combination with other Materials and Processes for Encapsulating Hydrophobic Components

Dairy proteins (caseinate, whey proteins) have been widely utilized in combination with various carbohydrates to formulate wall systems that encapsulate a range of fats, oils, and oleoresins (Table 19.5). In dairy-based emulsion systems formulated with a blend of protein and carbohydrate, the milk proteins act as effective emulsifiers and film-formers, while the carbohydrates usually act as a filler or matrix-forming material.

Lactose and anhydrous milkfat (AMF) are widely utilized in combination with caseinate or whey protein to prepare both dry dairy- and nondairy-based emulsions. The incorporation of lactose into sodium caseinate-based wall systems has been shown to improve the encapsulation efficiency of AMF during spray drying. This has been attributed to the ability of lactose to form a glassy state on dehydration (Vega and Roos, 2006). Fäldt and Bergenståhl (1996) have suggested that in whey protein-stabilized soy oil spray dried emulsions, the presence of lactose aids in encapsulation. The microencapsulation efficiencies of WPI (0% lactose), WPC75 (10% lactose), and WPC50 (37% lactose) were compared (Young et al., 1993). Irrespective of the AMF loadings (up to 75% wt), WPC50 showed superior microencapsulation efficiency, which was attributed to its high lactose content. An increase in the lactose:WPI ratio significantly decreased free fat of microencapsulated AMF but did not significantly decrease surface fat (Keogh and O'Kennedy, 1999).

The molecular state of casein also affects its encapsulation properties in the presence of sugars. Sodium caseinate was more effective than micellar casein (SMP) when the proteins were used in combination with sugars for AMF encapsulation (Vega et al., 2007). This may be due to the high flexibility and strong amphiphilic characteristics of the individual caseins, which impart better surface-active properties than aggregated (micellar) casein. However, micellar casein provided superior oxidative stability to microencapsulated fish oil than sodium or calcium caseinate and lactose (Keogh et al., 2001). The low vacuole volume of micellar casein was a critical factor, minimizing the amount of entrapped air in dried emulsions.

TABLE 19.5 Examples of Dairy Protein and Carbohydrate Formulations as Encapsulant for Powder Delivery Formats

Core	Dairy–Protein-Based Wall Material	Nondairy Wall Material	Encapsulation Technique	Benefit(s) of Encapsulation	Reference
Hydrophobic Component					
Corn oil	β-LG	Maltodextrin and either pectin or ι-carrageenan	Protein–polysaccharide coated lipid droplets; freeze drying	Improved freeze–thaw and freeze–dry stability of the emulsions	Mun et al. (2008)
Vitamin D epicatechin gallate	Casein–maltodextrin conjugate	Maltodextrin	Coassembly	Protection of active at low pH	Markman and Livney (2012)
Orange oil	Sodium caseinate	Lactose	Double emulsion; spray drying	Maintained stability of the double emulsion after drying	Edris and Bergnståhl (2001)
Avocado oil	WPI	Maltodextrin	Spray drying	Improved oxidative stability of the oil at high temperature (60°C)	Bae and Lee (2008)
Lemon myrtle oil	WPC	Maltodextrin	Spray drying	Good retention of lemon myrtle oil	Huynh et al. (2008)
Ginger essential oil	WPI	Maltodextrin	Spray drying	Improved oxidative stability of the oil during storage and lowered surface oil content	Toure et al. (2007)
Caraway essential oil	WPC	Maltodextrin	Spray drying	Increased retention of encapsulated component during drying and storage, and improved oxidative stability	Bylaitë et al. (2001)
Palm-based oil	Sodium caseinate	Maltodextrin	Spray drying	Low surface oil content	Dian et al. (1996)
Squid oil	Caseinate	Gelatin and maltodextrin	Spray drying	Improved oxidative and thermal stability	Lin et al. (1995)
Red chili oleoresin	WPC	Mesquite gum and maltodextrin	Spray drying	Improved thermo-oxidative stability	Pérez-Alonso et al. (2008)
β-Carotene	Casein	Dextran	Maillard conjugation/ vacuum drying	Protected β-carotene against pH change, dilution, oxidation, and during storage	Pan et al. (2007)
Hydrophilic Component					
Lipase	SMP	Gum arabic	Spray drying	Improved retention of lipase activity during storage	Alloue et al. (2007)
Alcohol dehydrogenase	BSA or β-LG	Trehalose	Spray drying	Improved retention of ADH activity during spray drying	Yoshii et al. (2008)

(*Continued*)

TABLE 19.5 (Continued)

Core	Dairy–Protein-Based Wall Material	Nondairy Wall Material	Encapsulation Technique	Benefit(s) of Encapsulation	Reference
β-Galactosidase	Low-lactose milk whey	Maltodextrin	Freeze drying	Improved retention of β-galactosidase activity during freeze drying and storage, and delayed both browning development and lactose crystallization	Burin et al. (2004)
Riboflavin	WPI	Alginate	Emulsification/internal cold gelation; freeze drying	Controlled release of riboflavin	Chen and Subirade (2006, 2007)
Thiamine	WPI	Pectin	Coacervation/freeze drying	High entrapment of thiamine	Bédié et al. (2008)
Vitamin B12	Casein–maltodextrin conjugate	Maltodextrin	Double emulsion	Improved encapsulation efficiency	O'Regan and Mulvihill (2010)
Lactoferrin	WPI (secondary emulsifier)	Butterfat, corn oil, and polyglycerol polyricinoleate (primary emulsion)	Double emulsification/ freeze drying	Improved retention of antimicrobial action of lactoferrin	Al-Nabulsi et al. (2006)
Probiotic					
Bifidobacterium sp.	Caseinate	Canola oil, fructo-oligosaccharides and either dried glucose syrup or resistant starch	Spray drying	Improved cell viability during nonrefrigerated storage and *in vitro* gastrointestinal conditions	Crittenden et al. (2006)
Lactobacillus and *Bifidobacterium* sp.	Casein	Pectin	Spouted-bed drying	Improved cell viability during drying and subsequent storage	Oliveira et al. (2007)
Bifidobacterium sp.	Milk proteins	Lactose	Spray drying	Improved cell viability during storage	Hsiao et al. (2004)
L. casei	Caseinate	Gellan gum	pH-induced gelation	Protection in simulated gastric fluid	Nag et al. (2011)
Bifidobacterium sp.	Milk proteins	Lactose	Spray drying	Improved cell viability on exposure to simulated gastrointestinal fluids	Lian et al. (2003)

Use of alternative carbohydrates to lactose, such as trehalose (Vega et al., 2007) or hydrolyzed starches (maltodextrin, dried glucose syrup, corn syrup solids) (Pedersen et al., 1998; Heinzelmann and Franke, 1999), in combination with a protein, improve the oxidative stability of microencapsulated lipid formulations. For example, in some protein-based spray-dried emulsions, the replacement of lactose with other carbohydrates (e.g., gum arabic or maltodextrin) enhances storage stability but this was at the expense of an increased particle size after reconstitution (Vega and Roos, 2006). A mixture of WPC and various maltodextrins improved volatile retention during spray drying and storage and increased oxidative stability of the microencapsulated powders containing caraway essential oils (Bylaitë et al., 2001). In contrast, a mixture of SMP and various maltodextrins had the opposite effect, decreasing both volatile retention and oxidative stability (Bylaitë et al., 2001).

Increasing the dextrose equivalent (DE) of the carbohydrate has been shown to improve oil encapsulation efficiencies of sodium caseinate (Hogan et al., 2001b; Danviriyakui et al., 2002) and improve oxidative stability of microencapsulated fish oil powders in sodium caseinate matrices (Kagami et al., 2003). Glucose syrups with high DE are less permeable to oxygen and afford better protection to encapsulated flavors than carbohydrates with low DE (Reineccius, 1991). However, DE is not the only determinant for improvement of oxidative stability. The oxygen permeability of the wall, as well as type of carbohydrate and its molecular weight distribution, play a key role in the stability of encapsulated core materials. For example, wall matrices formulated with sodium caseinate and cyclodextrin (DE 11.3) or maltodextrin (DE 18) protected microencapsulated fish oil against oxidation to a similar extent despite cyclodextrin having a lower DE (Kagami et al., 2003). Moreover, cyclodextrin was crucial to prevent Maillard browning and agglomeration of the protein-based, spray-dried microcapsules. Other studies show that blending maltodextrins with low molecular weight carbohydrates, such as mono- and disaccharides, can further limit oxygen permeability (Desorby et al., 1999). For the encapsulation of oils that are very prone to oxidation (e.g., fish oils), Maillard reaction products formed by heating caseins and reducing carbohydrates have been found to be superior to corresponding physical blends as encapsulants (Augustin et al., 2006).

Microencapsulation of aroma and flavor compounds is an important application in the food industry. In this context, whey protein−carbohydrate blends have been effectively used as carrier matrices (Table 19.5). For example, ethyl butyrate and ethyl caprylate were microencapsulated in wall systems formulated with WPI or WPI:lactose (1:1) (Sheu and Rosenberg, 1995; Rosenberg and Sheu, 1996). Retention of the volatiles was strongly influenced by their initial ester load, wall solids concentration, and ester and wall type. WPI:lactose was more effective than WPI. Wall systems consisting of WPI:maltodextrin (DE 5, 10, 15) and WPI:corn syrup solids (DE 24) were also effective for microencapsulation of volatiles, providing 70 to 91% ester retention (Sheu and Rosenberg, 1998). Combinations of WPI with high DE carbohydrates were more effective than those with low DE carbohydrates in limiting surface cracks of the spray-dried microcapsules (Sheu and Rosenberg, 1998). The absence of surface cracks is crucial to maximize protection of the core, with regards to limiting deterioration and/or losses of the encapsulated component on storage.

β-Carotene is an antioxidant with anticancer properties and is the precursor of vitamin A. It is highly sensitive to light, heat, and oxygen and has poor solubility in water (Burton and Ingold, 1984). Retention of β-carotene in a sodium caseinate:maltodextrin or gum arabic:maltodextrin wall system was evaluated following microencapsulation in palm-based oil-in-water emulsions using a spray-drying technique (Dian et al., 1996). Sodium caseinate:maltodextrin wall systems performed significantly better than those based on gum arabic:maltodextrin. In an alternative approach, the high hydrophobicity and relatively high lysine content of β-casein was used to construct nanoparticle encapsulant systems based on simultaneous formation and encapsulation of β-carotene within casein−dextran conjugates (Pan et al., 2007). The authors took advantage of the hydrophobic interaction between β-carotene and β-casein to form the capsule core. The shell comprised of the β-casein−dextran conjugate afforded stability and dispersibility to the particles over a wide pH range (pH 2−12), ionic strength, and presence of ferric ions. Encapsulated β-carotene could be released by the action of pepsin or trypsin. The nanoparticles could also be stored in a dried form.

19.4.2 Milk Proteins in Combination with other Materials and Processes for Encapsulating Hydrophilic Components

Formulating milk protein/carbohydrate blends for microencapsulation is applicable for delivering a host of water-soluble ingredients (Table 19.5). The use of whey proteins as carriers of sensitive bioactives relies on the formation of cold-set gels, which offers interesting opportunities to develop whey proteins as carriers for hydrophilic, heat-sensitive bioactives. In this approach, a combined emulsification-internal cold gelation method has been applied to prepare whey protein−alginate microspheres that delay the release of riboflavin, pending degradation of the microspheres in simulated

intestinal fluid (Chen and Subirade, 2006, 2007). The microspheres were converted into dry powders by freeze drying. Coacervation is another means of encapsulating water-soluble ingredients. For example, WPI-pectin coacervates provided a carrier for thiamine entrapment (Bédié et al., 2008). The freeze-dried powders contained sufficient vitamin to fulfill the recommended daily intake of thiamine for an adult male based on just 70 to 80 mg of the dry complex.

Dairy proteins may be combined with other food constituents to protect enzymes during drying by entrapment within an amorphous matrix. Protection of β-galactosidase in a freeze-dried matrix of low-lactose milk whey. The addition of maltodextrin improved retention of enzyme activity and delayed both browning development and lactose crystallization during storage of the powders at different relative humidities (RH) (Burin et al., 2004). In another example, the retention of the enzymatic activity of alcohol dehydrogenase (ADH) on spray drying was examined in the presence of trehalose with and without added whey proteins (Yoshii et al., 2008). Though trehalose is sufficient to encapsulate enzymes during freeze drying it was not sufficient to overcome the thermal damage to ADH on spray drying. The addition of whey proteins, such as bovine serum albumin (BSA) and β-LG, provided an additional improvement of the retention of ADH activity. The increase in ADH activity in the presence of whey protein was attributed to possible interaction of the proteins with ADH, which protected the enzyme against denaturation.

Loss of enzyme activity on spray drying could also be explained by inactivation of the enzyme absorbed at the surface of the powders. It was demonstrated that the surface concentration of trypsin in spray-dried lactose powders could be efficiently controlled by adding a low molecular weight surfactant to the mixture prior to drying. In general, the residual enzyme activity was maintained at >90% (Millqvist-Fureby et al., 1999). The activity of entrapped enzymes in spray-dried matrices has also been studied during storage (Alloue et al., 2007). The enzymatic activity of a microbial lipase was studied following storage of spray-dried powders prepared from SMP and gum arabic. The powders were stored for 18 months at 4 or 20°C without loss of lipase activity, providing an efficient method of enzyme preservation (Alloue et al., 2007).

19.4.3 Milk Proteins in Combination with other Materials and Processes for Encapsulating Probiotics

Skim milk itself has also shown to be an effective wall material for encapsulating bifidobacteria to enhance their survival through the gastric environment (Lian et al., 2003) as well as during storage of the spray-dried microcapsules (Hsiao et al., 2004). Of the various wall materials studied (skim milk, soluble starch, gelatin, gum arabic), encapsulation in skim milk provided the best matrix under which *Bidiobacterium longum* B6 and *Bifidobacterium infantis* CCRC 14633 populations remained most viable during storage (42 days at 4°C) (Hsiao et al., 2004). For the production of freeze-dried probiotics, reconstituted SMP, disaccharides, and disaccharide mixtures were evaluated for their ability to enhance survival of lactobacilli during storage of the powders at room temperature at various RH (Miao et al., 2008). The disaccharides improved cell viability during freeze drying and storage. Trehalose and a blend of lactose and maltose were the most effective carriers, especially notable at 0 and 11.4% RH. Crystallization of the matrix adversely affected cell survival.

An alternative approach utilizes milk protein coacervates. Coacervates between casein and anionic polysaccharides are formed at pH below the pI (isoelectric point) of casein. Complex coacervates of casein and pectin were employed to microencapsulate probiotics (*Bifidobacterium lactis* and *Lactobacillus acidophilus*) by spouted bed drying (Oliveira et al., 2007). While there was little loss of viability of the probiotics during drying (0.3 log reduction), the microencapsulated bacteria were not protected at low pH.

Casein−carbohydrate conjugates have been shown to provide a protective environment for probiotics encapsulated by spray drying of probiotics within an oil-in-water emulsion (Crittenden et al., 2006). *In vitro* experiments showed that the probiotic cells (*Bifidobacterium infantis*) were protected from digestion in simulated gastric fluid and were released in simulated intestinal fluid.

19.5 PATENT-BASED STRATEGIES

There is considerable patent literature related to the use of milk proteins as encapsulating materials for food ingredients. Table 19.6 provides a selection of examples from the patent literature. This demonstrates the interest in the field and potential commercial opportunities for encapsulated food ingredients formulated with various food ingredients where there is an example of a formulation with milk proteins.

TABLE 19.6 Selection of Patents Relating to Food Ingredient Encapsulation That Includes Milk Proteins within the Formulation

Active	Comments	Reference
Oil containing polyunsaturated fatty acid	Oil emulsified with denatured whey protein and reducing sugar added; dispersion heated at 80°C for 30 min and spray dried	Van Seeventer et al. (2011)
Bacterial cells	Active entrapped within gelled whey protein matrix	Brodkorb and Doherty (2010)
Polyunsaturated fatty acid	Active coated with protein (e.g., dairy protein) and incorporated into matrix comprising protein and starch	Van Lengerich and Walther (2010)
Citrus phytochemical composition	Active encapsulated within shell containing protein (e.g., casein, whey protein) and polysaccharide	Crouse et al. (2010)
Antioxidant	Stabilized liposome containing one biopolymer around the liposome and an oppositely charged biopolymer; one biopolymer may be a milk protein	Decker et al. (2009)
Lipid-based material (e.g., phytosterol, vitamin in triglyceride)	Active emulsified with protein (e.g., whey protein, casein) and mixed with carbohydrate	Nakhasi et al. (2009)
Lipid with flavorants and/or colorants	Active within oil emulsified with gel-forming component (e.g., milk protein) and calcium ions	Huisman et al. (2009)
Food ingredient (e.g., color, taste additive, flavor)	Active encapsulated within complex coacervate of protein and polyanionic polymer crosslinked with transglutaminase	Lee (2009)
Lipophilic nutrient	Active encapsulated within complex coacervate of cationic protein (e.g., whey protein, casein) and anionic polymer	Given et al. (2009)
Food grade substance (e.g., bacteria, metal ion, bioactive)	Active entrapped in denatured proteins (e.g., β-lactoglobulin) coated with lipid	Pouzot et al. (2008)
Labile compound (e.g., vitamin, probiotics, polyunsaturated fatty acid)	Active encapsulated within primary and secondary encapsulant and Maillard reaction product	Subramanian et al. (2008)
Omega-3 fatty acid, phytosterol ester of omega-3 fatty acid	Active encapsulated within primary shell and outer shell; composition may contain casein, whey protein or coacervate of whey protein and carbohydrates	Yan et al. (2008)
Lipid-soluble vitamins, polyphenols, antioxidants, and long chain polyunsaturated fatty acids	Active formulated with whey protein micelles	Schmitt and Bovetto (2007)
Oxidizable material (e.g., omega-3 oils)	Food product containing oxidizable lipid, protein (e.g., casein, whey protein) and water impermeable shell wall	Kolar and Moore (2007)
Hydrophobic bioactive (e.g., peptide, phytochemical, vitamins, omega-3 acids)	Active carried by reassembled casein micelles	Livney and Dalgleish (2007)
Oxidizable lipid and vitamins	Emulsion comprised of complex of casein and whey protein protects oxidizable active	Singh et al. (2006)
Probiotics	Probiotics encapsulated in a matrix comprising protein (e.g., whey protein, casein) and carbohydrate	Crittenden et al. (2005)
Various oxygen sensitive agents (e.g., omega-3 oils, citrus oils, vitamins)	Active encapsulated within modified starch/casein mixture	Trubiano and Makarious (2005)

(*Continued*)

TABLE 19.6 (Continued)

Active	Comments	Reference
Various bioactive molecules	Active embedded in gelled whey protein matrix by contacting with salt solution	Subirade et al. (2002)
Oxygen-sensitive oil (omega-3 oil)	Active emulsified within heated protein (e.g., whey protein, casein)−carbohydrate matrix	Sanguansri and Augustin (2001)
Ferric salt	Iron fortification system based on ferric−caseinate complex	Sher et al. (2000)
Flavors	Actives encapsulated in protein (e.g., whey protein, caseinate) or other biopolymer and high pressure treated	Mandralis and Tuot (2000)

19.6 CONCLUSION

Dairy proteins are highly valued ingredients in the food industry and perform multifunctional roles when used in food applications. In many food applications, milk proteins contribute to the physical structure, sensory properties, and functionality of foods. Their attributes have been exploited for the formulation of encapsulated ingredients because of their capacity to protect various food components and deliver them through food without detracting from the sensory properties of the product. The challenge that has yet to be adequately addressed is the development of encapsulated formulations for controlled delivery of bioactive ingredients to target sites of the gastrointestinal tract, to cause a desirable physiological effect in the body. This is an active area of research (Garti, 2008), and dairy-based encapsulated ingredients feature as key constituents of encapsulation matrices with potential to protect and deliver bioactive components.

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Chapter 20

Gelatin and Other Proteins for Microencapsulation

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20.1 INTRODUCTION

As nutritious food ingredients, proteins, such as gelatin, soy protein, zein, pea protein, and so on, have many superior properties making them good and widely used microencapsulation shell materials in the food industry.

Emulsifying property is important for microencapsulation shell materials, especially for microencapsulation of oil-soluble ingredients, such as flavor oil, oil-soluble vitamins, and fish oil, and so on. Due to its amphiphilic nature, protein can orient at the oil−water interface with the hydrophilic groups oriented toward the water phase and the hydrophobic groups oriented toward the oil phase to lower the interfacial tension and form the emulsion. Protein can also build the protective barrier around the oil droplet to prevent the emulsion coalescence and maintain the stability of the formed emulsion (Zayas, 1997).

Through microencapsulation, the volatility, flavor, odor, and reactivity of food ingredients are suppressed. The protection of the microencapsulated contents from degradation or loss is provided by the barrier properties of the shell materials. Oxygen is involved in many degradation reactions in foods, such as fat and oil rancidity and vitamin loss. As a group, proteins appear to have lower oxygen permeability (Avena-Bustillos et al., 2011). Table 20.1 lists the oxygen permeabilities of different protein films.

Crosslinking can enhance the barrier properties of protein films, which can be achieved by either chemical or enzymatic treatment. Aldehydes, such as glutaraldehyde and formaldehyde, are generally used to chemically crosslink proteins. However, the toxicity of aldehydes enormously limits the exploitation of their use in food applications. To overcome the restriction in using toxic crosslinkers, microbial transglutaminase (MTGase) has been proposed as a crosslinking reagent for proteins. MTGase catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues, to form an ε-(γ-glutamyl)lysine isopeptide bond (Motoki and Seguro, 1998). Crosslinking protein via Maillard reaction is also widely used in microencapsulation (Graham, 1996). The "Maillard crosslinks" can be formed by heating a protein in the presence of a reducing sugar. The Maillard reaction could also generate antioxidative compounds that can be useful for retarding the oxidation of core material (Amarowicz, 2009).

In addition to the emulsifying and barrier properties, most proteins have high water solubility, good drying property, and are widely available and generally regarded as safe (GRAS), making them ideal shell materials for microencapsulation in the food industry.

20.2 GELATIN

20.2.1 Gelatin Manufacture: From Collagen to Gelatin

As a substantially pure protein food ingredient, gelatin is obtained by partial hydrolysis of collagen derived from the skin, white connective tissue, and bones of animals (Haug and Draget, 2011).

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00020-0

TABLE 20.1 Oxygen Permeability of Protein Films

Protein Type	Test Conditions	Oxygen Permeability, $cm^3\ mm\ m^{-2}\ d^{-1}\ kPa^{-1}$
Pork skin[a]	23°C, 55% RH	0.0055
Cattle hide[a]	23°C, 55% RH	0.0048
Warm water fish skin (catfish)[a]	23°C, 55% RH	0.0035
Cold water fish skin (Alaska pollock)[a]	23°C, 55% RH	0.0023
Soy protein isolate[b]	25°C, 50% RH	0.0518
Corn zein[c,d]	25°C, 0% RH	0.0120

[a] *Avena-Bustillos et al. (2011).*
[b] *Cho et al. (2010).*
[c] *Krochta (2002).*
[d] *Glycerol used as plasticizer.*

The primary structure of collagen consists of approximately 1050 amino acids called alpha chain. Collagen consists of three alpha chains and each alpha chain is coiled into a left-handed helix to form the secondary structure of collagen. The three chains are twisted around each other to form a right-handed triple helix, which is a rigid rod-like structure that makes the tertiary structure of collagen. These rods assemble together in a quarter-stagger arrangement to form the quaternary structure of the collagen (Schrieber and Gareis, 2007).

The triple-helical structure of collagen arises from its unique amino acid composition and sequence. Table 20.2 shows the amino acid composition of gelatin compared with globular protein (soy protein isolate). Glycine constitutes around 33% of the amino acid component, proline and hydroxyproline together about 22%. The amount varies among different species although the high content of proline and hydroxyproline seems to be characteristic of collagen (Boran and Regenstein, 2010). The predominant sequence of amino acids in alpha chain is Gly-X-Y, where X and Y could be any amino acids but are often proline and hydroxyproline, respectively. This unique sequence allows for the feasible helix formation. Proline and hydroxyproline display a lateral group that loops back to reattach the main chain and results in a ring, which limits the rotation of the polypeptide backbone and thus explains the stabilization of the collagen triple helix and gives collagen rigid structure to serve as the main stress-bearing component of tissues. The entire structure of the collagen is stabilized and reinforced by crosslinks in the form of covalent bonds (Schrieber and Gareis, 2007).

In the manufacture of gelatin, collagen containing raw material is first pretreated with acid or alkali to partially cleave the crosslinks, resulting in two types of gelatin. Type A gelatin is produced by the acid process, which is mainly used with pig skin and fish skin and are not heavily crosslinked (Schrieber and Gareis, 2007). In the acid process, the raw material is soaked in dilute mineral acid for up to 24 h. Type B gelatin is pretreated by the alkali process, in which the raw material is subject to alkali treatment. The alkali process is used for heavily cross-linked collagen, such as bovine hide, and takes longer time (Haug and Draget, 2011). During the pretreatment, the regular triple-helix structure of collagen is hydrolyzed to form random gelatin coils, which can then be extracted with hot water. After extraction, the gelatin solution is usually filtered, deionized, concentrated by evaporation, and then sterilized. The gelatin solution is then cooled to gel. The gels are extruded into "noodles" that are fed onto conveyor belts for drying. The dried gelatin noodles are then ground into particles of 0.1 to 10 mm in diameter, which is usually translucent, brittle, colorless or slightly yellow, nearly tasteless, and odorless (Haug and Draget, 2011).

Type A gelatin has an isoionic point of 7 to 9 based on the severity and duration of the acid treatment, which is hardly different from collagen. For type B gelatin, the alkali hydrolyzes the asparagine and glutamine side chains and almost completely converts them to aspartic and glutamic acid, respectively. As a result, type B gelatin has an isoionic point of 4.8 to 5.2 (Schrieber and Gareis, 2007).

Gelatin is generally regarded as safe (GRAS). Bovine spongiform encephalopathy (BSE) is virtually impossible to survive the gelatin production process (Haug and Draget, 2011).

TABLE 20.2 Amino Acid Composition (% mole) of Gelatin and Globular Proteins

Amino Acid	Pork Skin[a]	Cattle Hide[b]	Warm Water Fish Skin (Catfish)[b]	Cold Water Fish Skin (Alaska Pollock)[c]	Soy Protein Isolate[d]
Aspartic acid	4.6	4.83	4.59	5.21	11.81
Hydroxyproline	9.1	10.39	7.72	5.3	N/A
Threonine	1.8	2.01	2.6	2.68	4.1
Serine	3.5	3.52	3.61	5.85	5.48
Glutamic acid	7.2	7.78	7.22	7.17	21.29
Proline	13.2	13.78	11.14	10.1	5.29
Glycine	33	28.74	34.01	35.7	3.86
Alanine	11.2	11.91	12.4	10.9	3.83
Valine	2.6	2.16	2.19	1.67	4.41
Cystine	0	0.04	0.1	0.14	0.06
Methionine	0.4	0.33	0.49	1.13	0.93
Isoleucine	1	1.28	1.17	1.07	4.48
Leucine	2.4	2.59	2.09	2.1	7
Tyrosine	0.3	0.22	0	0.24	3.71
Phenylalanine	1.4	1.33	1.3	1.2	5.3
Hydroxylysine	0.6	0.86	0.58	0.61	N/A
Lysine	2.7	2.77	3.1	2.78	5.39
Histidine	0.4	0.47	0.6	0.8	2.9
Arginine	4.9	4.98	5	5.18	7.57
Tryptophan	0	N/A	N/A	N/A	N/A
Homocystine	N/A	0.02	0.09	0.16	N/A

[a] *Zhou et al. (2006)*.
[b] *Avena-Bustillos et al. (2006)*.
[c] *Chiou et al. (2006)*.
[d] *Tang et al. (2006)*.

20.2.2 Gelation of Gelatin

Gelatin is insoluble in cold water and swells or hydrates when stirred into cold water. The swollen gelatin particles can hold water 5 to 10 times their weight. When the temperature is raised above 40°C, the swollen gelatin particles dissolve and form a colloid solution or sol, in which the gelatin molecules behave as random coils in water. When the sol temperature is lowered the sol-gel transition happens, during which the gelatin molecules partly revert to the triple-helix structure of collagen as some crosslinks are formed. This sol-gel transition is thermoreversible because the bonding energy in gelatin is relatively weak (Schrieber and Gareis, 2007).

Forming thermally reversible gels is the most remarkable characteristic of gelatin. Gelatin solution will start gelling when the temperature drops below a certain point, which is called gelling temperature. The formed gel will start melting when the temperature increases above a certain point, which is called the gel melting point, and is usually lower than human body temperature. This melt-in-the-mouth property makes gelatin superior as the shell material to encapsulate

TABLE 20.3 Gelling and Melting Temperature of Gelatins

Gelatin Type	Gelling Temperature °C	Melting Temperature °C
Pork skin[a]	27	29
Cattle hide[b]	24.7	32.3
Warm water fish skin (catfish)[a]	22	25
Cold water fish skin (Alaska pollock)[c]	11.9	21.2

[a] *Liu et al. (2008)*.
[b] *Gudmundsson (2002)*.
[c] *Zhou et al. (2006)*.

flavors and nutrients in the food industry (Baziwane and He, 2003). Table 20.3 shows the gelling and melting temperatures of different gelatins. In general, mammalian gelatin has the highest gelling temperature, followed by warm water fish gelatin; the cold water fish gelatin has the lowest gelling temperature.

Gel strength is the most important attribute of gelatin. It depends on the gelatin type, molecular size, concentration, temperature, and setting time. Gelation of gelatin is a slow setting process and the full gel strength is attained after 15 h. When determined by the standard method (GMIA, 1986), gel strength is graded by the Bloom value. Gelatin gel is made by cooling 6.67% gelatin solution at 10°C for 17 h. The gel strength, or Bloom value, is measured as the force, in grams, required to impress a 0.500 in diameter plunger to a depth of 4 mm into the surface of the gel. Commercial gelatins have Bloom values from 50 to 300 Bloom grams, varying from low Bloom (<150), medium Bloom (150−220), to high Bloom (>220) types. Gelatin is usually priced according to the gelling ability; consequently, the higher the Bloom, the higher the price.

Gelatin is a mixture of polymers with different chain lengths. The molecular weight distribution of gelatin is important for its gelation properties. For example, gelatin with high molecular weight normally has high gel strength and high gelling temperature. In the gelatin manufacturing process, with the control of pretreatment and extraction conditions, the desired molecular weight distribution of gelatin is obtained (Haug and Draget, 2011). For example, lower temperature extraction produces high molecular weight gelatin because less hydrolysis of the polypeptide backbone occurs.

Gelatin type is another important factor for gelation properties. Normally, fish gelatin from warmer waters has a similar gel strength to mammalian gelatins and they share similar hydroxyproline content. Fish gelatin from cold water species has low or no gel strength, which is due to its low hydroxyproline content (Table 20.2) (Karim and Bhat, 2009). But there are advantages to using low Bloom gelatin as a stabilizer of emulsions. The emulsion can be produced at or below room temperature, which allows the loading materials (e.g., fish oil) to avoid harsh conditions (e.g., high process temperatures), which can be detrimental to the stability of the loading substance.

As shown in Table 20.1, gelatin has better oxygen barrier properties than globular proteins because of its more linear structure, leading to higher cohesive energy density and lower free volume (Miller and Krochta, 1997).

20.2.3 Gelatin as Shell Material in Microencapsulation

Gelatin has been used as shell material to encapsulate different food ingredients through different technologies due to its superior physical and chemical properties.

20.2.3.1 Spray Drying

In the food industry, spray drying is the most commonly used microencapsulation method. Gelatin microcapsules prepared by spray drying are good carriers for many food ingredients due to the emulsifying and film-forming properties of gelatin.

Lipophilic food ingredients are normally emulsified before being encapsulated by spray drying. Compared with globular proteins, such as casein and whey protein, gelatin is a relatively weaker emulsifier (Dickinson and Lopez, 2001). But gelatin has very good film-forming properties during spray drying. Matsuno and Adachi (1993) interpreted the drying curve of different microencapsulation wall materials in terms of the ability to form a dense network. They suggested that the gelatin drying curve showed that the drying speed decreased rapidly at the early stage of drying,

which implied the rapid formation of a dense film and a good protection of core ingredient. Gelatin is thus considered as the most suitable for microencapsulation using a spray-drying process (Gharsallaoui et al., 2007). Liu et al. (2001) used gum arabic and maltodextrin to encapsulate ethyl butyrate by spray drying. The retention of ethyl butyrate was quite low. By adding 1% gelatin they found that the formation of crust on the surface of the droplets was improved and the retention of ethyl butyrate was appreciably increased.

Gelatin is normally used together with hydrocarbon compounds as shell materials in microencapsulation by spray drying. Shu et al. (2006) prepared lycopene microcapsules using spray drying with gelatin and sucrose as shell materials. Sucrose was used as a plasticizer, which was also believed to promote the formation of spherical and smooth-surfaced microcapsules (Ozgur and Mustafa, 2005). Bruschi et al. (2003) developed propolis ethanolic extraction powder encapsulated in gelatin by spray drying. When mannitol was added in the formulation, the dried powder had a very smooth and uniform surface, and spherical shape. Mannitol retains some water molecules linked to its own structure and could fill the empty space of the microparticles, preserving the hydration, avoiding depressions on the surface, and assuring a more uniform wall of the obtained microparticles (Bruschi et al., 2003).

20.2.3.2 Gelation

Gelatin has been used for microencapsulation based on its gelation property. Different approaches can be used to encapsulate food ingredients in gelatin gels. Gelatin gels show high permeability and dissolve under warm conditions, so the crosslinking of gelatin matrix is normally essential.

A simple way to use gelatin gel for encapsulation is to directly incorporate the core materials in the gelatin solution and then induce the gelation and crosslink the gel.

In patent US 5153177 (Bower et al., 1992), a crosslinked gelatin hydrogel was prepared to encapsulate fat-soluble vitamins. The gelatin was dissolved in 60°C water together with a reducing sugar, like fructose, and a salt, like calcium acetate. The oil containing fat-soluble vitamin was mixed into the solution and the mixture was allowed to gel. The gelled slab was dried at ambient condition and then kept at 70°C for 6 h for Maillard reaction crosslinking. The crosslinked slab was kept insoluble in boiling water. US Patent 4670247 (Scialpi, 1987) revealed a process called powder catch to make water-insoluble beadlets to encapsulate fat-soluble vitamins. The first step was the emulsification of the fat-soluble vitamins in gelatin and reducing sugar solution. The emulsion was then sprayed into collecting powder, which converted droplets to particles. The gelatin gelled during this process. The collecting powder can be starch or modified starch, such as starch esters. The particles were then separated and dried with air stream. The dried beadlets were heated to high temperature (90−180°C) to induce a Maillard reaction to make insoluble beadlets. US Patent 6444227 (Leuenberger et al., 2002) reported a similar process to prepare beadlets but crosslinked the gelatin matrix by radiation or enzyme treatment. Patent WO 2005/089569 (Diguet et al., 2005) applied these beadlets containing polyunsaturated fatty acid in the extruded foods with excellent sensory properties.

Another way to encapsulate food ingredients in gelatin gel constitutes two steps. Gelatin microspheres are first prepared, which are then loaded with the food ingredients.

Gelation-induced phase separation is normally used to prepare the gelatin microspheres. Iwanaga et al. (2003) used the water-in-oil emulsion technique to prepare gelatin microspheres. Gelatin aqueous solution (10%) was preheated to 40°C and added drop-wise into olive oil under stirring to form the water-in-oil emulsion. The emulsion was cooled to induce the gelation of the gelatin droplets under stirring. The collected gelatin microspheres were crosslinked using glutaraldehyde and then freeze dried. Kong et al. (2011) prepared gelatin microspheres by the water-in-water emulsion technique. Gelatin was dissolved in water under stirring at 60°C to form a homogeneous solution, which was used as the dispersed phase. This gelatin solution was added drop-wise into polyethylene glycol (PEG) aqueous solution under agitation at 60°C. The PEG solution formed the continuous phase in this water-in-water emulsion system. Then the gelatine microspheres were solidified by rapidly cooling to 5°C. The gelatin microspheres were purified by multiple washing with chilled water. These microspheres were then crosslinked with glutaraldehyde and dried. Morita et al. (2001) prepared gelatin microspheres by freeze drying aqueous solutions containing gelatin and PEG. Phase separation between gelatin and PEG during freezing was assumed to be the mechanism of the formation of gelatin microspheres.

The prepared gelatin microspheres can be loaded with ingredients by diffusion. Dinarvand et al. (2005) loaded gelatin microspheres with lactic acid by immersing them in an aqueous solution of lactic acid. Yamamoto et al. (2001) prepared an aqueous solution of the protein to be loaded and dropped it onto the prepared dry gelatin microspheres. Iwanaga et al. (2003) discussed the *in vitro* release rate of insulin from gelatin microspheres prepared with various crosslinking densities and concluded that the release rate could be controlled by the density of the crosslinking.

20.2.3.3 Coacervation

Microencapsulation by proteins can be obtained by the formation of an insoluble layer of protein around fine droplets of core material. Usually, the formation of the insoluble layer is achieved by simple or complex coacervation. The first approach is based on the addition of compounds with high affinity for water, such as alcohols. The second approach is based on the formation of a protein-rich layer around the core material, by interaction with oppositely charged polymers (Magdassi and Vinetsky, 1995). The gelatin crosslinking process is often used to fix the shell structure and the coacervates can be dried by different ways, such as spray drying and freeze drying.

Patent US 5035896 (Apfel et al., 1991) described a simple coacervation process using low melting point fish gelatin being soluble at 5 to 10°C. The coacervation of fish gelatin can be brought by lower alkyl alcohols such as ethanol and inorganic salts such as sodium sulfate. In the gelatin-based microencapsulation process, normally gelatin must be heated to approximately 40°C or above to dissolve the gelatin. This temperature may adversely affect heat labile core materials. The process of this invention is particularly useful for heat-sensitive core materials to be encapsulated in gelatin shell.

In a complex coacervation process, gelatin has been widely used as positively charged polymer. Patent US 7727629B2 (Yan, 2010) revealed a complex coacervation process to make multicore microcapsules. Type A gelatin with 275 of Bloom and isoelectric point of 9 was dissolved in 50°C deionized water. Fish oil was dispersed into the gelatin solution followed by homogenization to form oil-in-water emulsion. The emulsion was diluted with deionized water at 50°C. The sodium polyphosphate solution was then added into the emulsion with agitation. The pH was then adjusted to 4.5 with a 10% aqueous acetic acid solution. During pH adjustment and the cooling step that followed, coacervates formed from gelatin and polyphosphate coated onto the oil droplets to form primary microcapsules. After cooling, the primary microcapsules started to agglomerate to form lumps under agitation. After further cooling, polymers remaining in the solution further coated the lumps of primary microcapsules to form microcapsules with an outer shell and average size of 50 μm. Once the mixture was cooled to 5°C, gluteraldehyde was added to crosslink the shell. The mixture was then warmed to room temperature and stirred for 12 h. Finally, the microcapsule suspension was washed with water. The washed suspension was then spray dried to obtain a free-flowing powder. The powder particle size would be a little bigger than the size of microcapsules in the slurry and vary according to the drying conditions.

Gelatin has been used in complex coacervation with different polyanions. With acacia as polyanion, Junyaprasert et al. (2001) prepared gelatin acacia complex coacervates with vitamin A palmitate in corn oil as the core material. Silva et al. (2012) used bovine gelatin and pectin as wall material to encapsulate lycopene by a complex coacervation process.

The gelatin coacervation process can be controlled by pH, polymer concentration, and agitation (Yan, 2010). Nakagawa and Nagao (2012) used a freezing process to control the gelatin complex coacervation. An oil-in-water emulsion stabilized by gelatin−acacia solution was prepared, and the pH of the emulsion was adjusted with acetic acid. When emulsion pH was adjusted to 3 and 4, clear phase separations were confirmed. When emulsion pH was adjusted to 4.7, the emulsion was visibly stable at ambient temperature. By freezing the pH 4.7 emulsion, complex coacervation was confirmed. The membrane properties were found to be dependent on the cooling rate during freezing. However, freezing the emulsions with pH levels of 3.0 and 4.0 caused damage to the membrane due to ice crystal formation.

Surfactants play an important role in the gelatin complex coacervation process. Bhattacharyya and Argillier (2005) and Li et al. (2009) enhanced the shell structure of the gelatin−acacia complex coacervation microcapsules using cationic surfactants. Li et al. (2009) enhanced the shell structure of the gelatin−sodium carboxymethylcellulose complex coacervate by using sodium dodecyl sulfate (SDS). Magdassi and Vinetsky (1995) prepared gelatin microcapsules using type A gelatin and anionic surfactant SDS with soybean oil as the core material.

20.3 SOY PROTEIN

Soybean is a species of legume that contains 35 to 40% of proteins. Ten percent of soy proteins are water-soluble albumins and around 90% of soy proteins are globulins (Fukushima, 2004). Globulins are soluble in diluted salt solutions. β-Conglycinin and glycinin are the two major globulins in soy protein. β-Conglycinin is a trimeric protein with the sedimentation coefficient (SC) of 7S and the molecular weight (MW) of 70 kDa, whereas glycinin is a hexameric protein and has the SC of 11S and the MW of 350 kDa (Fukushima, 2004). The ratio between β-conglycinin and glycinin, which varies with the species and production process, has a significant effect on protein functionality (Swanson, 1990). For example, the higher hydrophobicity and more easily unfolded structure in β-conglycinin make its emulsifying ability much stronger than that of glycinin (Fukushima, 2004).

In the food industry, soybean attracts people's attention partly due to the nutritional value of soy proteins and other components in soybean, such as genistin, and partly due to the functional properties of soy proteins in food systems. Soy protein has superior functional properties, such as fat absorption, gelation, emulsification, film forming, and good barrier property, which make soy protein a good microencapsulation shell material. However, off-flavors (grassy, beany, bitter, astringent, and chalky) and allergen issue limit the application of soy proteins (Okubo et al., 1992). Soy protein isolate (SPI) is the most widely used soy protein product used for microencapsulation in the food industry. Aqueous alkaline extraction followed by isoelectric precipitation and salt extraction are commonly used for producing SPIs.

20.3.1 Spray Drying

Soy protein has been used in microencapsulation through different techniques. Spray drying is the commonly used one. Solubility is important for a microencapsulation shell material used in the spray-drying method. Protein solubility results from the hydrophilic groups on the surface of the protein interacting with water. The pH of the system is a major factor in determining solubility of a legume protein, as solubility decreases around the isoelectric point of the protein. In general, most legume proteins have very low solubility between pH 4 and 6, but outside this range, solubility increases dramatically (Boye et al., 2010). Soy protein possesses good solubility in water at pH higher than 9 (Fukushima, 2004). Soy protein has good drying and film-forming properties (Nesterenko et al., 2012) and good fat-absorbing property, which make it widely explored in microencapsulation of fat-soluble ingredients by spray drying.

With flavor compounds as core materials, Charve and Reineccius (2009) evaluated the microencapsulation performance of SPI, whey protein isolate (WPI), sodium caseinate, modified starch, and gum arabic by spray drying. The proteins effectively reduced the oxidation of the core material, which was pronounced in modified starch and gum arabic shells. WPI and SPI were good materials for the production of dried flavors based on volatile retention during spray drying. However, they performed poorly during storage as nonenzymatic browning occurred. Sodium caseinate performed poorly due to the low infeed solids limited by viscosity and its susceptibility to browning.

Casein hydrolysate has high biological activity for nutraceutical food formulation, but its application is limited by its intense bitter taste and high hygroscopicity. Ortiz et al. (2009) encapsulated casein hydrolysate by spray drying with SPI as wall material, which significantly attenuated the bitter taste of the casein hydrolysate. The bitter taste of casein hydrolysate is directly related to the existence of hydrophobic groups, which were probably less exposed due to hydrophobic interactions with the SPI in the encapsulating process. But the microcapsules had much higher hygroscopicity. Favaro-Trindade et al. (2010) used SPI and gelatin mixture as the shell and made the spray-dried casein hydrolysate microcapsules with lower hygroscopicity.

Rascón et al. (2011) evaluated the performance of SPI and gum arabic on encapsulating carotenoid by spray drying. When gum arabic was used as shell material, the microcapsules were stable at low water activities during storage. But if water activity (a_w) is above 0.318, the degradation rate of carotenoid increased substantially, and if a_w is above 0.743, microcapsules became unable to keep their structural integrity and led to the gradual dissolution of the walls. The microcapsules with SPI as wall material were very stable at high water activities from 0.721 to 0.851.

Emulsification is critical for microencapsulation by spray drying. Soy proteins exhibit high emulsifying properties compared to other plant proteins (Zayas, 1997). Like other proteins, SPI is an ineffective emulsifier near the isoelectric point, where charges and solubility are minimal.

Kim et al. (1996) investigated the abilities of gum arabic, sodium caseinate, WPI, and SPI to form orange oil emulsion. Although SPI generated the largest orange oil emulsion droplets, SPI-emulsified orange oil droplets were found the most stable against creaming during 10 days of storage at room temperature, which could be due to SPI-emulsified orange oil droplets being surrounded by the thickest membrane structures. The spray-drying study (Kim and Morr, 1996) showed that SPI was the most effective wall material for retaining orange oil during spray drying.

Soy protein has been investigated through different modification processes to improve the emulsifying property. Protein hydrolysis results in peptides with smaller molecular size and less secondary structure than the original proteins, which give increased solubility and decreased viscosity. A strictly controlled hydrolysis usually improves the emulsifying property of the protein. Wu et al. (1998) prepared soy protein peptides by papain hydrolysis and obtained high emulsifying properties and protein solubility. Nesterenko et al. (2012) modified SPI by enzymatic hydrolysis and N-acylation. The oil-in-water emulsions prepared with native or modified SPI and hydrophobic α-tocopherol were spray dried to produce α-tocopherol microparticles. The results demonstrated that emulsions prepared with modified SPI had smaller droplet size and lower viscosity compared to those prepared with native proteins. The efficiency of oil retention decreased after protein hydrolysis from 79.7 to 38.9%, but the grafting of hydrophobic chain by acylation improved efficiency of α-tocopherol retention up to 94.8%. Molina et al. (2001) reported that the emulsifying ability index (EAI)

reached the highest level after treatment under 400 to 600 MPa. Wang et al. (2008) suggested that unfolding of proteins and subsequent exposure of hydrophobic groups during the high pressure treatment improved the emulsifying property of SPI. But high pressure treatment resulted in a gradual and significant decrease in emulsion stability index (ESI).

20.3.2 Coacervation

SPI is amphoteric and has an isoelectric point of pH 4.5, similar to that of gelatin. Its potential to be used as a polycation in complex coacervation has been explored. Mendanha et al. (2009) encapsulated casein hydrolysate by SPI−pectin complex coacervation. The bitterness was significantly masked. Compared with the spray-dried casein hydrolysate microcapsules (Ortiz et al., 2009), the complex coacervates had much lower hygroscopicity. Nori et al. (2011) encapsulated propolis extract with SPI and pectin by the complex coacervation process to obtain it in powder form. The microencapsulation also preserved the phenolic and flavonoid compounds present in free propolis from oxidation. Gan et al. (2008) used SPI as shell material and prepared fish oil microcapsules by simple coacervation. Microbial transglutaminase (MTGase) and ribose were tried to crosslink the microcapsules. It was found that compared to the use of ribose, the use of MTGase to crosslink SPI did not satisfactorily yield sufficient protection to the fish oil.

20.3.3 Gelation

Similar to gelatine, soy protein has been used as microencapsulation shell materials based on its gelation property.

Heat denaturation is normally regarded as a prerequisite for gel formation of globular proteins (Chen and Subirade, 2009). In a stable soy protein solution, the surface of the soy protein micelles is predominantly occupied by the hydrophilic group. Heating protein dispersion causes protein molecular unfolding, in which functional groups (such as sulfhydryl groups or hydrophobic groups) become exposed and interact with each other through disulfide interchange reaction or hydrophobic bonding leading to protein aggregation (Zhang et al., 2012). When the protein concentration is high enough, aggregation leads to formation of a gel. At lower concentrations, aggregation leads to precipitation of the protein. For SPI, the minimal protein concentration for gelation is around 6.6% (Bikbov et al., 1979). Disulfide bond formation on heating makes the gelation process irreversible. In contrast, gelatin gels are stabilized mainly by hydrogen bonds and the gelation process is thermoreversible. The gelation and characteristics of soy protein gel depend on the protein species and concentration, pH, and ionic strength. Minerals, especially calcium, can serve as a salt bridge to enable polypeptide chains to approach one another, facilitate the protein aggregation, and therefore enhance the strength of the gel. In the case of β-conglycinin and glycinin, the larger numbers of sulfhydryl groups and their topology in glycinin make glycinin gel much harder and more turbid in comparison with β-conglycinin gel (Fukushima, 2004). Chen and Subirade (2009) prepared SPI microspheres using the gelation method and loaded the prepared microspheres with riboflavin.

Excluding heat-induced gelation, enzyme-induced soy protein gelation was also explored for microencapsulation purposes. Cho et al. (2003) encapsulated fish oil using the double emulsification and subsequent enzymatic gelation method. SPI was used as shell material and it showed higher reactivity with MTGase.

20.4 ZEIN PROTEIN

Commercial zein is essentially a by-product of the corn wet-milling industry. The protein content of corn varies from 6 to 12% on a dry basis. According to the solubility in selected solvents, corn proteins primarily include albumin (soluble in water), globulin (soluble in salt), glutelin (soluble in alkali), and zein (soluble in alcohol). Depending on the protocol used for protein fractionation, zein content ranges from 35 to 60% of the total protein of corn (Shukla and Cheryan, 2001). Zein contains a rich amount of glutamic acid (21−26%), leucine (20%), proline (10%), and alanine (10%), but lacks basic and acidic amino acids. The high amount of nonpolar amino acid residues and lack of basic and acid amino acids are responsible for the insolubility of zein (Shukla and Cheryan, 2001). Biologically, zein is a mixture of proteins with various molecular size, solubility, and charge. Esen (1986) separated zein into three fractions labeled α-zein, β-zein, and γ-zein, based on the solubility in isopropyl alcohol (IPA) solutions. α-Zein can be extracted using only aqueous alcohol, whereas the other zeins need a reducing agent in the solvent to be extracted. α-Zein accounts for 75 to 85% of the total zein. Commercial zein is mainly α-zein (Anderson and Lamsa, 2011) extracted by 86% aqueous IPA, which selectively dissolves α-zein and minimizes the other fractions of zein.

Zein has been employed as an edible coating for foods and pharmaceuticals because of its low moisture absorption, high thermal resistance, and good mechanical, oxygen, and aroma barrier properties (Shukla and Cheryan, 2001).

From a nutritional point of view, zein is deficient in essential amino acids, such as lysine and tryptophan. At the same time, its insolubility in water limits its use in food products. However, zein has good potential as a shell material in microencapsulation due to its unique hydrophobic properties. For example, it is interesting to use zein as a food grade biopolymer carrier of antimicrobials because zein is insoluble in conventional food beverages (Zhong and Jin, 2009).

20.4.1 Spray Drying

Zein can form tough, glossy, hydrophobic, greaseproof films that are resistant to microbial attack, with excellent flexibility and compressibility. Therefore, zein has been explored for microencapsulation through spray drying. Zhong and Jin (2009) prepared spray-dried zein microcapsules to encapsulate lysozyme, an antimicrobial. Sustained release of lysozyme was obtained, which is important for an antimicrobial to enhance its efficacy. Quispe-Condori et al. (2011) prepared flax oil microcapsules using zein as a coating material by spray and freeze drying. It was demonstrated that spray drying had much higher microencapsulation efficiency than freeze drying.

20.4.2 Zein Microspheres by Solvent Evaporation

Zein is soluble only in aqueous ethanol, so aqueous ethanol may be used as a solvent to dissolve both lipophilic bioactive compounds and the carrier zein. Ethanol is easily removed through evaporation, which makes it possible to prepare zein microspheres by phase separation in a nonsolvent followed by solvent removal by evaporation.

Parris et al. (2005) reported a process to make zein microspheres to encapsulate essential oils. In the process, the oil and corn zein were dissolved in aqueous alcohol, and then rapidly sheared in water containing a small amount of dispersant. The decrease of overall ethanol concentration resulted in the precipitation of oil-loaded zein particles. The suspension of particles was freeze dried. Zhong and Jin (2009) used a similar method and prepared fish oil-loaded zein microspheres. Su (2012) encapsulated citral flavor in zein by the solvent evaporation method. Patent WO 91/06286 prepared zein microspheres containing insulin. Zein was dissolved in 90% ethanol to form the encapsulating phase. Insulin was added to the zein solution. The mixture was dispersed into an immiscible phase (corn oil) with agitation. The first solvent (ethanol) is then removed by heating the mixture to 45°C, then cooled down to room temperature. Zein is insoluble in corn oil, and precipitates after evaporation of ethanol, forming a suspension of microspheres. The resulting microspheres were repeatedly washed with petroleum ether to remove the oil and filtered. The washed microspheres were then dried. Low temperature is one of the advantages of this process. Zein was also found promising in microencapsulation by other techniques, such as electrospinning (Torres-Giner et al., 2010).

20.5 PEA PROTEIN

Pea is a leguminous plant. Like other legume seeds, pea seeds contain high amounts of protein (20−30%). Pea proteins mainly exist as globulins (65−80%), which are also the main components in pea protein isolate (PPI) products. Globulins are composed of legumin 11S and vicilin 7S. Legumin is a hexameric oligomer consisting of 6 subunits with a molar mass of 60 kDa. Each subunit has one basic and one acidic subunit associated via a disulfide bond. Vicilin is a trimer and each subunit has a molar mass of about 50 kDa. The ratio of legumin to vicilin ranges from 0.2 to 1.5 depending on the genetics as well as the processing involved (Casey et al., 1982). The use of pea protein in the food industry for the formulation of new food products is very interesting because of its nonallergenic characteristic, despite the high nutritive value and good functional properties (Gharsallaoui et al., 2009).

20.5.1 Spray Drying

Pea protein has good film-forming properties (Viroben et al., 2000) and has been used as shell material in microencapsulation by the spray-drying method.

Gharsallaoui et al. (2012a) encapsulated medium chain triglyceride oil using PPI and maltodextrin by spray drying. They found that microcapsules with lower dextrose equivalent (DE) maltodextrin were shallow and presented rough surfaces, and microcapsules with higher DE maltodextrin were circular and uniform showing minimum cracks and dents on the surface. Qi (2004) encapsulated β-carotene in a PPI and corn syrup solid (DE40) system using emulsification and spray drying. They found that corn syrup solid had significant effect on the β-carotene retention during storage. Pea protein-stabilized emulsions can be very sensitive to environmental factors such as pH and ionic strength. For this reason, they are usually combined with another emulsifier/stabilizer such as high methoxyl pectin (Gharsallaoui et al., 2010).

Gharsallaoui et al. (2012b) prepared multilayered emulsions using PPI and pectin to encapsulate flavor compounds (ethyl esters) and spray dried the emulsions to obtain dry powders. The results were compared with spray-dried powders from monolayered emulsion stabilized only by PPI. They suggested that the addition of pectin markedly improved the retention of the flavor compounds, which was possibly due to pectin being able to improve physical integrity of emulsion oil droplets during spray drying by preserving the β-sheet secondary structure of pea protein when pea globulins/pectin complexes are heated. Pea proteins were also successfully used for microencapsulation of hydrophilic ingredients by spray drying (Pereira et al., 2009).

To be used as a shell material in spray-dried emulsion, emulsifying property is important. Ducel et al. (2004a) reported that pea globulins were highly surface active at the oil−water interface, even more than gelatin at the same concentration. Karaca et al. (2011) compared emulsifying properties of PPI with SPI. PPI had lower emulsion capacity and stability, which was attributed to its higher surface hydrophobicity, lower surface charge, and lower solubility. They suggested that isolates produced by isoelectric precipitation had higher surface charge and solubility compared to those produced by salt extraction. Gharsallaoui et al. (2009, 2012a) evaluated the pH dependence of the stability of PPI emulsifying capacity. They reported the lowest emulsifying capacity at pH around the isoelectric point (pI $\approx$ 4.3), and both acidic and alkaline pH improved the emulsion capacity indicating the positive correlation between solubility and emulsifying capacity of PPI. The high emulsifying capacity of PPI at pH values below 4 and above 5 is due to intermolecular repulsion of charged amino and carboxyl groups, respectively; this aided dispersion of the protein molecules and increased repulsions between protein-stabilized oil droplets. At pH 4 to 5, the lack of electrostatic repulsions among emulsion particles promoted rapid coalescence and this decreased the volume of the emulsified fraction. They also stated that emulsions were more stable to creaming at acidic pH than at alkaline pH ranges, which was interpreted in terms of pea protein solubility, globulin dissociation, and oil-droplet surface electrostatic charge. Koyoro and Powers (1987) reported similar results. They found that at pH 4, pea protein globulins had minimum solubility and emulsifying capacity, which was improved when pH went higher or lower. They reported higher emulsifying capacity of pea protein globulins at pH 3 than pH 7, which followed their solubility profiles. As pea protein contains a large amount of reactive amino groups (lysine residue), chemical modification reactions onto the amino group, such as acetylation or succinylation, could effectively be carried out. These reactions were powerful to improve, for instance, emulsifying properties (De Graaf et al., 2001).

20.5.2 Coacervation and Gelation

Ducel et al. (2004b) demonstrated the potential of using PPI as polycation in complex coacervation to encapsulate medium chain triglyceride. Gum arabic was used as polyanion in the process and the optimum coacervation condition was obtained at pH 2.75 with a protein:polysaccharide ratio of 3:7.

PPI shows good potential as a matrix material for the microencapsulation of β-carotene, by means of supercritical CO_2 technology (De Graaf et al., 2001). In this process, microspheres based on PPI were first prepared by a water-in-oil emulsion technique. Stable PPI microspheres were obtained by gelation of the pea protein by heat treatment. The PPI microspheres were isolated and dried to give a free-flowing powder. β-Carotene was then loaded in the microspheres via supercritical CO_2 treatment.

20.6 CONCLUSION

Gelatin and other plant source proteins, such as soy protein, corn zein, and pea protein, have good emulsifying and oxygen barrier properties. Proteins, especially gelatin, have excellent drying property, film-forming property, and unique gelation property. Based on those physical and chemical properties, different techniques, including spray drying, coacervation, gelation, and so on, have been used to microencapsulate food ingredients with proteins as shell materials.

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Chapter 21

Hydrocolloids and Gums as Encapsulating Agents

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21.1 INTRODUCTION

Hydrocolloids are a diverse group of polysaccharides that have come to be appreciated for, among other things, their function as wall materials in microencapsulation processes. This chapter covers a number of hydrocolloids commonly used for this purpose, as well as some of the most frequent applications of hydrocolloid encapsulation.

21.2 MATERIALS

Gum arabic and sodium alginate are by far the most studied encapsulating hydrocolloids and are each described in detail in this section. However, many other hydrocolloids are also in use; the properties of these are summarized in Table 21.1. Some materials that are often grouped with hydrocolloids but that have dedicated chapters in this book—gelatin, for example—are not included here.

21.2.1 Gum Arabic

Gum arabic, also known as gum acacia, is a plant exudate that originates from some plants in the *Acacia* family, in particular the *Acacia senegal* and *Acacia seyal* trees. Production of the gum occurs naturally as a response to tissue damage or environmental stress. It is artificially encouraged by carefully removing sections of bark from the trees so as not to damage the plants themselves. The resulting ooze eventually hardens into nodules on the surface of the trees and is harvested by hand, at which point it can be divided into grades and undergo further processing. This processing could be as simple as breaking the nodules into smaller, more manageable pieces, or it could include purification of the gum followed by spray or roller drying. Although *Acacia senegal* is traditionally considered to produce the more premier form of gum arabic, blends of the two species are common due to the lower cost of *Acacia seyal* and the slightly different properties each holds (Williams and Phillips, 2009).

The basic building blocks of gum arabic are sugars (galactose, arabinose, and rhamnose), glucuronic acid, and amino acids (primarily hydroxyproline and serine, with significant content of aspartic acid, leucine, and glycine) (Williams and Phillips, 2009). The material has been found to be a blend of three different polymer fractions: carbohydrate-based arabinogalactan (88%), the low-protein arabinogalactan−protein complex (10%), and high-protein glycoprotein (1%) (Nussinovitch, 2003). The arabinogalactan molecules are responsible for the film-forming properties of the gum, while the protein-containing portions, in particular the glycoprotein molecules, lend its emulsification abilities. Stabilization of emulsions results from the amino acids, which are largely hydrophobic, embedding themselves in the oil droplets of an oil-in-water mixture, and from the slight negative charge contributed by glucuronic acid, which creates a repulsive force that prevents these droplets from coalescing. Additional properties of gum arabic include very high water solubility, often as much as 50% (Thevenet, 1995), and a viscosity that starts low and increases relatively slowly up to

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00021-2

TABLE 21.1 Selected Information about the Most Common Hydrocolloids used for Microencapsulation in the Food Industry

Name	Source	Monomers	Structure Notes	Uses	References
Alginate	*Laminaria* spp. (brown algae)	Mannuronic acid Guluronic acid	Linear (1→4) linkages Homo- and heteropolymeric blocks	Ionic gelation, spray drying	Roy et al. (1986), Anker and Reineccius (1988), Lencki et al. (1989), Bhandari et al. (1992), Sheu et al. (1993), Sheu and Marshall (1993), Mishra and Kar (2003), Nussinovitch (2003), Reilly and Subramanian (2003), Leclercq et al. (2009), Azarnia et al. (2011), Burin et al. (2011), Sohail et al. (2011), Frascareli et al. (2012), Nualkaekul et al. (2012), Perrechil et al. (2012), Sarkar et al. (2012), Silva et al. (2012)
κ-Carrageenan	*Kappaphycus alverezii* (red seaweed)	Galactose 3,6-Anhydrogalactose	Linear polymer Alternating α-(1→3) and β-(1→4) linkages	Ionic gelation	Champagne et al. (2000), Sun and Griffiths (2000), Özer et al. (2009), Idham et al. (2012)
Gellan gum	*Sphingomonas elodea* (microorganism)	Glucose Glucuronic acid Rhamnose Glyceryl, acetyl	Linear repeating unit is tetrasaccharide (glu, gluA, glu, rha) One glyceryl substituent per repeat unit One acetyl substituent per two repeat units	Ionic gelation	Idham et al. (2012), Sobel (2012)
Guar gum	*Cyamopsis tetragonoloba* (plant seeds)	Mannose Galactose	Linear mannose backbone connected with β-(1→4) linkages Galactose side branch attached to every other mannose with α-(1→6) bonds	Spray drying	Rivera et al. (2010)
Gum arabic	*Acacia* spp. (tree exudate)	Galactose Arabinose Rhamnose Glucuronic acid	Main fraction consists of (1→3)-linked galactose units with large side branches made of other carbohydrate monomers Also contains protein fractions	Spray drying, coacervation	Klein et al. (1983), Thevenet (1995), Sultana et al. (2000), Kailasapathy and Lam (2005), Drusch et al. (2007), Homayouni et al. (2008), Schaffner and Schäfer (2008), Tsen et al. (2008), Al-Assaf and Phillips (2009), Bhandari (2009), Brownlee et al. (2009), Draget (2009), Prasad and Kadokawa (2009), Williams, and Phillips (2009), Champagne et al. (2010), Tonon et al. (2010), Rascón et al. (2011), Adamiec et al. (2012), Cilek et al. (2012), Ortakci et al. (2012)

(*Continued*)

TABLE 21.1 (Continued)

Name	Source	Monomers	Structure Notes	Uses	References
Konjac glucomannan	*Amorphophallus konjac* (plant tuber)	Glucose Mannose	Main chain is repeating unit of (1→4)-bonded glucose and mannose residues Branches are attached approximately once every 80 sugar monomer units	Spray drying	Tonon et al. (2010), Adamiec et al. (2012)
Mesquite gum	*Prosopis* spp. (tree exudate)	Galactose Arabinose Glucuronic acid	Linear backbone of galactose attached by (1→3) bonds Large and frequent branches attached with (1→6) linkages	Spray drying	Ibarguren et al. (2012), Ortakci et al. (2012)
Pectin	Citrus peels Apple pomace	Galacturonic acid Rhamnose Galactose Arabinose	Galacturonic acid is the majority monomer Contains linear galacturonic acid segments and highly branched segments with the other sugars	Ionic gelation, spray drying	Burin et al. (2011), Cilek et al. (2012), Garcia et al. (2012)
Xanthan gum	*Xanthomonas campestris* (microorganism)	Glucose Glucuronic acid Mannose	Linear backbone of (1→4)-linked glucose Side chain every other glucose with one glucuronic acid residue followed by two mannose residues	Ionic gelation	Sobel (2012)

Information not found in the cited references is taken from Phillips and Williams (2009). For additional information about specific applications, sources from the reference list are given for each material.

25% solids or more (Sobel, 2012). At low shear, a gum arabic solution at 30% solids has a viscosity that is less than one-tenth that of a 10% pectin solution and less than one-hundredth that of a 1% xanthan gum solution (Al-Assaf and Phillips, 2009). Gum arabic thus forms an encapsulating solution that is much easier to work with than those made with other, more viscous materials.

This combination of properties makes gum arabic the go-to choice for two different methods of encapsulation. It is traditionally the most popular wall material for spray drying and, when paired with gelatin, can also be used for complex coacervation. Gum arabic is suitable for spray drying because it can initially form stable emulsions in relatively high concentrations, it maintains a low enough viscosity to easily move through the pump of a spray dryer, and it is able to form films after drying. In coacervation, it is preferred because of its slight negative charge above pH 2.2 (Burgess and Carless, 1984), which results in an attraction to positively charged gelatin molecules.

Because gum arabic is a natural substance with some variation, especially between the two species, but also as a result of factors such as location of the harvest and age of the tree, it is possible to select for the properties that are most important for a given application, and many producers sell a variety of gum arabic blends that take advantage of this. Variation is not always a positive thing, however, and inconsistency in the product is often listed as one drawback. In addition to variable product quality, the primary countries of production—Sudan, Nigeria, and Chad—have historically encountered problems with price stability due to unfavorable environmental conditions or political unrest (Williams and Phillips, 2009). For this reason, a number of studies have looked into possible replacements, but so far no material has been found that can mimic all of the unique properties possessed by gum arabic, so, for now, gum arabic remains an essential material for encapsulation by both spray drying and coacervation.

21.2.2 Alginates

Alginic acid is a polymer that is found in the cell walls of brown algae as well as some bacterial cells. Although it can be used as is, for reasons of stability it is more commonly found as one of its salts, with sodium alginate being the most common of these (Brownlee et al., 2009). Alginate for large-scale industrial use is taken from algae, because an economically feasible way to extract alginate from bacteria has not yet been devised. The production process for alginate requires first setting up an ion exchange solution with the milled particles of algae to replace the diverse mixture of sea water counterions with protons, creating pure alginic acid. Once this has occurred, a neutralizing sodium source can be added, substituting the hydrogen atoms with sodium to create the water-soluble sodium alginate salt. This allows the remaining solid material from the algae to be removed through one of a number of mechanical processes, leaving pure sodium alginate (Draget, 2009).

Alginic acid is made up of two monomer units: α-L-guluronic acid and β-D-mannuronic acid. These are connected in a linear chain in distinct segments: homopolymeric blocks of either guluronic or mannuronic acid, and heteropolymeric blocks, which contain both. The heteropolymeric blocks, although not made up of strictly alternating units, do contain almost equal concentrations of each acid, with many of these in guluronic−mannuronic dimers (Brownlee et al., 2009).

Alginates are most noted for their gelling abilities. In the presence of di- or trivalent cations, linkages are made between the guluronic acid blocks of one polymer chain and another, creating a network (Prasad and Kadokawa, 2009). For this reason, varieties containing a high ratio of guluronic to mannuronic acids and a large concentration of homopolymeric blocks are often desired for gel applications. In particular, those alginates produced from *Laminaria hyperborea* tend to have high guluronic acid levels and are therefore well suited to gel applications (Draget, 2009).

The ability to undergo ionic gelation makes alginates attractive wall materials for encapsulation. Although the kinetics of gel formation change with temperature, heat is not required to set alginate gels, so it is particularly well suited for protection of heat-sensitive materials (Draget, 2009). Alginate gels are porous, so they are not a good choice for particularly small molecules such as flavors, but for encapsulating sensitive bacteria or enzymes, they have often been found to be effective (Brownlee et al., 2009).

Unlike gum arabic, the supply of alginic acid is very stable; even with an average annual production of 30,000 tons, only 10% of the standing crop must be processed each year, and the algae can also be cultivated if natural sources become less abundant. Additionally, as knowledge of the polymer and ways to modify it increase, it is becoming more possible to tailor the polymer structure, including size and concentration of each monomer, to a chosen application. For this reason, alginate appears to be a microencapsulation material that will continue to be in regular use in the food industry in the coming years (Draget, 2009).

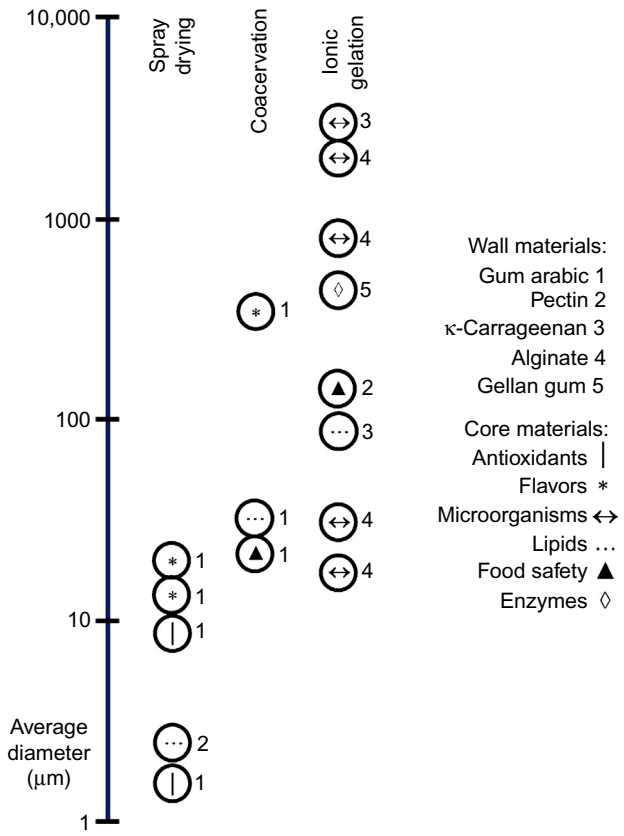

FIGURE 21.1 A graphic summarizing information about method, core, and wall materials and the resulting size of encapsulates for select references from this chapter. Sources mentioned throughout this chapter were included here if they measured particle size and included this information in the form of a mean particle diameter.

21.3 APPLICATIONS

The following subsections discuss some of the most frequent and notable applications for hydrocolloids as wall materials. Also included in this section is Figure 21.1, summarizing properties of hydrocolloid microencapsulates produced using a variety of encapsulation methods and wall materials.

21.3.1 Antioxidants

Antioxidants are compounds that are able to inhibit the process of oxidation and have been linked to numerous health benefits for consumers. They are popular additives to foods, both for their functionality as health-promoting compounds and for their often bright pigments. Unfortunately, these compounds are also sensitive to harsh conditions, including light, heat, and extreme pH levels. Continued exposure to any of the above can lead to compounds that are no longer able to confer a health benefit and that take on unpleasant off-colors. For this reason, encapsulation has received a lot of attention as a means of protecting antioxidants from contact with their surroundings, thus preserving them. Spray drying is the most common method for encapsulating antioxidants in hydrocolloid matrices and is commonly paired with gum arabic as a wall material. A typical formula for spray drying fruit or plant extracts that contain antioxidants is shown in Table 21.2.

The standard method for encapsulation of antioxidants involves dissolving the wall material in water, creating an emulsion of this solution with the chosen antioxidant material, and feeding this emulsion through a spray dryer. The spray-drying process draws off the water, creating antioxidant particles with a protective coating of wall material. With the low water solubility of some antioxidants, however, such particles can be difficult to incorporate into beverages, leading one group to patent an alternative process. This patent implements additional steps in the encapsulation process

TABLE 21.2 Appropriate Ranges of Hydrocolloid Carrier and Antioxidant Extract Core Concentrations for an Emulsion Intended for Spray Drying

Item	Percent (wt)/(vol water)
Gum arabic	6–24
Maltodextrin	0–12
Extract solids	3–11.5
Total solids	9–30

Although maltodextrin is not covered in this chapter, it is often used to supplement gum arabic in these types of emulsions. These figures do not apply to pure antioxidant compounds, which are added to the carrier solution in much smaller quantities; formulas for many of these can be found in Rodrigues et al. (2012). Compiled from Sun and Griffiths (2000), Homayouni et al. (2008), Prasad and Kadokawa (2009), Ibarguren et al. (2012), and Ortakci et al. (2012)

to create smaller particles that will disperse properly in beverages. First, the antioxidant is mixed with a water-immiscible solvent and at least one fat or oil, such as medium chain triglycerides; this mixture is added to the solubilized wall material, homogenized as usual, and the solvent is allowed to evaporate. Following evaporation of the solvent, the emulsion is fed through a filter with several layers of increasingly smaller grade. Only the smallest emulsion droplets—those less than 1 μm in diameter—are sent to the spray dryer to become a finished encapsulate. The patent claims that this finished powder provides the benefits of antioxidants without the problems associated with low solubility, such as ringing around the necks of beverage bottles (Schaffner and Schäfer, 2008).

Much of the literature focuses on testing the impact of different formulas, spray-drying conditions, and storage conditions on the overall stability or antioxidant capacity of the chosen core materials. One group looked at the ability of various chemically pure antioxidants and encapsulating matrices to scavenge radical species. They found that gum arabic is a better scavenger than maltodextrin and that a combination of apo-8′-carotenal as antioxidant with gum arabic as the matrix created capsules with the best scavenging abilities (Rodrigues et al., 2012). A study on açai extracts in different carriers found the opposite result, however: not only were açai extracts in maltodextrin found to be more stable over their shelf-life when exposed to high temperatures and water activity levels, but they were also found to have greater overall antioxidative activity (Tonon et al., 2010). In another storage stability study, it was shown that, at most water activities, gum arabic provides greater stability to paprika oleoresin carotenoids than soy protein (Rascón et al., 2011). Optimal mixtures of carriers also receive quite a bit of literature attention: with both his biscus extracts and sour cherry pomace, a mixture of gum arabic and maltodextrin was found to provide optimal protection to the antioxidants contained within (Idham et al., 2012; Cilek et al., 2012).

Although gum arabic is by far the most commonly studied hydrocolloid carrier, it is certainly not the only possibility for encapsulation of antioxidant materials. Mesquite gum, for instance, has received attention as a possible replacement for some or all of the gum arabic in spray-drying formulations. In the case of blueberry extracts, mesquite gum alone was found to increase stability of phenols, anthocyanins, and total antioxidants when compared with unencapsulated powdered extracts (Jiménez-Aguilar et al., 2011). A comparison of the protective abilities of gum arabic–maltodextrin blends with those of mesquite gum–maltodextrin found that mesquite gum outperformed gum arabic at all temperatures tested when used to encapsulate the extract of muitle, a tropical plant native to Mexico (Pavón-García et al., 2011).

Knowing that ingredients with health benefits are often incorporated into products rather than sold alone, some groups have worked on determining an optimal formulation for antioxidants for use in beverages. In 2010, a patent was published for a sports drink containing antioxidants derived from citrus fruits. The patent is broad, but it claims that addition of such citrus phytochemicals can be valuable for the consumer's well-being, and that encapsulation prevents a bitter taste sensation while drinking the beverage. Preferably, this encapsulation would use as shell materials a combination of a protein and a polysaccharide; most of the cited polysaccharides, which include gum arabic, are hydrocolloids (Rivera et al., 2010). A separate study looked at creating a grape anthocyanin powder to incorporate into soft drinks. These compounds were to serve the dual functions of providing both a vivid color and strong antioxidant functionality to the beverage. When given the choice between maltodextrin alone or in combination with gum arabic or γ-cyclodextrin, researchers found that the gum arabic and maltodextrin blend in a ratio of 1.5:1 offered the most protection. Using this matrix, degradation rates of anthocyanins were the most favorable under all tested conditions (Burin et al., 2011).

21.3.2 Flavors

Encapsulation of flavors in hydrocolloid matrices follows many of the same patterns as encapsulation of antioxidants. Flavors are often encapsulated to improve stability, thereby extending shelf-life, and this is most commonly achieved by spray drying with gum arabic. Older research often focuses on the common model system of citrus oils and character compounds, nearly always spray dried with gum arabic (Anker and Reineccius, 1988; Bhandari et al., 1992). Research has since branched out into a number of other systems, and some of the most recent looks at spray-dried flavors in gum arabic include coffee oil (Frascareli et al., 2012), basil oil (Garcia et al., 2012), and mussel protein hydrolysates for seafood flavor (Silva et al., 2012).

Konjac glucomannan is one alternative to gum arabic that has been looked at for spray drying of flavors. Again using citrus oil—in this case, sweet orange—researchers compared encapsulation efficiency and surface morphology of particles made with konjac glucomannan, gum arabic, maltodextrin, and starch sodium octenyl succinate, as well as binary blends. Konjac glucomannan offered encapsulation yields and volatile profiles comparable to those of gum arabic and starch sodium octenyl succinate and better than that of maltodextrin. Blends, except with maltodextrin, increased these values even more. The particles also displayed smooth, nonporous surfaces, an attribute that is often lacking in gum arabic encapsulates (Yang et al., 2009). Another possible substitute is depolymerized guar gum, which has its long polymer chains broken up into smaller ones, lowering the naturally high viscosity of guar gum solutions such that they are suitable for spray drying. When partially substituted for gum arabic in mint oil microencapsulation, this material performed better than gum arabic alone (Sarkar et al., 2012).

Coacervation is an additional method that often utilizes hydrocolloids and has received some attention for flavor encapsulations because of its good control over release properties of the core material. This method takes advantage of the opposing charges between two compounds—for food, most commonly gelatin and gum arabic—and the resulting attractions between them. This type of coacervation begins with a solution of gelatin and gum arabic. The chosen flavor is added to this, and it is mixed at a high speed to create an emulsion. This emulsion is heated, and the pH is adjusted from neutral to acidic, such that gelatin and gum arabic take on charges that are equal and opposite. Strong attractive forces form between the two, and a separation occurs into a polymer-rich layer, which contains small droplets of flavor oil surrounded by gelatin and gum acacia, and an aqueous layer. These can be further stabilized by adjustment to a basic pH followed by application of a chemical or enzymatic crosslinking agent (Leclercq et al., 2009).

One of the drawbacks of coacervation for food ingredients is the necessity of using a crosslinking agent for thermal stability. This had generally been a chemical agent such as glutaraldehyde or formaldehyde, which are considered unsafe for consumption and therefore restricted for use in food products. However, in 2000, Soper and Thomas received a patent for the use of trans-glutaminase as an enzymatic crosslinking agent for coacervation of flavor oils. Not long after, in 2003, Reilly and Subramanian patented the use of plant extracts rich in polyphenols as crosslinking agents for coacervation. Both trans-glutaminase and polyphenols allow for the elimination of hazardous chemicals from the coacervation process, making it more commercially viable for food products.

Recent work in flavor coacervation has examined release rates and storage stability of gum arabic−gelatin coacervates. Yeo et al. (2005) looked at the impact of homogenization rate and concentration of wall materials on the final release rate of a herb-infused oil, finding that coacervates produced at low homogenization rates were more likely to contain a single oil droplet and release quickly, while higher homogenization rates created shells that contained a number of slow-releasing cores. Leclercq et al. (2009) compared the storage stability of coacervated limonene to that of the same molecules in a spray-dried powder form. Limonene oxide content stayed almost completely stable in coacervates but increased by more than half in spray-dried particles.

21.3.3 Microorganisms

Microorganisms are prevalent throughout the food industry, both as food ingredients and processing aids. Many food producers have found, however, that there are often unwanted interactions with the surroundings, interactions that lead to either a loss of product quality or death of the organisms themselves. Keeping microorganisms walled off from their surroundings is, therefore, one of the most studied applications for hydrocolloid microencapsulation. Typically, these microencapsulates are formed through the ionic gelation of sodium alginate after contact with calcium chloride, but other materials, including κ-carrageenan and pectin, may be used instead, and a number of additional hydrocolloids have been investigated as secondary matrix components. Sun and Griffiths (2000), for instance, found a mixture of gellan and xanthan gums to be very effective at protecting *Bifidobacteria* species.

TABLE 21.3 For Methods Based on Sheu and Marshall (1993), a Solution of Sodium Alginate and Cells Is Emulsified in Oil with Polysorbate

Dispersed Phase (aqueous)	
Sodium alginate	2–3%
Cells	9–10 log CFU/mL
Continuous Phase (oil)	
Oil	Vegetable, canola, soy
Polysorbate 80	0.20%
For Emulsion	
Solution: oil ratio	1:5
Gelling Solution (aqueous)	
Calcium chloride	0.05–1 M

Calcium chloride solution is added in the necessary volume to break the emulsion. Information for this table is from Bhandari et al. (1992), Frascareli et al. (2012), Nualkaekul et al. (2012), and Sarkar et al. (2012).

Initial forays into trapping bacteria in microstructures involved the controlled release of drops of sodium alginate–bacteria solutions through a syringe into a calcium chloride bath (Klein et al., 1983). This was advantageous when compared with traditional methods of encapsulation, such as spray drying, because no heat is required that might threaten bacterial viability, and the finished beads can be incorporated into liquid products without dissolution of the matrix. However, control of attributes such as bead size was limited in this method, and scaling the operation up would be nearly impossible at any reasonable cost.

More recently, many researchers have been investigating the use of alternative methods that require an emulsification step and then break this emulsion by inducing gelation. One such method begins by creating a mixture of sodium alginate solution, the desired core organisms and an insoluble source of calcium ions, such as calcium citrate, and emulsifying this in vegetable oil. Following emulsification, acetic acid is added, breaking the emulsion and creating particles with a polymer matrix surrounding a liquid core (Lencki et al., 1989). A similar and more widely applied method was introduced by Sheu and Marshall a few years later (1993). Here a mixture of sodium alginate solution, core material, and emulsifier is agitated in oil until an emulsion of the desired particle size is formed. Addition of calcium chloride, as with the acetic acid just mentioned, breaks the emulsion and forms gelled beads. These methods allow for more control over the size of encapsulates compared to a syringe, and the operation is less labor-intensive and therefore more suitable for scale-up. A formula for creating microbeads compiled from a number of adapted versions of Sheu and Marshall's methodology is given in Table 21.3.

While the academic literature focuses on applications using these methods, some attempts have been made at creating systems that incorporate ionic gelation, but on a scale more suitable for industrial applications. In 2000, Champagne et al. published a theoretical look at one such possible setup. This incorporated a rotating disc atomizer recessed in a vortex bowl; the atomizer would spray a solution of sodium alginate directly into a climbing film of ion-rich solution created by the spin of the bowl, with the resulting beads eventually spilling over the edges. If this mechanism is placed at the top of a fermentation tank, the reaction vessel below can receive a continuous supply of microorganism-containing beads.

Moving beyond the world of theory, Bhandari (2009) has created and patented a dual aerosol system for creation of gel particles. The aerosols are positioned such that they cross paths, and the materials are chosen so as to form a gel matrix after contact; suitable pairs include sodium alginate with calcium chloride and κ-carrageenan with potassium chloride. The benefits of such a system include its suitability for large-scale production, but also the ability to create much smaller gel beads than most methods, on the range of 10 to 40 μm, which is below the sensory detection threshold. Such gels have a number of possible applications, including those for microorganism encapsulation, and Bhandari and his colleagues have looked into some of these since publication of the patent. In 2011, they showed that microbeads provided protection to *Lactobacillus rhamnosus* that was comparable to that of larger beads prepared using a traditional ionic gelation method. Some more finicky microorganisms may be less successful in such small particles, however, as

Lactobacillus acidophilus died more quickly in small gel beads than it did in larger ones (Sohail et al., 2011). In 2012, the group again explored bacterial encapsulation, incorporating encapsulated *L. rhamnosus* and *L. acidophilus* into both fruit snacks and orange juice. Here they found that the beads did not always protect the microorganisms from their external environment but that the environment was protected from the microorganisms, in this case limiting the acidification of the juice and snacks by the *Lactobacilli* (Sohail et al., 2012).

Incorporation into food products is, in many cases, the end goal for probiotic cells. To this end, a number of studies have looked into the impact of adding encapsulated cells to real food systems. The starting place for many of these applications was in the world of dairy, where many products, such as yogurt, have traditionally been fermented by some of the same bacteria that are now used as probiotics. These applications included not only yogurt (Sultana et al., 2000; Brinques and Ayub, 2011), but also white-brined (Özer et al., 2009) and mozzarella cheeses (Ortakci et al., 2012), and frozen desserts (Sheu et al., 1993; Homayouni et al., 2008). In general, the findings were that microencapsulation of the probiotic organisms had some protective effect for the organisms and limited to some degree changes in the quality of the finished food product, but that in many cases the survival and quality were both still less than desired. Outside the world of dairy, microencapsulated probiotics were incorporated into pomegranate juice for those who are unwilling or unable to drink dairy, and bacterial survival was found to increase by several log scales over the shelf-life (Nualkaekul et al., 2012). Another novel nondairy application involved incorporating encapsulated probiotics into soy-based bars with the dual purpose of breaking down indigestible soy oligosaccharides and conferring a health benefit to the consumer (Chen and Mustapha, 2012).

Another important set of applications involves the use of microorganisms for fermentation processes. Immobilizing the organisms in gel beads rather than adding them to a fermentation vessel as free cells allows for reuse of the organisms. Some initial investigations into this technology deemed encapsulated cells unsuitable for industrial use in fermentation of whey permeate because of the loss of control afforded by free cells and problems with leakage out of beads (Roy et al., 1986). In fermentation of cream, however, it was found to provide a number of benefits, including a fermentation time that was less than 30% of that for free cell fermentation and the ability to extend the product shelf-life by limiting acid-producing bacteria content in the finished product (Prevost and Divies, 1992). While dairy is a natural choice for fermentative studies, it is not alone in this type of research: vegetable juices have received some attention as well. Carrageenan-encapsulated cells in tomato juice were found to have higher viability following the fermentation process and to produce a product with better overall sensory properties (Tsen et al., 2008). More mixed results were obtained when alginate- and pectin-based particles were incorporated into mixed vegetable juices, but researchers were still able to obtain acidification comparable to free cells for some encapsulates (Champagne et al., 2010).

21.3.4 Other Applications

Antioxidants, flavors, and microorganisms have received as much literature attention as applications for hydrocolloid encapsulation. There are a number of other arenas, however, where these types of processes can also be extremely useful; these include applications with lipids, enzymes, and materials intended to improve food safety.

Encapsulation is often required for lipids, especially those that are unsaturated and therefore particularly prone to oxidation and other degradation. In many cases, one or more hydrocolloids are an appropriate choice as the wall material for this application. The most popular oils to study are those with high omega-3 fatty acid content. These have been touted for their health benefits but are currently typically consumed in the form of a large capsule that can be difficult to swallow and lead to fishy breath and repeating; a desired solution is to find a way to incorporate these oils into small microcapsules and, from there, possibly food and beverage products. One study examined a number of different encapsulating materials—gum arabic, pectin, modified starch, glucose syrup, and sodium caseinate—alone and in combination and measured oxidative stability of fish oils in these matrices over time. Gum arabic and pectin alone did not perform well after 3 to 4 weeks of storage, but in combination with modified starch, gum arabic provided significant production from oxidation (Drusch et al., 2007). Others studied the combination of alginate with modified starch in a spray-dried matrix and found that of six different formulas incorporating alginate, all six resulted in smoother, more spherical particles, higher encapsulation efficiencies, and better protection against oxidation when compared to encapsulates using starch alone (Tan et al., 2009). Microalgal oil, a vegan source of omega-3 fatty acids, has also received some attention; gum arabic−gelatin coacervates made under a number of different conditions were used to study release rates of this material (Zhang et al., 2012). Although omega-3 fatty acids have been studied most thoroughly, not all microencapsulated oils include them. Soybean oil, for instance, was found to be well protected in certain solutions when encapsulated in κ-carrageenan gels (Perrechil et al., 2012).

Enzymes are an excellent candidate for hydrocolloid encapsulation because the encapsulating material can simultaneously protect the enzymes from denaturation and control the rate at which they work. These are often incorporated into gels, similar to those used for microorganisms. One of the most studied applications of alginate-encapsulated enzymes is in the production of cheese. Aging of cheese is a lengthy process that accounts for a significant portion of the overall cost. Addition of a high concentration of the enzymes that already work naturally during aging was proposed to shorten the ripening time, but free enzymes are often lost in the cheese-making process, and the ones that remain act too quickly, compromising cheese quality. An initial study of enzymes encapsulated in gellan and κ-carrageenan as well as milkfat found that some test cheeses aged for 5 months, especially those with gellan-encapsulated enzymes, received overall acceptability scores that were comparable to or better than those given to control cheese aged for 6 months (Kailasapathy and Lam, 2005). Another group determined that in cheeses with alginate-encapsulated aminopeptidase, soluble nitrogen and free amino acids took only 2 months to reach the 6-month levels of control cheese (Azarnia et al., 2010). Azarnia et al. (2011) also found that when all cheeses were subjected to sensory testing after 4 months, panelists overwhelmingly preferred the ones prepared with the highest concentrations of encapsulated enzymes over those with free or no enzymes. Cheese is not the only application in which hydrocolloid-encapsulated enzymes can be useful: alginate-encapsulated naringinase was proposed as a replacement for resins used to debitter grapefruit juices (Mishra and Kar, 2003).

Another useful application for hydrocolloid matrices is encapsulation of anti-bacterial ingredients to improve food safety and preservation. Kimchi, for instance, has a tendency to continue to ferment during storage, causing it to spoil quickly. Allyl isothiocyanate limits growth of the bacteria responsible for this fermentation, extending the product's shelf-life, but it also lends an unpleasant off note. Spray drying allyl isothiocyanate on gum arabic was found to preserve its ability to slow down kimchi fermentation while also masking the strong flavor of the compound itself (Ko et al., 2012). In another example, capsaicin was encapsulated using a coacervation process. It had been previously characterized as an antimicrobial compound, but was difficult to apply to foods in a uniform way; encapsulation allowed the antimicrobial properties to be harnessed, impeding growth of two common food spoilage fungi (Xing et al., 2005). Techniques used for encapsulating probiotics, such as ionic gelation with calcium alginate, were also applied as a means of improving the long-term viability of *Lactobacilli* and *Bifidobacteria*, which are added to fermented sausages to prevent growth of *E. coli* (Muthukumarasamy and Holley, 2007). Spray drying kaffir lime oil in a mixture of gum arabic and konjac glucomannan preserved the oil's antibacterial activity against a wide range of pathogenic bacteria while also protecting it from oxidative deterioration (Adamiec et al., 2012). Finally, an improved delivery system for bacteriocins that placed them in gelled microspheres of pectin and butter allowed for a more even distribution of the compounds across the food surface and fewer interactions between the food and bacteriocins while still inhibiting growth of *Listeria monocytogenes* (Ibarguren et al., 2012).

21.4 CONCLUSION

Hydrocolloids are useful materials in the field of microencapsulation. Although gum arabic and sodium alginate are the best characterized as wall materials, a number of other promising polysaccharides are emerging as possible encapsulants. Current applications include encapsulation of antioxidants, flavors, microorganisms, and a number of other food ingredients.

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Chapter 22

Fats and Waxes in Microencapsulation of Food Ingredients

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22.1 INTRODUCTION

Microencapsulation is a broad area of research with applications in drug delivery, cosmetics, and prepared foods. It is a process in which a substance (solid, liquid, or gas) is incorporated into a matrix forming fine particles. The particles generated can be suspended in liquid media (emulsions) or in dry form. Microencapsulation allows for the protection of sensitive ingredients, the controlled release of food and pharmaceutical actives, and the transformation of liquids into more manageable solid forms. Depending on the desired encapsulated product architecture, a number of techniques including emulsification, spray drying, spray cooling/chilling, freeze drying, fluidized bed coating, centrifugal extrusion, centrifugal suspension-separation, coacervation, and liposomal entrapment have been utilized and are discussed in detail elsewhere in this book. In food, one or more materials from the following categories can be used: carbohydrates, proteins, gums, and lipids. Material selection is dictated by the encapsulation techniques, the active ingredient, the release mechanism needed, and the properties of the carrier material itself. Since selection depends in part on the properties of the carrier material, an understanding of these basic properties is necessary. In this chapter, we will focus on lipids as carriers. Lipids are often chosen because of their melt properties and their moisture barrier properties. These properties are important in both microcapsule fabrication and release mechanism. In this chapter, we will discuss the impact of chemical structure on physical characteristics such as crystallization, melt property, and chemical barrier property. We will also discuss applications where lipid carriers are employed.

22.2 STRUCTURAL DIVERSITY IN LIPIDS

Fats and waxes are part of the broader lipid category. This category is made up of a diverse group of compounds related by their solubility profile. In general, lipids are soluble in non-polar solvents and insoluble in water. The lipids that will be covered in this chapter fall into three groups:

1. Hydrocarbon-rich substances (saturated long-chain fatty acids and waxes), which may or may not have ester linkages but have melting points higher than ambient temperature.
2. Simple lipids (mono-/di- and triglycerides), which are esters of glycerin and fatty acids.
3. Lipid-derived substances (polyol-esters, modified mono-/diglycerides, and phospholipids), which are esters with additional polar groups.

22.2.1 Hydrocarbon-Rich Substances

The unifying theme for this group, comprising fatty acids and waxes, is melt properties. Structurally, the members of this category are different (Figure 22.1). Fatty acids are carboxylic acids with an aliphatic tail. Commercially, they are obtained from both vegetable and animal sources (O'Brien, 2003). They can be rendered from animal parts, pressed, or extracted from vegetable sources. The resulting oil is either hydrolyzed in the presence of water to yield fatty acids or transesterified with an alcohol (often methanol) to yield fatty acid methyl esters (Gunstone et al., 2007). Hydrolysis

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00022-4

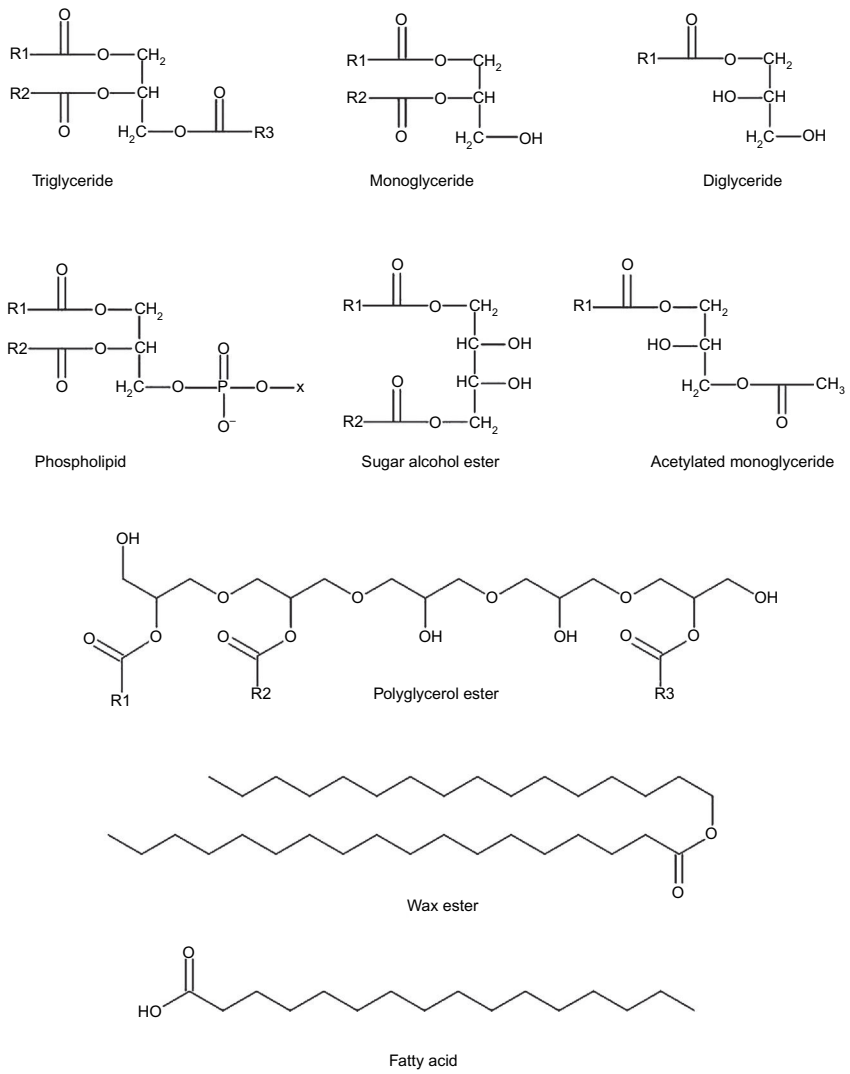

FIGURE 22.1 Selected structures in the lipid category. R denotes fatty acid moiety and X represents head group functionality of phospholipids.

or transesterification generates products that have much lower boiling points than their parent oils. Hence, the resulting fatty acids or methyl esters can be separated by carbon chain length using distillation. Often, methyl esters are generated because they can be separated at much lower temperatures compared to the corresponding acid. When methyl esters are generated, they have to be hydrolyzed to yield fatty acids following distillation. Fatty acids can be grouped according to structural features:

- Carbon number: (C4 to C24)
- Chain length: short (C4 and C6), medium (C8 to C12), long (C14 to C24)
- Double bonds: saturated, mono- or polyunsaturated
- Double bond location: Omega-3, -6, or -9
- Geometric configuration: *cis* or *trans*.

These features impact the properties of the fatty acids (Gunstone and Padley, 1997). The longer the carbon chain, the greater the hydrophobicity of the acid. Double bonds impose geometric restrictions leading to kinks in the molecule, while saturated fatty acids are devoid of kinks. These structural differences lead to differences in crystal and melt properties. Finally, the oxidative stability of the fatty acid is inversely related to the number of double bonds present.

Waxes are derived from many sources: animal, vegetable, microbial, and synthetic. There is no clear definition on what constitutes a wax, but it is normally taken to be a mixture of compounds with high melting point. The wax mixture can contain monoesters, diesters, sterol esters, hydroxy-monoesters, triglycerides, long chain fatty acids, aldehydes, ketones, alcohols, and hydrocarbons (Akoh and Min, 2008) The hydrocarbons in waxes are mostly long chain paraffinic

species. The rest of the species also have high carbon numbers, which explains the large hydrophobic character of waxes. There are many waxes, but only a few are of importance in food application: carnauba wax, candelilla wax, beeswax, rice bran wax. Carnauba wax has high ester content (as much as 85%) and low paraffinic hydrocarbon (below 3%) while candelilla wax is quite high in paraffinic hydrocarbon (greater than 50%) with less than 30% esters. Rice bran wax appears to be a good alternative to carnauba wax in most applications and has been reported to have 97% ester content.

22.2.2 Simple Lipids

The diversity in this group comes from the fatty acids found in nature (Table 22.1). In oil or fats, fatty acids form ester linkages with the hydroxyl groups on the glycerin backbone. Examples of fats/oil and their fatty acid compositions are given in Table 22.2.

Partial esterification yields diglycerides (two esters per molecule) or monoglycerides (one ester per molecule). Esterification at all the hydroxyl groups yields triglycerides. In typical fats and oils, the most prevalent compounds are triglycerides with limited mono-/diglycerides. Monoglycerides and diglycerides are produced industrially via the trans-esterification of triglycerides with additional glycerin. In triglycerides, if only one type of fatty acid (i.e., stearic acid) is involved, a single structure is obtained (i.e., tristearin), often referred to as a monoacid triglyceride. However, most fats and oils are not monoacid triglycerides but contain a number of fatty acids. This makes the pool of possible compounds

TABLE 22.1 Common Fatty Acids in Food

Fatty Acid (FA)	Chemical Structure	Carbon Length	Double Bonds	C:D Notation	State at 25°C
Butyric	$CH_3(CH_2)_2COOH$	C-4	0	C4:0	Liquid
Caproic	$CH_3(CH_2)_4COOH$	C-6	0	C6:0	Liquid
Caprylic	$CH_3(CH_2)_6COOH$	C-8	0	C8:0	Liquid
Capric	$CH_3(CH_2)_8COOH$	C-10	0	C10:0	Liquid
Lauric	$CH_3(CH2)_{10}COOH$	C-12	0	C12:0	Solid
Myristic	$CH_3(CH2)_{12}COOH$	C-14	0	C14:0	Solid
Palmitic	$CH_3(CH2)_{14}COOH$	C-16	0	C16:0	Solid
Stearic	$CH_3(CH_2)_{16}COOH$	C-18	0	C18:0	Solid
Arachidic	$CH_3(CH_2)_{18}COOH$	C-20	0	C20:0	Solid
Behenic	$CH_3(CH_2)_{20}COOH$	C-22	0	C22:0	Solid
Lignoceric	$CH_3(CH_2)_{22}COOH$	C-24	0	C24:0	Solid
Myristoleic	$CH_3(CH_2)_3CH{=}CH(CH_2)_7COOH$	C-14	1	C14:1	Liquid
Palmitoleic	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	C-16	1	C16:1	Liquid
Oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	C-18	1	C18:1	Liquid
Gadoleic	$CH_3(CH_2)_9CH{=}CH(CH_2)_7COOH$	C-20	1	C20:1	Liquid
Erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	C-22	1	C22:1	Liquid
Linoleic	$CH_3(CH_2)_4[CH{=}CHCH_2]_2(CH_2)_6COOH$	C-18	2	C18:2	Liquid
Linolenic	$CH_3CH_2[CH{=}CHCH_2]_3(CH_2)_6COOH$	C-18	3	C18:3	Liquid
EPA	$CH_3CH_2[CH{=}CHCH_2]_5(CH_2)_5COOH$	C-20	5	C20:5	Liquid
DHA	$CH_3CH_2[CH{=}CHCH_2]_6(CH_2)_5COOH$	C-22	6	C22:6	Liquid

TABLE 22.2 Fatty Acid Composition of Selected Fats and Oils

	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C20:0	C16:1	C18:1	C18:2	C18:3	FA	TG
Cocoa					26.4	33.9	0.6	0.2	35.9	3.0		6	216
Coconut	5.8	6.5	51.2	17.6	8.5	2.7			6.5	1.2		8	512
Cotton seed				0.9	25.8	1.9		0.5	16.7	54.2		6	216
Palm			0.2	1.1	44	4.5	0.3	0.1	39.2	10.1	0.4	9	729
Palm olein			0.5	1.2	39	4.4	0.3	0.2	42	12.2	0.5	9	729
Palm stearin			0.2	1.4	55.7	4.8	0.3	0.1	31.0	6.6		8	512
Palm kernel	3.6	3.5	47.3	16.4	8.1	2.3			16.2	1.8		8	512
Soybean					11.3	3.4			23.1	55.8	6.4	5	125

large, with the number of triglycerides given by n^3 where n is the number of fatty acid types (Gunstone and Padley, 1997). Some of the same descriptors used for fatty acids can be employed to describe triglycerides (i.e., chain length, saturation level, unsaturation location). When more than one type of fatty acid is on the glycerin backbone, positional descriptors are needed to differentiate between species. A fatty acid can be at position 1 (sn-1), position 2 (sn-2), and position 3 (sn-3) in the triglyceride. For example, a triglyceride with both stearic acid (S) and oleic acid (O) on the backbone can have multiple mixed fatty acid composition: SOO, OOS, SSO, OSS, OSO, and SOS. Although OOS and OSO have the same chemical formula, they are different species with different properties. In OSO, a saturated chain is at the sn-2 position while in OOS an unsaturated chain is at the sn-2 position.

Commercially, the fatty acids on the backbone of triglycerides can be statistically redistributed via the interesterification process. This manipulation leads to differences in the melting point of the resulting fat. Interesterification of cottonseed oil randomizes the fatty acids in the backbone to an equilibrium composition in which more saturated long chain triglycerides are generated, causing an increase in melting point of more than 20°C. Interesterification of coconut oil, a short chain fatty acid-rich oil, results in a lowering of melting point (O'Brien, 2003).

22.2.3 Lipid-Derived Substances

This category includes the polyglycerol esters, modified mono-/diglycerides, sugar alcohol esters, and phospholipids. In Figure 22.1, an example from each of these subgroups is given. The compounds in this category are not used as shell/matrix material but tend to be used as surfactants that stabilize interfaces. Notable exceptions are phospholipids and polyglycerol monostearate (PGMS).

Unlike triglycerides, which are found in nature, polyglycerol esters are commercially produced (Hasenhuettl and Hartel, 2008). Since they have some of the same functional groups as simple lipids, they can undergo similar chemical transformations (i.e., transesterification, acetylation, interesterification). Polyglycerol esters are amphiphilic, comb-like molecules. The backbone of the polyglycerol esters consists of an oligomer of glycerin with typically three to ten condensed units. These units provide a significant number of hydroxyl groups and make up the hydrophilic moiety of the molecule. The hydroxyl groups of the polyglycerol can form ester linkages with any of the fatty acid mentioned in Table 22.1. The teeth of the comb are the pendent fatty acids and make up the hydrophobic moiety of the molecule. In these molecules, the balance between hydrophobic and hydrophilic moieties can be tailored depending on the length of the condensed glycerin, the number of ester linkages, and the type of fatty acid (Hasenhuettl and Hartel, 2008). This tailoring determines how the molecules will behave at an interface.

Monoglycerides can be modified with acetate, lactate, phosphate, succinate, citrate, or diacetyltartrate. These modifications are done to enhance the hydrophilic character by increasing the overall size and/or polarity of the head group. Aside from the acetate and the lactate, which have no ionizable groups, all the head groups of the modified monoglycerides have the ability to become anionic at the appropriate pH. The acetate modified monoglycerides are the only members of this group in which the hydrophilic character has been lessened. Because of its reduced hydrophilicity compared to the other modified monoglycerides, acetate-modified monoglycerides have been incorporated in formulations where moisture barrier attributes are necessary.

Phospholipids are amphiphilic molecules and are commercially derived from animal and vegetable sources (egg, soy, sunflower, and canola). They owe their hydrophobic character to their non-polar fatty acid chains and their hydrophilic character to the phosphate head group (Figure 22.1). Diversity in the phospholipids comes from the fatty acids that can be esterified at positions 1 and 2 of the glycerol and from the head group at position 3 (Akoh and Min, 2008). Table 22.3 depicts the most common classes of phospholipids utilized in the food industry. Some of the head groups are ionizable, causing the resulting phospholipid to develop a charge under certain conditions.

22.3 PHYSICOCHEMICAL PROPERTIES OF LIPIDS

The discussion above showed the diversity of structural elements that exist within the lipid category. In this segment, the impact of molecular structure on melt behavior, crystallization behavior, film properties, and chemical stability will be presented. The relationship between structure and surface activity will also be discussed.

22.3.1 Melt and Crystallization in Lipids

Fats have many triglycerides, which makes their phase transition complex. Melting in fats is directly related to structural features such as chain length and saturation: the lower the unsaturation, the higher the melting point; the longer

TABLE 22.3 Phospholipids Classification

Head Group Identity		Phospholipid Class	Phospholipid Abbreviation
Chemical Formula	Name		
$-H$	Proton	Phosphatidic acid	PA
$-CH_2-CH_2-NH_3^+$	Ethanolamine	Phosphatidylethanolamine	PE
$-CH_2-CH_2-N(CH_3)_3^+$	Choline	Phosphatidylcholine	PC
$-CH_2-CH(COO^-)-NH_3^+$	Serine	Phosphatidylserine	PS
$-(CH-(CHOH)_5$	Inositol	Phosphatidylinositol	PI
$-CH_2CH(OH)CH_2OH$	Glycerol	Phosphatidylglycerol	PG

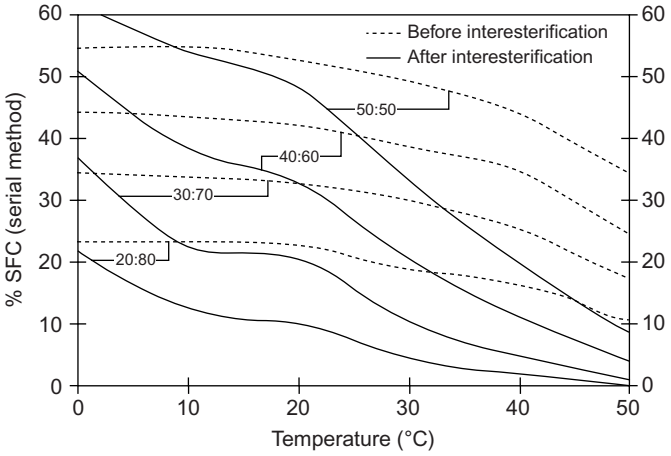

FIGURE 22.2 Impact of triglyceride composition on solid fat content. Solid fat content of mixtures of hydrogenated soybean oil and soybean oil before and after interesterification. *(Reprinted with permission from Petrauskaite et al., 1998.)*

the chain, the higher the melting point. One of the particularities of fats is that they solidify along a continuum and they have liquid domains at temperatures far below their melting points. This gives them plastic character (O'Brien, 2003). The percent solid can easily be obtained by pulse-NMR (nuclear magnetic resonance), and a solid fat content (SFC) curve as a function of temperature can be drawn (Figure 22.2). Melting in this context is defined as the point where 0% solid is achieved. The melting profile is a direct consequence of the mixtures of species in the fat. In Figure 22.2, interesterification, which changes the species present in the mixture, causes a shift in the SFC and a decrease in melting point (Petrauskaite et al., 1998). The plasticity of a fat is important in microencapsulation because it determines the brittleness of the fat. Fats with broad melt curves are less brittle than those with sharp SFC curves. The factors that impact the plasticity of fat are ratio of solid to liquid domains, the size and shape of the solid domains, and the interaction between these solid domains, as well as the underlying crystal structure of the solid domain. Crystallization behavior in fats is discussed below and illustrated in Figure 22.3.

The individual molecules in fats interact and pack into different forms (polymorphs) during crystal growth. Triglycerides can take on two configurations: chair (sn-1, sn-2 on the same side and sn-3 on the opposite side of glycerin) or tuning fork (sn-1, sn-3 on the same side and sn-2 on opposite). These configurations can order in chair-like fashion to yield two distinct stacking patterns: head-to-head or head-to-leg. The head-to-head stacking pattern generates trilayer (triple) structures while the head-to-leg pattern creates bilayer (double) structures. These molecular aggregates (embryos) are transiently created and return to the melt before any crystal growth can begin (Himawan et al., 2006; Akoh and Min, 2008).

To transition to crystallization, these aggregates must reach a critical size, which is highly dependent on the saturation of the melt. In addition, crystallization cannot take place if appropriate supercooling has not been reached. Once these conditions are met, nucleation takes place and crystal growth begins. Discussion on fat crystallization has been

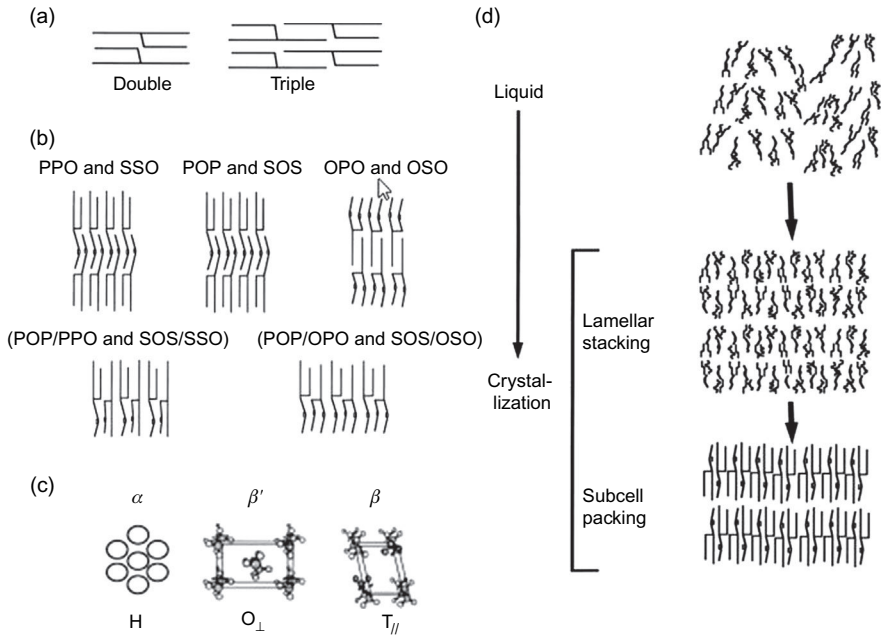

FIGURE 22.3 Triglyceride crystallization. (a) Triglyceride configuration in the melt. (b) Lipid stacking in embryo formation. (c) Crystal polymorphs. (d) Model of fat crystallization from the melt. *(Reprinted with permission from Sato, 1999.)*

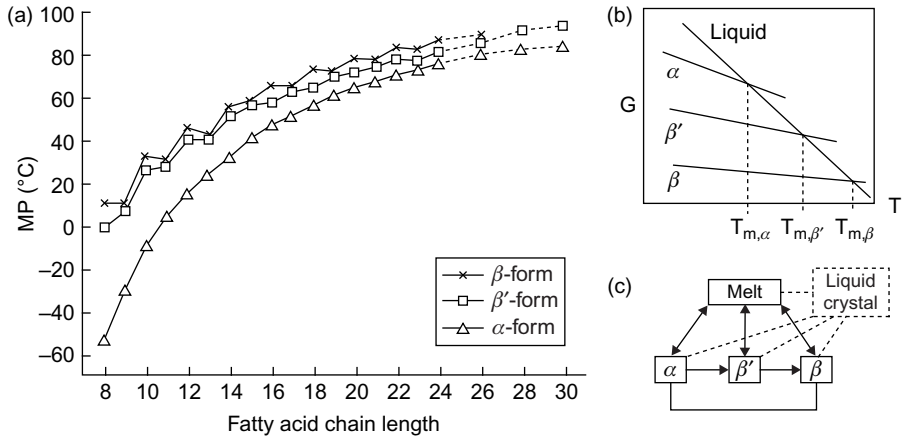

FIGURE 22.4 Crystal morphology impact on melt behavior. (a) Polymorph melting point with varying fatty acid chain length. (b) Polymorph thermodynamic stability. (c) Model of polymorph transition. *(Reprinted with permission from Himawan et al., 2006.)*

extensively presented in the literature (O'Brien, 2003; Martini et al., 2006; Akoh and Min, 2008). The growth rate and shape of crystals depends on cooling rate: faster cooling leads to smaller crystals of less stable polymorph, but slow cooling leads to larger crystals of more stable polymorph. The achievable polymorph for a given fat is determined by its structure. Triglycerides crystallize into three crystal classes or polymorphic groups: alpha (α), beta prime (β′), and beta (β). Within each of these groups other polymorphs can exist. The subclassification is a result of differences in the lamellar layer spacing. The α-polymorphic group can only have bilayer aggregates and has no angle of tilt while the β′ and β can have both bilayer and trilayer aggregates in addition to tilt angle in the crystal. These differences make for different degrees of openness between polymorphic groups but also within polymorphic groups.

Fat polymorphs differ in crystal size, morphology, density, melting point, melting enthalpy, and thermodynamic stability (Figure 22.4) (Sato, 1999; Garti and Sato, 2001; Himawan et al., 2006). The β-polymorphs are the most stable, more difficult forms to achieve from the melt since the change in Gibbs free energy (ΔG) associated with this transition is the greatest. The β-polymorphs yield the largest crystal with the densest packed crystal lattice (triclinic parallel T subcell with strong spacing near 0.46 nm and additional strong lines between 0.36 and 0.39 nm). On the other hand, the α-polymorph is the least stable and easiest form to achieve since its G is closest to that of the melt. This polymorph yields the smallest crystals and the least dense lattice (hexagonal H subcell with one strong lattice spacing near 0.42 nm). When only saturated fatty acids are on the triglyceride backbone, they easily align into the most compact

crystal structure (β-polymorph). Double bonds when present disrupt the crystal packing, with their kinks causing steric hindrance and precluding the formation of the most stable and more dense β-polymorphs. All fats crystalize first to the α-polymorph, most would be able to achieve the β′, and only some can get to the β-polymorph (O'Brien, 2003). Fat can transition from the least stable to the most stable form without going through the melt, but the reverse is not possible. Once at the most stable polymorph, the system needs to go through the molten state to have access to the other polymorphs.

Like the triglycerides, the other lipids also exhibit structure-driven polymorphism (Friberg et al., 2004). Distilled monoglyceride exhibits multiple polymorphs: sub-α, α, and β. The β-polymorph is the most stable and the sub-α the least stable, and accessible only with cooling of the α form. The polymorphs of the monoglycerides tend to exhibit higher melting point than their triglyceride equivalents, a phenomenon stemming from the presence of the hydroxyl groups, which provide stronger inter-crystal cohesive forces. Diglycerides can also take various forms but the most stable crystal form attainable is governed by the positioning of the fatty acid on the backbone. Diglycerides with acids at the sn-1 and sn-2 positions are most stable in the β′ form while those with acids at the sn-1 and sn-3 positions have stable β crystals. Finally, modified mono-/diglycerides undergo crystallization to form α-stable crystals. The above discussion is relevant to microencapsulation because in many encapsulation techniques fats and waxes crystalize and define the property of the microstructure.

22.3.2 Moisture Barrier in Lipids

It is sometimes necessary to limit diffusion of a specific chemical across a barrier to protect a product from degradation. Chemical barriers are constructed when the following attributes are fulfilled: diffusivity control, film-forming ability, and mechanical strength. All three of these attributes are not often found in a single material. The material may be an effective barrier for one class of compounds but ineffective in others. Fats and waxes have been used as coating for fruits and vegetables to prevent moisture loss and are often used in microencapsulation for their moisture barrier properties (Zambrano-Zaragoza et al., 2013; Han, 2014). The attribute that contributes the most to barrier property in lipids is their hydrophobicity, which limits water diffusivity. The hydrophobicity of lipids is due to features in their molecular structure: the higher the paraffinic characters, the higher the hydrophobicity; the higher the number of polar groups, the lower the hydrophobicity. Aside from chemical composition, factors impacting film strength (Bourlieu et al., 2006), such as intermolecular forces, and crystalline domain arrangement, size, and shape, will impact barrier properties. While dense crystalline domains have barrier properties because they limit diffusion, they also cause poor ductility. In a study by Bourlieu et al. (2009), acetylated monoglycerides of various acetylation levels were compared to tristearin. The study found that acetylation level impacts crystal habit, disorder, fluidity, melt behavior, and moisture barrier properties. High acetylation leads to a looser and more ductile interconnected crystal network but lower moisture barrier properties. To take advantage of the hydrophobic character of fats as well as their ability to self-plasticize, many researchers have used them in combination with proteins and starches (Sothornvit and Krochta, 2005; Muscat et al., 2013).

22.3.3 Surface Activity in Lipids

Lipids play a large role in the process of emulsification. Emulsions arise when two immiscible phases are mixed and large numbers of interfaces are generated. The process is not thermodynamically favored and strong energy penalties are associated with forming these interfaces. In general, to overcome the thermodynamic barrier, energy must be applied. The magnitude of the energy penalty is defined by the interface composition. To achieve fine dispersion and limit energy penalties, lipids such as mono-/diglycerides, polyglycerol esters, modified monoglycerides, and phospholipids are used. These surface active agents (surfactants) migrate to the interfaces, lower the surface tension, and stabilize the dispersion to prevent reversion to the more favorable two-phase system.

Dispersions can be stabilized sterically, electrostatically, or via formation of complex interpenetrating networks. Polar lipids stabilize an interface by forming a densely packed fluid monolayer around the dispersed droplets (Friberg et al., 2004). This monolayer is constructed such that the hydrophobic moiety of the surfactant is oriented toward the lipid phase and the polar head is in the water phase. In a number of microencapsulation techniques, an emulsion must first be generated defining the microcapsules before any drying or setting methods can be applied. The quality of the emulsion tends to impact the property of the finished product. In addition, polar lipids in solution interact with water to generate many ordered states (liquid−crystalline phases) (Friberg et al., 2004). In the fabrication of liposomes from phospholipids, this ability to self-assemble in water and form ordered structures is central. The phase behavior of phospholipids is well documented and has been used to engineer liposomes with specific release mechanisms (Lasic, 1998).

The formation and type of crystalline phase depends on factors such as concentration, temperature, molecular shape, ionic strength, and molecular structure. The cylindrically shaped PC molecules organize in bilayers and pack into a lamellar cubic phase, while the conically shaped PA molecules organize into reversed micelles and pack into a hexagonal-2 phase. Each one of these structures has a different radius of curvature and different stability profile.

22.3.4 Chemical Stability of Lipids

The stability of lipids is quite important for their usage in food. The quality of a lipid can degrade due to oxidation or hydrolysis. Degradation via oxidation occurs in unsaturated lipids but not in saturated ones. The high melting lipids such as waxes and hydrogenated fats used in microencapsulation have limited double bonds, and hence are at lower risk of oxidation. The lipids with unsaturation such as lecithin and polyglycerol oleate can become oxidized. When unsaturated fats oxidize, they form hydroperoxides, which in the presence of metal and heat further decompose to aldehydes, ketones, acids, alcohols, and short chain hydrocarbons (Akoh and Min, 2008). These species yield compositional changes that can disturb packing and make the coating material more hydrophilic, hence increasing moisture diffusivity in a manner analogous to the acetylation work by Bourlieu presented above.

Ester linkages are prone to hydrolysis making this degradation path important in all ester containing lipids. The degree of hydrolysis depends on the environmental factors such as pH, temperature, and water activity. Not all the members of this group are equally afflicted. The higher the ester content, the more important this degradation path becomes. As the polarity of the lipid increases, water diffusivity increases, bringing with it higher risk of hydrolysis. Dry encapsulation products will not suffer hydrolysis until they are in the end-use formulation. But in liquid systems such as liposomes, hydrolysis can begin at manufacture. Phospholipids, which make up liposomal systems, are prone to hydrolysis and in the presence of acid they can be hydrolyzed to glycerol, fatty acids, phosphoric acid, and their respective head groups. Once hydrolysis begins, liposomes go from lamellar to micellar structures. During this transformation, these vesicles spill their load, resulting in a burst release profile (Heurtault et al., 2003).

22.4 LIPIDS IN MICROENCAPSULATION APPLICATIONS

22.4.1 Techniques

Microencapsulation is a vast field with varying structures, composition, process, and applications (Risch and Reineccius, 1995; Nedovic et al., 2011; Garti and McClements, 2012). It is typically used to provide protection for active ingredients against environmental factors (i.e., moisture, oxygen, and other reactive components). Microencapsulation extends formulation shelf-life and improves the stability of sensitive ingredients. The type of vesicle generated by encapsulation can be classified into two groups: reservoir (shell with distinct homogeneous solid/liquid or gas phase), and matrix (solid core with dispersed particles). In encapsulation with lipids, the two types of vesicles can be generated and are determined by the encapsulation technique utilized. A brief discussion with focus on the use of lipids in these techniques is presented in this section. Detailed discussions on these techniques are presented elsewhere in this book. The techniques most often used in conjunction with lipids are fluidized bed coating, spray cooling/chilling, centrifugal suspension separation, centrifugal extrusion, emulsification, liposomal entrapment, and solid lipid nanostructures (Table 22.4). The release mechanisms for these techniques that are most relevant to lipids are the following:

- Melt → hard fat systems in heated applications
- Lipase digestion → lipid systems in intestinal delivery
- Temperature, pH, salt-driven phase transition → liposome
- Interface destabilization → emulsion
- Diffusion → liposome, solid lipid nanostructures.

In fluidized bed coating, the lipid material constitutes only the shell as it is sprayed at temperatures above its melting point onto a template. Once cooled, the fat mass solidifies around the template and provides a protective coat. In selecting a material for this encapsulation technique, the following should be considered: viscosity, thermal and oxidative stability, film forming, and adsorption behaviors. Molten lipids are suitable for this technique because they have low viscosity, can easily be sprayed and adsorbed, and spread easily on surfaces. Depending on the lipid, oxidation and heat degradation may be an issue.

TABLE 22.4 Lipids in Microencapsulation Techniques

Technique	Lipid Material	Material Select Criteria	Vesicle Type
Spray chilling/cooling	Fatty acids Waxes Solid triglycerides Solid polyglycerol esters	Melt profile Viscosity Crystal properties	Matrix
Centrifugal extrusion	Fatty acids Waxes Solid triglycerides Solid polyglycerol esters	Melt profile Viscosity Crystal properties	Reservoir
Centrifugal suspension separation	Fatty acids Waxes Solid triglycerides Solid polyglycerol esters	Melt profile Viscosity Adsorptivity Film properties Crystal properties	Reservoir
Homogenization (solid lipid structures)	Fatty acids Waxes Solid triglycerides Solid polyglycerol esters Emulsifiers: • Mono-/di-glycerides • Phospholipids • Polyglycerol esters	Melt behavior Surface activity Phase behavior in water Interfacial properties Crystal properties	Matrix
Homogenization (fluid emulsions)	Liquid triglycerides Emulsifiers: • Mono-/di-glycerides • Phospholipids • Polyglycerol esters	Interfacial properties Phase behavior in water	Reservoir
Liposomal entrapment	Phospholipids	Phase behavior in water Interfacial properties Molecular structure	Reservoir

There are three spray techniques: spray drying, spray cooling, and spray chilling. Lipids have minor applications in spray drying where they are used as emulsifiers in preparing emulsions for atomization. They are, however, extensively used in spray chilling and spray cooling. These two techniques are essentially the same except for the temperature in the solidification tower (Zuidam and Nedović, 2010). The technique is referred to as cooling when high melting fats are used and chilling for low melting fats. In spray chilling and cooling, the active is embedded within a molten lipid with homogenization. The homogenized mixture is sprayed in a low temperature chamber and allowed to solidify. Depending on what is incorporated, the crystallization behavior of the matrix can be altered. If small molecules are incorporated into the matrix, they may act as plasticizers and retard crystallization, negatively impacting encapsulation.

Centrifugal suspension separation (CSS) allows for coating of particles with a thin shell of fat or wax (Rahman, 2007). In this technique, the particles are suspended in molten shell material and poured onto a spinning disk. As the particles spread on the disk, a thin film of shell material is applied. When they reach the edge of the rotating disk, the particles break off from the fat film to form core−shell microcapsules. When used with a cooling or chilling tower, CSS can be considered as a spray cooling/chilling technique with a spinning disk nozzle. In centrifugal extrusion, liquids are coextruded with a nozzle consisting of concentric holes mounted on the outer circumference of a rotating cylinder (Risch and Reineccius, 1995). The concentric holes allow physical separation of the core (inner orifice) and shell (outer orifice) material, until they are pumped through their respective holes. The rotation of the supporting cylinder forces the core−shell jet to break into small droplets, affording a reservoir-like microcapsule.

The remaining techniques in Table 22.4 (emulsification, solid lipid structure fabrication, and liposomal entrapment) rely on phase behaviors between immiscible liquids. Solid lipid nanostructures (SLN) differ from normal emulsions because the core is in the solid state. SLN are emulsions created at high temperatures where both dispersed and continuous phases are liquid. As the temperature of the system goes down, the inner core solidifies, generating a solid

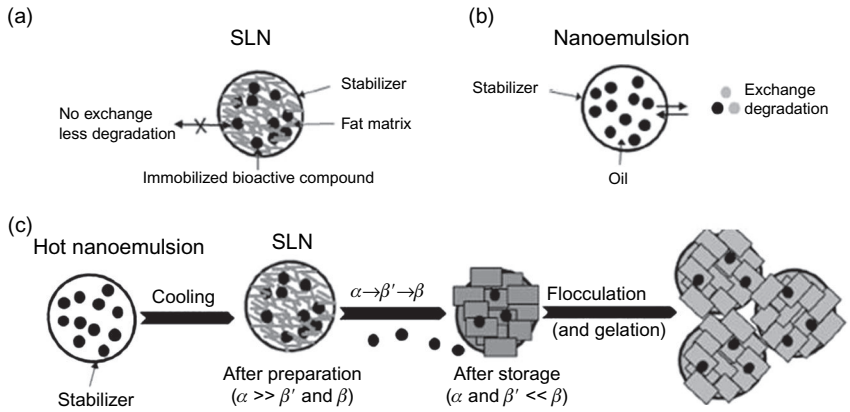

FIGURE 22.5 Structure and stability of solid lipid nanostructure. (a) SLN post cooling, (b) SNL pre-cooling, (c) polymorphism-mediated destabilization in SLN. *(Reprinted with permission from Tamjidi et al., 2013.)*

suspension that can be isolated. SLN during their fabrication are subject to the same destabilization mechanism as normal emulsions and rely on emulsifiers to stabilize the interface. During processing and storage, irreversible changes due to crystal behavior impact the performance of SLN. Changes in polymorphs lead to a denser crystal lattice and cause squeezing of the active from the matrix (Figure 22.5). This results in premature delivery and a shortened shelf-life (Müller et al., 2000; Tamjidi et al., 2013).

22.4.2 Applications

Lipids are used to encapsulate a wide range of food, cosmetic, and pharmaceutical ingredients. In this section, applications where lipids are used to encapsulate sensitive food ingredients such as vitamins, minerals, acidulants, enzymes, and microorganisms are presented.

22.4.2.1 Vitamins and Minerals

There is a push to fortify foods and beverages with vitamins and minerals to improve their health benefits. Vitamins and minerals are not always stable in formulated products; hence, encapsulation offers a way to deliver these active ingredients while protecting them from the formulation environment. Vitamin C, a water-soluble vitamin, loses its potency in solution where increases in temperature, pH, and the presence of certain metals accelerate its oxidation. Oxidation of the vitamin yields undesired color and flavor profiles and a reduction in active content in the formulation. Use of carnauba wax (Uddin et al., 2001) and phosphatidylcholine liposomes (Kirby et al., 1991) has been reported for vitamin C encapsulation. Because carnauba wax is hydrophobic, it limits moisture diffusion and slows down the release profile of the vitamin in aqueous media, thus limiting the degradation that can arise in solution. Liposomes protect the vitamin across a wide pH range and prolong its shelf-life. With liposomes, 60% potency remains after 50 days under refrigeration, while no active remains after just 20 days with unprotected vitamin. In a recent review (Velikov, 2012), the encapsulation of lipid-soluble vitamins (A, E, D, and K) in SLN and in liposomes is presented. The SLN are effective because they can incorporate the lipophilic vitamins in their core. Surface active agents such as polysorbates (Tween) or phospholipids (lecithin) are used to stabilize the interface and limit agglomeration.

Iron fortification in food and beverage is challenging because metal species are active in the oxidation of unsaturated fatty acids, leading to sensorial deterioration of fortified products containing these lipids. In addition, the metallic taste associated with iron makes consumer acceptance difficult. To limit these sensorial problems, encapsulated iron has been proposed. Kwak et al. (2003), Lee et al. (2004), and Li et al. (2009) reported iron encapsulation in lipids (medium chain triglycerides, polyglycerol monostearate, hydrogenated fat) and that this showed good sensory and oxidative stability while maintaining bioavailability. Wegmuller et al. (2006) demonstrated the fortification of salt with vitamin A and ferric pyrophosphate by using a spray cooling technique with hydrogenated palm fat. Kosaraju et al. (2006) used liposomes in encapsulating a combination of ferrous sulfate and ascorbic acid.

Kuslys and Sohier (2005) reported the co-encapsulation of PUFA (poly-unsaturated fatty acid) and MUFA (mono-unsaturated fatty acid) in the presence of trace metal micronutrients such as iron and copper in pre-term, infant, and toddler formulae, and many other nutritional dry mixes for groups such as pregnant or lactating women, elderly people, and hospitalized patients. In the aggregate premix, the metals and fats are both essential components; however, metals

catalyze the oxidation of double bonds resulting in poor taste and shorter shelf-life. By encapsulating the metals within an oxidatively stable lipid matrix (hydrogenated fats or waxes), Kuslys and Sohier prevented them from acting as catalysts in the oxidation of PUFAs and MUFAs in the dry mix, hence limiting sensorial deterioration and yielding extended shelf-life for the products.

22.4.2.2 Food Additives

Acidulants like citric, acetic, malic, sorbic, fumaric, and lactic acid are food additives that are used to provide flavor modification, prevent microbial growth, and impact texture in a variety of applications ranging from meat processing to baking (Garti and McClements, 2012). In baking, both acidulants and leavening agents are sometimes needed. The acidulants are used as mold inhibitors but they can negatively impact dough workability and the chemical leavening. At low pH, gluten loses its extensibility and higher energy is needed to work the dough. If the acidulant is not protected, it can prematurely reduce the pH of the formulation, causing the above-mentioned condition. Navarro (2001) showed that lipid-coated fumaric acid can be used to extend the shelf-life of tortillas without impacting dough extensibility and finished tortilla texture or appearance. Dockendorf and Gross (1975) showed that sourdough bread can be effectively made with lipid-coated fumaric acid without any negative impact on the dough proofing and crust formation.

Restrictions such as those discussed in baking are also at play in the production of sausages. Sausages are made using emulsified meat protein, fats, seasoning, and curing agents. Because of the high water content, the resulting emulsion is prone to microbial deterioration and can be preserved with acidulants. For effective microbial protection, the emulsion must be at a pH lower than 5, but under these conditions, the proteins in the emulsion toughen, making it difficult to process, which ultimately impacts the texture of the sausage. Ueno et al. (1973), Sato et al. (1981), Weiss and Reynolds (1988), and Camelot and Bontenbal (2010) reported the use of fat-coated acidulants such as lactic and sorbic acid in the reduction of microbial growth in meat products. The controlled release afforded by the encapsulation helps keep the pH high early in the process to protect the texture, but effectively brings the pH down later in the process allowing for proper microbial protection.

Customers enjoy the convenience of refrigerated or frozen dough products, which cut down on the time to bake goods in the home. However, it can be challenging for manufacturers to deliver non-developed dough under prolonged refrigerated or frozen storage conditions with enough leavening to give the finished product the necessary rise for proper texture. Often, reaction of the chemical leavening agent causes premature gas evolution, product expansion, packaging problems, and diminished rise of dough during baking. Encapsulation of the leavening agent with lipids such as hydrogenated fat has been utilized to prevent interaction between the acid and basic portion of the chemical leavening, which limits such problems. Domingues (2004a,b, 2013) and Bhatia and Morad (2007) reported using fat encapsulated chemical leavening agent applying fluidized bed coating to extend the self-life of frozen ready-to-bake products such as pizza dough, dinner rolls, biscuits, bread sticks, crescent rolls, and cinnamon rolls.

22.4.2.3 Enzymes and Microorganisms

The catalytic activity and nutritional benefit of many biological agents have been leveraged for centuries. They include ingredients such as yeasts, enzymes, and probiotics that are deployed in the production of bread, cheese, yogurt, wine and beer, and nutritional formulations. Microencapsulation has been used to modify these ingredients and increase their industrial performance and shelf stability.

Yeast, a key biological leavening agent in bread making, is sensitive to moisture, heat, and pH. Often, these factors are not easily controlled because water, heat, and acidulant are all important for bread fabrication. To maintain the yeast viability for proper activity during proofing, protection is often needed. Lee and Richardson (2004) reported the development of monoglyceride-coated yeast produced by fluidized bed coating and demonstrated that superior bread rise is obtained following storage of the yeast under ambient conditions. Narayanaswamy and Daravingas (2001) reported the protection of yeast and other leavening agents in high water activity dough and batter formulations. In this application, β- and β′-forming lipids provided moisture barrier and helped prolong shelf stability of the finished product.

Enzymes can digest biological polymers and convert them to oligomers and monomers (i.e., proteins to amino acids, complex carbohydrates to sugars, and triglycerides to fatty acids and glycerin). In bread and cheese, texture and flavor are obtained as a result of these digestion processes. Düsterhoft et al. (2002) presented the utilization of coated amylase granules in baking. These microcapsules were made using fluidized bed coating with hydrogenated vegetable oil as the shell. The coated amylase helps break down starch into sugar to fuel fermentation, yielding increased loaf volume as well as improved crumb structure. Luebering and Hair (1971) reported using a protease, encapsulated via spray chilling with hydrogenated vegetable oil, to limit the shrinkage of pie crust during baking. Picon et al. (1994) showed that with

liposomal entrapment of chymosin (the main enzyme in the coagulation step of cheese making), a marked increase in casein degradation can be obtained during the ripening stage thereby yielding softer cheeses. Kheadr et al. (2003) demonstrated shorter ripening time, more mature texture, and higher flavor intensity for cheddar cheese made using a cocktail of liposomes containing peptidases, proteases, and lipases. These enzymes were added to the milk prior to curd formation and were effectively retained. Typically, when unprotected enzymes are added to milk prior to coagulation, these enzymes are largely lost to the whey fraction during curd formation, slowing down flavor development during ripening. el Soda et al. (1989) reported milk fat-coated enzymes as well as liposomal systems that led to accelerated ripening and increased flavor profiles. They also showed that liposomal stability depends on factors such as pH, salt concentration, and temperature, all of which can be used to trigger delivery in the cheese formulation.

Okuro et al. (2013) showed up to 120 days of storage stability for a co-encapsulated mix of pro- and prebiotic. In the formulation, the probiotic (*L. acidophilus*) and prebiotic (inulin or polydextrose) were mixed in triglycerides obtained via interesterification of hydrogenated palm oil and palm kernel oil. Spray chilling of the mix generated solid lipid structures that allowed release of the probiotic in the intestine during normal fat digestion. Selection of an interesterified fat allows for coating at low temperature, hence limiting heat-related damage. The coat also prevents moisture attack by providing a hydrophobic moisture barrier.

22.5 CONCLUSION

Lipids are complex systems due to the diversity in their chemical compositions. The impact of structure on polymorphic crystallization, melting behavior, and permeability has been presented. The melt behavior of lipids allows for heat activated release, while hydrophobicity makes lipids ideal for protection of moisture-sensitive materials. Lipids in conjunction with many encapsulation techniques offer protection and extend shelf-life of sensitive food ingredients. To illustrate these benefits, applications were presented from fortification to commercial processing of food staples such as bread and cheese. Although fluidized bed and spray chilling/cooling are the most prevalent techniques used in encapsulation with fats, liposomes that have been exploited in the pharmaceutical industry are seeing increasing application in the food and nutrition industry. Finally, there is growing interest in SLN as novel delivery systems for food ingredients, but their application is still more prevalent in the pharmaceutical industry.

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Chapter 23

Yeast Cells and Yeast-Based Materials for Microencapsulation

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23.1 INTRODUCTION

Yeast cells have comprised a vital role in the brewery and bakery industry for thousands of years. Recently, yeasts have become commercially available from global ingredient suppliers or even biofuel (ethanol) producers. The structure of yeast cells, along with their presence in human nutrition (also recognized as GRAS material), has made them an attractive and novel encapsulation matrix (vehicle) for the food industry. Both hydrophobic and hydrophilic active food ingredients and pharmaceutical substances can be encapsulated in yeast cells for protection, masking and targeted drug delivery. Yeast encapsulation mainly involves whole cells or cell walls of *Saccharomyces cerevisiae* (baker's and brewer's yeast), although *Saccharomyces bayanus* (wine yeast), *Candida utilis*, *Kluyveromyces fragilis* (dairy yeast), *Torulopsis lipofera*, *Endomyces vernalis*, and oleaginous yeast (*Cryptococcus curvatus*) have also been used for encapsulation.

23.2 DESCRIPTION OF THE YEAST CELL AS ENCAPSULATION MATERIAL

Yeast cells are spherical or ellipsoidal in shape with a diameter of approximately 2−5 μm. The main cell parts responsible for the yeasts' excellent encapsulating agent properties are the rigid cell wall and the inner plasma membrane (Figure 23.1).

The wall of *S. cerevisiae*, the predominant yeast used for encapsulation, is approximately 100−200 nm thick and comprises 15−25% of the dry mass of the cell. It consists of a β-1,3-glucan network crosslinked to β-1,6-glucans, a mannoprotein layer, and a small amount of chitin. In dried cells the protein phase is approximately 10% of the cell wall volume (15 ± 2 nm thick) (compared to 30% in living cells) and the polysaccharides are the remaining 90% (130 ± 17 nm thick) (Zlotnik et al., 1984). The outer 10 nm of both sides of the cell wall are comprised of the glycosylated mannoprotein layer. The mannoproteins are extended both at the surface and in deeper layers, thus protecting the glucans from external attacks and providing a hydrophilic environment. The β-1,3-glucans and the chitin are responsible for the mechanical rigidity while the mannoproteins are responsible for the porosity and thus permeability of the cell wall (Scherrer et al., 1974; Zlotnik et al., 1984). In general, the cell wall provides mechanical strength to the cell, and thus to the encapsulated compound, and allows small polar and apolar molecules with molecular weight up to 760 Da to diffuse freely (Scherrer et al., 1974). However, even molecules up to 400,000 Da can enter the cell after proper prior treatment to increase cell wall porosity (De Nobel et al., 1989).

The much thinner (<10 nm) plasma membrane mainly consists of phospholipids that are organized in lipid bilayers, in addition to sterols and globular proteins. A major role of the plasma membrane is selective permeability to what enters or leaves the cell. Thus, it is the major permeability barrier for a permeating molecule (Zlotnik et al., 1984; De Nobel et al., 1990), but it has also an attractive role for encapsulation (Dardelle et al., 2007). The membrane's permeability is highly associated with its fluidity, as expressed by the phase transition temperature: the temperature at which the membrane transits from the gel to the more fluid liquid crystalline phase.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00023-6

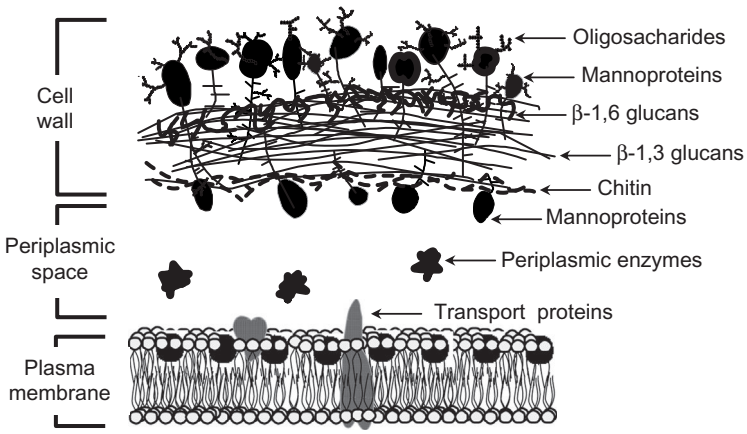

FIGURE 23.1 The cell envelope of *Saccharomyces cerevisiae*. The outer layers of both sides of the cell wall are comprised of mannoproteins and are responsible for the porosity, while the inner layer of β-1,3 and β-1,6-glucans and chitin, provides mechanical rigidity. The role of the plasma membrane, consists of phospholipid bilayers, is selective permeability.

23.3 THE YEAST CELL ENCAPSULATION PROCESS

One of the main advantages of the yeasts as encapsulation material is the simple and low-cost encapsulation process, which, in its simplest form, can be accomplished with no additives, with only the involvement of the yeast, water, and the active ingredient. Bibliographic data (Tables 23.1 and 23.2) mention that the encapsulation process generally involves mixing of the yeast cells (alive, plasmolyzed, or non-plasmolyzed, wet or dried) or the yeast walls with the active ingredient in a water or water/organic solvent solution, followed by stirring for several hours under controlled temperature (usually 20−60°C) (Figure 23.2). The stirrer speed is also controlled to obtain the desired droplet size for optimum diffusion.

During the encapsulation process, the cells lose their viability, particularly within 2 hours, while the cell wall and the membrane remain intact (Bishop et al., 1998). The non-viability of the loaded cells is desirable in order to avoid possible regrowth of the cells following their application. Moreover, the loss of viability of yeast cells does not influence their ability as encapsulant, since the encapsulation process is mostly driven by passive diffusion, with negligible relevance of active transport (Bishop et al., 1998; Ciamponi et al., 2012). In non-pretreated (non-plasmolyzed) cells the displacement of cell components occurs simultaneously with the uptake of the active ingredient (Bishop et al., 1998). The major parameter in encapsulation kinetics is the solubility of the hydrophobic compound in the cell wall, which is inversely related to partition coefficient (log P) (Ciamponi et al., 2012). Furthermore, the molecular size and shape of the active compound in addition to the presence of hydroxyl or other polar functional groups (which are attractive to the polar headgroups of the membrane phospholipids and promote their passage through them) affect the process (Bishop et al., 1998). Afterwards, hydrophobic interactions, van der Waals attractive forces, and hydrogen bonds between the yeast cell components and the active compound act synergistically to maintain the ingredient within the cell.

At the end of the encapsulation process, the loaded non-viable cells are washed with water or organic solvent in order to remove the non-encapsulated material. The last step in the encapsulation process is drying. In order to secure the encapsulation of the active compounds and avoid losses, the water is preferably rapidly removed from the loaded yeasts by spray drying, fluidized bed, or freeze drying (Dardelle et al., 2007). A typical spray-dried product contains spherical agglomerates of yeast cells with a diameter of 30 μm, each cell having a 5 μm diameter. The agglomerates are non-hygroscopic and insoluble in water, with good shelf-life properties.

An alternative encapsulation process for application in coatings of chewing gum, fried, baked, or extruded products (cereals, French fries) has recently been patented by Marty and Zampieri (2012), where the pre-preparation step in which the cells are loaded with the active compound has been omitted. Instead, the active compound (in a liquid form) is mixed simultaneously with the yeast cells and with the edible material (syrup, batter, dough). According to the inventors, the encapsulation process is carried out during the mixing process, and drying of the capsules before incorporation in the food product is omitted to avoid yield loss.

23.4 PARAMETERS THAT AFFECT YEAST ENCAPSULATION PERFORMANCE

The two main quantitative parameters used to evaluate the loading capacity of yeast cells and encapsulation performance are "*%Encapsulation Yield*" *(%EY)* and "*%Encapsulation Efficiency*" *(%EE)*. *%EY* refers to the % w/w content

TABLE 23.1 Published Examples of Encapsulation of Active Ingredients in Cells of *Saccharomyces cerevisiae*

Active Ingredient	Cells' Plasmolysis	Encapsulation Process	Comment	Reference
Orange peel & peppermint oil *(% EY up to 40)*	Non-plasmolyzed dead or alive compressed yeast cells	– oil:cells:water (1:2:4) – mixing (4 h, 40°C) – centrifugation – washing (water) – freeze-drying	• Yeast encapsulation *(%EY)* enhanced by: – encapsulation temperature >40°C – pre-treating cells with sodium azide or sterilization – compounds with -OH groups or amphiphilic properties • cells lose viability within 2 h of encapsulation • dead cells effective for encapsulation • encapsulation mechanism: passive diffusion	Bishop et al. (1998)
Enzymes: β-galactosidase, adenylate kinase & pyruvate kinase	Permeabilized cell walls (prior extraction of cell membrane)	permeabilization by: – detergent – freeze-thaw – ethanol or osmotic stress	• Zymolyase treatment increases wall permeability • Encapsulated enzymes offer kinetic advantages • Decreased enzyme activity in encapsulated cells, but reuse of the enzymes for over 15 times • Advanced system for large enzymes that act on small reactants	Chow and Palecek (2004)
Limonene *(% EY26.7)*	Plasmolyzed spray-dried cells	Inoue et al. (1991) Patent 0 453 316 B1	Release mechanism: • T >260°C or • $a_w > 0.7$	Normand et al. (2005)
Chlorogenic acid (CGA), *(%EE up to 12.6)*	Plasmolyzed (NaCl), freeze-dried cells	– CGA: yeast: water, (1:3:50) – stirring, (25 MPa, 40°C, 4 h) – centrifugation – washing (water) – freeze-drying	• Yeast encapsulation *(%EE)* enhanced by plasmolysis of cells & increased CGA purity (5, 85, 98%) • Encapsulation increased: – storage stability of CGA – CGA release: Simulated Gastric Fluid (SGF) > phosphate buffer > water	Shi et al. (2007)
98 substances with $1.09 < \log P < 6.39$ & MW < 300 Da	Plasmolyzed spray-dried cells	Inoue et al. (1991) Patent 0 453 316 B1	• No release from a dried powder in oil or air, unless water is present • Application in battered foods and instant noodles: yeast capsules were superior regarding flavors' boost effect and long lasting in the mouth	Dardelle et al. (2007)
Resveratrol *(% EY4.52)*	Plasmolyzed (NaCl), freeze-dried cells	– resveratrol: yeast: ethanol: water (1:3:50:50) – stirring, 25 MPa, 40°C, 4 h – centrifugation – washing (ethanol) – freeze-drying	Yeast encapsulation enhanced: – solubility – antioxidant activity and – stability of resveratrol against moisture, light and heat	Shi et al. (2008)

(*Continued*)

TABLE 23.1 (Continued)

Active Ingredient	Cells' Plasmolysis	Encapsulation Process	Comment	Reference
Conjugated linoleic acid rich oil, fish oil	Dead and viable *Cryptococcus curvatus* yeast cells	– fermentation of cells – mixing with the oil, 72 h – freeze-drying	• Encapsulation protects fatty acids from oxidation for more than 7 weeks • Non-encapsulated oils oxidized faster than encapsulated in either viable or dead cells	Iassonova et al. (2008)
Curcumin *(max %EY35.8) (max %EE 88.0)*	Plasmolyzed, non-plasmolyzed freeze-dried and live compressed cells	– curcumin: yeast in mass ratio from 10 up to 0.2, in water or 50% v/v ethanol – stirring, 25/35/45 &55°C, 48 h – centrifugation – washing (ethanol or water) – freeze-drying	• Temperatures >35°C favor encapsulation • Cells' plasmolysis is redundant and live cells are also effective • Preparation in water instead of 50% v/v ethanol increases both *%EE* and *%EY* by at least 2-fold • For high *%EY* the mass ratio curcumin:cells ≥ 2.0 • For high %EE the mass ratio curcumin:cells <0.5 • Curcumin is integrated in lipid membrane but also interacts with cell wall components	Paramera et al. (2011a)
Curcumin *%EY:* – *yeasts: 31.8,* – *6β-CD: 3.4,* – *MS: 2.9*	Plasmolyzed and non-plasmolyzed yeast cells, β- cyclodextrin (β-CD), modified starch (MS)	Paramera et al. (2011a,b)	• Encapsulation in yeast cells, β-CD and MS enhances curcumin's water solubility • Rapid dissolution of curcumin from β-CD and MS capsules, prolonged release from yeast capsules in SGF, and low degradation rate in Simulated Pancreatic Fluid (SPF) • For yeast capsules better storage stability at %RH >75.5, better light and heat stability at 200°C	Paramera et al. (2011b)
Terpenes: limonene, carvone, linalool *(max % EY4,9,9)*	Non-plasmolyzed wet cells	– Suspension of 5,10, 20, 30% w/w_{total} hydrophobes – agitation, 2–8 h, 20–60°C – centrifugation – washing (water)	• Encapsulation process: passive diffusion with negligible relevance of active transport • Major parameter in encapsulation kinetics: the solubility of the hydrophobe in the cell wall, which is inversely related to partition coefficient *(log P)*	Ciamponi et al. (2012)

of the encapsulated compound in the yeast microcapsules. *%EE* expresses the quantity of the encapsulated compound over its initial total quantity available for encapsulation. Yeast cells have a significantly high loading capacity (*%EY*) and the microcapsules that are produced may contain up to 70% w/w of the encapsulated compound, most commonly 30–40% w/w, depending on the characteristics of the active ingredient and on the encapsulation process. The most important parameters that affect yeasts' encapsulation performance are analyzed below.

23.4.1 Origin and Pretreatment of Yeast Cells Used for Encapsulation

The yeast cells are easily available in large quantities since they can either be grown under controlled conditions (cultures), or are commercially available in the form of compressed wet cells and as spray or freeze-dried powders,

TABLE 23.2 Patents of Encapsulation of Active Ingredients in Yeast Cells

International Publication Number - Date	Title	Inventors	Applicant
US 4001480—04/01/1977	Encapsulation process utilizing microorganisms and products produced thereby	Shank J.L.	Swift & Company
EP 0085805 A1—17/08/1983	Process for encapsulating substances by means of microorganisms, and the encapsulated products obtained thereby	German R.J., Morris G.G., Pannell N.A.	Dunlop Ltd
US 4696863—29/09/1987	Biocapsule	Matsushita T., Morishita S., Sekine M., Hiraishi S.	Mitsubishi Paper Mills Ltd
EP 0242135 A3—21/10/1987 **EP 0242135 B1**—04/07/1990	Microbial encapsulation	Pannell N.A.	AD2 LIMITED
EP 0453 316 A1—19/04/1991	Process for preparation of microcapsules	Inoue C., Ishiguro M., Ishiwaki N., Yamada K.	Mitsubishi Paper Mills Ltd; Kirin Brewery Company Ltd
EP 0414282 A1—27/02/1991	Bleach compositions containing microorganism encapsulated perfumes	Behan J.M., Birch R.A., Perring K.D.	Quest Int.
JP 3202141 A—03/09/1991	Preparation of microcapsule	Ishiguro M., Inoue T.	Mitsubishi Paper Mills Ltd
EP 0460945—11/12/1991	Process for producing microcapsules	Ishiguro M., Shimura Y., Ishiwaki N.	Mitsubishi Paper Mills Ltd; Kirin Brewery Company Limited
US 5078904—07/10/1992	Fabric softening compositions containing microorganism encapsulated perfumes	Behan J.M., Perring K.D.	Unilever Patent Holding B.V.
EP 0 528 466 A1—24/02/1993	Chewing gum with improved flavor release	Burger J.J., Sloot E.A., Jansma M.J., Glasius M.E., Koolhass W.E.	Quest International Nederland
WO 9311869—24/06/1993 **EP 0672113 A1**—20/09/1995 **US 5496728**—05/05/1996	Encapsulation of liquids in microorganisms	Hardy F.E., Willey A.D., Scialla S.	The Procter & Gamble Co.
US 5288632—22/02/1994	Encapsulation of material in microbial cells	Pannell N.A.	AD2 LIMITED
US 5521089—28/05/1996	Process for treating yeast with β-1,3-glucanase to produce microcapsules for enclosing hydrophobic liquids	Ishiguro M., Shimura Y., Ishiwaki N.	Mitsubishi Paper Mills Ltd; Kirin Brewery Company Limited
WO 1996/036433—21/11/1996 **EP 0844909 A1**—03/06/1998	Encapsulated product	Hobson J.C., Greenshields R.N.	CPC International Inc.
US 5660769—26/08/1997	Method of encapsulating substances in biocapsules	Sagar B.F., Sagar A.J.G., Graham S.G., Wragg R.T.	CPC International Inc.
US 5,798,252—25/08/1998	Encapsulated product containing essential oil and dyed microbial cell wall material	Hobson J.C., Greenshields R.N.	CPC International Inc.
EP 1171103 B1—16/01/2002 **US 7399475 B1**—15/07/2008	Inactivated microorganisms comprising substances having pharmacological activity	Grabitz E.B.	Toner Enterprise Inc.

(*Continued*)

TABLE 23.2 (Continued)

International Publication Number - Date	Title	Inventors	Applicant
EP 1246602 A2—09/10/2002	Use of microbially encapsulated materials in cosmetic and formulations	Baschong W., Hueglin D.	Ciba Specialty Chemicals Holding Inc.
GB 2394416 A—28/04/2004	Targeted delivery of microbially encapsulated drugs	Nelson G., Duckham S.C., Round A.E.	FLUID TECHNOLOGIES PLC MINCAP PLC
WO 2004/037232 A1—06/05/2004 **EP GB2396107**—16/06/2004 **US 2006/0127489 A1**—15/06/2006	Targeted delivery	Crothers M.E.D., Nelson G.	MICAP PLC
GB 2395124 A—19/05/2004 **WO 2004/045588 A1**—03/06/2004	Palatable micro-capsules	Nelson G., Duckham S.C., Round A.E.	FLUID TECHNOLOGIES PLC MINCAP PLC
US 2005/0118273 A1—02/06/2005	Microcapsules and oral composition containing the same	Yasushi S., Orikoshi H., Hideo H.	—
WO 2005/067733 A1—28/07/2005 **EP 1 681 944 B1**—12/11/2008	Edible product comprising flavoring microcapsules/ Process for the preparation of an edible product comprising flavoring microcapsules	Hahn A., Trophardy G.	FIRMENICH SA
WO 2005/102508—03/11/2005 **UK GB 2418654 (A)** — 05/04/2006 **US 2007/0269473 A1**—22/11/2007	Microbial encapsulation	Nelson G.	MICAP PLC
WO 2006/006003 A1—19/01/2006 **US 2007/0122398 A1** — 31/05/2007	Encapsulated hydrophilic compounds	Benczedi D., Hahn A., Trophardy G., Cantergiani E., Wagner R.	—
US 2006/0165614 A1—27/07/2006	Palatable micro-capsules	Nelson G., Duckham S.C., Round A.E.	—
WO 2006/085240 A1—17/08/2006	Heated food product with coating of encapsulated flavors	Le A., Barra J., Maurel C, Gordon J.F., Chiaverini M., Normand V.	FIRMENICH SA
GB 2424408 A—27/09/2006	Encapsulation using microbial cells	Round A., Nelson G.	MICAP PLC
WO 2006/100308 A2—28/09/2006	Compositions comprising microcapsules consisting of autolyzed microbial cells and encapsulated material		
WO 2007/066295 A2—14/06/2007	Instant food comprising flavor capsules	Katada J., Nicolas L, Sushida T.	FIRMENICH SA
EP 1 879 470 A1—23/01/2008	Fat, wax or oil-based food ingredient comprising encapsulated flavors	Barra J., Le A., Maurel C, Chiaverini M., Gordon J.F., Normand V.	FIRMENICH SA

(*Continued*)

TABLE 23.2 (Continued)

International Publication Number - Date	Title	Inventors	Applicant
EP 1891866 Al – 27/02/2008 **WO 2008/023271 A2**–28/02/2008 **US 2008/01566336 A1**–03/07/2008	Smoking article with encapsulated flavor	Wyss-Peters A., Jordil Y., Kuersteiner C.	Philip Morris Products
WO 2009/053711 A2–30/04/2009	Method of Encapsulation	Tirelli N., KilcherG., Ciamponi F.	The University of Manchester
WO 2011/001318 A1–06/01/2011 **US 2012/0076892 A1**–29/03/2012	Process for encapsulating an active ingredient	Marty M., Zampieri D.	FIRMENICH SA
US 7,914,825–29/03/2011	Encapsulated vaccinium extracts with balanced gastrointestinal release	Siegel S., Mavric E., Krammer G.	–
WO 2012/024229 A1–23/02/2012 **US 2012/0070376 A1**–22/03/2012	Yeast cell wall particle for receptor-targeted nanoparticle delivery	Ostroff G.R., Soto E.	University of Massachusetts

provided by global suppliers or even biofuel producers. One milligram of a freeze-dried powder of *S. cerevisiae* cells contains approximately 4.7×10^7 cells (Paramera et al., 2011a).

As regards the type of the cells used, viable (alive) as well as non-viable cells can effectively be used as encapsulation vehicles and the cells' viability does not have a significant effect on yeast loading, since the encapsulation process, as referred to in Section 23.3, is mostly driven by passive diffusion (Bishop et al., 1998; Ciamponi et al., 2012). The freeze drying or spray drying process that is applied for pretreatment of cells or in the last step of the encapsulation process is lethal for nearly the total number of cells (Paramera et al., 2011a).

The composition of the yeasts' cell wall and plasma membrane plays a vital role in the encapsulation performance, and the synergistic effects of proteins, polysaccharides, lipids, and nucleic acids affect the encapsulation properties of the yeast cells (Shi et al., 2010). The yeast cells' composition can be modified by several means. One way is to control the yeasts' growth conditions (culture medium composition, incubation temperature, etc.). For instance, Shi et al. (2010) reported that the encapsulation characteristics of *S. cerevisiae* cells used for the encapsulation of chlorogenic acid (CGA) were largely affected by the composition of the culture medium.

However, the most common method used to modify yeast cells' composition and affect their encapsulation performance is chemical pretreatment or plasmolysis (autolysis) of yeast cells, which can potentially increase the available encapsulation intracellular space or the encapsulation rate and decrease the protein and nucleic acid content of the cells. Chemical treatments, including enzymes (pepsin, trypsin, chymotrypsin, chitinase, β-glucanase) and magnesium salts, can be used to increase the permeability of the cell wall or an aldehyde solution to decrease it. Chemical pretreatment of yeast cells is generally carried out by surfactants, enzymes, sugars, salt, and organic solvents. For instance, chemical autolysis of yeast cells by ethanol, sodium chloride, Triton X-100, and sodium dodecyl sulfate destroyed the intermolecular network between the mannoprotein chains and disrupted the plasma membrane, facilitating the permeation of the hydrophilic CGA into the yeast cell; thus, as regards water-soluble compounds the loading capacity of yeast cells can be enhanced after prior cells' plasmolysis where the water-soluble cell components have been removed (Shi et al., 2007, 2010). Bishop et al. (1998) reported that the encapsulation rate of orange peel oil was enhanced significantly using non-viable *S. cerevisiae* cells after prior pretreatment with sodium azide or sterilization. However, as summarized in Table 23.1 and described in numerous patents (Table 23.2), in many cases of yeast encapsulation, prior plasmolysis or chemical treatment of yeast cells is rather redundant or is avoided in order to facilitate the encapsulation process. For instance, plasmolyzed cells with sodium chloride were as efficient as non-plasmolyzed cells for the encapsulation of the hydrophobic curcumin, although plasmolysis modified their cells' lipid, protein, and polysaccharide content (Paramera et al., 2011a).

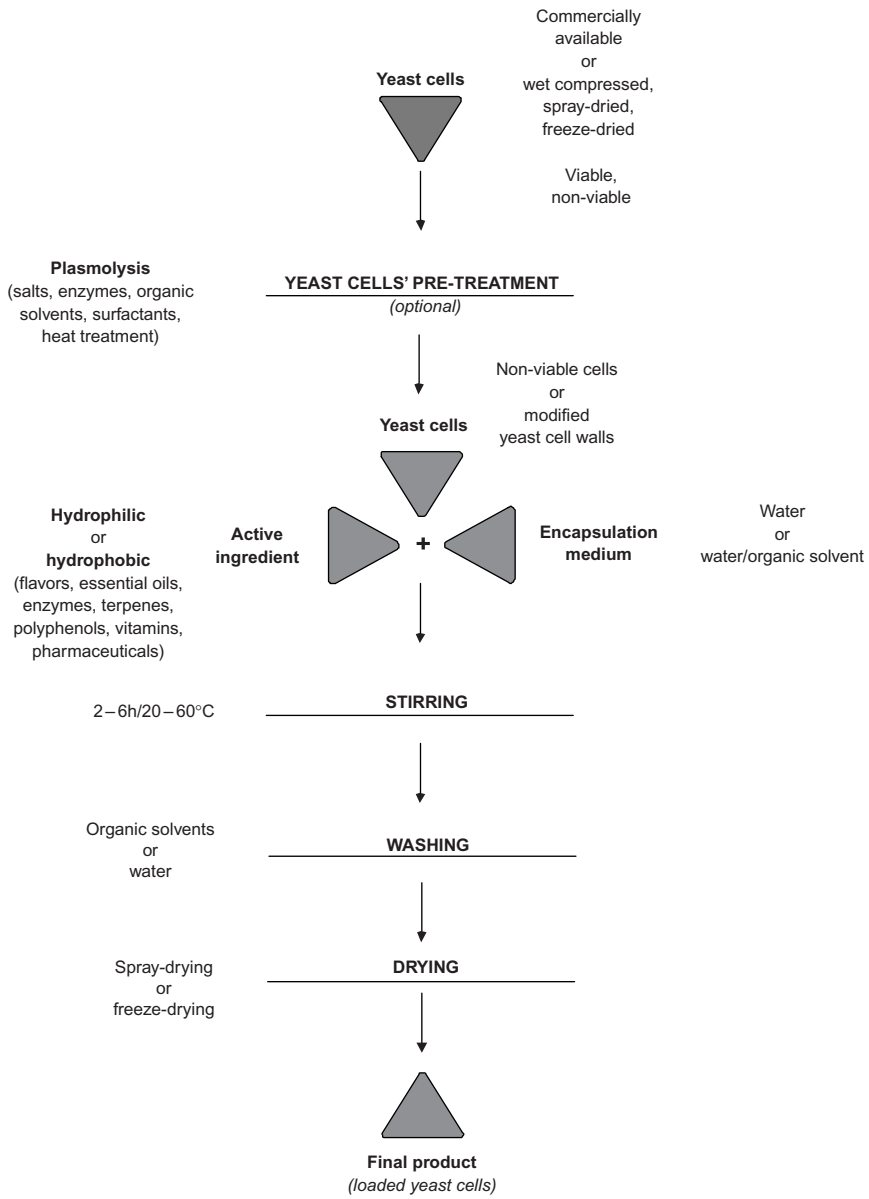

FIGURE 23.2 Typical yeast encapsulation process. A yeast cells' aqueous suspension is mixed with active compound solution (aqueous or organic) for several hours, under controlled temperature, followed by drying.

In general, the loss of proteins, polysaccharides, and other cellular components due to autolysis can empty the cells and promote their loading capacity; however, it can make the retention and interaction of the active ingredient more difficult. Thus, a compromise between the wall permeability and the cells' ability to preserve the active ingredient is important for a high loading capacity of yeast cells (Shi et al., 2010).

In the cases where the yeast flavor or color is undesirable due to possible interference with the encapsulated compounds, a method for elimination of yeast flavor/odor has been developed, by their pretreatment with peroxygen bleach (Hardy et al., 1993).

23.4.2 The Active Ingredient

Regarding the active compound, the penetration yield is correlated both to its size (molecular weight) and shape, in addition to its polarity (hydrophobic properties). Both hydrophilic and hydrophobic compounds can effectively be encapsulated in yeast cells. *%EE* can reach a value of 50% when log P (where P refers to the partition coefficient) of the active compound is higher than 2.0 (Dardelle et al., 2007). The presence of hydroxyl or other polar functional

groups in the active compound, which allow interaction with the polar headgroups of the phospholipids' bilayer and promote their passage through them, affect the encapsulation process and result in higher *%EY* values (Bishop et al., 1998). Ciamponi et al. (2012) also reported that the solubility of a hydrophobic compound in the cell wall, which is inversely related to partition coefficient (log P), is the major determinant of the encapsulation kinetics. For instance, the *%EY* of the volatiles limonene, carvone, and linalool capsules were found to be 4, 9, and 9%, respectively, and the internalization rate was dependent on the hydrophobe: carvone showed the most rapid internalization and limonene the slowest one. Regarding the water-soluble compounds, the encapsulation performance is significantly enhanced along with the purity of the active ingredient. For instance, the *%EE* of CGA increased from 5.2 to 12.6%, when CGA's purity was 35% and 98%, respectively (Shi et al., 2007).

23.4.3 Medium of Encapsulation

The medium of encapsulation can either be exclusively water or a water/organic solvent/emulsifier solution (Tables 23.1 and 23.2). For water-soluble compounds, encapsulation is usually performed in an aqueous environment. On the contrary, the solid hydrophobic compounds can optionally be dissolved in an organic solvent (carrier) before being added in an aqueous cell suspension. A polar organic solvent can also act as a lipid-extending agent and promote the penetration and passive retention of the active ingredient into the cell (German et al., 1982). Nevertheless, independently of the presence of an organic solvent, the presence of water is necessary for the encapsulation process to hydrate the membrane and swell the yeast. By changing the polarity of the encapsulation environment, i.e., by partially replacing water with a polar aprotic solvent such as dimethylsulfoxide (DMSO), larger hydrophobes (of molecular weight higher than 400 up to 3800 Da) are able to penetrate into the cell, due to the increased solubility in the yeasts' cell wall (Kilcher et al., 2008; Tirelli et al., 2009).

The presence of an organic solvent in the (aqueous) encapsulation medium does not necessarily enhance the encapsulation performance. For instance, when resveratrol was encapsulated in 50% v/v ethanol, the *%EY* was 4.52 (Shi et al., 2008). Low encapsulation performance has also been reported for curcumin when the encapsulation medium was 50% v/v ethanol compared exclusively to water: under identical encapsulation conditions both *%EY* and *%EE* values were at least two-fold higher when encapsulation occurred in water (max. *%EY* 33.8, *%EE* 88.0), instead of 50% v/v ethanol (max. *%EY* 16.6, *%EE* 18.0) (Paramera et al., 2011a).

23.4.4 Encapsulation Temperature

The temperature at which encapsulation occurs plays an important role in the encapsulation performance. Usually, temperatures from 20°C up to 60°C have been used to perform encapsulation; however, temperatures around 40°C, above the membrane's phase transition temperature (around 30−37°C, depending on the phospholipid composition, water activity, etc.), where membrane transits from gel to the more fluid liquid crystalline phase, enable penetration and increase the permeation of the compound in the cell (Bishop et al., 1998). A dependence on the encapsulation rate on the encapsulation temperature has been reported for orange peel oil by Bishop et al. (1998). The encapsulation rate was almost identical between 6°C and 40°C, whereas between 40°C and 50°C it increased significantly and the *%EY* reached 56.0 after 4 days' incubation at 40°C. Ciamponi et al. (2012) also reported an increase of encapsulation rate along with temperature, which was more noticeable for carvone and less so for limonene. Similarly, both *%EY* and *%EE* values of curcumin yeast microcapsules were favored when encapsulation occurred at the highest temperatures (45°C, 55°C) rather than the lower (25°C, 35°C) ones. For instance, the *%EY* of microcapsules prepared at 25°C and 35°C was 14.0 and 14.6, respectively, whereas (under identical conditions) at 45°C and 55°C the corresponding values increased to 25.0 and 27.6, respectively (Paramera et al., 2011a).

23.4.5 Mass Ratio Compound: Yeast Cells

The mass ratio of the active compound to the yeast cells used for the preparation of yeast microcapsules is also a crucial parameter for both *%EY* and *%EE* values. As depicted in Table 23.1 and described in many patents (Table 23.2) in most cases of yeast encapsulation, excess of yeast cells rather than the active ingredient is used (mass ratio compound: cells 1:2 or 1:3). Nevertheless, the bibliographic data that evaluate the mass ratio compound:cells for optimum encapsulation performance are limited. In a patent by Nelson (2006) where essential oils are encapsulated, it is pointed out that a ratio of compound:cells lower than 1:1 by weight, thus an excess of yeast cells rather than the ingredient, can improve the loading and the total *%EE* of the method by more than 50%. Wang et al. (2006) also studied the effect of

compound:yeast ratio on the encapsulation performance of methyl salicylate and found that under encapsulation at 70°C for 6 hours and a methyl salicylate:yeast ratio of 2 ml/g, the *%EY* reached 48.58. The importance of mass ratio compound:cells on the *%EY* and *%EE* (in relation to plasmolysis of cells, encapsulation temperature, and medium) has also been studied for curcumin, where microcapsules were prepared with mass ratios curcumin:cells from 10.0 to 0.2 (Paramera et al., 2011a). It was shown that when the aim is to produce microcapsules with high curcumin content (high *%EY*), curcumin should be in excess (mass ratio curcumin:cells ≥ 2.0). On the contrary, when the aim is to exploit as much of the initial quantity of curcumin as possible (high *%EE*), excess of yeast cells should be reasonably used (mass ratio curcumin:cells ≤ 0.5). Nevertheless, a mass ratio 1.0 can provide considerable values of both *%EY* and *%EE*.

23.5 PROPERTIES OF YEAST MICROCAPSULES

23.5.1 Encapsulation of Both Hydrophilic and Hydrophobic Compounds and High Loading

The baker's yeast cells (*Saccharomyces cerevisiae*) have been used for the encapsulation of both hydrophobic and hydrophilic molecules, as summarized in Table 23.1: resveratrol (Shi et al., 2008), curcumin (Paramera et al., 2011a), flavor compounds including limonene (Normand et al., 2005; Dardelle et al., 2007), essential oils (Bishop et al., 1998), as well as the water-soluble chlorogenic acid (CGA) (Shi et al., 2007). Yeast walls, available after extraction of the lipid bilayer membrane of yeast cells, have also been used for immobilization of the enzymes β-galactosidase, adenylate kinase, and pyruvate kinase, in order to permit their recovery and reuse (Chow and Palecek, 2004). Oleaginous (*Cryptococcus curvatus*) yeast cells have also been used for the encapsulation of the susceptible-to-oxidation polyunsaturated triacylglycerols: linoleic acid-rich oil and fish oil containing omega-3 long-chain fatty acids (Iassonova et al., 2008).

Besides cells of *S. cerevisiae*, *Candida utilis*, *Kluyveromyces fragilis*, *Torulopsis lipofera*, and *Endomyces vernalis* cells (dead or alive) in addition to cell walls, such as yeast glucans particles, have also been used for the encapsulation of vitamins, flavors, essential oils (lavender, eucalyptus, mint, cedar, peppermint, mustard, garlic, lemon, spearmint, oregano, onion, rosemary), fragrances, drugs, nutraceuticals, dyes, and even nanoparticles as described in a number of patents (Table 23.2).

In Table 23.1 the *%EY* values of yeast microcapsules are also presented. As was described in detail in Section 23.4, yeast cells have a significantly high loading capacity and the microcapsules most commonly contain 30−40% w/w of the encapsulated compound. In comparison to other encapsulation matrices, yeast microcapsules are characterized by comparable and even superior *%EE* and *%EY* values. For instance, when curcumin was encapsulated in yeast cells, β-cyclodextrin (β-CD), and modified starch (MS), the capsules prepared had *%EY* 35.8, 3.4, and 2.9, and *%EE* up to 88.2, 22.8, and 60.4, respectively (Paramera et al., 2011b).

23.5.2 Yeast Encapsulation and Protection

Microencapsulation in yeast cells can be applied to enhance the stability of the active ingredient. The particular structure of the yeast cells, which includes the mechanically strong cell wall with the reticulated network of mannoproteins and fibrous β-1,3-glucans, along with the lipid bilayer, inhibits the destructive effects of moisture, heat, light, and oxygen. This structure is extremely beneficial for volatile compounds, where this double carbohydrate wall/lipid membrane capsule provides a highly stable product with extended shelf-life. During heat food processes (extrusion, baking, roasting, frying, boiling), the yeast capsules provide particular thermostability.

For instance, the yeast capsules of *Cryptococcus curvatus* with linoleic acid/fish oil managed to improve the oxidative stability of the polyunsaturated omega-3 fatty acids for more than 7 weeks (Iassonova et al., 2008). Encapsulated enzymes in cell walls of *S. cerevisiae* were also protected from stresses and could also be reused for many reactions (over 15 times) with minor, but not significant, loss of activity. The advantages of the encapsulation increased along with enzyme size (small enzymes diffuse rapidly in and out) and as reactant size decreased (Chow and Palecek, 2004).

The yeast (*S. cerevisiae*)-encapsulated CGA exhibited good stability during storage at 60°C, and under wet and thermal stresses (relative humidity, %RH 75 and 90, 25°C) (Shi et al., 2007). The same observation refers to hydrophobic compounds, such as resveratrol, which after yeast encapsulation increased its stability under wet stress (%RH 75 and 90) and under light irradiation (Shi et al., 2008). The photosensitive curcumin also increased its photochemical (by 2.6-fold) and storage stability after encapsulation in *S. cerevisiae* cells, and to a higher degree than did β-CD and MS capsules. Yeast-encapsulated curcumin was also better protected against heat decomposition at high temperatures (150−200°C) than when non-encapsulated (at least 5% and up to 30% higher stability) or when encapsulated in β-CD and MS (at least from 2−10%, and 20% higher stability, respectively) (Paramera et al., 2011b).

23.5.3 Release Properties and Controlled/Targeted Delivery of Yeast Encapsulated Compounds

Yeast cells can effectively be applied for controlled and targeted drug delivery. The release mechanism of the yeast system has been studied by Normand et al. (2005), using the hydrophobic volatile limonene as a model, in correlation with temperature and water uptake in the cell wall. It was shown that the yeast cells provide particular thermostability and the cell wall remains stable up to 260°C (temperatures higher than those usually applied in food processing, including cooking), whereas the flavor starts to release at temperatures higher than 243°C, a property superior to fried foods. The flavor release is also water activity dependent; below a water activity of 0.7, flavor release cannot occur and the rate of release depends on the degree of swelling of the external wall (Normand et al., 2005).

Water is necessary to trigger the diffusion (release) of the encapsulated compound toward the outside medium; the polar groups of the proteins and polysaccharides (hydrogen bond donors) attract the water molecules and the cell wall swells, creating holes in the protein layer, thus permitting diffusion (Dardelle et al., 2007). Therefore, dried cells provide a well-"locked" system, impermeable to molecules, whereas wet cells permit the reversible transfer of molecules from the outside environment (Dardelle et al., 2007). When a dried powder of encapsulated limonene was immersed in an oily phase or stored in a dry environment, no flavor release occurred, unless water came in contact with the yeast powder (Dardelle et al., 2007).

Yeast cells also provide slow release of the encapsulated compound, which is favorable in numerous food processes. A kinetic hysteresis between water sorption and water desorption isotherms in the cell wall has been found, explaining the reduced cell wall permeability at elevated temperatures and during water loss. During desorption (drying), the water moves from the β-glucan layer towards the protein layer, rehydrating it, resulting in persistence of flavor diffusion, until the glucan layer is completely dried. Therefore, the whole release process is limited by the dehydration of the external protein layer (Dardelle et al., 2007).

In simulated gastrointestinal conditions, yeast microcapsules are able to release their content, although they provide a well-"locked" system. The acidic (gastric fluid) or basic (pancreatic fluid) conditions can be destructive for the cell wall structure, allowing the release of the encapsulated compound. A gradual and prolonged release has been reported for both hydrophilic and hydrophobic compounds. The water-soluble CGA is almost totally (95%) released in simulated gastric fluid (SGF) (pH 1.2) within 2 hours, with enhanced dissolution compared to phosphate buffer solution (pH 7.4) or water (Shi et al., 2007). The hydrophobic resveratrol is also almost totally released in SGF within 90 minutes (Shi et al., 2008). Slow and prolonged release from yeast microcapsules has also been reported for curcumin (Paramera et al., 2011b). Its release profile in SGF revealed that yeast encapsulation enhanced curcumin's dissolution and thus solubility, but to a lower degree than did β-CD and MS capsules, where a rapid disintegration and dissolution was obtained. Moreover, yeast encapsulation lowered the degradation rate of curcumin in the alkaline conditions of the simulated pancreatic fluid (SPF) to a higher degree than did encapsulation in β-CD and MS.

Yeast encapsulation can also be successfully applied for oral delivery, since yeast cells can offer targeted flavor release on the mucosal surfaces, such as the tongue and the nose, without breaking the cells. The flavor is immediately released into the mouth during mastication, producing an enhanced flavor impact on contact with the lipophilic sites on the tongue, possibly assisted by the enzymatic action of saliva (Crothers and Nelson, 2004).

Due to the bioadhesive properties of yeasts, a long-lasting release is achieved without loss of flavor profile. By coating the yeast particles with an acid-stable enteric coating system that breaks only in alkaline conditions, a targeted delivery of the yeast content is available from the mouth to the small intestine (Nelson et al., 2004). Once released, the cells disperse within the gastrointestinal fluids and at the mucosal surface, and deliver their content for absorption into the systemic circulation system. Thus, yeast encapsulation enables prolonged delivery of the active ingredients and can enhance their bioavailability.

Finally, yeast capsules can be incorporated in conventional cream bases for topical delivery, since contact with other live microbes can also trigger release.

23.5.4 Yeast Encapsulation and Sensory Evaluation

Sensory analysis of yeast-encapsulated flavors revealed enhanced flavor intensity and prolonged flavor perception, possible due to the characteristics of the yeast cell surface. The dimensions of the yeast capsules (similar to in-mouth wrinkles) and their adhesive properties due to the external protein layer, give longer residence time and long-lasting flavor in the mouth (Dardelle et al., 2007; Marty and Zampieri, 2012).

For instance, battered products containing yeast-encapsulated beef flavor and instant noodles prepared with yeast garlic flavor were sensorially evaluated. Comparison with conventional beef and garlic spray-dried powders produced

on the basis of identical cost revealed that the smell, the boost effect, the impact after first chew, and the lasting after swallowing were superior for yeast-encapsulated flavors. Moreover, the integrity of the flavor was not affected by yeast encapsulation (Dardelle et al., 2007).

In the cases where the yeast flavor or color is undesirable due to possible interference with the encapsulated compound, its elimination is possible (Hardy et al., 1993).

23.5.5 Antioxidant Properties and Solubility of the Yeast Encapsulated Compound

The antioxidant properties of antioxidant compounds are sustained after yeast encapsulation. Although few bibliographic data are available in this field, the measured antioxidant activity, i.e., of yeast-encapsulated resveratrol, was found to be higher than non-encapsulated (Shi et al., 2008). Yeast encapsulation also enhances the aqueous solubility of poorly soluble compounds, but to a lower degree than other encapsulation materials, such as cyclodextrins. Yeast-encapsulated resveratrol has three-fold higher aqueous solubility than non-encapsulated resveratrol, possibly due to the interactions between resveratrol and yeasts' proteins and polysaccharides (Shi et al., 2008).

23.5.6 Nutritional Value and Anticancer Properties of Yeast Cells and Yeast Microcapsules

Yeast cells, contrary to other encapsulants, have superior properties regarding the nutritional value and health benefits, which make them more attractive for the food and drug industry. Yeast cells are used in cGMP-based processes for the production of vaccines and biopharmaceuticals. Live yeast cells of *S. cerevisiae* have been shown to assist in the therapy of ulcerative colitis and irritable bowel syndrome (IBS). Dead cells of *S. cerevisiae* have shown positive effect against cancer cells (breast, tongue, colon) in experiments with mice, with no adverse side effects (Herberman, 2002; Ghoneum et al., 2008). The yeast cell wall polysaccharides and particularly the water soluble β-1,3 and β-1,6 D-glucans have shown significant protective antibacterial, wound-healing, antioxidant, antimutagenic, and antigenotoxic activities with potent application in anti-infective and anticancer therapy (Kogan et al., 2008; Jaehrig et al., 2008).

Yeast cells are also commercially available in the form of capsules and tablets, as a nutrition supplement. They are comprised of a natural diet source of amino acids, vitamins (mostly of B complex), and minerals (phosphorus, iron, zinc, sulfur, potassium, manganese, magnesium).

23.6 APPLICATIONS OF YEAST MICROCAPSULES IN THE FOOD INDUSTRY

Flavors encapsulated in yeast cells can survive cooking processes including frying, boiling, roasting, baking, and extrusion, but can be released during mastication when hydration occurs. Therefore, in addition to its long-lasting flavor release, targeted delivery, and GRAS properties, the yeast system finds numerous applications: in confectionery products, soups, fried foods, chewing gums, cereals, syrups, sauces, hot beverages, and brewery and bakery products. Moreover, since the yeast capsule relatively retains encapsulated the active ingredient in water and other aqueous mixtures, it is possible to produce not only a dry powder or tablet but syrup or a water-based formulation. The interest of the food industry in yeast encapsulation and its potential applications are seen by the numerous patents available, as summarized in Table 23.2.

Other than the food industry, yeast-encapsulated fragrances have been used in textiles, fabric conditioners, skin softeners, and even filter cigarettes. Yeast encapsulation is also attracting interest from the medical sector for the encapsulation of antibiotics, hormones, nanoparticles, and pharmaceuticals. Other possible applications of the yeast system include the encapsulation of agrochemicals and cosmetics (perfumes, dyes, insect repellents, shoe liners) (Nelson, 2002; Nelson and Crothers, 2003).

23.6.1 Yeast Encapsulation Patents

The industrial uses of yeast cells were first focused on carbonless copy paper and flavor delivery. In the first patent reference for yeast encapsulation in early 1973 (Serozym Laboratories, 1973) water-soluble flavor compounds were encapsulated in plasmolyzed yeast cells. Some years later (Shank, 1977) fat-soluble compounds (dyes, lubricants, drugs, vitamins, flavors) were used for encapsulation in yeast cells that had been grown in proper medium so as to have a lipid content higher than 40% w/w and a yeast leuco dye was applied in carbonless carbon paper. Thereafter, a European patent (German et al., 1982) described the yeast encapsulation of dyes with the employment of a carrier: an organic lipid-extending substance in order to extend the lipid content of the cell. This was later proved not necessary (Pannell, 1987): cells of normal lipid content (<5%) and non-pretreated with a plasmolyzer or a carrier provided high (50−75% w/w)

encapsulation efficiency values for essential oils (lavender, eucalyptus, mint, peppermint, mustard, lemon, etc.). The produced yeast microcapsules were applied in perfumed draw liners, fragranced stationery, pesticides, and drugs (Pannell, 1987, 1994), in addition to perfumed fabric softening compounds (Behan and Perring, 1992), perfumed bleach, and detergent compositions (Behan et al., 1991). For the rapid encapsulation of large amounts of hydrophobic liquid dyes, Inoue et al. (1991) described a method by prior elution of the intracellular components, comparing ethanol, acetone, autoclavation, and pH adjustment as elution promoting agents, whereas Ishiguro et al. (1996) pretreated the cells with alkaline solution or the enzyme β-1,3 glucanase.

Nelson (2006) patented a method for yeast encapsulation of food lipophilic essential oils and flavors with increased total *%EE* by more than 50%, by using excess of yeast cells rather than excess of the active ingredient. A recent patent (Tirelli et al., 2009) described the encapsulation of active compounds (flavors, nutraceuticals, agrochemicals) of molecular weight higher than 400 Da up to 5000 Da in yeast cells by pretreatment of the cells with DMSO. Hydrophilic and hydrophobic compounds (fragrances, food flavors, essential oils, colorants) have also been effectively encapsulated in *S. cerevisiae* processed autolyzed cell walls with polysaccharide content at least 70% w/w and with membrane completely or partially damaged or not present at all (Round and Nelson, 2006). However, intact yeast cells have been used for targeted delivery of lipophilic ingredients to a mucous surface (oral or buccal cavity, tongue, stomach, small−large intestine, vagina, cervix, nose, pulmonary system). By a simple contact of capsules with the mucous surface a targeted release occurred without cell wall breakage. Thus, yeast capsules were incorporated in mouth tablets (encapsulating menthol, tea tree oil for antibacterial effect, bergamot oil for ulcers), nasal sprays (loratidine-terpene oil), in formulations for delivery of drugs in the stomach (triacetic, ibuprofen), in the digestive track (ketoprofen), or for gastric ulcer treatment (omeprazole-nonanol) (Crothers and Nelson, 2004; Nelson et al., 2004). Moreover, yeast cell autolysates (fractions of both water-soluble and water-insoluble yeast constituents after incomplete autolysis) have been used for a particular targeted gastrointestinal release of *Vaccinium* (berries) extracts (rich in anthocyans); a continuous release was achieved with a balanced ratio in both the stomach and in the intestine. The capsules prepared were applied in chewing gums, candies, yoghurt, muesli, cake, cookies, snacks, sauces, and soups (Siegel et al., 2011).

Yeast cell wall particles (YCWPs) and particularly glucan particles (YGPs), chitosan particles (YCPs), and chitosan/mannan particles (YCMPs) have also been applied for receptor-targeted delivery of nanoparticles encapsulated in them (styrenes, quantum dots, ferromagnetic or gold particles, polymeric, silicon, glass, plastic). The nanoparticles were loaded with drugs and were further trapped either on the surface or inside yeast wall glucans (Ostroff and Soto, 2012). Yeast cell walls have also been used for the encapsulation of food and fragrance essential oils in addition to a dye; the dye was absorbed on the surface of the wall and gave a color visible in the bulk product for application in, for example dressings, confectionery products, colored toothpaste, and copying paper (Hobson and Greenshields, 1996).

Nelson et al. (2006) used yeast capsules containing food flavorings and essential oils for the production of palatable formulations that are orally administered (mouthwash, toothpaste, tablet, syrup, gel, chewing gum). The encapsulated flavors were able to mask, disguise, or neutralize the unpleasant taste or odor of formulations of drugs, vitamins, and nutraceuticals. In addition, the encapsulated flavor could be combined with an additional flavor, i.e., incorporated into the formulation's matrix, a technique termed "dual masking." This technique improved taste and acceptability during and after swallowing and is particularly applicable for strong tasting ingredients, such as fish or garlic oil, therefore eliminating the need for a deodorizing process.

Yeast cells have also been combined with matrix components (proteins and carbohydrates such as dextrins, corn syrup, gums) to enhance significantly the efficiency of encapsulation of both hydrophobic and hydrophilic food compounds, which, in this way, could be simultaneously present in the capsule: flavors not entrapped in yeast cells could be recovered by the matrix (Benczedi et al., 2007). Moreover, the combination of yeast capsules with a matrix for the preparation of food coatings has reduced the loss of flavors during heat treatments, but allowed the easy release of the flavor once arrived in the oral cavity (Le et al., 2006). Furthermore, by applying a combined yeast−matrix capsule in foods with lipid content or coating, the encapsulated flavors, even the hydrophobic ones, were retained and not diffused into the surrounding lipid environment, even at heating temperatures, unless hydration occurred (Barra et al., 2008).

Hahn and Trophardy (2008) applied yeast flavoring microcapsules in edible compositions that were subjected to thermal treatments at high temperatures. The yeast cells were combined with at least one carbohydrate carrier material (maltodextrin, starches) that formed a layer around yeast flavor capsules and enhanced the flavor's retention. Beef flavored French fries, garlic flavored crackers, and pasta flavored with herbs that contained encapsulated rather than free flavor were superior regarding smell and particularly long-lasting effect. Yeast capsules have also been applied in instant food products with a water content 13% w/w or less (soup, noodles, seasoning, topping) for particular release: some flavor was released in a short time during the food's preparation, whereas the rest of the flavors were released afterwards during consumption (Katada et al., 2007).

Finally, in the patent of Marty and Zampieri (2012), several food applications of yeast microcapsules that alternatively were prepared directly in an edible coating are given. Chewing gums that were coated with syrups containing encapsulated flavors were found to contain higher amounts of flavors compared to those prepared with free flavors. Sugar syrup containing yeast-encapsulated berry flavor was also used as a coating for corn flake cereals. The sensory analysis showed that the flakes covered with the yeast-encapsulated flavor had significantly superior berry aroma intensity in comparison to those with free berry flavor. Similarly, in the same patent, the preparation of batter-containing yeast-encapsulated garlic flavor is described, which was used to coat "French fries." Sensory analysis revealed that the perceived flavor was significantly superior for the yeast encapsulation form.

Yeast flavor technology has been brought to the market by Mincap PLC (Wigan, UK) and by Firmenich SA, a Swiss-based flavor and fragrance company. Mincap PLC specializes in using yeast cells as capsules for application in drug delivery, insecticides, herbicides, cosmetics, textiles, and food ingredients, including flavorings. Moreover, the trademark Thermarome® owned by Firmenich SA refers to a carrier that involves flavors encapsulated in yeast for use in the manufacture of foodstuffs, beverages, and pharmaceuticals.

23.7 CONCLUSION

Yeast cells are a cost-effective microencapsulation technology system. The encapsulation process is simple and can be accomplished with no additives, whereas it results in high loading capacity (most commonly 30–40% w/w). Yeast encapsulation can provide a long-lasting flavor release, which occurs when the capsule contacts mucus membrane surfaces (tongue, nose) without breaking the cells and, thus, can offer controlled delivery in the mouth. The double protection provided by the polysaccharide thick cell wall (which prevents cell rupture and provides mechanical strength) and the lipid membrane allows the stabilization of the active compounds and reduces the loss of volatile compounds from evaporation and from light or oxygen damage. In addition, the yeast capsules comprise a thermostable system which protects the contents from heat damage caused during food processing or cooking (baking, boiling, roasting, frying, extrusion, spray drying). Therefore, yeast-encapsulated active ingredients can provide a highly stable product with extended shelf-life.

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Chapter 24

Pollen and Spore Shells—Nature's Microcapsules

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24.1 INTRODUCTION

Pollen and plant spore walls have evolved to be highly efficient and robust microcapsules able to protect their delicate payloads from the rigors of the natural environment, which includes oxygen, sunlight, and in some cases compression (Brooks and Shaw, 1968). Of particular note is the outer shell wall polymer, known as sporopollenin (Brooks and Shaw, 1972). It is composed of carbon, hydrogen, and oxygen and its stability is illustrated by its being found intact in sedimentary rock some 500 million years old. The fundamental principle behind this new type of microencapsulation is to harness the unique properties of the pollen and spore walls for protection and delivery of a wide range of actives, with particular application to food additives. No other single naturally occurring polymer used in microencapsulation has such an extensive armory of protective properties. For the purpose of this review, plant spores will be synonymous with pollen unless a specific spore is being referred to. Pollen is found in a variety of foods as contaminants, with honey being the most obvious example (Linskens and Jorde, 1997). Bee pollen is consumed in many parts of the world as a herbal remedy and stimulant; also, spores such as *Chlorella vulgaris* are eaten in large quantities, particularly in Asia. The main uses of this type of microencapsulation in food applications are: taste masking of unpleasant additives; prolonging shelf-life as an antioxidant and light shield; protection of volatiles and flavors for release in the mouth; and the enhanced delivery of functional ingredients such as omega-3 and vitamins in the gut.

24.2 CONCEPT BEHIND USING POLLEN SHELLS FOR MICROENCAPSULATION

Evolution in respect of gene transfer in plant reproduction has produced a most remarkable microcapsule in the form of a pollen grain (Heslop-Harrison, 1971). Parallels between the properties of pollen grains and desirable design features of commercial microcapsules include the following:

- Protection: Pollen walls have evolved to be highly protective for the active components by possessing physical strength, elasticity, shielding capability, and antioxidant activity. A key aspect of microencapsulation is to protect an active against physical and chemical damage until it is required to be delivered.
- High loading: For efficient microencapsulation it is important to minimize the amount of materials used to encapsulate an active and maximize the amount carried within the microcapsule. The pollen wall constitutes about 20–25% of the mass of the grain and has evolved to be easily transported by water, wind, or insect; hence, it is advantageous for pollen particles not to carry excess mass that is not useful for the function of pollination. Figure 24.1 shows a microtome cross-section of a *Lycopodium clavatum* spore shell that was first emptied and then filled with an acrylic resin monomer that was heat polymerized. Thus, it can be seen that the pollen wall is relatively thin ($\approx 2\ \mu m$) compared to the diameter of the resin-filled internal cavity ($\approx 26\ \mu m$).
- Ease of dispersion: Pollen particles are monodispersed to aid their transport by insect wind or water and can be an important factor to aid mixing and dispersion in fluid environments such as food and beverages.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00024-8

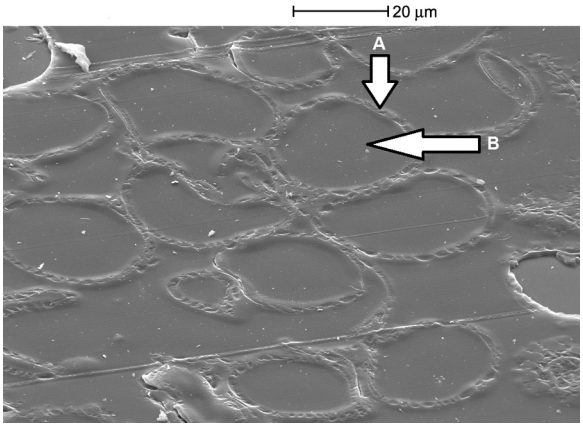

FIGURE 24.1 Scanning electron microscopy (SEM) micrograph of *Lycopodium clavatum* spore exine shells, first emptied, then acrylic polymer filled, and finally microtome sectioned. ("A" points to exine shell wall; "B" points to acrylic polymer filling.)

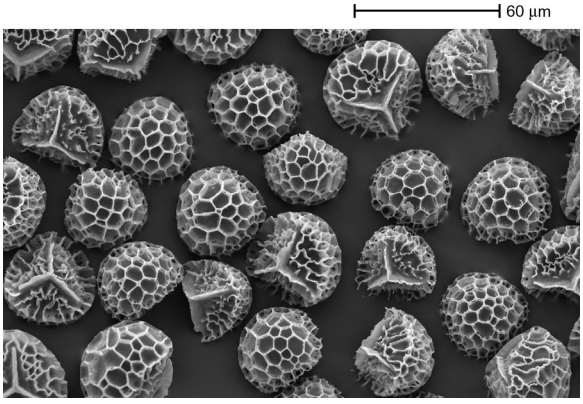

FIGURE 24.2 SEM micrograph of *L. clavatum* spores showing homogeneity of size and morphology.

- Size and morphological uniformity: Pollen particles from any one plant species are incredibly uniform in morphology and size as illustrated for the spores of *L. clavatum* in Figure 24.2. Such features, for example, as texture consistency of foods, are important. The outer surface of pollen particles is normally highly sculptured and particularly characteristic of the plant species; note, for example, the difference between the exine shells of *L. clavatum* and *Ambrosia trifida* (Figures 24.3a and b, respectively). These features have proved to be of enormous importance in forensic science, archeology, and palynology and could be important in food traceability (Holt et al., 2011). The protruding surface features on the exine shells, as shown for example in Figures 24.3a and b, are possibly important in aiding flight in wind or attachment to insects and could also aid attachment to biological surfaces such as mucoadsorption for delivery of an active.
- Ease of loading and release: The pollen shell wall (Figure 24.4) has a many very narrow channels ($\approx$20 nm) that penetrate it, which are important for transport across the walls of the natural pollen grain (Pettitt, 1976; Rowley et al., 2003). Such channels have been shown to allow a variety of substances to be loaded into and released from extracted shells (Barrier et al., 2011).

24.3 STRUCTURAL AND CHEMICAL FEATURES OF POLLEN SHELLS USEFUL FOR MICROCAPSULE FORMATION

24.3.1 Structural Features

Pollen and spore grains are the mobile reproductive particles of plants and contain the male gametophyte for plant reproduction. In most cases, the term "spore" is reserved for cryptogams (e.g., algae, mosses, and fungi), while "pollen"

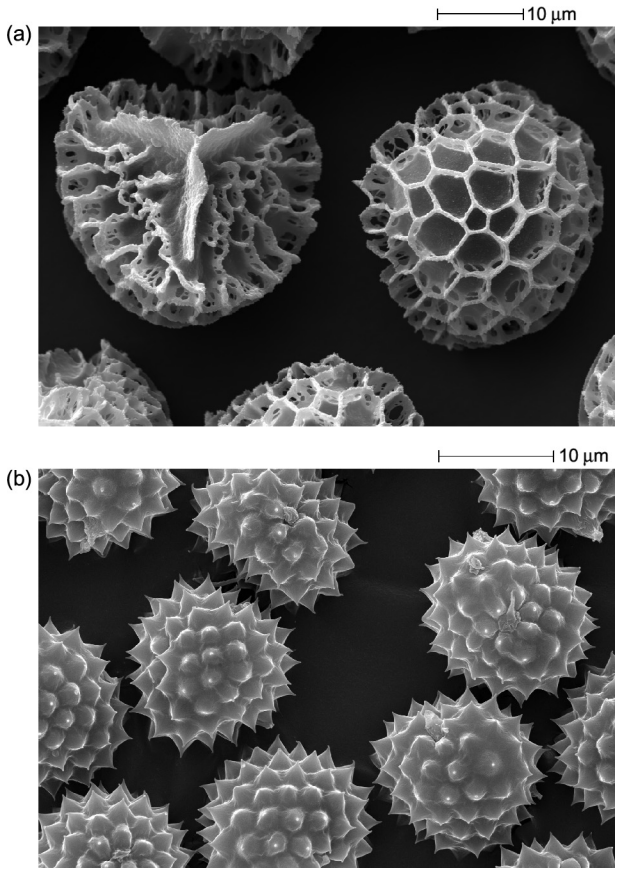

FIGURE 24.3 SEM micrographs of exine shells of (a) *L. clavatum* and (b) *Ambrosia trifida*, showing differences in the characteristic architectures of the two species. (NB in (a), the left-hand exine shows the "trilite" scar feature on one face while the right-hand spores reveal the rounded face.)

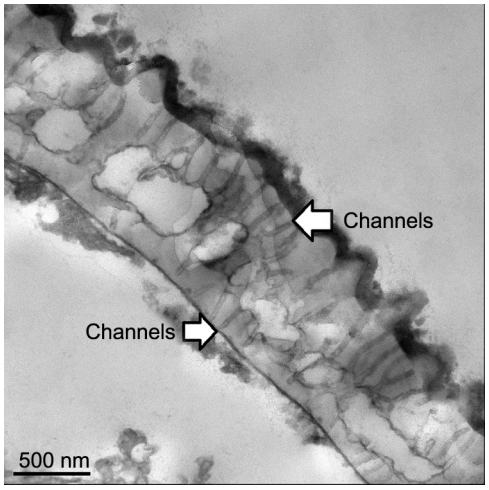

FIGURE 24.4 Transmission electron microscopy (TEM) micrographs of exine shell of *Secale cereale* showing nano-diameter ($\approx$ 20 nm) sized channels penetrating the shell wall.

is reserved for spermatophytes (e.g., flowering plants) (Moore et al., 1991). For the purpose of this review, pollen will be used as a general term to include spores unless a specific type of spore is being described. Their role is to disperse the haploid nuclei (i.e., containing only one set of chromosomes), constituting the fragile genetic information that is fundamental for reproduction. The fact that pollen constitutes a moveable stage of the sexual cycle of the organism has

led to specialized mechanisms for dispersal and to a specific morphology. The grains are between 5 (e.g., myosotis) and 250 μm (pumpkin) but of the same size and morphology from any one species of plant. They are protected by a remarkably complex and robust wall with two principal layers known as the "intine" and "exine." The intine is the innermost of the major layers, underlying the exine and bordering the surface of the cytoplasm (Punt et al., 1999, 2007). It is mainly composed of cellulose and a few other polysaccharides. The intine is divided into two sublayers: endintine is the inner thicker layer adjacent to the cytoplasm and of cellulosic character; exintine, the outer, thinner layer, stains positively for pectin.

A key and distinguishing feature of pollen grains is the exine since it consists largely of the unique polymer in Nature known as sporopollenin. The exine is subdivided into an outer, sculptured zone, sexine, which lies above an inner, non-sculptured part, nexine. Sporopollenin is one of the most resistant natural organic materials known and has been described as "one of the most extraordinarily resistant materials known in the organic world" and in the plural sense "sporopollenins are probably the most resistant organic materials of direct biological origin found in nature and in geological samples" as intact microcapsules found in ancient sedimentary rocks (Feagri and Iverson, 1964; Brooks and Shaw, 1978a). In contrast to the intine, the sporopollenin exine is acetolysis resistant. Such stability is important in the preparation of microcapsules for food applications since all allergenic proteins, which may be present in the bulk pollen, can be removed readily and cheaply by harsh treatment with strong acid and base.

24.3.2 Chemical Features

Sporopollenin, the fabric of the exine, has been defined based on the chemical characteristic of being "the resistant non-soluble material left after acetolysis" (Heslop-Harrison, 1971). The exact chemical structure is still unknown but much is known about the functional groups and elemental components (carbon:hydrogen ratio $\approx$5:8 mol:mol) (Zetzsche et al., 1937). The chemical structure of sporopollenin may be summarized as follows to distinguish it from other naturally occurring polymers. It is a mixed copolymer (van Bergen et al., 2004; De Leeuw et al., 2006) with an aliphatic core. Aromatic groups are also involved by extending from the core as side chains and also possibly act as bridging units within the core. Crosslinking functional groups are likely to be carbon−carbon, either aromatic or aliphatic and very probably sterically hindered ethers, which all together might explain the resilience of sporopollenin to chemicals. The large presence of aliphatic character along with olefinic and aromatic groups contributes to the lipophilic character but the involvement of different types of hydroxyl groups (carboxylic acids, phenols, and alcohols) imparts some amphiphilicity, thus enriching sporopollenin with a multiplex of properties such that the sporopollenin exines can act as microcapsules capable of loading a range of materials with different polarities (Barrier et al., 2011; Diego-Taboada et al., 2012) and as particle surfactants (Binks et al., 2011). A more detailed review of the structure of sporopollenin is presented in the appendix.

24.4 EXTRACTION OF POLLEN SHELLS

Depending on the choice of reagents, double (exine + intine) or single (exine) layered microcapsules can be obtained cleanly due to their being the most stable components of pollen grains.

24.4.1 Single Layered Shells

24.4.1.1 Chemical Methods

Early studies to obtain sporopollenin exines were aimed at simply isolating the polymer for study elucidation and not for any particular application of the material. The inertness of sporopollenin and its resistance to chemical, physical, and biological reagents was advantageous for isolating it free of other natural materials, including lipids, polysaccharides, proteins, and nucleic acids and still be morphologically intact as a microcapsule. Zetzsche et al. (Zetzsche and Huggler, 1928; Zetzsche and Kälin, 1931; Zetzsche and Vicari, 1931; Zetzsche et al., 1937) reported the first method that involved a logical sequential treatment with organic solvents to remove lipids, alkali to remove nitrogenous material, and aqueous acid to remove polysaccharides, yielding $\approx$20−25% by mass of empty exine shells from the parent *L. clavatum* spores. Several workers (Shaw and Yeadon, 1964, 1966; Brooks and Shaw, 1971; Shaw, 1971; Green, 1973; Guilford et al., 1988; Shaw and Apperley, 1996; Kettley, 2001; Boasman, 2003) followed up with modified versions of Zetzsche's extraction protocol using a range of acids and bases. More recently, similar extraction conditions have been used to obtain sporopollenin exine capsules from *L. clavatum* spores (Atkin et al., 2005) for taste masking

(Barrier et al., 2010b), drug delivery (Wakil et al., 2010), and sequestration of oils (Diego-Taboada et al., 2012) from aqueous media. Strong mineral acids and alkalis are non-toxic and therefore useful for obtaining exines for food and pharmaceutical applications. A one-pot method has been reported that also uses a non-toxic agent involving treated pollen with 6M hydrochloric acid at 110°C for 24 h (Amer and Tawashi, 1991, 1994; Tawashi, 1997). Hydrochloric acid hydrolysis has the advantage of being a one-step process and is suitable for extraction of exines from a variety of spores and pollens including *Zea mays* (maize), *Secale cereale* (cereal rye), *L. clavatum* (club moss), *Pinus* spp. (pine), *A. trifida*, and *A. artemisofila*. Another universal method applicable to a wide variety of pollen and spores has been extensively used to extract sporopollenin, particularly for palynological sample preparation (Hesse and Waha, 1989), developed by Erdtman (1960). It also involves a one-pot process, which involves acetolysis using acetic anhydride and sulfuric acid. Other chemical methods reported include using anhydrous hydrofluoric acid (Domínguez, et al., 1998) in pyridine at 40°C and aqueous 4-methylmorpholine-*N*-oxide and sucrose under alkaline conditions, heated at 70 or 20°C for 1 h.

24.4.1.2 Enzymic Methods

Several workers (Schulze Osthoff and Wiermann, 1987; Herminghaus et al., 1988; Gubatz et al., 1993; Jungfermann et al., 1997; Ahlers et al., 1999) have employed enzymatic digestion to isolate sporopollenin. For instance, removal of intine without the use of a strong acid was made possible with cellulase. Using a sequence of enzymes (protease, lipase, amylase, pectinase, cellulases, and hemicellulose) used in gastric digestion followed by exhaustive washing with hot methanol gave exines of *Corylus avellana* L. (hazelnut) (Gubatz et al., 1986; Herminghaus et al., 1988) and *Pinus mugo* Turra (mountain pine) (Schulze Osthoff and Wiermann, 1987) pollens. The enzyme sequence was composed of protease, lipase, amylase, pectinase, cellulases, and hemicellulase.

24.4.2 Double-Layered Shells

The double-layered microcapsules can be obtained in a one-pot process of hydroxides of either sodium or potassium (Diego-Taboada et al., 2012), thus avoiding the need for strong acid for removal of the cellulosic intine. Several reporters have obtained the double-layered microcapsules but only as an intermediate to obtain the single-layered sporopollenin microcapsule. A recent report describes its isolation from *L. clavatum* spores by a modified method of Zetzsche and Kälin (1931) and its characterization by a solid-state NMR, FTIR (Fourier transform infrared spectroscopy), and combustion elemental analysis (Diego-Taboada et al., 2012). A one-pot preparation can also be achieved by refluxing *L. clavatum* spores in (10% w/v) for 6 hours in hydroxides of either sodium or potassium. If the concentration of alkali is reduced, longer reflux times are recommended.

24.4.3 Quality Control and Analysis of Extracted Shells

24.4.3.1 Routine Quality Control of Extracted Shells

The quality of the extracted microcapsule is relatively easy to assess by physical and chemical means. First, the capsules are examined routinely by light microscopy to ascertain that the microcapsules are discrete and free of debris. Also, scanning electron microscopy (SEM) is used to look for any cracking of the shells. However, it has been found that there is incredible consistency of exine quality from batch to batch from any one plant species; hence, for routine purposes, light microscopy is often sufficient. Combustion elemental analysis for carbon, hydrogen, and nitrogen is important for determining either the absence of nitrogen, as occurs in most cases, or, in some milder extractions, the level of traces of nitrogen. The ratio of carbon and hydrogen gives an indication of batch consistency. Further support for batch consistency is achieved using FTIR, which provides a characteristic fingerprint spectrum of the polymer.

24.4.3.2 Non-Toxicity and Non-Allergenicity of Extracted Shells

It has been long reported in the literature (Barrier et al., 2010a, 2011; Diego-Taboada et al., 2012) that shells extracted from *L. clavatum* spores have zero %N by combustion elemental analysis, which is indicative of the absence of allergenic proteins. However, it has been recommended by the UK's Food Standards Agency's Advisory Committee on Novel Foods and Processes that more sensitive and better defining analytical techniques are required since, for example, calculating % protein from %N can be misleading due to the presence of non-protein nitrogenous products such as chitin. Particularly, recommended methods include mass spectrometry (ESI-QqTOF-MS, CID-MS/MS, and MALDI-TOF-MS)

and electrophoresis (SDS-PAGE). Recent studies using these techniques on exhaustive extractions of *L. clavatum* shells failed to detect the presence of any protein (Diego-Taboada et al., 2013). Human patch tests on sporopollenin extracted from *L. clavatum* and *Chlorella vulgaris* showed there to be no adverse effects upon human skin (Maack, 2003).

Toxicity testing of shells on growing human endothelial cells is a well-established preliminary assessment of toxicity (Ekwall et al., 1990), and therefore exines were exposed to two types of cells: (1) human umbilical vein endothelial cells (HUVEC), which are endothelial cells extracted from the vein of an umbilical cord; and (2) the immortalized endothelial cell line EAhy 926. Four different assays were used: (1) WST-1 proliferation assay; (2) cell death ELISA for apoptosis; (3) LDH assay for necrosis; and (4) microscopic examination of the cells to look for cell damage and apoptotic bodies. None of the tests showed any reduction in the proliferation or evidence of cell death in the human endothelial cells *in vitro* at concentrations between 100 ng and 10 μg. Furthermore, no cell damage was observed by SEM within this concentration range. It is of note that rats fed with sporopollenin from *Chlorella vulgaris* showed no signs of toxicity when fed with a single 2000 mg oral dose (Maack, 2003). Also, there is evidence to show that exine shells are unchanged when they pass through the GIT in rodents and humans (Sobolik, 1988).

24.5 MODIFICATIONS TO POLLEN SHELLS

Shells extracted by enzymes (Section 24.4.1.2) or hot alkali are light brown in colour (Atkin et al., 2009) and acid extracted exine shells are a slightly darker brown. However, both types of shells can be lightened considerably in color by bleaching with sodium hypochlorite or sodium chlorate. Hot alkali extracted shells have been lightened to show an L* value of 91.3 and hot acid extracted shells have been lightened to show an L* value of 91.2. The lightened shells can be readily colored with food dyes to obtain a wide color choice of exine. In the food industry this can be important when adding the microencapsulated supplement as a powder to the final product. Whitening of exine shells can also increase their hydrophilicity, presumably due to an increase in the hydroxyl groups on the surface of the sporopollenin capsules. Sporopollenin exines, whether bleached or not, possess carboxylic and phenolic groups on their surfaces, which can be present either in their protonated forms, following acidification, or their anionic forms as salts following alkali treatment, thus allowing some control of amphiphilicity. This was demonstrated when it was found that the exines became increasingly negatively charged with an increase in pH and adsorbed to both air−water and oil−water interfaces, stabilizing liquid marbles and emulsions, respectively (Binks et al., 2011). Also "water marbles" were formed at all pH values between 2 and 10 and for salt concentrations up to 1 M NaCl: transitional phase inversion of emulsions was found to occur from water-in-oil to oil-in-water with increasing pH. The increased hydrophobicity of exines in their protonated form has been demonstrated by their ability to sequester food oils from oil-in-water emulsions with high yields ($\approx$90%) of oil recovery in a one-step process (Diego-Taboada et al., 2012). Furthermore, almost quantitative yields of recovery of oil from oil-in-water emulsions could be obtained by forming acetyl esters or methyl ethers of the appropriate hydroxyl groups on the exines.

24.6 LOADING OF ACTIVES

Loading of actives into shells relies on the porosity of the shell walls due to the channels shown in Figure 24.4; hence, filling shells requires the active to be in a liquid form either as a melt or solution in a suitable solvent that can be removed easily by evaporation (Barrier et al., 2011). Alternatively, sparingly soluble materials can be encapsulated by first encapsulating solutions of reactants, which then combine within the exine shells to precipitate the desired active within the shell. As such the shells are acting as micro-reaction vessels (Paunov et al., 2007). For small-scale to pilot plant operations, loading of the microcapsules has been achieved by simply mixing and then imposing a force such as a vacuum or centrifugation if required. Vacuum is frequently used for aqueous solutions in combination with freeze drying, and thus vacuum-aided filling and solvent removal can be achieved concomitantly. Shells as powder or compressed as tablets can be used filled with active (Atkin et al., 2005, 2010; Beckett et al., 2009; Paunov et al., 2007). The shells have good elasticity, and hence after compression they can return to their microcapsule form. Scaling up to plant-sized loading could possibly be achieved by vacuum tumbling, which could offer advantages over spray drying due to the simplicity of procedures and equipment. The shells of *L. clavatum* (club moss), in particular, along with those obtained from *Zea mays* (maize), *Secale cereale* (cereal rye), pine, *A. trifida*, and *A. artemisofila* can be loaded with polar and non-polar materials over a wide range of molecular masses since the channels penetrating the exine wall are $\approx$20 nm in diameter. Most studies have been undertaken using exine shells obtained from *L. clavatum* spores. The types of techniques used to fill shells are outlined as follows.

24.6.1 Passive Encapsulation

The channels passing through the exine shells readily allow low viscosity lipophilic materials to enter the inner cavity by capillary action. This technique involves simply mixing the active with the empty exines as a powder and allowing the active to be absorbed (Barrier et al., 2011). However, an essential feature of this type of encapsulation is to ensure homogeneity of mixing using a Thermomix TM31 blender; the best results are with fluids with low viscosity. For example, Histoclear® and limonene can be seen by light microscopy to speedily enter the inner cavity of the exines. Air bubbles in the exines, seen as dark circles, can be seen to escape the capsule giving a much lighter translucence to the microcapsule. Lipophilic materials such as cod liver oil and molten cocoa butter have also been encapsulated by this method with loadings reaching a favorable 3 $g.g^{-1}$ active:exine. At 1 $g.g^{-1}$ cod liver oil:exine, the shells provide excellent taste masking for the oil (Barrier et al., 2010b).

24.6.2 Vacuum-Aided Encapsulation

This technique is similar to the passive method but is aided by a vacuum of $\approx$25 hPa and is helpful for encapsulation of more viscous substances such as syrups and waxes (Barrier et al., 2011). The technique has been used to encapsulate an acrylic monomer (LR White), which was heat polymerized (80°C). Confirmation of complete filling of the exine's chamber was undertaken by SEM of microtome sections of the filled exines as exemplified in Figure 24.1. Oils such as sunflower oil, soybean oil, rapeseed oil, echium oil, and molten waxes such as carnauba wax, cocoa butter, and beeswax are encapsulated readily by this method with a loading of up to 3 $g.g^{-1}$ active:exine. Other materials encapsulated by this method have included polar materials such as dyes (malachite green, Evan's blue, and Nile red) and enzymes (amylase, galactosidase, and sHRP) at a loading of $\approx$0.2 $g.g^{-1}$ active:exine. The dyes were shown to be encapsulated in the inner chamber by confocal laser scanning microscopy (CLSM). Importantly, enzymes extracted from the exines still retained most of their catalytic activity. A limitation to loading levels in a single-step operation is the concentration of the solution of the active, since the solution has to be drawn through the porous shell walls into the inner cavity. For aqueous solutions of active, vacuum filling and solvent removal are achieved by a one-step lyophilization. Where high loadings are required, a series of filling procedures can be used to accumulate the loading within the exine shell.

24.6.3 Centrifugal-Aided Encapsulation

This technique follows the aforementioned techniques in the mixing but the mixture is subjected to centrifugation (10,000 $\times$ g). In this way, alkaline phosphatase in glycerol has been encapsulated at 4°C with a loading of $\approx$0.2 $g.g^{-1}$ active:exine (Barrier et al., 2011).

24.6.4 Compression-Aided Encapsulation

As an alternative to using empty exines as a loose powder, it is possible to compress them to form a pellet, which can be added to a fluid of the active either as a solution or as a melt (Atkin et al., 2005; Paunov et al., 2007). The pellets can be $\approx$1 g and compressed ($\approx$5 tonnes.cm^{-3}) to give cylindrical forms 0.5 cm deep by 1 cm diameter. Since the shells are particularly elastic, they absorb the liquid over a few minutes and return to almost the same morphology as observed prior to compression.

24.6.5 Encapsulation of Sparingly Soluble Substances

Since the sporopollenin exines are stable to heating to at least 250°C, substances such as molten waxes can be loaded using a suitable choice from the aforementioned techniques (Barrier et al., 2011), thereby avoiding solvents. An alternative strategy involves precipitation of the insoluble active within the shells by mixing two reagents within the shell to produce an insoluble product. For example, the sparingly soluble inorganic salt calcium hydrogen phosphate, which can find application in fortified foods, has been encapsulated by filling the shells, sequentially, with aqueous solutions of $CaCl_2$ and Na_2HPO_4 aided by small amounts of ethanol to obtain a precipitate of the calcium hydrogen phosphate within the shell (Paunov et al., 2007). In this manner, the exine microcapsule is being used as a micro-reactor.

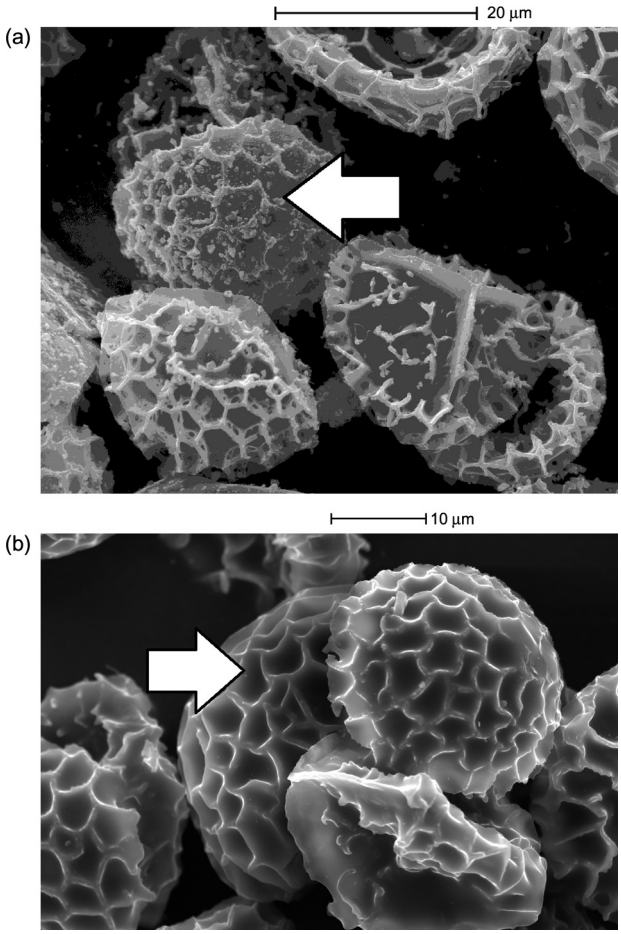

FIGURE 24.5 (a) and (b) SEM micrographs of exine shell of *L. clavatum.* Arrows are pointing to crystalline sodium chloride and cocoa butter deposits, respectively, on the surface of the exines indicating incomplete encapsulation. (NB coatings such as waxes and triglycerides on the surface of the exines have a similar surface appearance of filling the decorative features: compare with empty exines shown in Figure 24.3a).

24.7 QUALITY CONTROL OF LOADED SHELLS

Efficient microencapsulation is readily observed by a clean exine surface and surrounding the capsules as viewed by SEM. The surfaces of the exine shells are highly characteristic for a particular species of plant and are often highly decorated (e.g., Figure 24.3a and b). Therefore, comparing SEM images before and after encapsulation is a reliable means to indicate that the entire active is below the surface of the shell, either in the cavity of the shell or absorbed within its wall; for example, Figure 24.5a shows crystalline deposits of sodium chloride on the surface of *L. clavatum* exine shells and Figure 24.5b shows an overloading and hence coating of cocoa butter on the surface of the exines. The exines are highly insoluble in most inorganic and organic solvents; hence, recovery by solvent extraction is a convenient method to assess loading efficiency. Solid-state NMR has been used to assay the relative amounts of materials encapsulated within the exines, such as water and oils. The non-quantitative presence of the active has been detected by FTIR and CLSM; for example, Figure 24.6 depicts a CLSM image of a pollen shell of *Secale cereale* filled with fish oil showing almost total containment of the oil.

24.8 RELEASE OF ACTIVES

The porosity of the exine microcapsules, due to submicron diameter ($\approx$20 nm) channels, permits materials to be released from the microcapsules. However, some control of the rate of release can be achieved. The types of diffusion are outlined below.

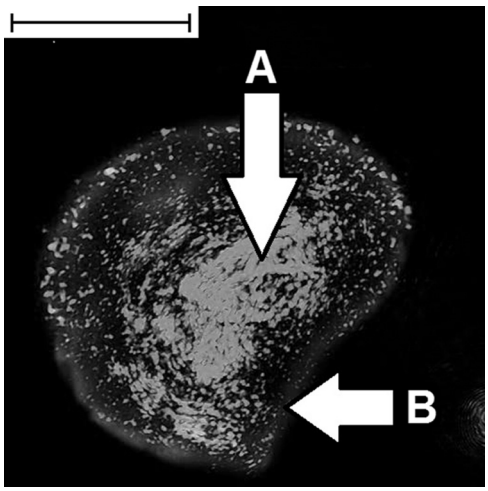

FIGURE 24.6 Confocal laser scanning microscopy (CLSM) image of exine shell of *Secale cereale* loaded with fish oil: the oil (pointed to by "A") appears to be well contained within the exine wall (pointed to by "B").

24.8.1 Release by Passive Diffusion

Passive release of an active can be triggered by factors in the environment surrounding the loaded shells depending on the nature of the active or protective additive used. For example, vigorous stirring of a loaded shell in a solvent that will dissolve the active can be used to extract the active. Vitamin D is crystalline inside the exines as determined by powder X-ray diffraction (XRD) but can be recovered from the exine by stirring in ethanol: the ethanol passes through the shell wall and dissolves the vitamin, which can then be removed by washing or pressing. Other materials such as encapsulated beeswax or cocoa butter can be removed by warming in a suitable lipophilic solvent or pressing. However, control of the rate of release can be affected by the choice of solvent relevant to the solubility of the active encapsulated. Changes in pH can also trigger release of an active. For example, a solid-state NMR study showed that when vitamin D is encapsulated in the exines incubated with lipids at pH 7.4, it partitions into a surrounding lipid bilayer environment, but not in acidic conditions at pH 1.5. This has implications in that absorption can occur post-gastric transit, which is in keeping with recent *in vivo* studies (Wakil et al., 2010). It was also shown that the magnetic resonance imaging (MRI) agent Omniscan™ was released much more rapidly in blood than in buffer of a similar pH.

24.8.2 Compression-Aided Release

Raw pollen particles have been shown to possess elastic character and it is interesting to observe that such characteristics are present in the extracted shells (Rowley and Skvarla, 2000; Liu and Zhang, 2004). As noted in Section 24.6.4 compressed tablets of shells can absorb an active and thereby load them into the shells. The reverse is also possible for liquids such as oils, which have been shown to release by repeated rubbing; e.g., between 70 and 80% of fish oil can be released by rubbing between a finger and a paper surface within 1−2 minutes (Atkin et al., 2007; Diego-Taboada et al., 2012). Using this mechanism, control of release can be effected in accordance with the amount of pressure and friction exerted upon the shells. This type of pressure takes place during chewing; hence, this type of release can be used for flavor enhancement. Additional control can be effected by coencapsulating (Section 24.8.3) materials with different viscosities.

24.8.3 Control of Release Using Coencapsulation and Coating

As indicated in Section 24.5 polarity of the exine can be adjusted in accordance with minor modifications to the functional groups on the sporopollenin exine, which in turn can influence release characteristics. However, controlled release can be assisted by the use of a protective excipient (additional substance), which can be on either (or both) the surface of the exine as a coating or encapsulated with the active ("coencapsulant") in the interior of the exine microcapsule (Atkin et al., 2008). Coencapsulation can be achieved either by coencapsulating a mixture of an active with a protective at the same time or sequentially loading with an active and then an excipient to form an inner protective layer

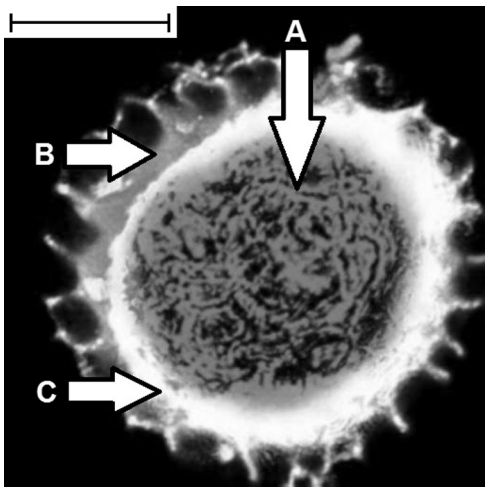

FIGURE 24.7 CLSM image of exine shell of *L. clavatum* loaded with Malachite green as the active (pointed to by "A"): the exine wall is pointed to by "B" and the coencapsulated protective of cocoa butter is pointed to by "C."

between the active and the exine shell as shown in Figure 24.7. However, it should be noted that coencapsulation also includes filling the narrow channels penetrating the exine shell wall itself. Coencapsulation of excipients has potential advantages over coatings on the outside of the exines as (1) complete outer coating of the shells can be difficult to achieve and (2) coatings are more exposed and therefore more easily damaged or removed. Coencapsulated excipients as protective additives such as those commonly used in the pharmaceutical industry have proved successful *in vitro*. For example, Eudragit®, a poly(meth)acrylate, has been shown to trigger release of a protein very efficiently with pH change such that >90% protein is retained in simulated gastric fluid (SGF at pH 1.5) for up to 1 h but released in phosphate buffer (PBS at 7.4) after 5 min. However, excipients of more interest to the food industry include fats and oils (esters or acids), waxes, and saccharides and their polymers. Useful retention of proteins in exines has been demonstrated *in vitro* over 45 minutes under simulated gastric fluid conditions (SGF) using a variety of coencapsulated protectives with specific food-related examples including gum arabic (80% protein retained), cod liver oil/1% lecithin (60% protein retained), gelatin (80% protein retained), starch (55%), and cocoa butter (60% protein retained).

24.9 APPLICATIONS OF POLLEN SHELLS FOR MICROENCAPSULATION RELEVANT TO THE FOOD INDUSTRY

24.9.1 Taste Masking of Actives

Some food additives which are considered to have a health benefit, have unpalatable tastes to humans such as the B vitamins and fish oil. While administering these types of actives in gelatin capsules can avoid an unpleasant initial taste and protect against oxidation they cannot prevent a repeat taste due to eructation, known as "burp-back." Therefore, microencapsulation of fish oil has been explored (Gibbs et al., 1999; Gharsallaoui et al., 2007) as a means to avoid the taste, protect against oxidation, provide the active as a powder, and attempt to deliver the oil into the small intestine to minimize burp-back. Sporopollenin exines extracted from the spores of *L. clavatum* have been shown to taste mask effectively in a double blind human taste trial involving 20 volunteers comparing the microcapsules filled with water, sunflower oil (0.5 g/g), and differing amounts of cod liver oil (cod liver oil per gram of sporopollenin exines: 0.5 g/g, 1.0 g/g, 2.0 g/g, 4.0 g/g) (Barrier et al., 2010b). The encapsulated oils were in the form of a flowing powder up to an oil loading of 1/1 (w/w). The tasters were not able to distinguish the cod liver oil preparation up to 1/1 (w/w) loading compared to the sporopollenin exines filled with either water or sunflower oil. Above these levels, the cod liver oil was uniformly identified.

24.9.2 Prolonging shelf-Life: Antioxidant, Light Shielding of Actives

An important feature of microencapsulation is to protect and thereby prolong the shelf-life of an active. Sporopollenin, the fabric of the exines, has evolved to protect the internal cytoplasmic materials of the pollen grain or spore against sunlight and air in transit by wind, water, or insect to the site of pollination. Also, it is of note that pollen grains can

store their cytoplasmic materials over long periods, thus demonstrating the levels of protection offered by pollen particle walls. Light absorbing properties of sporopollenin were observed by Rozema et al. (2001) who quoted values of the relative UV-B absorbance (integrated over the wavelength range 280−320 nm) of suspensions of sporopollenin in glycerol. In addition, the same authors stated that, "the pollen wall is effective in screening out more than 80% of the incident UV radiation." Another study (Atkin et al., 2011) describes sporopollenin comprising exine shells having approximately a flat spectrum over the wavelength range 190−900 nm with an absolute value of the absorption coefficient of approximately 0.02 μm^{-1} for the exine shells of *L. clavatum* and *A. trifida*. It was found that the optical transmission of a single exine shell transmits approximately 50% of light at 450 nm and varies only slightly with particle type but with little sensitivity to wavelength. Therefore, the shielding capabilities of sporopollenin exines coupled with the ease by which they can be loaded and enhancing bioavailability (Section 24.9.3) of the lipophilic active make them an attractive option for microencapsulating omega oils (Atkin et al., 2010). Exines extracted from spores and pollens of different sizes and species of *L. clavatum* (25 μm diameter), *Secale cereale* (45 μm diameter), and *Zea mays* (80 μm diameter) have all been shown to extend the shelf-life of omega oils; however, the larger exines have the attraction of potentially being capable of carrying larger loadings. *L. clavatum* spores and *Secale cereale* pollen are accessible in bulk quantities and have been shown (unpublished) to improve the oxidative stability and hence extend the shelf-life of commercial cod liver oil (Seven Seas) over 2 months with consistently lower peroxide values (PV) than the control (Atkin et al., 2010; Thomasson et al., 2010). It was found that, after low energy UV irradiation for 2 h, the PV of fish oil that had been encapsulated within sporopollenin exines was significantly lower than that of fish oil that had been irradiated without sporopollenin protection. Interestingly, the protection was still in evidence up to ratios of 1:6 (exines: oil; w/w), even though the oil had not been fully encapsulated. Furthermore, when only small amounts (2, 1, and 0.2% [w/w]) of the sporopollenin exines were added to commercial cod liver oil and exposed to air and daylight for up to 11 weeks, a significant improvement in stability was observed in comparison with controls. These findings were therefore indicative of the sporopollenin possessing antioxidant properties as an additional means to protect the oil, as well as light screening. In support of this phenomenon, a study by Thomasson et al. (2010) showed that the oxidative electrochemistry of sporopollenin from *L. clavatum* and *A. trifida* possesses redox activity that likely stems from a mixture of conjugated phenol functionalities within the sporopollenin. Such are known to be present in the polymer; for example, coumaric and ferulic acids (Wiermann and Gubatz, 1992) are known to possess antioxidant properties (Arrieta-Baez et al., 2012). In conclusion, sporopollenin exines have a built-in shielding and antioxidant capacity to protect and prolong shelf-life of actives, with oils in particular, and thus hold potential advantages over conventional materials used in microencapsulation.

24.9.3 Enhancing Bioavailability of an Active

An important benefit of using sporopollenin exines is the potential to improve the bioavailability of actives (Beckett et al., 2009; Wakil et al., 2010). In a recent study with healthy human volunteers, it has been shown that the bioavailability of eicosapentaenoic acid (EPA) can be enhanced significantly in the bloodstream by ingesting EPA as its ethyl ester encapsulated into *L. clavatum* exines. In the study, the volunteers ingested 4.6 g of fish oil containing 20% of the EPA ester, first alone and then as 1:1 w:w microencapsulated powder of exines and fish oil. The serum bioavailability of EPA was measured by area under curve (AUC 0−24 hours). The mean AUC 0−24 of EPA from ethyl ester with exine ($M = 19.7$, $SD = 4.3$) was significantly higher by a 10-fold order than for ethyl ester without exine encapsulation ($M = 2$, $SD = 1.4$, $p < 0.01$). Other studies (unpublished) involving human volunteers ingesting structurally different lipophilic actives have shown similar multi-fold levels of enhanced bioavailability. Very preliminary *in vitro* work has shown that exines have gut mucosal adhering properties and *in vivo* studies using rodents have shown exines to be located between villi in sections taken from intestinal walls. Such preliminary data are in support of a putative mucosal adhesion mechanism. In conclusion, sporopollenin exines with their highly decorative surfaces, chemical resilience, and elasticity offer a difference to more conventional means of microencapsulation, which might contribute to the phenomenon of enhancing bioavailability of actives.

24.10 PERCEIVED ADVANTAGES OF POLLEN SHELLS FOR MICROENCAPSULATION

In summary, microcapsules extracted from pollen and plant spores have the following possible advantages over more conventional forms of microencapsulation:

- Natural and renewable microcapsules from plant spores and pollen;
- Uniform-sized microcapsules from one plant source that are smooth to the touch and taste;

- Microcapsules that are extremely resilient both physically and chemically;
- Microcapsules monodispersed and available in multi-tonne quantities for some sizes;
- Microcapsules extracted using non-toxic agents and standard food processing equipment and microcapsule filling processes simple;
- Easily filled with high loading of active ingredients;
- Antioxidant activity and light shielding protection of encapsulated actives to prolong shelf-life;
- Controlled release of active ingredients from microcapsules;
- Microcapsules available in a wide range of colors.

APPENDIX

Chemical Structure of Sporopollenin

The building blocks and functional groups on sporopollenin are outlined as follows.

1. Crosslinked aliphatic framework: Sporopollenin has an aliphatic core or framework involving saturated and unsaturated oxygenated hydrocarbon building blocks (Brooks and Shaw, 1977, 1978a,b). It was suggested that since sporopollenin from different species has similar infrared spectra it most probably shares a common biosynthetic pathway in each species (Shaw and Yeadon, 1964, 1966). Later studies in the late 1980s, using solid-state NMR studies (Guilford et al., 1988; Espelie et al., 1989) were in support of unsaturated units involving a crosslinked aliphatic backbone with aromatic side chains (Rittscher and Wiermann, 1988a,b; Gubatz et al., 1993; Bubert et al., 2002). Kawase and Takahashi (1995) FTIR spectral data were also in agreement with a predominance of aliphatic building blocks (Hayatsu et al., 1988), and it was proposed that the aliphatic backbone of sporopollenin should be universal among different taxa (possibly all, but at least vascular plants), each species then producing its own aromatic (or conjugated) side chains, which could cause diversity in macroscopic morphology and properties (Rittscher and Wiermann, 1988a,b; Gubatz et al., 1993; Wilmesmeier, et al., 1993; Meuter-Gerhards, et al., 1995; Bubert et al., 2002). Further detail on the structure obtained from solid-state NMR data in conjunction with destructive methods (pyrolysis and oxidation by ruthenium oxide) led to the suggestion that the backbone is based on tetra-, hexa-, and octacosanes. In addition, tracer studies (Couderchet et al., 1996) using a ^{14}C-tagged precursor, showed that the 18:1 *cis*-9 fatty acid oleic acid was incorporated into sporopollenin. More unsaturated fatty acid involvement in the structure of sporopollenin was proposed (Dominguez et al., 1999) to include linoleic and linolenic acids as putative precursors. Other workers have agreed with the involvement of shorter alkyl chains (16−18) included in the structure of sporopollenin (van Bergen et al., 2004; De Leeuw et al., 2006). Reports on a number of pollen species added weight to the involvement of ether linkages, the structure of sporopollenin also having a role in crosslinking to strengthen polymer framework (Dominguez et al., 1999; Ahlers et al., 2000; Bubert et al., 2002).
2. Aromatic and phenolic groups: The presence in sporopollenin of aromatic (Shaw and Yeadon, 1964, 1966; Potonié and Rehnelt, 1971) and oxygenated aromatic units, either as phenols or ethers, have been detected. More detail was offered in the late 1980s by several workers (Schulze Osthoff and Wiermann, 1987; Herminghaus et al., 1988; Wehling et al., 1989), who found the presence of *p*-coumaric and derivatives in degradation studies. Tracer experiments (Gubatz et al., 1993; Gubatz and Wiermann, 1992) and NMR spectra showed the presence of 4-hydroxy and 4-methoxy cinnamic acids (Ahlers et al., 2003). Similar studies also showed that phenylalanine was an important precursor of sporopollenin in biosynthesis to provide a phenyl propane unit, which is kept intact in the final polymer (Schulze Osthoff and Wiermann, 1987; Kawase and Takahashi, 1995; Watson et al., 2007). Infrared spectroscopy also played its role in supporting the presence of phenolic groups in sporopollenin (Watson et al., 2007). There is support for the involvement of phenols (especially *p*-coumaric and ferulic acids) as side chains (Wiermann and Gubatz, 1992; Kawase and Takahashi, 1995) as well as crosslinking units (Schulze Osthoff and Wiermann, 1987; Herminghaus et al., 1988; Rittscher and Wiermann, 1988a, b; Wehling et al., 1989; Gubatz et al., 1992; Gubatz and Wiermann, 1993).
3. Hydroxyls: Hydroxyl (Fawcett et al., 1970; Shaw, 1971; Ahlers et al., 2000; Barrier et al., 2010a) content of sporopollenin has been estimated by acetylation of ^{14}C-labeled acetic anhydride acetyl groups and assaying loading of ^{14}C on the polymer, which was found to be significant at 6.6 OH groups per 90 C atoms (Fawcett et al., 1970) in the case of sporopollenin from *L. clavatum* spores.
4. Ketones: Ketones have been detected by NMR, infrared spectroscopy (Watson et al., 2007), and cytochemical studies although it is difficult to quantify their presence (Brooks and Shaw, 1977, 1978b).

5. Carboxylic acid esters: Spectroscopic analysis of enzymatic extracted sporopollenin, obtained by Wiermann et al. (Ahlers et al., 2000; Bubert et al., 2002), showed that carboxylic acid and hydroxyl groups were present in sporopollenin. A later study estimated loading by derivatizing with ammonia to form primary amides of the polymer, which were easily quantified by combustion elemental analysis to be between 1 and 2 mmol/g (Barrier et al., 2010a).

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Chapter 25

Mesoporous Solid Carrier Particles in Controlled Delivery and Release

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25.1 INTRODUCTION

Silicon (Si) is the second most abundant element in Earth's crust, at about 27.7 wt%. Hence, it is a material which should not be at risk of supply for many years to come. Silicon in the form of silica, i.e., SiO_2, can be found both in crystalline form (sand) and in amorphous form (glass), and both types of materials have found many different uses. While the crystalline form may have some adverse effects on human health (e.g., silicosis), the amorphous form is generally considered as safe, and is an ingredient in food products and pharmaceutics, as well as in many technical applications. Amorphous silica can be found both as a dense solid material and as manufactured porous or mesoporous materials, which may be characterized by low density, high specific surface area, and large pore volume. These materials, especially in particulate and microparticulate form, are of specific interest for controlled delivery and release applications.

The availability on the market of porous and mesoporous particles is limited to hydrophobic aerogels, which are fairly expensive and sold by companies such as Cabot, Grace Davison, and PQ Corporation. Both food-grade and non-food-grade qualities are supplied. If cheaper processes for aerogel-like porous silica particles can be developed, it is anticipated that access to these types of materials will be huge since they have potential in isolation, molecular filtering, and dehumidification. Furthermore, as described in this chapter, they are also an interesting class of materials for controlled delivery and release. Mesostructured porous particles have been around since the early 1990s (Kresge et al., 1992; Inakagi et al., 1993). It is an interesting class of material for controlled release purposes as the material can be designed to have a narrow pore size distribution and the exterior dimension can vary depending on how the particles are produced. An important difference between the aerogels and the mesostructured materials is the non-structured and varying pore size of the former and the well-structured architecture and narrow pore size distribution of the latter.

Actives aimed at providing a specific function (flavors, antioxidants, nutrients, vitamins, etc.) might be difficult to disperse as they are difficult to store in a formulation until use, difficult to release at a specified time, or may need taste masking. For these types of actives, it is useful to store them in a carrier material that can provide all of the attributes described above. In addition, if different actives are encapsulated in the same type of particle, the dispersion method need not be adjusted to different actives but only to the properties of the carrier particles. The mesoporous and porous silica particles have high capacity for carrying actives due to the properties of the material.

The pore volume of mesoporous silica particles can vary from 0.1−0.2 ml/g to commercial aerogel particles with 2 ml/g or more. The internal surface area is in the range of a few hundred m^2/g to about 1000 m^2/g. The external surface area only makes up about 1−2 m^2/g. The pores of mesoporous particles can be varied from a few nanometers to about 20−30 nm. It is possible to fill the internal pore volume in the carrier particles with actives.

The aim of encapsulation in mesoporous silica particles is to facilitate formulation, to protect the active during storage against degradation, and to release the active at a chosen time interval and release profile. The extra cost of adding actives to a food formulation as filled mesoporous carriers must be motivated by performance benefits (cost in use must be improved) and without any additional health risks.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00025-X

There is a wealth of studies mainly in the pharmaceutical sector of loading actives such as ibuprofen into mesoporous silica particles and releasing the actives in water. However, there is a lack of systematic studies on loading strategies, i.e., how to fill the particles effectively with the active and the release properties of the filled particles. The availability of commercial mesoporous particles at a low price is also limited. Finally, the processes of scaling up the production of such filled materials, which are also potentially coated to achieve a well-defined release profile, still need to be developed. This chapter aims at providing a technology platform for loading and release of actives in mesoporous solid silica particles without coatings by systematically studying how different chemical functional groups influence loading and release properties. The model actives are then extended to include more complex molecules of interest for food use.

25.1.1 Factors Affecting Loading and Release

25.1.1.1 Pore Size Versus Molecule Size

Several papers deal with the effect of molecule size versus pore size, and release rates for differently structured materials are compared. For example, the adsorption of chlorophyll A extracted from natural spirulina is strongly dependent on the pore diameter of the adsorbent (e.g., Hartmann, 2005). FSM-16 with pore diameters below 2 nm hardly adsorbs anything while ca. 30 wt% was adsorbed in a pore diameter of 4 nm (Figure 25.1). The chlorophyll molecule has a porphyrin ring of 1.5 nm by 1.5 nm and a phytol tail of about 2.0 nm length. Hence, correlating this to the size of the pore shows that the molecular dimensions need to be smaller than the pore diameter for the molecule to get inside the pore.

The adsorption of ibuprofen in mesoporous silica with columnar and circular (onion type of structure) pore geometry showed that the columnar geometry adsorbed twice as much ibuprofen as the circular one did (e.g., Cauda et al., 2009). The reason for this is most likely the low accessibility of circular geometry pores for ibuprofen, compared to the free access to the columnar pores.

The adsorption of globular proteins (cytochrome c, trypsin, and papain) in MCM-41 (pore size 4 nm) shows a clear dependence on the enzyme size on the achieved loading (e.g., Hartmann, 2005) (See Figure 25.2). The enzyme horseradish peroxidase was too large ($4.0 \times 4.4 \times 6.8$ nm^3) to be adsorbed into the pores.

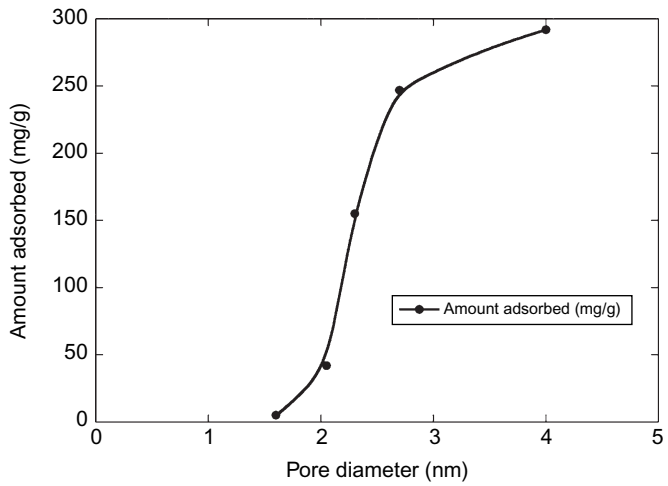


FIGURE 25.1 Relationship between the amount of chlorophyll adsorbed and the pore diameter of FSM-16. *Reproduced from Hartmann (2005) with permission.*

25.1.1.2 Effect of Exterior Particle Size on Loading

It has been shown (e.g., Huber et al., 2007) that mass uptake scales with $t^{\{1/2\}}$. The time it takes for an active to leave a particle depends very much on the particle size. For an active with an apparent diffusion coefficient of 10^{-12} m^2/s leaving the center of a particle, it would take 1 min for an 11 μm particle, 1 hour for an 85 μm particle, and 1 day for a 410 μm particle (Hedin, 2007). Hence, longer time for loading is needed for bigger particles using the solvent adsorption method. Bigger particles will also have a more extended release of active.

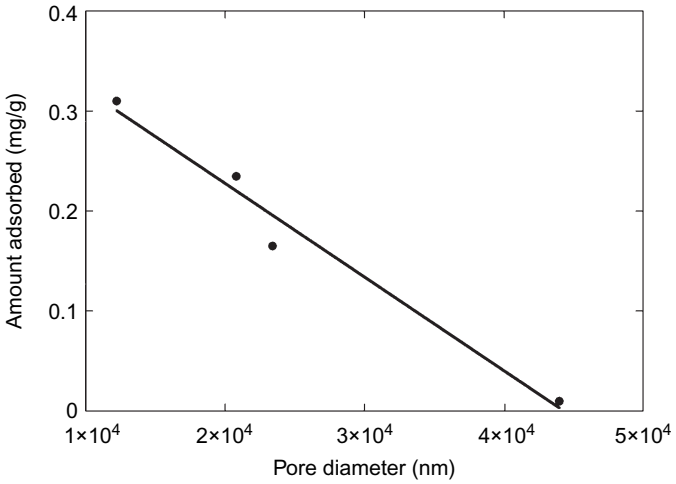


FIGURE 25.2 Effect of enzyme size on the immobilization efficiency of MCM-41 as support. *Reproduced from Hartmann (2005) with permission.*

25.2 CARRIER PARTICLES

Various organic and inorganic materials with mesoporosity have been developed by a large number of different research groups. Interesting examples besides mesoporous silica are calcium carbonates (Zhao et al., 2011; Yamanaka et al., 2014), metal-organic frameworks (MOFs) (Horcajada et al., 2008, 2010; An et al., 2009; Huxford et al., 2010), mesoporous titanium (Antonelli and Ying, 1995; Zhang et al., 2012), mesoporous organosilica (Asefa and Tao, 2012; Buyuan et al., 2012; Guan et al., 2012; Jin et al., 2012), hybrid organic/inorganic silica (Agostini et al., 2012), mesoporous carbon (Rammohan et al., 2013), or degradable polymers (e.g., Goltner and Weissenberger, 1998; Weber and Bergström, 2010).

Important for when choosing a suitable carrier material are stability in the formulation of choice, food-grade approval, cost, total loading volume, pore size, ease of surface modification, and (internal) surface energy. Amorphous mesoporous silica has for some time now been one of the most important materials in the carrier field because it can be made food-grade quality, has a wide range of possible pore sizes and geometries, can be surface modified, has a reasonable total internal pore volume, and is relatively inert in most common formulations. However, also other materials can arouse interest if they give advantages over mesoporous silica materials, e.g. in the form of favorable interactions, dissolution rates, and environmental footprints.

Mesoporous carbons can be synthesized via different methods, which all use a template for porosity (either surfactant micelles or mesoporous silica particles). Organic compounds like sucrose (Ryoo et al., 1999; Jun et al., 2000) or resorcinol/formaldehyde (Tanaka et al., 2005) are shaped around the template and later carbonized to form the mesoporous carbon particle. The syntheses of Zhang et al. (2005) in watery solutions make mesoporous carbon production economically possible.

Terasaki also used zeolites to create mesoporous materials (Ruan et al., 2009). By tiling nano-sized zeolitic species using surfactant templates, a material is made that contains mesopores, but also micropores (<2 nm) from the zeolitic species and intercrystalline uniform mesospaces.

Very small mesopores (or micropores) can be created by calcining hybrid inorganic/organic silicas. These materials are made using precursors containing one or more alkyl groups per Si atom. The pore structure and size depends on the size of the alkyl group, which leaves a pore when it is calcined away (Shimojima and Kuroda, 2002).

Periodic mesoporous organosilicas can be made when di-silanes are used that contain an organic linker that is bound via Si—C bonds rather than through Si—O bonds. These materials will have Si—R—Si (where R is an organic linker group) besides the normal Si—O—Si bonds. Pluronic block copolymers can also here be used as pore templates (Cho and Char, 2004; Zhao et al., 2005; Guo et al., 2006; Liu et al., 2010).

Another interesting class of mesoporous carriers is that of the calcium carbonates. In CODIRECT[1] these materials are prepared using different polymers to direct the crystallization and adhesion of nanocrystals. The pores here consist of the void between these primary particles (Gebauer et al., 2013).

1 CODIRECT (Controlled Delivery and Release Centre) was an Institute Excellence Centre hosted by YKI Institute for Surface Chemistry, Stockholm, Sweden, during 2007–2012. The center was financed by VINNOVA Swedish Governmental Agency for Innovation Systems, the Knowledge Foundation, Swedish Foundation for Strategic Research, and participating companies.

25.2.1 How is Porosity Created?

Ordered mesoporosity can be created using organic template materials. In mesoporous silicas these template materials are surfactant molecules that form micelles with nanometer length scales. Depending on the type and concentration of these template molecules, different structures will be formed in the liquid precursor solution. The order in these structures can be two dimensional (2D) or three dimensional (3D) and further can be cubic or hexagonal or even lamellar. The type of structure is of course very important for the diffusion of loaded molecules into and out of the particle.

Figure 25.3 shows a schematic diagram of a surfactant solution with a certain concentration and temperature. When the concentration of the template molecules increases, differently shaped micelles or liquid crystalline structures will be formed. Silica species in the precursor solution (oligomers or gel particles) will form around the surfactant structures creating a composite material of organic molecules surrounded by a crosslinking silica sol. When dried and fully crosslinked, a stable solid particle is obtained. To create porosity, the template should be removed; removal of the template can be done by calcination of the particles or by solvent extraction, as schematically illustrated in Figure 25.4. The porosity is best analyzed using nitrogen adsorption, where the amount of adsorbed nitrogen as a function of the pressure is recorded, both during adsorption and desorption.

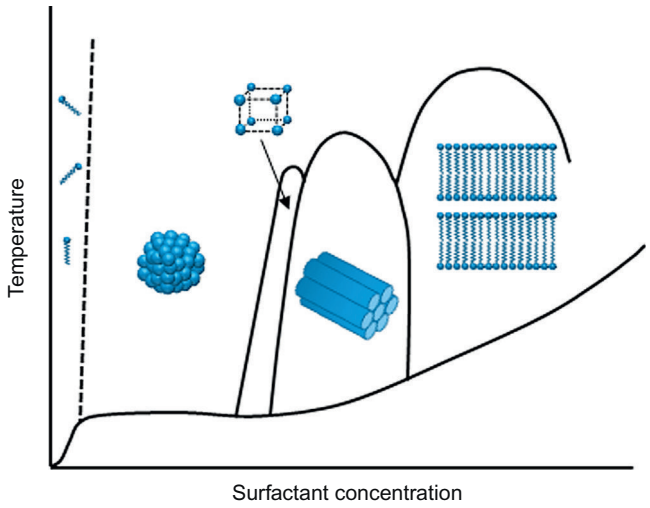


FIGURE 25.3 Schematic graphs depicting liquid crystalline phases as function of surfactant concentration and temperature. From the left: individual surfactant molecules, micelles, cubic phase, hexagonal phase, and lamellar phase.

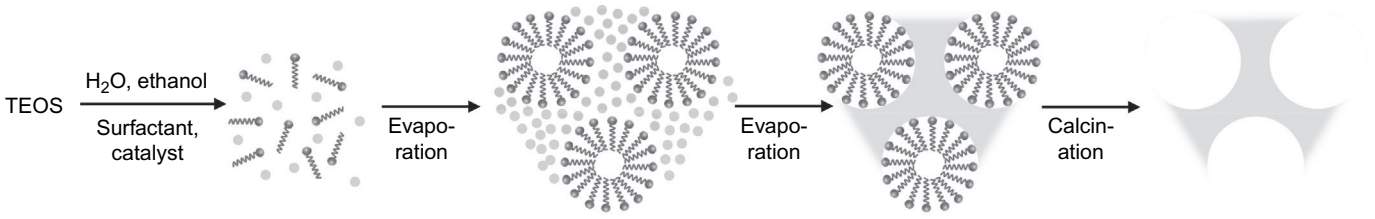


FIGURE 25.4 Schematic representation of pore formation in mesoporous silica. *Redrawn from Cademartiri and Ozin (2009).*

25.2.2 Particle Structures

Spherical carrier particles are usually of most interest in any application where the particles need to be formulated in liquid media. Spherical powders will flow more easily when dry and be easier to disperse than particles with other shapes. When spray drying or emulsion processes are used for the synthesis of carrier materials, the results are mainly spherical particles. Precipitation processes might form other shapes because the particles are built up in the energetically most favorable shapes rather than being forced into a spherical shape.

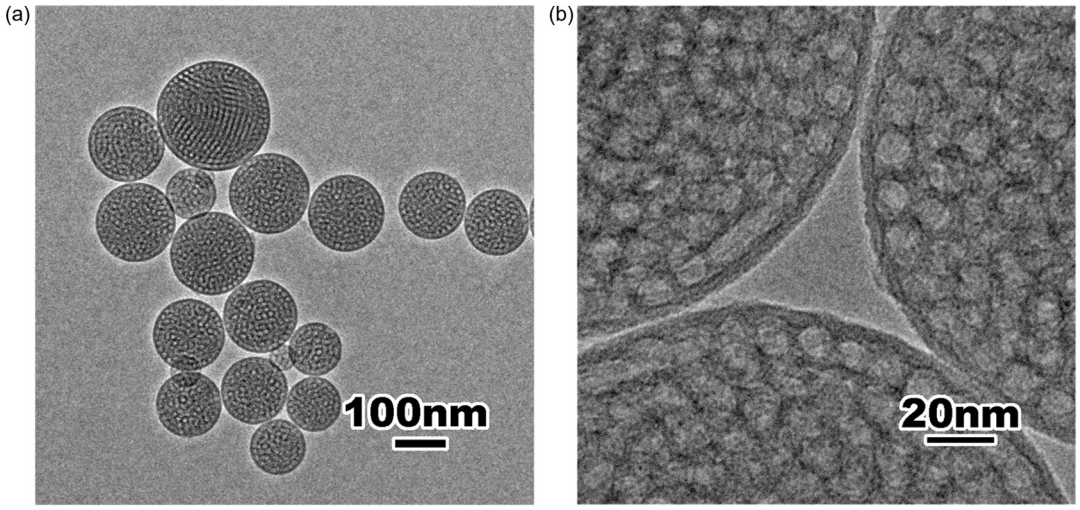


FIGURE 25.5 Transmission electron micrographs of spray-dried spherical mesoporous silica particles: left low resolution; right: high resolution.

Particle size homogeneity can be important for certain applications and when using spray drying or emulsion processes, the control of the droplet size is very important. Spray drying typically results in a rather broad particle size distribution, which to some degree can be controlled by choosing particular atomizer or nozzle designs.

Mesoporous particles with large internal cavities have higher loading capacity while having similar release profiles to those of normal mesoporous materials (Zhao et al., 2008). These types of particles are interesting for applications in which one wants to minimize the carrier content in the formulation and for applications that require lower carrier material costs. Cavities can be made via several methods. Basically, an organic template for a cavity is needed, which then, together with the pore template, is removed during calcination. Guo et al. used PMMA spheres as a cavity template to make hollow silica, titania, and alumina particles via a precipitation route (Guo et al., 2009), and Li et al. used surface modified PS as cavity template (Li et al., 2009). Another elegant way to create hollow particles is to use an oil droplet as template (Schacht et al., 1996; Lin et al., 2001; Zhang et al., 2009). Using an emulsion and solvent evaporation process, hollow particles were made in our laboratory. A double emulsion is created in which a hexadecane droplet acts as cavity template and around this droplet a shell of silica precursor solution containing pore templates will form the mesoporous silica shell. The silica condensation is pushed by the evaporation of ethanol and water from the silica precursor solution. The formed particles after calcination have considerably higher loading capacities than comparable non-hollow mesoporous silica particles (Figure 25.5).

25.3 LOADING METHODS

Several different aspects determine which loading method is the most suitable in a given combination of carrier and active, such as physical properties of the active and the carrier, and the intended release profile. In principle, four major methods are used:

- loading by capillary action (incipient wetness)
- melt method
- adsorption from solution
- *in situ* loading during particle synthesis

When loading from a liquid medium, the carrier must be wetted by the liquid, otherwise a counter-pressure will occur, which prevents the liquid from entering into the pores in the carrier material. This is important both for loading by capillary action and by adsorption, since in both cases the liquid must penetrate into the pores. The penetration of liquid into the pores is governed by capillary pressure, i.e., the pressure difference between the inside of the pore and the surroundings, which can be derived from Young−Laplace's equation and written as:

$$\Delta p = \frac{2\gamma}{r} = \frac{2\gamma\cos\theta}{r} \tag{25.1}$$

TABLE 25.1 Properties of Test Liquids

	Water	Ethylene Glycol	Medium Chain Triglyderide Oil	Rapeseed Oil	α-Tocopherol
Surface tension (mN/m)	72.8	48	29	32	31
Viscosity (mPa s)	0.89	16.9	28	86	4265
Density (g/cm^3)	1.0	1.11	0.93	0.93	0.95

where Δp is the pressure across the liquid meniscus in the pore, γ is the surface tension of the liquid, θ is the contact angle between the liquid and the pore surface, and r is the radius of the pore. The equation shows that the capillary pressure will be positive for contact angles less than 90°, i.e., for wetting liquids, and such liquid can then be loaded through the capillary pressure into the pores of the carrier material. In practice, this is generally the case for both polar and non-polar liquids, and hydrophilic silica. As an example, the contact angle for hexadecane on silica is 5°. In the case of hydrophobized silica, this will not be true for very polar liquids (e.g., water), and imbibition will then not occur. The imbibition of a liquid in the pores is described by Lucas−Washburn's equation, from which it is clear that viscosity, surface tension, and pore radius are the important parameters. For a straight, cylindrical capillary, the penetration distance (x) is linearly dependent on the square root of time, as described in Eq. 25.2:

$$x = \sqrt{\frac{r\gamma\cos\theta}{2\mu}}\sqrt{t} \tag{25.2}$$

where μ is the liquid viscosity. The penetration into a uniformly porous media can be described by a modified Washburn equation:

$$x = \frac{M}{A\cdot\rho\cdot\varepsilon} = \sqrt{\frac{2C\in\gamma\cos\theta}{S(1-\in)\mu}}\sqrt{t} \tag{25.3}$$

where M is mass of liquid that has penetrated into the pores, A is the cross-section of the porous matrix, ρ is the liquid density, ε is the porosity of the material, S is the surface area, and C is a constant that depends on the pore geometry (Mamur, 2003). The mean pore diameter is given by:

$$r_{\text{mod}} = \frac{4C\in}{S(1-\in)} \tag{25.4}$$

The imbibition of liquids of different viscosities and surface tensions was studied in order to confirm the usefulness of the relations (25.1)−(25.3) for loading of liquid into mesoporous silica materials of uniform pore diameter. To this end, thin porous silica glass plates (controlled pore glass (CPG) membranes) with pore diameters 12 and 60 nm were used. The glass has a hydrophilic surface. The test liquids included oils, ethylene glycol, and water, chosen to cover a range of viscosities and surface tensions, and the specific data for the liquids are shown in Table 25.1.

The imbibition was studied gravimetrically with a tensiometer (KSV Sigma 70), where the porous glass plates were mounted as a Wilhelmy plate. The porous glass plate was brought into contact with the test liquid, and the increase in mass was recorded as a function of time. A plot of the mass increase versus square root of time for imbibition of the test liquids into the glass membrane with 60 nm pore diameter is given in Figure 25.6. The fast mass increase at short time scales is due to the influence of the initial contact of the plate with the liquid. The good linearity of the curves after this initial phase shows that the imbibition obeys Lucas−Washburn type of kinetics. The difference in slope corresponds to the difference in viscosity of the liquids. However, for the highly viscous α-tocopherol, the linearity of the plot is poor, which indicates that the measuring time of 2 hours is too short (data not shown). The penetration into the 12 nm pore silica was slower, as should be expected from the pore size effect in Eq. 25.2. An example of imbibition of rapeseed oil in porous glass of 12 and 60 nm is shown in Figure 25.7, where the slower imbibition in the smaller pores is evident (see also Figure 25.8).

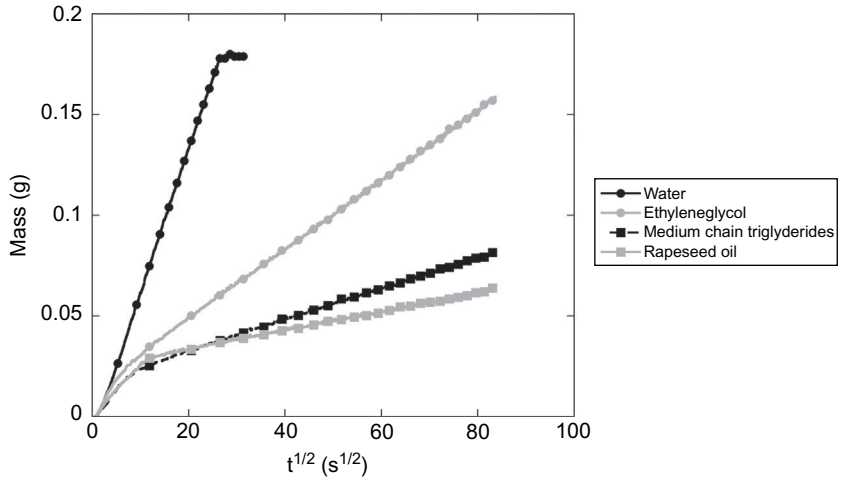


FIGURE 25.6 Imbibition of test liquids into porous glass membrane with pore diameter of 60 nm.

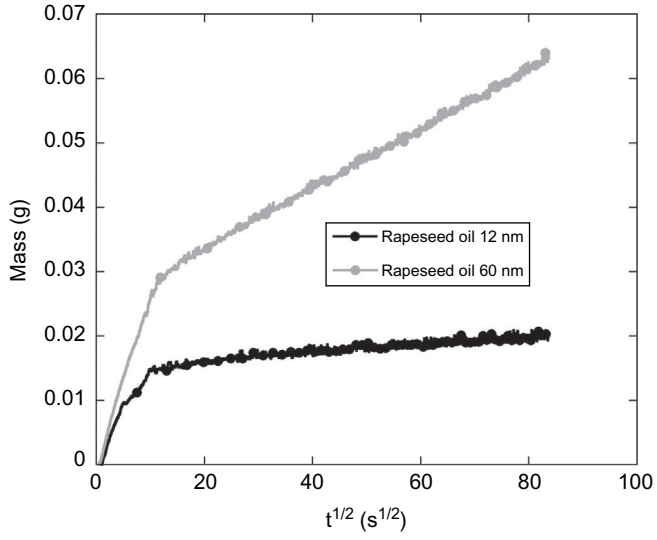


FIGURE 25.7 Imbibition of rapeseed oil into porous glass of different pore size.

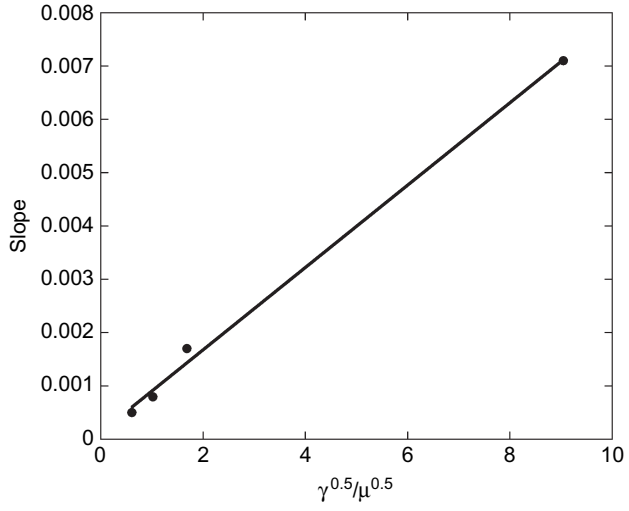


FIGURE 25.8 The slope of the mass increase versus $\sqrt{t}$ plotted versus $\sqrt{\gamma/\mu}$.

Since the imbibition agrees well with Lucas−Washburn kinetics, the slope of the curves in Figure 25.7 was further evaluated. The contact angle is assumed to be zero for all liquids, which is a valid assumption in the case of a high-energy material like glass. Since the pore characteristics are constant (Eq. 25.4), the difference in slope between the liquids is related to their difference in viscosity and surface tension. This is supported by the good linear relationship when the slopes are plotted against $\sqrt{\gamma/\mu}$ see Figure 25.8. This means that, when the imbibition rate follows the Lucas−Washburn equation and when the contact angle is assumed to be zero, the imbibition rate for a particular liquid can qualitatively be estimated from the surface tension and the viscosity. Thus, from the imbibition rate and the particle size, the necessary loading time can be estimated.

There are several ways of loading porous particles based on capillary action, and these are divided depending on whether surface adsorption or pore filling mechanisms are utilized.

25.3.1 Incipient Wetness

In the loading method called incipient wetness (or incipient wetness impregnation), no solvent is used for the active component if the active is liquid. An advantage with this method is that difficulties with strong competition of the solvent for adsorption to the carrier surface can be circumvented. The method is very efficient and easy to use, provided that the viscosity of the liquid is not too high, which then can be remedied by adding a solvent that reduces the viscosity. In principle, the porous carrier powder is mixed with the liquid active under vigorous stirring. The liquid is added in portions to the powder, and the loaded powder will act as a powder until the pores are filled. If too much liquid is added to the powder, it will start to stick. The loading capacity is determined by the pore volume, and in the case of using a solvent, the concentration of active in the solution. For solid actives, a solution is prepared and loaded into the particles in the same way and the solvent is evaporated. In this case, the loading capacity is also affected by the concentration in the solution.

The incipient wetness method was used by, for example Van Speybroeck et al. (2009) in a study of poorly soluble pharmaceuticals encapsulated in mesoporous silica. Several different drugs were loaded into the particles, using different solvents and drug concentrations depending on the individual systems. In all cases, a loaded system with about 20% loading was obtained, and the loaded drug was amorphous as determined by X-ray.

25.3.2 Melt Method

A physical mixture of the active and the particles is prepared, and the solid active is first ground to a fine powder. The mixture is heated so that the active melts (but does not decompose), and the mixture is stirred by some means, and kept above the melting point of the active for an additional period of time (a few minutes). Finally, the mixture is left to cool (e.g., Mellaerts et al., 2008). The adsorption is driven by capillary pressure, and the method is thus similar to the previously described incipient wetness method but differs in the way the carrier and the active are contacted. A prerequisite for filling the pores is that the melt viscosity of the active is not too high. It has, for example, been shown (e.g., Mellaerts et al., 2008) that itraconazole is unable to fill pores of a size of 8.4 nm while ibuprofen easily fills the pores. Previous work within CODIRECT (e.g., Larsson and Köhler, 2008) has shown that melt viscosity as well as pore size are important for the penetration of waxes and fatty acids into mesoporous silica. It appeared in this study that a pore size of 7 nm was necessary for high loadings of wax. Furthermore, it was shown that the addition of wax would fill the pores completely before wax covered the exterior surface.

25.3.3 Adsorption from a Solution

The loading of actives can also be carried out by physically adsorbing the active to the carrier material surface by adsorption from a solution. This method is suitable both for liquid and solid active. In general, the active is dissolved in the solvent, and the carrier particles are added. The dispersion is mixed, e.g., by ultrasonication, for a period of time, and then the loaded particles are collected by filtration and remaining solvent is removed by evaporation. While employing the loading method by adsorption from a solution, there is a clear risk of precipitation of the active material on the exterior surface of the particles after removal of the solvent, since it is difficult to wash off any excess active without releasing the adsorbed active at the same time.

Depending on the solvent properties in relation to the carrier properties and the chemical functionalities of the active molecule, different loading efficiencies are achieved. These correspond to the equilibrium situation for the combination of particles, solvent, and active. If the active and solvent compete for specific interactions with the carrier

(e.g., hydrogen bonding to silanol groups), the efficiency of the binding of solvent in relation to that of the active will determine the loading capacity in this system (e.g., Hillerström et al., 2009). It is typically not possible to load more than a monolayer of the active into the pores (unless these interact with themselves to build additional layers). It has, however, been reported that repeated fillings can increase the loading of actives into mesoporous materials and this is attributed to the increased active intermolecular interactions within the pore voids, whereby larger pore volumes may result in greater active loading (e.g., Vallet-Regi et al., 2007).

The effect of different solvents and concentrations will be discussed in Section 25.7.

25.3.4 Effects of Chemistry of the Active on Loading Efficiency

In the cases of the incipient wetness method and the melt method, the chemistry is not expected to have a major influence on the loading efficiency since the liquid and the active are dragged into the pores via capillary pressure. On the other hand, if the solvent method is used the loading will depend on the chemistry of both the active and the liquid, as well as the surface chemistry of the porous particles. The achieved loading will at a maximum correspond to a monolayer covering the internal and external surfaces. Specific interaction e.g., hydrogen, bonding between active and surface, increases loading. Addition of a non-solvent to the mixture of solvent, active, and particles can enhance the loading efficacy (e.g., Hillerström et al., 2009).

25.3.5 *In situ* Loading

A loading approach that is fundamentally different to the ones described above is *in situ* loading (also called the one-pot synthesis method), in which the active component is built into the carrier structure while it is formed. Spatial separation of active molecules is then accomplished by the introduction of actives (or dopants) in the initial sol. The dopants will assemble in a separate region during material synthesis. We will below discuss three one-pot strategies: philicity, bonding, and bifunctional strategy.

25.3.5.1 Philicity Strategy: Physical Immobilization Based on Differences in Solubility

The deliberate placement of active molecules is based on the "philicity" (or local solubility) of the molecules (e.g., Angelos et al., 2008). Hydrophobic molecules and ionic molecules will stay in the organic region and in the ionic region, respectively. Neutral polar molecules will partition to the silica precursor solution and end up in the silica framework of the final material. The appropriate molecules are simply added in the initial sol and self-assemble into the desired region during material synthesis.

25.3.5.2 Bonding Strategy: Active Molecules as Framework Structured Materials

The "functional molecule" is itself a building block, which is covalently linked to the silica network (e.g., Angelos et al., 2008). The active molecule is precisely positioned because it is directly incorporated into the inorganic framework of the material. This strategy requires that the molecule undergo hydrolysis and condensation and it must also allow templating by micelles to occur before the final framework is formed. This strategy is used, for example, in energy transfer systems, but to our knowledge not used for controlled delivery and release as such.

25.3.5.3 Bifunctional Strategy: Chemical Bonding at the Framework–Ionic Interface

This strategy places a "functional molecule" at the surface between the silica framework and the ionic region by chemical bonding of the active to the outside of the framework. This type of functionalization is achieved utilizing bifunctional molecules with one end that is a trioxysilane and one end that is hydrophobic. This strategy has been used to functionalize the pore interior with azo-benzene-based molecular machines (e.g., Angelos et al., 2008). These molecules can undergo a wagging motion upon light excitation acting as a nanoimpeller, which can impel unbound guest molecules.

25.4 CHARACTERIZATION OF UNLOADED AND LOADED PARTICLES

When the particles have been loaded with the active material, characterization of the particles is necessary to determine the amount that has been loaded into the particles, the physical state of the active, and also where in the particles the active is located. In order to acquire this information, different characterization methods are available, some of which are rather advanced.

Different imaging techniques can be used to observe the internal as well as external structure of the carrier particles. Light microscopy can be used to investigate the internal structure on a low resolution level (down to 0.5 μm) by simply dispersing the powder in an oil, which enhances the contrast between the solid materials and occluded air. To investigate the particle structure in more detail, different types of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can be used. A very high resolution SEM with a resolution down to about 5 nm can also give some information on how the pores are open toward the surface of the particles. This technique was used to study the pore geometry in different mesoporous silica materials (Tüysüz et al., 2008).

In order to determine the loaded amount in porous carriers, thermogravimetric analysis (TGA) is very useful. In this technique, a small amount of sample (a few mg) is weighed into a crucible that is placed on a fine balance. Temperature controlled gas is passed by the sample, which is continuously weighed, and the weight loss is recorded. The total weight loss corresponds to the loaded amount, and in addition the weight loss profile versus time gives information on how the active is bound and also indicates the presence of, for example residual solvent or moisture.

By comparing the porosity as measured by nitrogen adsorption of unloaded and loaded particles, it can be determined whether the pores are (partially) blocked by the loaded active. The shape of the pores will influence the adsorption and desorption curves and the hysteresis. The pore volume will also carry information about the loading of the particles.

X-ray powder diffraction (XRD) can be used to determine the spatial organization of the pores in the structured mesoporous materials, which then can be described using the nomenclature of crystallography. Further, XRD is also used to determine the amorphicity/crystallinity in the silica material, as well as in the loaded active. Typically, the loaded active remains amorphous for a long time (years), since the space in the pores is so small that crystallization is delayed or even impossible. Potentially, nanocrystals smaller than the pore diameter may form, which are not detectable by XRD. In larger pores, crystallization can occur (e.g., Guo et al., 2013).

25.5 RELEASE MEASUREMENTS

The release of active components from the carrier is a major interest from an application point of view, but not always easy to measure in an appropriate way. Depending on the intended use of the loaded particles, different release profiles may be desired, which is discussed below. Further, use of a simplified release medium for testing may give different results compared to the "real" release medium in an application situation. Therefore, it is important to consider release conditions and release results together.

25.5.1 Immediate Release

As soon as the filled particles are placed in the release medium, being a solvent or gas phase, the release of the active is initiated. The solubility of the active in the release medium will affect the release rate. A compound adsorbed in mesoporous silica, which is highly soluble in the release medium, will release almost immediately. On the other hand, a compound adsorbed in mesoporous silica with a much lower solubility in the release medium will be released much more slowly (e.g., Larsson and Köhler, 2009).

25.5.2 Extended/Slow Release

In a situation where the actives must perform over a longer time interval, an extended release of actives from a carrier material is beneficial. Factors that can affect the release rate are wetting of the pores by a solvent, diffusion path length through the particle, surface interaction between the active and the pore, and addition of a diffusion barrier on the carrier particles. All these factors can contribute to a slower release of the active. It has been reported that functionalization of the pore wall of calcined SBA-15 with hydrophobic silanes can affect the release rate of erythromycin (e.g., Doadrio et al., 2006). Not only has the presence of these silanes seemed to be important in this process but also the nature of the solvent used for release (toluene or acetonitrile).

In another study conducted at our institute (e.g., Alberius et al., 2005), a commercially available biocide was loaded into mesoporous silica. The loaded particles were protected by a thin non-porous silica skin of about 9 nm to delay the release of the active. Exterior acrylic emulsion paints were prepared from these particles and compared to standard formulations including the biocide and also a formulation without biocide. The paints were applied on filter paper and subjected to water leakage cycles corresponding to 2 years' weathering. The filter papers were placed in Petri dishes containing agar and the painted papers were tested against growth of three different molds, namely, *Aspergillus niger*,

Cladosporium sphaerospermum, and *Penicillium funiculosum*. It was clearly demonstrated that proper encapsulation slowed down the release rate significantly and resulted in prolonged protection against mold growth, projected to last for several years.

25.5.3 Triggered Release

Triggered release can be achieved if an active can be strongly bound to the internal pore surface and released as a response to external stimuli. Alternatively, an active can be moderately hard bound to the internal pore surface and instead a skin/coating on the particle surface hinders the release of the active. The skin/coating can be removed or opened as a response to external stimuli such as temperature, pH, salt, light, and enzyme action, and then the active can be released. This approach is heavily investigated in pharmaceutical as well as food applications.

25.5.4 Choosing Release Medium and Conditions

The choice of release medium is very decisive for the results that will be obtained. In laboratory studies, it is common to use relatively simple release media, such as a buffer, or a buffer with added surfactant for improved solubilization of poorly soluble materials. In order to have comparable conditions in different experiments, it should be ensured that sink conditions are used. This means that the solubilization capacity of the active in the release medium should be 5−10 times higher than the concentration of the active corresponding to complete release (Phillips et al., 2012). The reason for this is that the release in this type of experiment should not be influenced by the concentration in the release medium. For water-soluble actives, sink conditions are relatively easily obtained, but for poorly soluble substances the release medium needs to be modified to solubilize the active. This can be accomplished by adding a surfactant that forms micelles that can solubilize the active, and SDS (sodium dodecyl sulfate) is commonly used in concentrations around 1% (w/w) in water or buffer (Phillips et al., 2012). It must be noted that if UV-VIS spectrophotometry is used for analysis, the spectrum of the active may be shifted due to the local hydrophobic environment when it is solubilized in micelles.

25.5.5 How to Handle Particles in Release Media

Standardized release equipment, such as that used in the pharmaceutical industry and specified in the pharmacopeia, is usually adapted for dissolution from tablets rather than powders. This type of equipment relies on spectrophotometric analysis, and if particles enter into the light beam, light will be diffracted and increase the measured light absorption. Thus, measures must be in place to prevent particles from entering the spectrophotometer. This can be accomplished by fitting different kinds of filters to the tubing leading into the spectrophotometer, or if measurements at discrete times are sufficient, samples can be withdrawn and the particles can be removed by filtration or centrifugation. The drawbacks are that the active substance may be adsorbed in the filter material, that particles are accumulated in the vicinity of the filter, and that the presence of a surfactant in the release medium can lead to formation of bubbles during filtration. Alternative solutions are to enclose the particles in, for example a filter bag or dialysis tubing, or to correct for the contribution of the light diffraction due to particles (Hillerström et al., 2009). The enclosure of particles in a cell or bag, or dialysis tubing gives rise to additional resistance to the diffusion and an observed delay in the release that is in fact an artifact due to the methodology.

25.5.6 Continuous Measurements

For release experiments where the release occurs during shorter time periods (up to a few hours), continuous measurements can be easily performed, if spectrophotometry is a suitable method of detection. Baseline drift in the instrument may be an issue for longer experiments. The continuous measurements can be done in a single sample fashion, by continuously passing the release medium through a flow-through cell in a spectrophotometer. Alternatively, the release equipment used in the pharmaceutical industry and standardized according to the pharmacopeia can be used. Typically, six or eight release vessels can be run in parallel with detection using a spectrophotometer. The instrument can record the continuous release profile in all vessels at the same time. A common practice is to use one of the vessels as the reference vessel in which the release medium is used but no loaded particles are added. This type of equipment is often run according to the pharmaceutical standard protocols, specifying vessel dimensions, volume, and stirring speed.

25.5.7 Point Measurements

For slowly releasing substances, i.e., usually actives with low or very low water solubility, discrete measurements are more suitable, for several reasons: in many labs it is undesired to keep the equipment in use for long periods, data files become very large, and there is also a baseline drift in spectrophotometers over time. Continuous data are most often not necessary for release over long periods (typically >24 h). When samples are withdrawn from the release medium, the volume can be topped up with fresh release medium, or the reduced volume is accounted for in the data evaluation.

25.6 THE EFFECTS OF CHARACTERISTICS OF THE ACTIVE ON LOADING AND RELEASE

The loading method must be selected to fit the active and the carrier material. In most instances, it is desired to achieve as high loading efficiency as possible, but at times, only physically adsorbed actives are desired since this can influence the subsequent release profile. For liquid actives, the incipient wetness method is suitable for achieving high loading levels. However, for actives with a high melting temperature, this is impractical and working at high temperatures may cause the active to deteriorate. These different aspects have been considered by several authors (e.g., van Spreybroeck et al., 2009; Fang and Bhandari, 2010; Belingheri et al., 2012), but usually different model molecules for pharmaceutical substances, food flavors, or nutraceuticals are studied.

In order to understand how different functional groups in the active molecules contribute to loading and release phenomena, a more structured approach was necessary. In the study presented below, a set of substances containing UV light adsorbing phenyl functionality was selected to represent different chemical functional groups; see Table 25.2. Further, to investigate the effect of hydrophobicity of the molecule, each functional group is also combined with a longer hydrocarbon chain. The functional groups selected include all the common functional groups of different polarity (state of oxidation), and both positive and negative charge. Due to the strongly varying water solubility of the compounds studied, different release media were used: either MilliQ water for water-soluble substances or 1% SDS in MilliQ water for the poorly soluble substances, where sink conditions otherwise could not be obtained. In addition, more complex test molecules were also used to investigate effects of multifunctional molecules. These molecules were vanillin, α-tocopherol, and quercetin; see Table 25.3.

Different physical properties of the test molecules are shown in Table 25.4, and these are the properties that are used when trying to correlate loading and release behavior with the properties of the different test molecules.

The actives in Table 25.2 were loaded in mesoporous silica particles with regular pore structure with pore diameter of either 2 nm (CTAB template) or 10 nm (swollen Poloxamer F127 template) and pores organized in hexagonal structure. Different loading methods were applied, either incipient wetness of the liquid actives or adsorption from solvent for solid actives. Whenever using different loading methods, it is important to keep in mind that these methods can give rise to different bonding between the active and the carrier, and can thus influence both loaded amounts and release profiles.

First, several of the actives in Table 25.2 were loaded by the incipient wetness method to an average loading level of 40%. In some cases, gentle heating was applied to melt the active in order to be able to load it using the melt method, which is very similar to the incipient wetness method. The release measurements were conducted using water

TABLE 25.2 Test Molecules Used to Map Behavior in Loading and Release Based on Chemical Functionalities

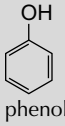
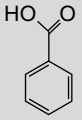
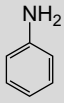
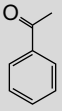
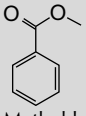
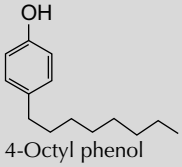
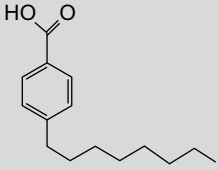
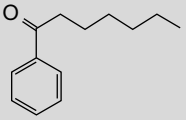
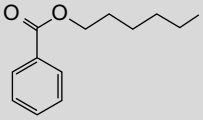
Functional Group	–OH	–COOH	$–NH_2$	–CO–R	–CO–O–R
Small polar	≈0.6 nm  phenol	 Benzoic acid		 Acetophenone	 Methyl benzoate
Large less polar	≈1.5 nm  4-Octyl phenol	 4-Octyl benzoic acid		 Octanophenone	 Hexyl benzoate

TABLE 25.3 Structure of Complex Test Molecules

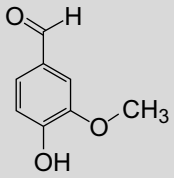
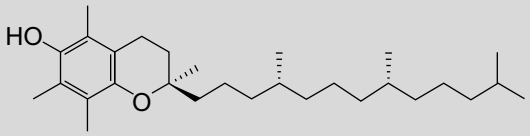
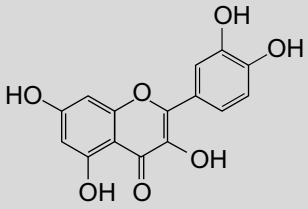
Substance	Structure
Vanillin	
α-Tocopherol	
Quercetin	

TABLE 25.4 Physical Properties of the Test Molecules

Compound	Molecular Weight (g/mol)	Melting Point (°C)	Solubility in Water (g/l)	Log *P*
Phenol			83	1.47
Benzoic acid	122.12	122	2.9	1.96
Acetophenone	120.15	19	5.5	1.58
Methyl benzoate	136.15	−12	2.1	2.12
4-Octyl phenol	206.3	83	0.0031	5.5
4-Octyl benzoic acid	234.3			3.6
Octanophenone	204.3	22	0.005	4.7
Hexyl benzoate	206.28		0.0089	4.85
Vanillin	152.15	81–83	10	1.208
α-Tocopherol	430.7	2	0.0000209	9.9
Quercetin	302.2	316	0.00044	1.82

as dissolution medium for the water-soluble actives, and 1% SDS solution in water for the poorly soluble actives. The effect of the active on the release rate is shown in Figure 25.9. A major factor affecting the release rate is the solubility of the active in the release medium. Comparing the water-soluble actives and the poorly soluble actives, we find that the high solubility in water promotes the release rate, and even though sink conditions are maintained also for the poorly soluble substances by means of SDS micelles, these release at much lower rates. Thus, it appears that the transfer of the actives through the pores and/or solubilization of the actives in the SDS micelles is a rate limiting step. If the actives are ranked by their water solubility (Table 25.4), the release rate tends to follow this order. Phenol releases the fastest of all actives, which indicates that the OH-group is more important for rapid release than the keto-group or

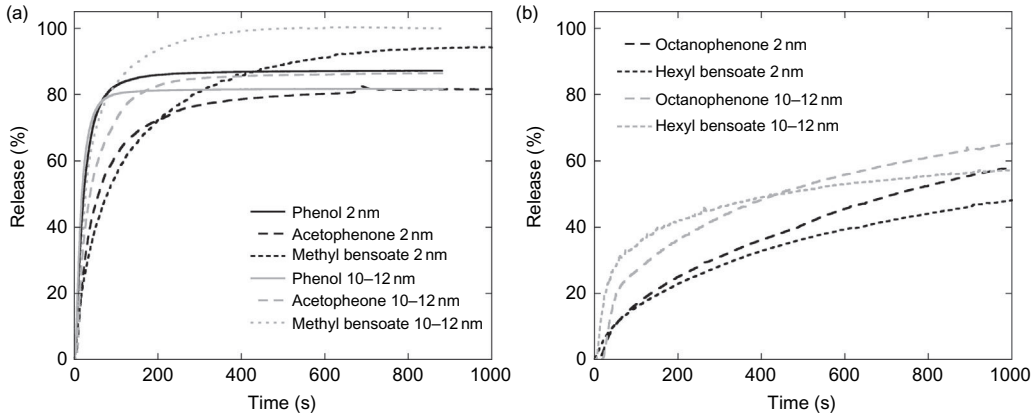


FIGURE 25.9 Release rate for different actives loaded using the incipient wetness method. Two different pore sizes in the silica particles were used, 2 nm and 10−12 nm. (a) Release of water-soluble actives in water with solid lines: phenol, dashed lines: acetophenone, dotted lines: methyl benzoate. Black lines: 2 nm pore size and gray lines: 10−12 nm pore size. (b) Release of poorly soluble actives in 1% SDS in water with solid lines: octyl phenol, dashed lines: octanophenone, dotted lines: hexyl benzoate. Black lines: 2 nm pore size and gray lines: 10−12 nm pore size.

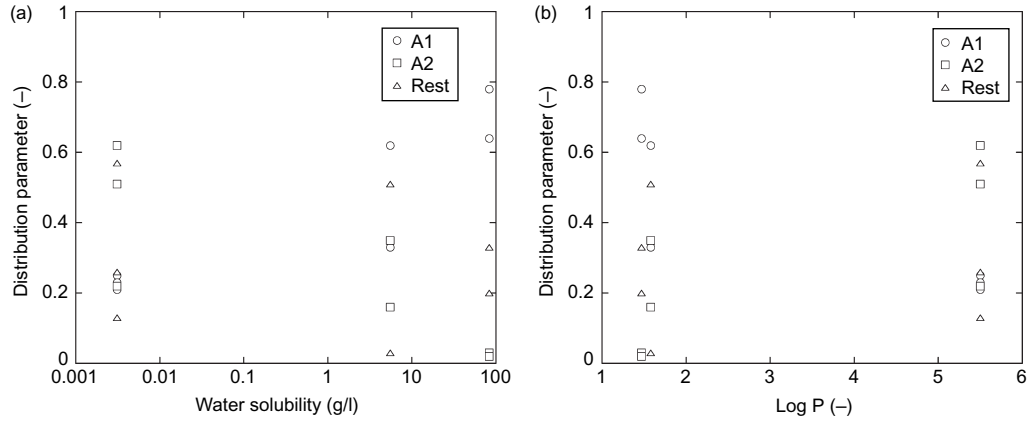


FIGURE 25.10 Release parameters (fractions released) as function of (a) water solubility and (b) log P_{ow}. (○) A1, (□) A2, and (Δ) rest.

the ester group. Further, the release is faster from the larger pore size particles irrespective of the character of the active, since the diffusion rate is improved by the larger pores.

The detected release profiles were fitted by a model based on diffusion controlled release. The diffusion was modeled as occurring from two populations, one loosely bound fraction and one strongly bound fraction; the model also takes an irreversibly adsorbed fraction into account. The following equation is used to describe the release profile:

$$y = \mathrm{A1} * (1 - \exp(-x/t_1)) + \mathrm{A2} * (1 - \exp(-x/t_2)) + (1 - \mathrm{A1} - \mathrm{A2}) * (1 - \exp(-x/t_3)) \tag{25.5}$$

where A1 and A2 denote the loosely and strongly adsorbed fractions, t_1 and t_2 the characteristic release times for the loosely and strongly bound fractions, and the rest (1 − A1 − A2) denoted in following text as ε corresponds to the residual fraction that is practically not possible to release.

The distribution of the active in three different populations with different characteristic release times can be interpreted as a consecutive release of loosely bound molecules, more strongly bound molecules, and very slow release of very tightly bound molecules.

The fitted results are summarized in Figure 25.10, where the different fitting parameters are shown as a function of water solubility of the active, and log P_{ow} (the partitioning coefficient between octanol and water). The parameter A1 and to some extent also the parameter A2 show a correlation with the water solubility and with log P_{ow} values; however, the amount of the residual fractions seems not to be related either to water solubility or to partition coefficient.

The distribution of the active in three different populations with different characteristic release times can be interpreted as a consecutive release of loosely bound molecules (A1), more strongly bound molecules (A2), and very slow

TABLE 25.5 Loading Level and Residual Amount of Phenol after Release (Residual Fraction from Fitted Data)

Sample	Loading (%)	Loading (g/g Carrier)	Residual (mg/mg Carrier)
Incipient wetness, 2 nm	31	44.9	0.06
Incipient wetness, 10–12 nm	31	44.9	0.08
2 nm, adsorption, acetone	19.6	24.4	0.15
10–12 nm, adsorption, acetone	15.3	19.2	0.17

release of very tightly bound molecules (ε). These fractions are formed during the adsorption or loading process, where a multimolecular layered loading is more likely in the case of incipient wetness. This could result in the layers furthest away from the carrier surface being desorbed and released more rapidly than the molecules that interact directly with the carrier surface.

As an example of the effect of loading method, incipient wetness and adsorption loading of phenol in mesoporous spherical silica particles with pore size 2 and 10–12 nm, respectively, were investigated. The loading level achieved (determined by TGA) using the incipient wetness method was 31% and by adsorption from a 10% solution of phenol in acetone, 15–20% loading was obtained (Table 25.5). In this case, the incipient wetness loading was carried out to a point where the loading was perceived as less than complete, i.e., it was stopped before carrier particles started to stick together. The pore size has at this loading level no effect on the capacity for loading via incipient wetness. However, it can be noted that the larger pore size (lower specific surface area) carrier results in lower loading when the adsorption method is used. The pore size influences the release rate to some extent, so that the release is slightly faster from the large pores, although the overall release rate is to be regarded as fast for all samples (Figure 25.11). Further, the smaller pore size results in higher total release in both cases. The relative residual amount is less in the case of adsorption loading, but if this is converted into absolute numbers, the residual amount of phenol in the carrier particles is higher in the samples where phenol was adsorbed from acetone (Table 25.5). This indicates that the interaction between the active and the carrier surface depends on the loading method, and the adsorption loading seems to result in a stronger interaction between the active and the silica surface in the pores. The release rates were similar and high in all cases, but slightly higher for the larger pores (Figure 25.11).

The studies of simple actives carrying one chemical functionality, but varying in size, hydrophobicity, and chemical structure, have shown that the loading protocol influences the amount of loaded active, but also the release rate and the total release. The major factors affecting the release profile are the log *P* of the active in addition to the water solubility, which indicates that the partitioning of the active between the pore surface, the liquid in the pores, and the bulk liquid in the release medium is important for the release behavior. Carrier properties such as the pore diameter also influence the loading rate (by capillary action), and to some extent the release rate.

The learning from the simple actives was also applied on loading and release of more complex actives, α-tocopherol (vitamin E), methyl vanillin, and the flavonoid quercetin, see Tables 25.3–25.5 for physical properties and chemical structure. Depending on the solubilities of these actives, different loading protocols and solvents were applied. Vanillin and quercetin have high melting points and were thus loaded by adsorption from 10% (w/w) solutions in organic solvents, while α-tocopherol was loaded both using the incipient wetness method and by adsorption from an organic solvent. The resulting loading levels are shown in Figure 25.12. It is clear that the loading solvent as well as the loading method influence the resulting loading. The data indicate that the more hydrophobic solvents give rise to higher loading levels in mesoporous silica (isooctane vs. acetone for α-tocopherol, and THF vs. acetone for vanillin). However, due to the different solubilities of the actives in the solvents, the comparison is not straightforward.

The model presented in the previous section was also applied to fit the release data for the complex actives, and it was found that the results correlate slightly better with log P_{ow} as the parameter to describe the actives than with the water solubility of the active (Figure 25.13). This is particularly the case when all investigated actives are considered together. This indicates that the log P_{ow} plays a role in the partitioning between the particle surface, the pore volume, and the release medium, but other factors, such as specific interactions, may also be of importance. When the different fractions where correlated separately to the water solubility and log P of the loading solvent, no clear correlations were observed, again an indicator of the complexity of the binding of actives to the surface and the release mechanism.

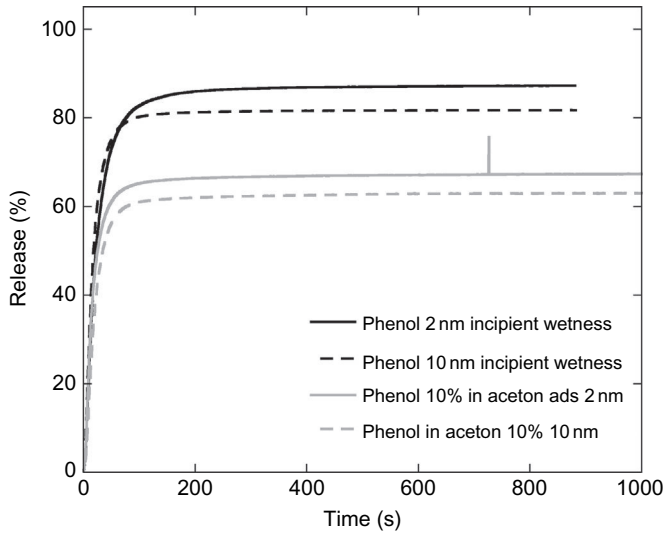


FIGURE 25.11 Release profiles in water of phenol in mesoporous silica particles loaded by different methods. Loading by (----) incipient wetness in 2 nm pore size silica, (- - - -) incipient wetness in 10 nm pore size silica, (----) adsorption in 2 nm pore size silica, and (- - - -) adsorption in 10 nm pore size silica from a 10% (w/w) phenol solution in acetone.

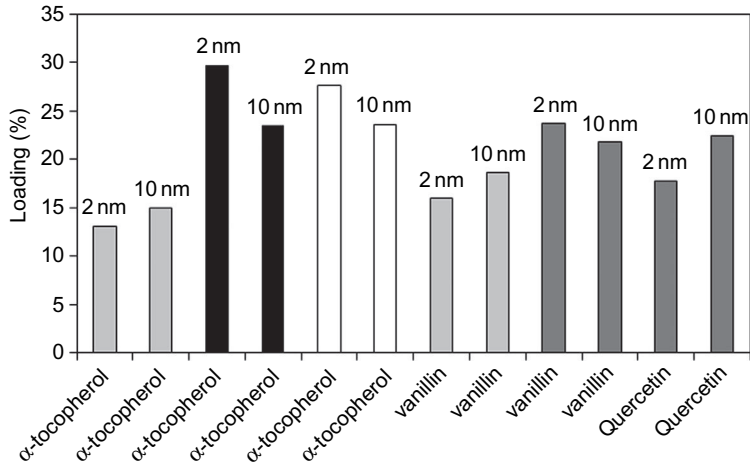


FIGURE 25.12 Loading levels in mesoporous silica particles for different actives using different loading solvents and methods. Light gray bars: acetone; black bars: isooctane; white bars: no solvent; and dark gray bars: THF. The pore size is indicated above each bar. The pore volume and area in the 2 nm pore particles was 0.73 ml/g and 1106 m^2/g, for the 10−12 nm particles it was 0.71 ml/g and 414 m^2/g, respectively.

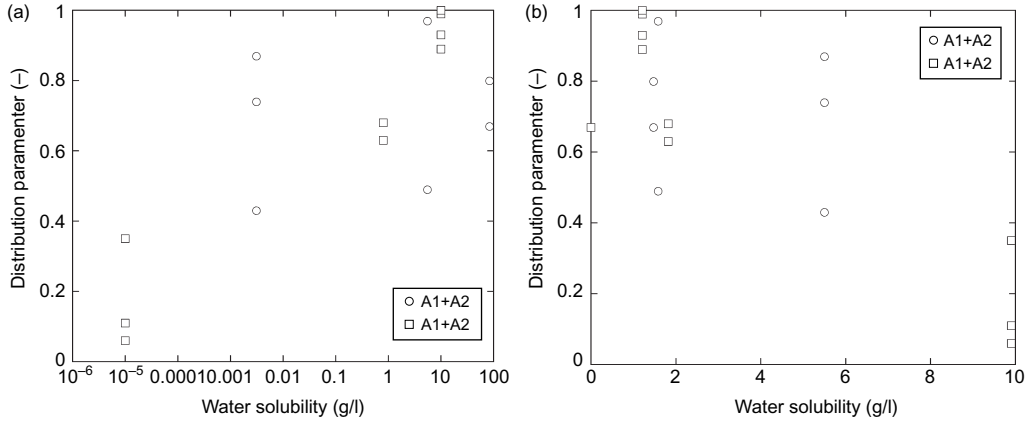


FIGURE 25.13 The adsorbed fractions A1 and A2 as a function of (a) water solubility of the actives, and (b) log *P* of the loading solvent. Green triangles represent the simple actives and the blue triangles represent the complex actives.

25.7 EFFECTS OF LOADING MEDIUM

The studies on simple and complex actives showed that the choice of solvent as well as the loading technique influenced loaded amounts as well as the release profiles. This was studied in some detail using more complex actives (vanillin) and one of the selected simple actives (hexyl benzoate). The effect of the concentration of the active in the loading medium was studied either by using different concentrations in relation to the saturation concentration, i.e., maximum solubility, in a solvent (vanillin) or by using a set of concentrations at which the active was miscible with the solvent (hexyl benzoate). The intention was to achieve as similar a chemical activity of the same active in different loading media as possible, as this is the driving force for the adsorption to the carrier surface. For vanillin, three different solvents were used, acetone, tetrahydrofuran (THF), and toluene, while hexyl benzoate was mixed with acetone and isooctane.

The loading level was evaluated using TGA, and the data are shown in Figure 25.14. It is clear that the loading level for vanillin correlates well with the relative solubility data, but not with the concentration in terms of g/l. This indicates that it is indeed the chemical activity of the active rather than the concentration that is the decisive factor for the loading by adsorption, which agrees well with theory. The chemical activity is related to the concentration and solubility, but this is a non-linear relationship for non-ideal solutions. This means that in most cases, the ideal case occurs only at very dilute conditions, and deviations are expected at higher concentrations. This non-ideal behavior can explain the observation of lower loading level of vanillin with toluene compared to the other solvents, but still the same curve shape was followed. In the case of hexyl benzoate, the loading level was less dependent on the concentration of the active in the loading medium, which may be due to the complete miscibility of hexyl benzoate with these solvents.

The release profiles of the different actives in water for vanillin and in 1% SDS for hexyl benzoate were recorded (Figure 25.15). For vanillin it was found that the release rate strongly depended on the loading solvent, so that the release was most rapid when adsorbed from toluene and slowest when loaded from THF. This suggests that the loading solvent influences how the active interacts with the surface in the particles, although the underlying properties are not yet fully interpreted. A part of the explanation may be that in the case of toluene, the amount of particles was increased due to the lower loading, and thus a larger surface area was available for the release. However, this does not explain the slower release from the particles loaded from THF in relation to the particles loaded from acetone. In the case of hexyl benzoate, the release was faster for the samples loaded from the hydrophilic solvent acetone than from the hydrophobic isooctane.

The release data were fitted to obtain the parameters for adsorbed fractions and characteristic release times using Eq. 25.5. The results are summarized in Figure 25.16 in terms of the total released fraction (A1 + A2) and the residual fraction as amount of residual drug per amount of carrier. The data for vanillin (Figure 25.16a) show that the released fraction is increased as the concentration in the loading solvent is increased. This increase is influenced by the solvent choice so that loading from THF provides the lowest released fraction at the lowest loading concentration, while the effect is much less pronounced for loading from acetone. Interestingly, the non-released fraction in absolute numbers is virtually constant in all cases, and highest for THF and lowest for toluene. This implies that the vanillin loaded from the less hydrophobic solvents binds more tightly to the silica surface as compared to the vanillin loaded from toluene.

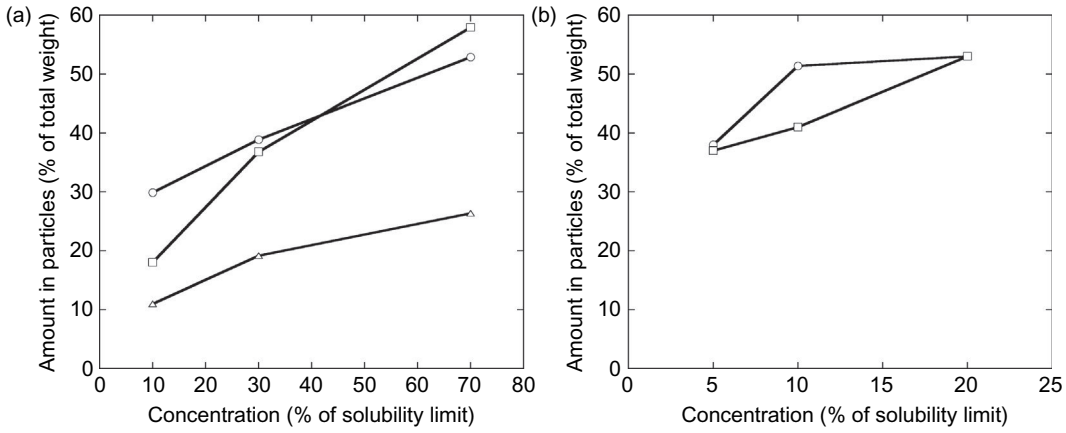


FIGURE 25.14 Loaded amounts of actives when using different loading media and in relation to the solubility of the active in the solvent. (a) Vanillin adsorbed from (○) acetone, (□) tetrahydrofuran, and (△) toluene, and (b) hexyl benzoate adsorbed from (○) acetone and (□) isooctane.

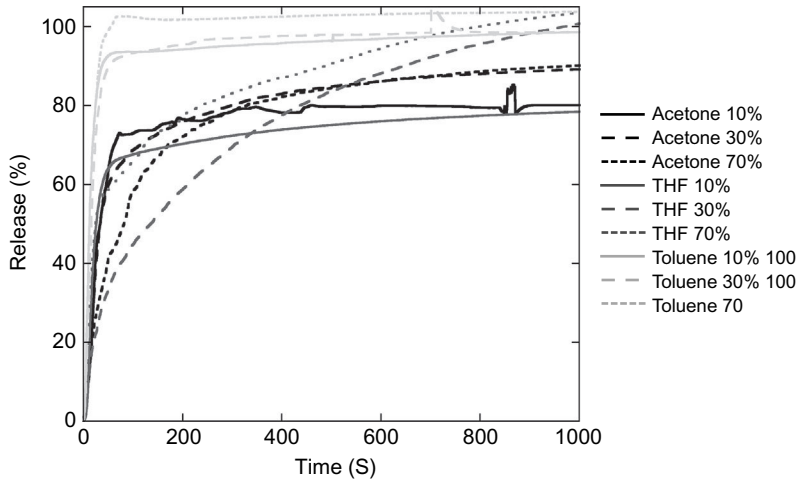


FIGURE 25.15 Release profiles for vanillin loaded from three different solvents at three different fractions of the saturation concentration of vanillin in each solvent.

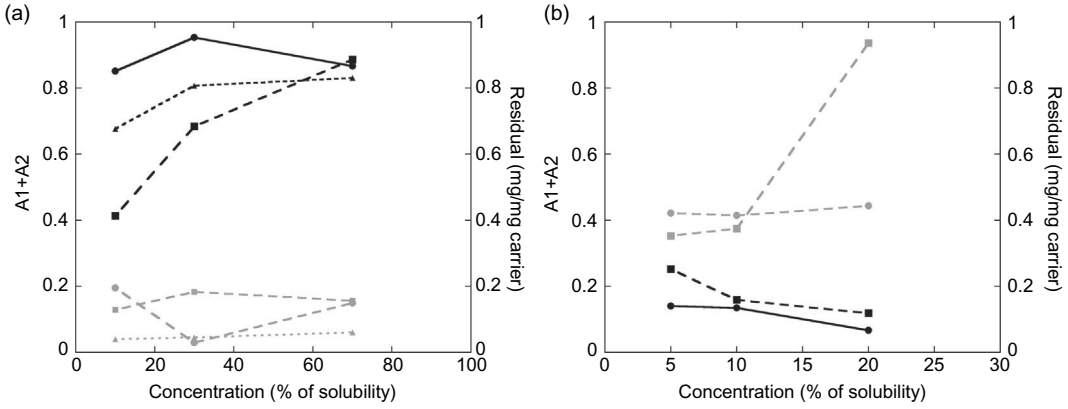


FIGURE 25.16 The combined released fractions (A1 + A2) of the active and the absolute residual fraction for different loading solvents and concentrations. The ratio of active:particles was constant at 3:1 on weight basis during the loading experiment. The amount of loaded particles in each release experiment was adjusted to give the same maximum concentration of released active.

The data for hexyl benzoate look quite different; the released fraction is much lower than for vanillin, and the released fraction is slightly reduced when the concentration in the loading solvent is increased. Further, the residual fraction both in relative numbers and in absolute numbers is much larger than for vanillin; however, release curves show that the release was still continuing when the experiment was interrupted (data not shown).

To conclude the effects of the loading medium and conditions on the loading level and release, we found that the solvent choice is of importance, and in particular the concentration of the active relative to the solubility limit when there is a limited solubility of the active in the solvent. It seems to be the chemical activity rather than the absolute concentration that is of importance for the obtained loading. The amount of loaded active was not reduced by the presence of solvents that can compete with the active of the adsorption sites on the silica surface through hydrogen bonding, such as acetone and THF. Moreover, it appears that the solvent also influences how the active binds to/adsorbs to the silica surface. The loading level achieved does not necessarily correspond to the absolute amount of active that can be released from the particles.

25.8 HOW CAN LOADING AND RELEASE BE CONTROLLED?

The mesoporous silica particles constitute a versatile platform for carrying various molecules, converting liquids into powders. The loading capacity is high (around 40−50%), and this can be increased further by generating hollow

particles with a mesoporous shell. The loading process is influenced by the pore size and the particle size, as well as the properties of the liquid that is loaded into the particles. Most liquids have the ability to wet silica surfaces, which is a prerequisite for loading, and the loading rate is described by the Washburn equation. Liquids of different properties in terms of contact angle and viscosity were investigated, and this relationship was found to be applicable for all liquids tested. Thus, the loading time can be estimated from the Washburn equation and the size of the particles. The loading level can be controlled by using different loading solvents, or by adding controlled amounts of the active.

The release occurs primarily through diffusion, where the release medium diffuses into the particles, and the active diffuses out of the pores and into the bulk. The particles themselves provide no other means for control of the release rate than variation of pore size and particle size. The residual amount that is left after release is influenced by the loading conditions (choice of solvent), and also the release rate is to some extent influenced by the loading conditions. However, these parameters are not sufficient to provide precise control of release properties. To accomplish this, other modifications of the particles are necessary, such as surface modification of the particles (e.g., Chang et al., 2010; Szegedi et al., 2011; Wani et al., 2012; Guo et al., 2013), or application of coatings that can be either slowly eroded or responsive to different kinds of stimuli (Alberius et al., 2005; Rammohan et al., 2013; Bhattacharyya et al., 2012). In the case of coatings, there is a need to develop efficient, robust, and scalable methods for application of coatings on small particles.

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Part V

Testing and Quality Control

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Chapter 26

Testing Tools and Physical, Chemical, and Microbiological Characterization of Microencapsulated Systems

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26.1 INTRODUCTION

Microencapsulation is a versatile technology and is the preferred means to protect volatile flavor compounds. In fact, encapsulated flavors comprise about 20−25% of the total flavor market (Ubbink and Schoonman, 2003). For this reason, much effort has focused on improving flavor encapsulation performance over the past few decades. There are two main objectives pursued through encapsulation (Ubbink, 2013): (1) protect the flavor compounds during storage (based on use of amorphous carbohydrates) and (2) control the release of the flavors during food processing or end use. Extensive encapsulation technologies (spray drying, spray cooling, coacervation, melt extrusion, fluidized bed, molecular inclusion, and freeze drying) are available, which can provide specific functionalities (physicochemical stability, safety stability, and organoleptic qualities). The distribution of encapsulated flavors according to the production technologies are 80−90% by spray drying, 3−9% by spray chilling, 2−4% by melt extrusion, and <1% by melt injection (Ubbink and Schoonman, 2003; Porzio, 2004, 2007, 2012), and <1% are produced by other technologies.

According to Ubbink and Krüger (2006), the choice of flavor encapsulation technology should be made using a retro-design approach based on the final application (food matrix conditions) and the nature of the core material (physical, chemical, and biological properties). It is well known that the process variables can impact the physical (e.g., internal and surface morphology, size and wall thickness), mechanical (mechanical strength), and structural properties of the encapsulated particles including flowability, dispersibility, density, glass transition temperature, degree of crystallinity, and permeability (Walton and Mumford, 1999; Hadinoto et al., 2007; Walzel and Furuta, 2011). The knowledge and understanding of the physical and structural properties are essential to reduce cost and to obtain an optimum process and product functionality (stability, release, and sensorial perception). Therefore, reliable analytical techniques should be used to characterize flavor encapsulates. This chapter reviews the general concepts and principles of existing tools used for physical, chemical, and microbiological characterization of encapsulated flavors and provides recommendations and examples leading to a better understanding of their performance characteristics. Table 26.1 classifies and summarizes methods commonly used for the characterization of important properties of encapsulated flavors.

26.2 PHYSICAL CHARACTERIZATION

Physical characterization provides a detailed understanding of the surface morphology and structural properties of flavor encapsulates. These characteristics may be triggered by the method of manufacture and processes variables as shown in Figure 26.1. A wide array of analytical techniques are available and utilized for the physical characterization encapsulates. The most common methods are microscopy techniques (Champion et al., 2007), including electron microscopy

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00026-1

TABLE 26.1 Testing Tools used for Characterization of Encapsulated Flavors

Purposes	Characterization Methods	Advantages	Disadvantages	Particle Size (μm)	References
Morphology and size distribution	Laser light scattering (LLS)	A large number of spherical encapsulates could be measured rapidly.	Refractive indexes of wall and active are required, not preferred for non-spherical encapsulates.	0.02−1000	Baranauskiené et al., 2006, 2007; Bhandari et al., 1992; Bringas-Lantigua et al., 2011; Chen et al., 2013; Choi et al., 2009; Finney et al., 2002; Jimenez et al., 2010; Fabra et al., 2012; Kaasgaard and Keller, 2010; Kim and Morr, 1996; Ko et al., 2012; Li et al., 2013; McNamee et al., 1998, 2001; Paramita et al., 2012; Penbunditkul et al., 2012; Shah et al., 2012; Sillick and Gregson, 2012; Soottitantawat et al., 2005, 2007; Sosa et al., 2013; Syamaladevi et al., 2012; Yang et al., 2012a,b; Yeo et al., 2005; Zandi et al., 2014.
	Scanning electron microscopy (SEM)	Simple sample preparation and operation, analysis of sample size from nm to cm.	No color-distinguishable capability.	0.02−700	Adamiec et al., 2012; Baranauskiené et al., 2006; Bhandari et al., 1992; Choi et al., 2009; Fieber et al., 2011; Finney et al., 2002; Kaasgaard and Keller, 2010; Kim and Morr, 1996; Ko et al., 2012; Li et al., 2013; Ma et al., 2013; McNamee et al., 1998; Paramita et al., 2012; Patel et al., 2013; Rodríguez et al., 2013; Shah et al., 2012; Soottitantawat et al., 2004, 2005, 2007; Yang et al., 2012a,b; Zandi et al., 2014.
	Optical microscopy	Accessibility, low cost, simple operation, color distinguishability ability.	Lower resolution than SEM and TEM, particles less than 0.2 μm will not be characterized.	2−2000	Bhandari et al., 1992; Chen et al., 2013; Onwulata and Smith, 1994; Patel et al., 2013; Sillick and Gregson 2012; Zandi et al., 2014.
	Environmental scanning electron microscopy (ESEM)	Appropriate to investigate wet encapsulates, requires low vacuum environment, hydrated samples can be observed as a "wet mode" image.	Limitation of the distance in the specimen chamber.	0.1−1000	Fabra et al., 2012; Marcuzzo et al., 2012; Syamaladevi et al., 2012.
	Transmission electron microscopy (TEM)	Higher resolution than SEM and optical microscopy.	High vacuum is required, complicated sample preparation.	0.1−2	Choi et al., 2009; Fieber et al., 2011.

	Confocal laser scanning microscopy (CLSM)	High resolution, three-dimensional fluorescent structure can be seen.	Limited number of excitation wavelengths, high irradiation, high cost.	0.1−1000	Kim and Morr, 1996; Sosa et al., 2013.
	Atomic force microscopy (AFM)	Three-dimensional surface profile, simple sample preparation, no vacuum environment is required, high resolution.	Small image size, relatively low scanning rate.	0.02−1.2	Choi et al., 2009; Shah et al., 2012.
	Others	NA	NA	100−1000	Ma et al., 2013; Marcuzzo et al., 2012; Patel et al., 2013; Penbunditkul et al., 2012; Yeo et al., 2005.
Mechnical strength	Fracture force	Essential information for production, storage, and application of encapsulates.	The difficulty increases when the size of microcapsules goes down to μm range.	NA	Patel et al., 2013; Yuliani et al., 2006a,b.
Glass transition and moisture sorption	Glass transition	Important information to design encapsulates for controlled release.	Special equipment is required, destructive test.	2−625	Cadwallader et al., 2010; Chen et al., 2013; Ramoneda et al., 2011; Soottitantawat et al., 2004; Syamaladevi et al., 2012; Yang et al., 2012a.
	Moisture sorption	Important factors to quality the stability of microcapsules.	Usually time-consuming, appropriate storage system is required.	40−2000	Cadwallader et al., 2010; Ponce Cevallos et al., 2010; Ramoneda et al., 2011; Sillick and Gregson, 2012; Soottitantawat et al., 2004.
Degree of crystallinity	DSC	Multiple properties can be measured from the same sample simultaneously.	Reference sample is required.	0.1−2000	Choi et al., 2009; Kalogeropoulos et al., 2010; Patel et al., 2013; Ponce Cevallos et al., 2010; Ramoneda et al., 2011; Sillick and Gregson 2012; Syamaladevi et al., 2012.
	Nuclear magnetic resonance spectroscopy (NMR)	Detailed structure of encapsulates and small molecules an be obtained.	Sensitivity is affected by magnetic field, cannot exam molecule size >64 kDa	0.1−2000	Fieber et al., 2011; Hafner et al., 2011; Kalogeropoulos et al., 2010; Sillick and Gregson, 2012; Sosa et al., 2013.
	X-ray diffraction	Non-invasive, non-destructive, high resolution, three-dimensional structure can be seen.	Fine pores in encapsulates not detectable.	0.01−4	Kasemwong and Itthisoponkul, 2013; Szente and Szejtli, 1986.

(*Continued*)

TABLE 26.1 (Continued)

Purposes	Characterization Methods	Advantages	Disadvantages	Particle Size (μm)	References
Flowability	Angle of repose	Important information in handling and processing operations of encapsulates.	Difficult to conduct appropriate tests for encapsulates.	0.1−1000	Bhandari et al., 1992; Jimenez et al., 2010; Fabra et al., 2012; Marcuzzo et al., 2012; Onwulata and Smith, 1994.
Composition analysis	GC	Widely used, accurate method.	Sample preparation is not simple, relatively expensive.	0.04−500	Adamiec et al., 2012; Badee et al., 2012; Baranauskiené et al., 2006, 2007; Bhandari et al., 1992, 1999; Bringas-Lantigua et al., 2011; Cadwallader et al., 2010; Charve and Reineccius, 2009; Finney et al., 2002; Giroux and Britten, 2011; Kim and Morr, 1996; Ko et al., 2012; Ma et al., 2013; Marcuzzo et al., 2010 and 2012; Paramita et al., 2012; Penbunditkul et al., 2012; Soottitantawat et al., 2005.
	GC-MS	Widely used, accurate method.	Sample preparation is complicated and expensive.	2−1000	Baranauskiené et al., 2006, 2007; Bhandari et al., 1999; Cadwallader et al., 2010; Kalogeropoulos et al., 2010; Penbunditkul et al., 2012; Yeo et al., 2005.
	HPLC	Widely used, accurate method.	Sample preparation is complicated and expensive.	0.02−1.200	Kalogeropoulos et al., 2010; Shah et al., 2012.
	Absorbance	Low cost, simple operation.	Relatively inaccurate.	15−1000	Li et al., 2013; Yeo et al., 2005.
	Liquid-gas chromatograph	Widely used, accurate method.	Sample preparation is complicated and expensive.	0.1−1000	Fabra et al., 2012.
Encapsulation efficiency	Total oil/surface oil	Widely used accurate methods.	Time consuming, extraction and GC are usually required.	0.02−500	Badee et al., 2012; Baranauskiené et al., 2006, 2007; Bringas-Lantigua et al., 2011; Chen et al., 2013; Goula and Adamopoulos, 2012; Paramita et al., 2012; Fabra et al., 2012; Kalogeropoulos et al., 2010; Ko et al., 2012; Li et al., 2013; Ma et al., 2013; Marcuzzo et al., 2010; McNamee et al., 1998, 2001; Penbunditkul et al., 2012; Shah et al., 2012.

	Thermal properties	Limited sample amount is required, accurate method.	Special equipment is required, destructive test.	0.1−1	Choi et al., 2009; Ponce Cevallos et al., 2010.
	Absorbance	Low cost, simple operation.	Relatively inaccurate.	NA	Ramoneda et al., 2011.
	Others	NA	NA	40−2000	Sillick and Gregson, 2012.
Flavor retention	Distillation, or extraction and evaporation followed by GC; absorbance measurement	Important information to design encapsulates for controlled release.	Multiple pathways, not easy to identify the best one for each encapsulate.	0.02−1000	Baranauskiené et al., 2007; Bhandari et al., 1992, 1999; Bringas-Lantigua et al., 2011; Cadwallader et al., 2010; Charve and Reineccius, 2009; Fabra et al., 2012; Kaasgaard and Keller, 2010; Li et al., 2013; Ma et al., 2013; Paramita et al., 2012; Penbunditkul et al., 2012; Ramoneda et al., 2011; Rodríguez et al., 2013; Shah et al., 2012; Sillick and Gregson, 2012; Soottitantawat et al., 2005; Yuliani et al., 2006a,b.
Flavor stability: flavor release	GC, DVS fast GC-FID, melting enthalpy, e-nose, liquid-gas chromatography	Essential information for the stability and shelf-life of encapsulates.	Multiple pathways, not easy to identify the best one for each encapsulate.	0.04−1000	Baranauskiené et al., 2007; Giroux and Britten, 2011; Fabra et al., 2012; Kim and Morr, 1996; Ko et al., 2012; Li et al., 2013; Marcuzzo et al., 2010; Patel et al., 2013; Ponce Cevallos et al., 2010; Rodríguez et al., 2013; Soottitantawat et al., 2004, 2005; Yeo et al., 2005; Zandi et al., 2014.
Flavor stability: oxidation	GC	Accurate method to quantify stability.	Not appropriate for every encapsulate system.	0.05−70	Chen et al., 2013; Charve and Reineccius, 2009; Kim and Morr, 1996; Soottitantawat et al., 2004, 2005.
Safety assessment	Toxicology	Essential information required in food industry.	Usually time consuming.	NA	Badee et al., 2012.
	Microbiology	Important safety indicator.	Not a guarantee of product safety, one component of an overall safety system.	NA	Adamiec et al., 2012.

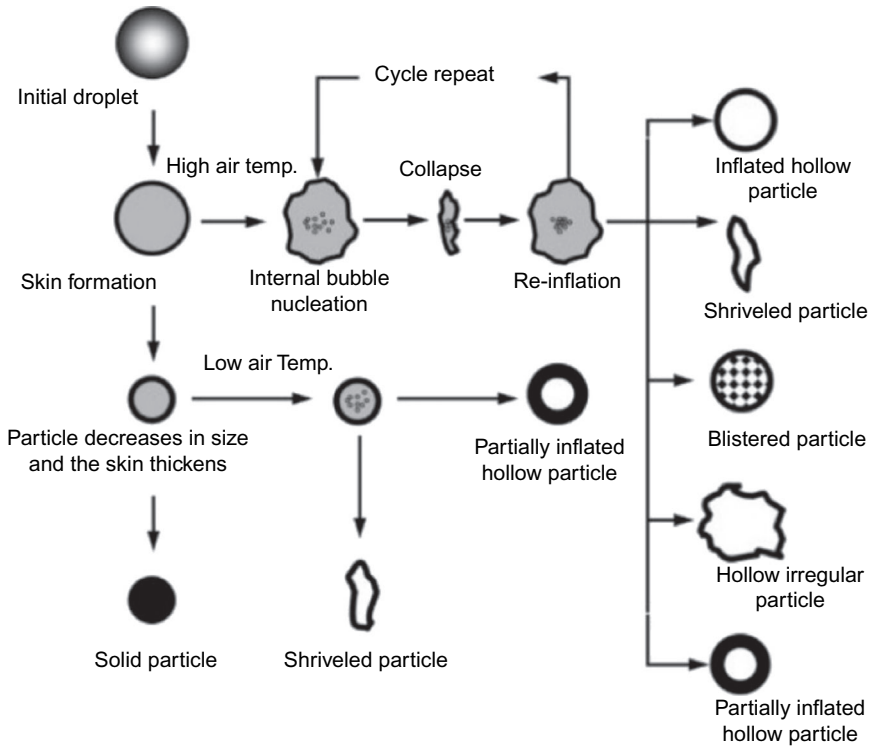


FIGURE 26.1 Schematic of effect of spray drying process parameters on the morphological characteristics of the encapsulate. *(Source: Walzel and Furuta, 2011; Reproduced with permission from John Wiley and Sons, Hoboken, NJ.)*

TABLE 26.2 Properties of the Most Common Types of Microscopes used for the Physical Characterization of Microencapsulates

Capabilities	Lens Imaging		Scanning Imaging	
	Optical Microscope	Transmission Electron Microscope	Scanning Electron Microscope	Atomic Force Microscope
Magnification	2 to 2000	200 to 2×10^6	20 to 1×10^5	1000 to 2×10^6
Sample observed	Surface or bulk if transparent	Bulk, thin films <0.2 μm transparent	Surfaces	Surfaces
Specimen environment	Ambient or transparent fluid	High vacuum	High vacuum	Ambient, high vacuum or fluid
Sample preparation	Easy	Very difficult	Easy	Easy
Radiation damage	None	Severe	Rarely serious	None
Chemical analysis	No, unless μ-Raman or IR	Yes, X-ray and electron loss	Yes, X-ray	No
Can detect molecular orientation?	Yes	Yes	No	Sometimes

Source: Sawyer et al., 2008; **Reproduced with permission from Springer-Verlag, New York, NY.**

(SEM, TEM), optical light or fluorescence microscopy (CLSM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM) (Table 26.2). Two key aspects should be considered when performing morphology studies: (1) selection of an adequate instrumental technique and (2) method used for specimen preparation.

26.2.1 Morphology and Size Distribution

Microscopy-based instruments have been widely utilized to evaluate various physical characteristics of microencapsulates. Electron microscopy (SEM, TEM), optical light (OM), or fluorescence microscopy (CLSM) are the most common instruments used to evaluate these characteristics. These methods can provide various kinds of information. Transmission electron microscopy (TEM) allows two- and three-dimensional imaging, while scanning electron microscopy (SEM) permits imaging of structures with a greater depth of focus than light microscopy, and confocal laser scanning microscopy (CLSM) enables optical sectioning of the specimen beneath the surface. Table 26.3 describes several microscopy characterization techniques that are most often used by the food industry to provide information about microencapsulates based on the nature of the size range of the specimens and encapsulation technologies used to produce them. Understanding the principles of these techniques is important in the selection of the most appropriate technique to analyze a specific type of microencapsulate.

26.2.2 Electron Microscopy

An electron microscope (EM) uses electrons for imaging of specimens, which permits better resolution than with light microscopy. Hence, smaller structural details can be visualized using electron-based microscopy. The most popular types of EM used for physical characterization of encapsulated flavors are SEM and TEM. There are three main imaging signals derived from the interaction of the specimen and an electron beam. Figure 26.2 shows the direction in which the signals are detected/generated during EM. These include backscattered electrons (BSE), secondary electrons (SE), and X-rays. Backscattered electron imaging (BSEI) provides a strong compositional contrast. Additionally, when combined with X-ray microanalysis, it is feasible to determine the chemical composition of a material. Secondary electron imaging (SEI), which measures the interaction of the primary beam close (a few nanometers) to the specimen, can produce a very high resolution image; thus, the process is efficient. This is the normal SEM imaging mode. Compared to most imaging methods, X-rays are larger in volume and have less resolution.

EM-based techniques require some sample preparation (pre-handling steps, including dehydration and coating of specimen) and analysis under high vacuum (to reduce radiation damage), which might alter the properties of a particle, including both its structure and chemistry. Consequently, this is a major limitation of most EM methods. In general, sample preparation for TEM is more tedious than for SEM. Different methods can be used for sample preparation for TEM (negative staining, freeze-fracture, and vitrification by plunge freezing) (Klang et al., 2012); each provides different kinds information about the structures examined. Negative staining is the most commonly used method (Harris, 2008): a drop of the sample is placed on a TEM grid (gold or copper of a defined mesh size) and stained with a solution (uranyl acetate) to provide high contrast (the sample appears brighter against the stained background). The freeze-fracture technique gives additional information about the internal structure of the particles (Severs and Robenek, 2008). With this method the sample is dropped on a TEM grid, which is subsequently placed between two plates (gold or copper) and is further vitrified by rapid freezing (liquid propane or melting nitrogen). Under constant cooling and in a vacuum, the frozen sample is fractured, with the fracture plane developing predominantly along the areas of the sample with weak molecular interactions (e.g., within lipid bilayers). The fracture plane can be further etched (e.g., using water sublimation in vacuum) and then shadowed with a thin platinum/carbon layer (about 2 nm thick) usually at an angle of 45° with respect to the fracture surface. This provides a "negative" replica of the fractured sample plane. An alternative to the above technique is cryo-TEM, which involves plunge freezing. Cryo-TEM allows for direct investigation in the vitrified, frozen-hydrated state, which is very close to the native state of the material. As with freeze-fracture TEM, information about the internal structure of the particles may be obtained with this method.

Proper sample preparation is critical for the acquisition of reliable and meaningful data. For proper interpretation of the images generated through EM techniques, it is important to base any interpretation on previous knowledge of materials, techniques used, specimen preparation, and potential artifacts. Following are a few examples of the use of different characterization tools which have been used to investigate physical characteristics of aroma encapsulates using EM:

- Scanning electron microscopy (SEM)**:** Soottitantawat et al. (2003) applied the SEM technique to observe the outer and inner structure of spray-dried particles containing core material (d-limonene emulsion) and the effect of emulsion droplet size and flavor retention (Figure 26.3).
- Transmission electron microscopy (TEM): TEM has been used to characterize morphology, particle size and shell thickness (Wang et al., 2013). In a recent study, Wang et al. (2013) successfully used TEM (freeze-fractured samples)

TABLE 26.3 Applicability of Various Microscopy Techniques Based on Size Range and Encapsulation Technology

		Physical Characterization				Particle Sizing	Glass Transition Temp. (T_g)	Degree of Crystallinity		
		Transmission Electron Microscopy (TEM) 0.2 nm–0.2 mm	*Scanning probe microscopy (SPM, AFM, etc.) 0.2 nm–0.2 mm*	*Scanning electron microscopy (SEM, ESEM) 4 nm–4 mm*	*Optical microscopy (OM) 200 nm–200 μm*	*Laser Light Scattering (LLS) 0.02–1000 μm*	*DSC/TGA 0.1–2000 μm*	*DSC 0.1–2000 μm*	*NMR 0.1–2000 μm*	*XRD NA*
Encapsulation technology	Spray drying 1–50 μm	+	+	+	+	+	+	+	+	+
	Spray chilling 20–200 μm	+	+	+	+	+	+	+	+	+
	Melt extrusion 200–2000 μm	−	−	+	+	+	+	+	+	+
	Fluidized bed 20–200 μm	−	−	+	+	+	+	+	+	+

Symbols: + = Feasible; ± = Challenging; − = Not possible.

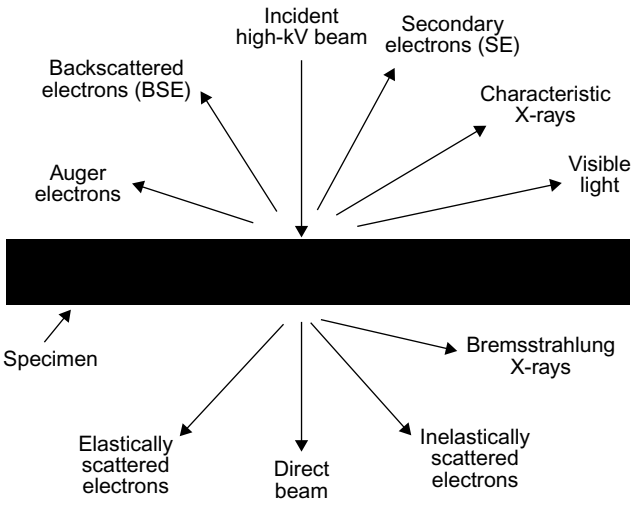


FIGURE 26.2 Schematic of the interaction of an electron beam with a solid specimen. *(Source: Williams and Carter, 2009; Reproduced with permission from Springer Verlag, New York, NY.)*

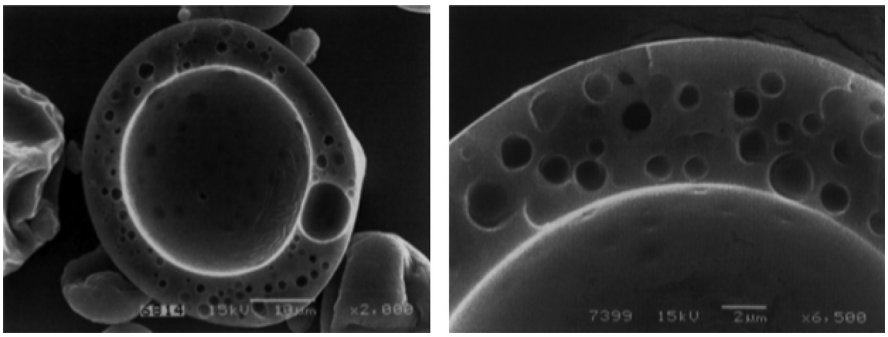


FIGURE 26.3 SEM micrographs of the surface and inner structure of spray-dried particles. *(Source: Soottitantawat et al., 2003; Reproduced with permission from John Wiley and Sons, Hoboken, NJ.)*

to characterize core−shell structures containing zein:lime oil or zein:citral and produced by evaporation induced self-assembly (Figure 26.4).

- SEM can be sometimes combined with energy dispersive X-ray spectroscopy (EDX) analyses to characterize elemental constituents of a sample. Its principle is based on the unique atomic structure of elements. Thus, to stimulate the emission of characteristic X-rays from a specimen (see Figure 26.2), a high-energy beam is focused into the sample that creates an EDX spectrum. Consequently, the frequency and energy level of the peaks are plotted; the higher a peak in a spectrum, the more concentrated the element is in the sample.
- Confocal laser scanning microscopy (CLSM): CLSM has become very popular over the past few decades since it affords visualization and characterization of both the inner structure and outer surface. This microscopic technique is based on point-by-point laser beam scanning of a fluorescent-labeled specimen (Lamprecht et al., 2000a,b) and enables a 3D image of specimen to be constructed using electronic-computer software. The CLSM technique was proven feasible for characterization of the cross-sectional structures of spray-dried powders of maltodextrin (DE = 2 and 20) when florescent markers were used to label the wall material (Soottitantawat et al., 2007). Yang et al. (2012a,b) used CLSM to study the internal structure of vanilla oil encapsulates, including oil distribution. The fluorescence ring of the microcapsules indicated that the vanilla oils were successfully encapsulated in the microcapsules by the complex coacervation process. This characterization tool (CLSM) was shown to be helpful for characterization of internal microparticles (Table 26.4) of whole milk powder, and it was also used to observe the protein structure in corn flour (Murrieta-Pazos et al., 2012).
- A wide array of micro-analytical techniques based on microscopy are available for the characterization of microparticles. A single characterization technique may not afford all the information needed for the improvement of processes and performance of flavor encapsulates, and, hence, assurance of quality and sustained stability to the end

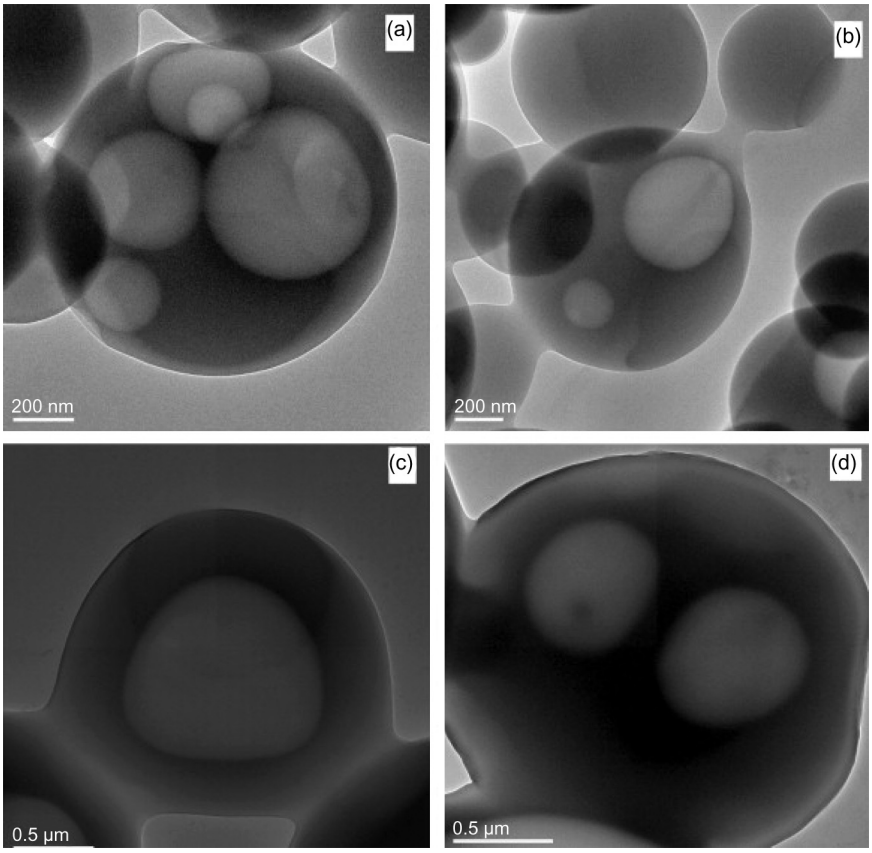


FIGURE 26.4 TEM micrographs of core–shell structures of (a) and (b) lime in zein and (c) and (d) citral in zein. *(Source: Wang et al., 2013; Reproduced with permission from Elsevier, Cambridge, MA).*

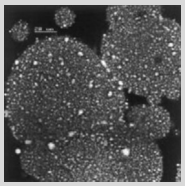
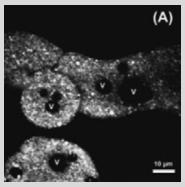
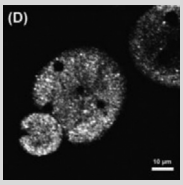
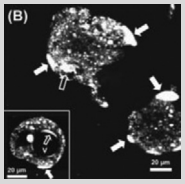
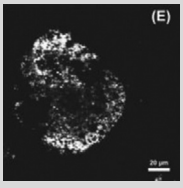
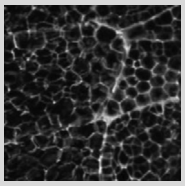
user/consumer. The combination of techniques could aid in the understanding of the impact and behavior of the properties–process variables relationship involved in encapsulation. For example, Soottitantawat et al. (2007) used both SEM and CLSM to study the internal and external morphology of spray-dried powders as a function of the process parameters (Figure 26.5a–f).

- X-ray micro-tomography (XMT)**:** XMT is a three-dimensional, non-destructive technique that enables visualization of the internal structure of a specimen (Perfetti et al., 2010). X-rays pass through the sample and according to the density and atomic number of an element they will be attenuated depending upon the X-ray energies used. Hence, cross-sectional slice images, which are obtained from different angles, can be used to reconstruct 3D images with high resolution of the internal structure of the object.
- Atomic force microscopy (AFM): AFM is a relatively recent technique that can be used to investigate the surface at the nanoscale/nanoemulsions (Klang et al., 2012; Murrieta-Pazos et al., 2012). It has been most often used to study the structures of biopolymers and food powders, and is especially useful for topographic surface determination. Sample preparation is needed and it may still be a challenge to interpret images. Murrieta et al. (2011) utilized AFM to characterize powder milks and evaluate the method's precision. SEM images of the same materials were in good agreement with those from AFM.

26.2.3 Particle Sizing Methods

Particle size and particle size distribution (PSD) are among the most important physical characteristics of microencapsulated flavors, and other kinds of food microparticles in general (O'Hagan et al., 2005). These are important characteristics of food matrixes (e.g., spreads), especially with respect to handling and storage. This information can be used to improve process design due to its inherent effect on flow, compaction, and/or segregation. There is a wide array of technologies that are used to measure particle size and PSD. These techniques can measure wet dispersed particles, dry dispersed particles, or both, and as for distribution, it could be either differential or cumulative. The choice of technique should be based on knowledge of materials used and information needed.

TABLE 26.4 Characterization of Food Powders by Confocal Laser Scanning Microscopy (Murrieta-Pazos et al., 2012)

Methods	Powder Systems	Instant Whole Milk Powder		
Spray drying		Whole milk powder		
		Milk model with low free fat		Milk model with low free fat after free fat extraction
		Milk model with high free fat		Milk model with high free fat after free fat extraction
Milling		Proteins in corn flour		

(Source: Murrieta-Pazos et al., 2012; Reproduced with permission from Elsevier, Cambridge, MA.)

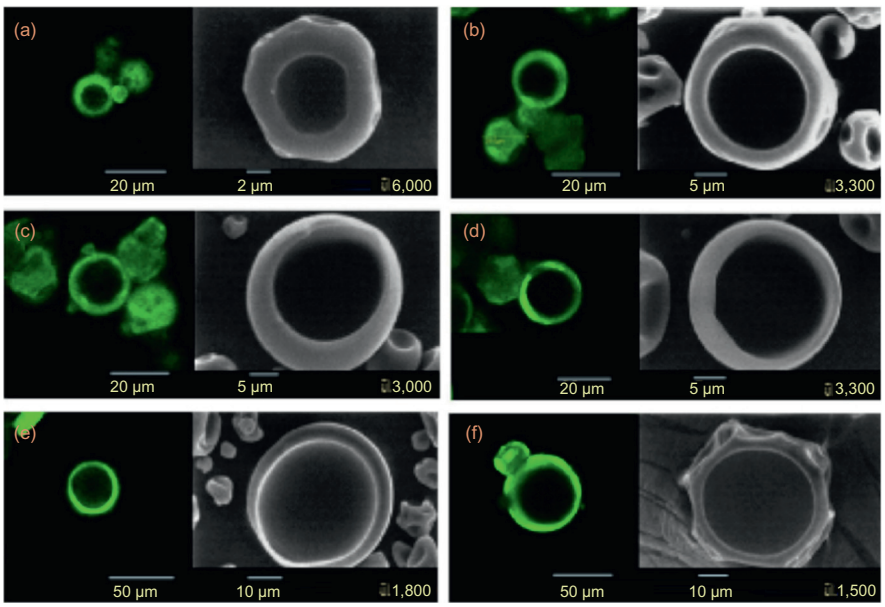


FIGURE 26.5 (a)–(f) SEM and CLSM images of inner and external particle morphology of spray-dried powder produced under different operating conditions. *(Source: Soottitantawat et al., 2007; Reproduced with permission from John Wiley and Sons, Hoboken, NJ.)*

TABLE 26.5 Characterization Methods of Mechanical Strength of Encapsulates

	Measured Properties	Technique/Equipment	Advantages	Disadvantages	References[a]
Indirect methods	Resistance to fluid shear force; percentage of intact encapsulated remaining; osmotic pressure	Cone and plate shearing apparatus	Less time-consuming	Difficult to interpret	Leblond et al. (1996); Peirone et al. (1998); Van Raamsdonk and Chang (2001)
Direct methods	Compression of a layer of encapsulates; elastic properties of single encapsulates	Two glass plates and weight; micropipette aspiration technique; atomic force microscope probe; texture analyzer; micro-manipulation technique	Practical and useful; relatively more accurate and reliable	More time-consuming	Chung et al. (2005); Ohtsubo et al. (1991); Sun and Zhang (2001, 2002); Zhao and Zhang (2004).

[a]*All of the references are for mechanical strength characterization of encapsulates, although flavors are not necessarily the active core materials in some cases.*

Laser light scattering (LLS) is the preferred method for the determination of the distribution of a large number of particles. The method measures particle size based on light scattering intensity, which changes with variation in scattering angle, size of particles, refractive indices of particles, and the medium. However, there are some limitations with this method. It is difficult to evaluate particles with a large size distribution, the particles should be spherical, and there should be a difference between particle refractive index and dispersion medium. Therefore, LLS is often combined with other techniques such as microscopy methods (OM, SEM, and TEM).

26.2.4 Mechanical Strength

Flavor encapsulates have considerable market potential and are widely used in the food industry. These encapsulates should have appropriate mechanical strength to fulfill various imaginative applications (Sun and Zhang, 2002). Also, mechanical strength is one of the most important required criteria for developing successful encapsulation technologies (King, 1995; Gharsallaoui et al., 2007).

There are two general methods used for determination of mechanical strength: direct and indirect (Zhang et al., 2010) (Table 26.5). Peirone et al. (1998) applied the indirect method to determine the mechanical strength of encapsulates by measuring the resistance to fluid shear force. A number of other studies made use of direct methodology because it provides relatively more accurate and reliable results. Ohtsubo et al. (1991) developed a classic method to determine the mechanical strength of encapsulates; a layer of encapsulates is compressed between two glass plates and the required force to break the encapsulate is based on a given weight on top of the plate (Figure 26.6). Yuliani et al. (2006a,b) examined the texture of an encapsulate extrudate using a direct three-point bend test, where the maximum force required to break the sample was measured. Patel et al. (2013) measured the mechanical strength by fracture force. It was noticed from results of the above studies that the larger particles always broke first followed by the smaller ones. For this reason, there is a need to quantify mechanical properties of a single encapsulate. Techniques such as micropipette aspiration and the atomic force microscope probe are capable of achieving this goal (Zhang et al., 2010).

However, the assessment of a single encapsulate is difficult when its size is in the μm range. In such cases, a micro-manipulation technique may be able to overcome this limitation (Sun and Zhang, 2002). The principle of this approach is to compress a single encapsulate between a probe and a glass plate while the probe is connected to a force transducer to which the data acquisition system is linked (Figure 26.7). Sun and Zhang (2002) applied this technique to determine the mechanical strength of encapsulates made of different wall materials: the melamine-formaldehyde (M-F) and urea-formaldehyde (U-F) encapsulates showed clear signs of bursting under compression, and the bursting force, deformation at bursting, and at pseudo-yield point were determined (Figure 26.8). To date, although not much work has been done on single encapsulates, the micro-manipulation technique is still believed to be a powerful tool to determine the mechanical properties of various kinds of encapsulates (Zhang et al., 2010).

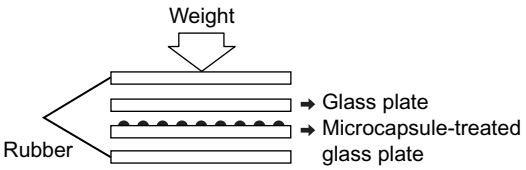


FIGURE 26.6 The schematic diagram of a direct method of mechanical strength characterization. *(Source: Ohtsubo et al., 1991; Reproduced with permission from Elsevier, Cambridge, MA).*

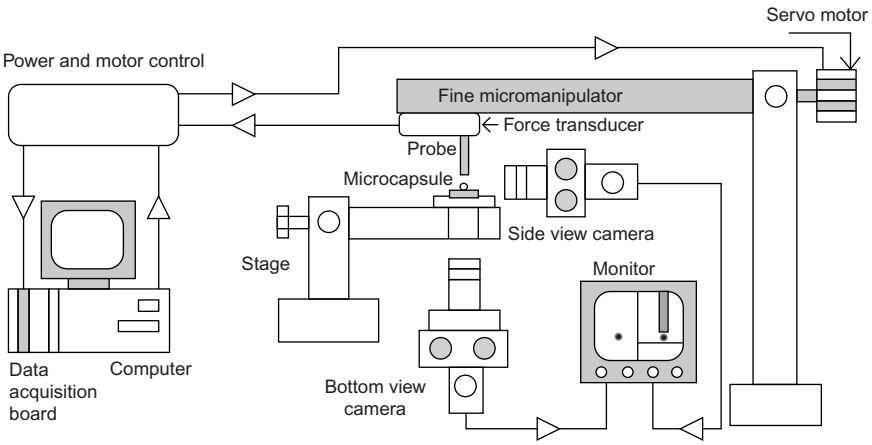


FIGURE 26.7 Schematic diagram of micromanipulation setup. *(Source: Sun and Zhang, 2002; Zhang et al., 2010; Reproduced with permission from Elsevier, Cambridge, MA.)*

26.2.5 Glass Transition Temperature and Degree of Crystallinity

In general, the structure crystalline or amorphous solids content of encapsulates is important relative to the stability of microencapsulates and can affect the material's flowability, dispersibility, and permeability (moisture sorption)—factors that can lead to caking. The glass transition temperature (T_g) and degree of crystallinity of wall materials can be used to help characterize the fine structure of encapsulates and can aid in design of encapsulates with specific functionalities.

T_g is an important property of wall materials, and is defined as the midpoint temperature at which a transition from a liquid to a glassy solid takes place during a drying process. Depending on the drying conditions (slow/fast), the structures formed could be crystalline, amorphous, or a mixture of the two. In general, the most desirable structure is crystalline, which consists of tightly packed molecules, which limits their interaction with environmental factors such as water. Below the T_g, the behavior of a material is similar to that of a glassy state and, therefore, more stable. Meanwhile, above the T_g, the material's behavior is similar to that of a rubbery state, and consequently molecule diffusion is faster. Information about the T_g can aid in the determination of the storage conditions and shelf-life stability of an encapsulate. Two types of thermo-analytical techniques usually are used to determine the T_g of wall materials: (1) thermo-gravimetric analysis (TGA) and (2) differential scanning calorimetry (DSC). DSC, along with X-ray diffraction (XRD) and nuclear magnetic resonance (NMR) techniques, can also be used to determine degree of crystallinity. Although both TGA and DSC can provide a measure of T_g, the two methods differ in how this is accomplished. TGA measures difference, in this case weight changes of the materials as they undergo heating rate. Meanwhile, DSC measures the heat flow difference between sample and a reference. The difference in the amount of heat required to increase the temperature of a sample and a reference are measured. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. DSC may be used to create heating profiles that give valuable information about the melting, temperature transition, and solidification properties and can also be used to determine crystallization profiles (degree of crystallization), which is the temperature the crystals start to form (Lai et al., 2000; Saadi et al., 2012). As mentioned above, XRD can be used to determine the molecular orientation in a lattice structure when X-rays are diffused through a crystal and can measure degree of crystallinity and crystal polymorphs.

Provided below are a number of examples where researchers have determined the T_g and degree of crystallinity in encapsulates using the above-mentioned methods:

- Aghbashlo et al. (2012) utilized DSC to analyze chemical interactions between spray-dried encapsulated fish oil and wall material (three different formulas) in the microstructure. Results revealed no significant differences and no interaction of the core and wall material.

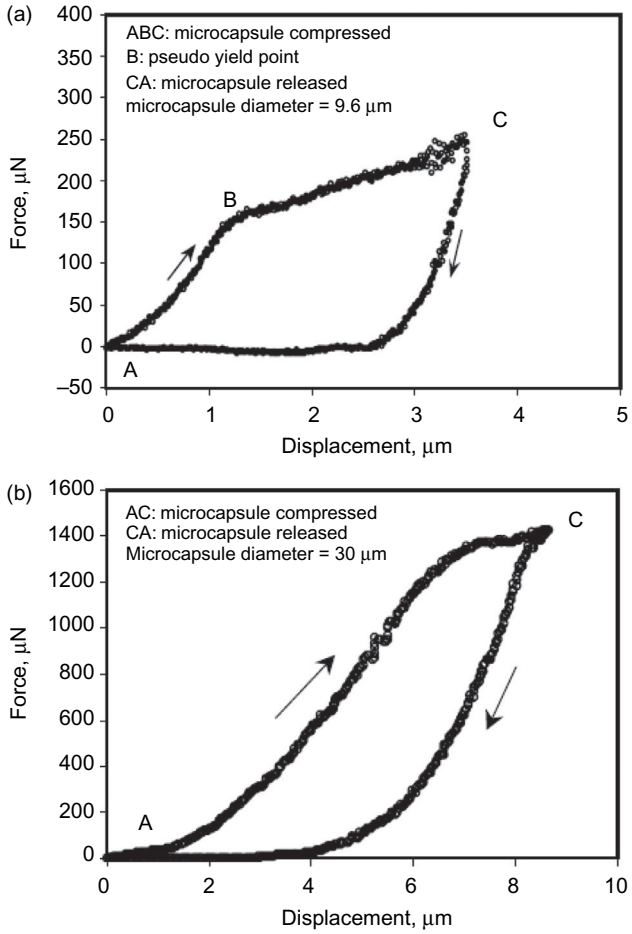


FIGURE 26.8 Force versus displacement curve when a single microcapsule was compressed to a relatively large deformation and then released. The compression speed was 1 m/s. (a) A melamine-formaldehyde resin encapsulate; (b) a urea-formaldehyde encapsulate. *(Source: Sun and Zhang, 2002; reproduced with permission from Elsevier, Cambridge, MA.)*

- DSC combined with the XRD analysis (Sato and Ueno, 2011) was used to study the polymorphic structures of natural fats. The analyses showed polymorph-dependent crystallization kinetics and indicated the appropriate rate of cooling necessary to enhance the desired crystalline structure.
- Saadi et al. (2012) utilized DSC, high performance liquid chromatography (HPLC) and proton-NMR for interpretation of the primary crystallization caused by combined low and high melting triacyl glycerides (TAGs). TAGs can be used as wall materials for encapsulation of hydrophilic flavor compounds by the spray chilling technique. Any change to the polymorphic structures of TAGs could alter the stability of the flavor encapsulates.

26.2.6 Flowability

The surface composition of encapsulates can have a great impact on particle−particle interactions, which can subsequently affect particle size, shape, bulk density, and chemical composition and lead to either good flowability or otherwise cause an adverse outcome (stickiness-T_g,/polymorphism). Flowability is an important property of food powders (Barbosa-Canovas and Juliano, 2005; Fitzpatrick, 2005; Ortega-Rivas, 2005); it affects processes (mixing, conveying), handling (transportation), and storage (silos) and it may be the cause of chemical/economical loss. Flavor encapsulates may be thought of as food powders and flowability may also affect their performance and stability. Therefore, it is important to characterize the flowability of encapsulates.

In general, flowability is a characteristic that is mainly measured by the Jenike shear test, which is considered the most accurate method (Fitzpatrick, 2005). It was developed in 1964 and although it is mainly used in food powders, it

could also be applied to flavor encapsulates. Although the results of this method have aided in the design of silos and processes, its application to flavor encapsulates is very limited.

26.3 CHEMICAL CHARACTERIZATION

26.3.1 Gas Chromatography and High Performance Liquid Chromatography

Gas chromatography (GC) is generally performed on all incoming (volatile) raw materials (e.g., purity, adulteration) and some finished aroma encapsulates (e.g., storage stability, encapsulation efficiency). On the other hand, high performance liquid chromatography (HPLC) has traditionally found little use with respect to flavor analysis, except in the case of some non-volatile components such as capsaicins, colorants, savory enhancers (MSG or the 5′-nucleotides), salts, or other flavor adjuncts (Reineccius, 2006). There also has been some use of HPLC for detecting adulteration in flavor ingredients. McHale and Sheridan (1988) reported the use of HPLC to detect the adulteration, as indicated by presence of ethyl p-dimethylaminobenzoate, of lemon oil on the UK market.

GC has been used for evaluation of volatile release as well as encapsulation efficiency. Whorton and Reineccius (1995) utilized the gas chromatographic (GC) static headspace method to evaluate flavor diffusion over time as a function of volatile molecular weight and the rate of flavor exhaustion from the encapsulated powders from maltodextrin matrices. Bohn et al. (2005a) evaluated the physicochemical changes by utilizing differential scanning calorimetry (DSC), dynamic vapor sorption (DVS) and DSC (DVS-DSC), and DVS-fast GC-flame ionization detection (DVS-fast GC-FID). Specifically, DVS-fast GC-FID was used to evaluate the volatile release over time that occurred in an artificial Cherry Durarome® when exposed to different humid environments. Bylaite and Venskutonis (2001) utilized GC-FID to determine storage stability and surface oil content, and dynamic headspace analysis (DHS) to determine release of volatiles of caraway (*Carum carvi* L.) essential oil encapsulated into milk protein-based matrices.

26.3.2 Flavor Active Dispersion

Flavor active dispersion is an important feature because it characterizes how an active is distributed inside and outside of the microcapsules and how it interacts or affects the physical properties, stability, and release mechanisms of an encapsulated flavor. Usually, the characterization of the flavor active dispersion is done in tandem with the quantification of the amount of oil or investigation of the microstructure.

Overall, the flavor active dispersion consists mainly of two aspects, one of them being distribution of active in the inside versus on the outside of the microcapsules (Figure 26.9), for which the internal oil and surface oil are usually applied as parameters to indicate the flavor distribution. The total oil is made up of surface oil plus internal oil; in other words, the internal oil content could be calculated by subtracting the surface oil from the total oil content. The total oil can be determined by distillation (Baranauskiené et al., 2006; Bringas-Lantigua et al., 2011; Chen et al., 2013), extraction and evaporation (McNamee et al., 1998, 2001; Chen et al., 2013; Li et al., 2013), GC analysis (Ko et al., 2012), and absorbance measurement (Ramoneda et al., 2011; Li et al., 2013). The surface oil was determined by washing, extraction, and evaporation, and followed by GC and GC-MS (Baranauskiené et al., 2006, 2007; Paramita et al., 2012), by gravimetric methods (McNamee et al., 1998, 2001; Badee et al., 2012; Goula and Adamopoulos, 2012), and by measuring absorbance (Li et al., 2013). Typically, the microcapsules are washed with a fixed amount of solvent to extract the surface oil (Bringas-Lantigua et al., 2011; Penbunditkul et al., 2012), sometimes with addition of an internal

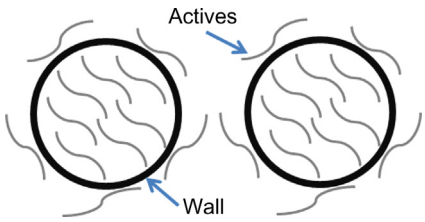


FIGURE 26.9 Flavor active dispersion inside versus outside of an encapsulate.

standard (Kalogeropoulos et al., 2010), prior to GC analysis. The flavor distribution inside and outside of the microcapsules is related to encapsulation efficiency via Eq. 26.1:

$$\text{Encapsulation efficiency} = \frac{\text{Total oil} - \text{Surface oil}}{\text{Total oil}} \times 100\% = \frac{\text{Internal oil}}{\text{Internal oil} + \text{Surface oil}} \times 100\% \quad (26.1)$$

Another aspect of flavor dispersion relates to the flavor profile within the microcapsules, as shown in Figure 26.10. The two main types of dispersions include the reservoir type, in which the active exists as a single core within the wall material (Figure 26.10), and the matrix type, in which the active agent is dispersed in the carrier material in the form of small droplets (Figure 26.10) (Uhlemann et al., 2002; Fang and Bhandari, 2010; Zuidam and Shimoni, 2010). There are a number of factors that affect the flavor dispersion within the microcapsule, the processing methods and parameters, properties of the core and wall materials, and the properties of the active agent. Usually, the method of production determines whether the encapsulate is a reservoir type or matrix type. For example, the morphology of spray-dried, freeze-dried, emulsified, and extruded encapsulates are normally matrix type, while coacervated encapsulates are reservoir type (Table 26.6). However, the processing parameters usually determine to what degree the active disperses for a certain morphology type. For example, increasing the drying air temperature in spray drying has a greater tendency to produce hollow particles (Walton, 2000), and, therefore, influences the distribution of active droplets (Figure 26.10).

To characterize the flavor dispersion profile within encapsulates, normally the processing methods are considered first, and then appropriate microscopy methods are used to indicate the morphology (Table 26.6). These characterizing technologies should be considered only as qualitative analysis tools for determination of the active dispersion inside the microcapsule. For the reservoir type, optical microscopy (OM) could fulfill the requirement of identifying the active dispersion. Hsieh et al. (2006) observed citronella oil embedded inside chitosan microcapsules using orifice processing under OM; the results indicated that good formation and dispersion of microcapsules was obtained using the combination of 0.5 wt% chitosan, 0.5 wt% NaOH, and 0.5 wt% natural coconut oil (Figure 26.11a). In fields other than flavor science, for instance cosmetics, OM has been a useful tool to characterize the distribution of actives. Fairhurst and

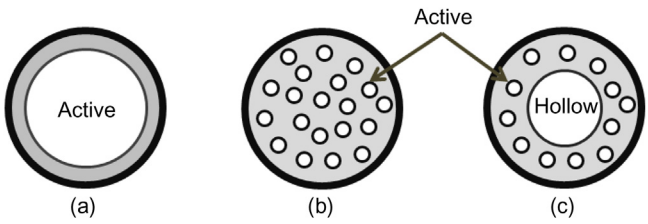


FIGURE 26.10 Flavor dispersion inside encapsulates: (a) reservoir type, (b) matrix type, (c) matrix type with hollow (only spherical-shaped encapsulates are shown here, but other shapes are also possible).

TABLE 26.6 Characterization Methods for Profiling of Flavor Dispersions Inside Encapsulates

Morphology	Processing Technologies	Characterization Methods	References	Example Figure
Reservoir type	Orifice method	Optical microscopy	Hsieh et al. (2006)	Figure 26.11a
	Coacervation	Optical microscopy	Fairhurst and Loxley (2008)	Figure 26.11b
Matrix type	Spray drying	SEM	Edris and Bergnståhl (2001);	Figure 26.11c
			Shah et al. (2012);	
			Soottitantawat et al. (2005)	
	Freeze drying	SEM	Kaasgaard and Keller (2010)	Figure 26.11d
	Extrusion	SEM	Fieber et al. (2011);	Figure 26.11e
			Zuidam and Shimoni (2010)	
Matrix type with hollow	Spray drying	SEM	Kim and Morr (1996); Paramita et al. (2012); Soottitantawat et al. (2005).	Figure 26.11f

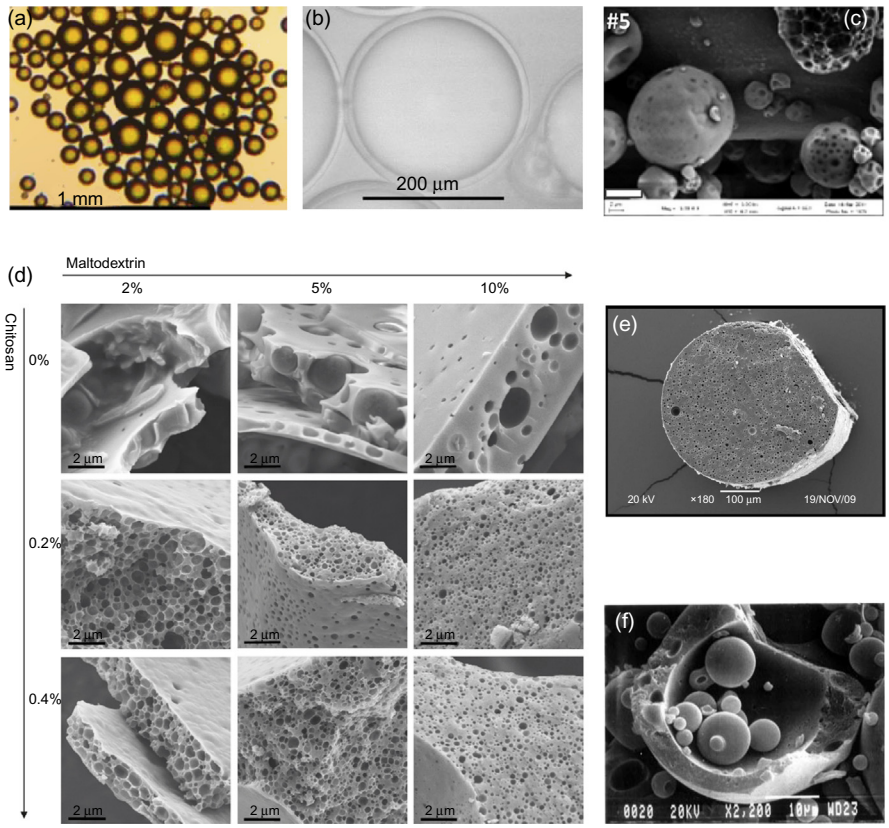


FIGURE 26.11 Images of active dispersion within the microcapsules: (a) optical microphotographs of chitosan microcapsules prepared at concentrations of 0.5 wt% chitosan and 1.0 wt% NaOH at 800 rpm stirring rate ($\times 80$) (Hsieh et al., 2006); (b) optical micrograph of gelatin–acacia microcapsule prepared by coacervation (Fairhurst and Loxley, 2008); (c) SEM micrographs of eugenol capsules prepared by spray drying of emulsions (Shah et al., 2012); (d) SEM micrographs showing the interior structure of freeze-dried chitosan-coated carvone emulsions containing different amounts of chitosan and maltodextrin (Kaasgaard and Keller, 2010); (e) SEM images of an orange oil-glassy encapsulation system prepared by incorporating natural orange oil into a sucrose–maltodextrin melt (Fieber et al., 2011); (f) SEM micrographs of soy protein isolate microencapsulated orange oil particles (Kim and Morr, 1996). *(Reproduced with permissions from Elsevier, Cambridge, MA, Allured Publishing Corporation, Carol Stream, IL, and ACS Publications, Washington, DC.)*

Loxley (2008) characterized gelatin–acacia microcapsules prepared by coacervation by OM and the shell and core materials were clearly indicated (Figure 26.11b). For the matrix type, OM and SEM are commonly used as qualitative analysis tools to indicate the flavor dispersion. Shah et al. (2012) produced SEM microstructure graphs of eugenol embedded in spray-dried microcapsules coated with whey protein isolate and maltodextrin—the active dispersion is shown in Figure 26.11c. Fieber et al. (2011) prepared the glassy encapsulation system by incorporating natural orange oil into a sucrose–maltodextrin melt and characterized the extrudates by SEM. They observed that the oil droplets (which do not deviate significantly from being spherical) were dispersed in the solid matrix discretely and the droplet size distribution was multimodal (Figure 26.11e). Kim and Morr (1996) encapsulated orange oil with soy protein isolate and characterized the oil dispersion by SEM. The results indicated that the spray-dried microencapsulated particles were hollow spheres with porous walls that were less than 10 μm in thickness (Figure 26.11f).

26.3.3 Flavor Retention and Stability

26.3.3.1 Flavor Retention

Flavor plays an important role in food quality and it greatly influences consumer acceptance of food products. Significant attention has been paid to flavor retention, since the presence of air, light, moisture, and high temperature are major factors responsible for loss of volatile flavors (Given, 2009; Ma et al., 2013). Also, considerable effort has gone into the improvement of flavor retention, including development of better encapsulation techniques (Sadafian and Crouzet, 1988; Kollengode and Hanna, 1997; Yuliani et al., 2006a,b).

Flavor retention is defined as the mass ratio of total flavor (oil) in the powder to the theoretical quantity in the powder assuming ideal retention (Finney et al., 2002; Charve and Reineccius, 2009; Penbunditkul et al., 2012; Ma et al., 2013) (Eq. 26.2); in other words, it accounts for the flavor losses during the production of encapsulates.

$$\text{Flavor Retention (\%)} = \frac{\text{Total flavor in encapsulates}}{\text{Flavor in the feed mixture}} \tag{26.2}$$

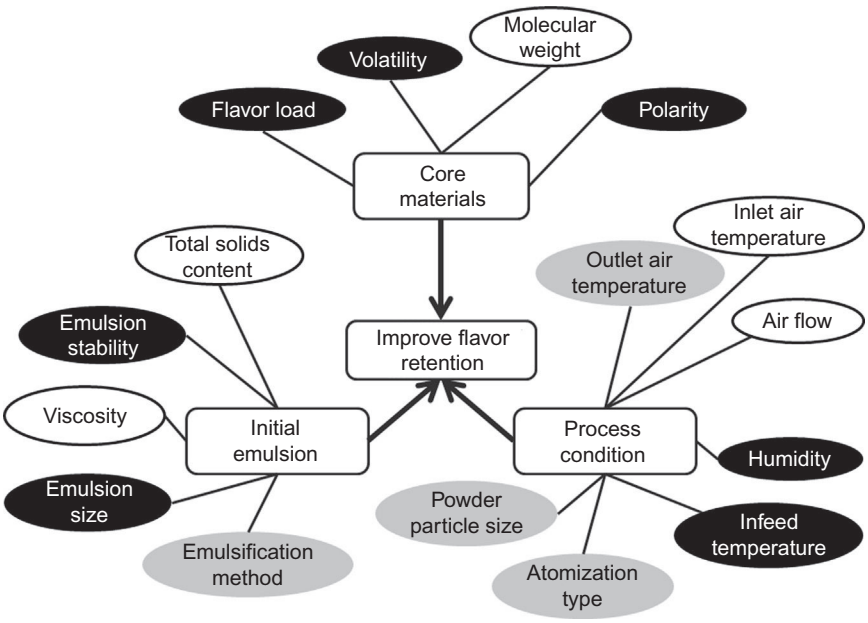


FIGURE 26.12 Factors influencing flavor retention: the general trend is that properties in the white ellipses positively correlate, while those of the black ellipses negatively correlate with retention. *(Summarized from Jafari et al., 2008 and Reineccius, 1989.)*

The total flavor in encapsulates has been determined by various techniques, including distillation (Baranauskiené et al., 2006; Bringas-Lantigua et al., 2011; Chen et al., 2013), extraction and evaporation (McNamee et al., 1998, 2001; Chen et al., 2013; Li et al., 2013), GC analysis (Ko et al., 2012), and absorbance measurements (Ramoneda et al., 2011; Li et al., 2013). Equation 26.2 is then used to calculate flavor retention based on the flavor load of the feed mixture. Some examples are given below. Bringas-Lantigua et al. (2011) determined total mandarin oil content by Clevenger distillation, where the volume of the distilled oil was read from the collection arm and converted to mass by multiplying by the density of the oil. Oil retention was found to be within 90–96%. McNamee et al. (1998, 2001) determined the total oil content of the encapsulated powders by a modification of the Mojonnier and Röse Gottlieb methods; the flavor oil was extracted with diethyl ether and petroleum ether, the ethers were evaporated, and the residues were weighed to give total oil. Ko et al. (2012) measured total allyl isothiocyanate retention by dispersing the encapsulate powder in distilled water, followed by the addition of diethyl ether with phenyl isothiocyanate as an internal standard; the organic phase and water were then centrifuged to separate the layers, and the flavor content of the organic phase was measured by GC—the highest retention reached was 84.66%. Ramoneda et al. (2011) measured total β-carotene in the hexane phase spectrophotometrically at 452 nm.

There are many important determinants of flavor retention. Taking spray drying as an example processing method for encapsulation, the factors that affect the flavor retention significantly could be properties of the core materials, initial emulsion composition, and conditions of the drying process (Jafari et al., 2008) (Figure 26.12). There are general trends that we could follow before design of an encapsulation system, for example, as the molecular weight of the core material increases, the flavor retention increases (Rosenberg et al., 1990; Voilley, 1995). These same trends were found for emulsion viscosity and air flow. On the other hand, as volatility and polarity of the core material increases, the retention will usually decrease (Bangs and Reineccius, 1982; Ré, 1998). However, there is always an optimal with respect to composition and physical/chemical properties for any encapsulant. If we take total solids content of the initial emulsion as the example, although high solids content increases the retention by reducing the time required to form the membrane at the particle surface, when the solids exceed the solubility, the undissolved wall materials cannot provide an encapsulating effect and this therefore leads to lower retention (Jafari et al., 2008). For more detailed information of improving flavor retention, one should refer to the publications of Jafari et al. (2008) and Reineccius (1989).

26.3.3.2 Flavor Stability

The stability of encapsulated flavors from the powder is important for estimating the shelf-life of the flavor and for controlled release applications in food (Anandaraman and Reineccius, 1986; Whorton and Reineccius, 1995; Bertolini et al., 2001; Yoshii et al., 2001; Soottitantawat et al., 2004, 2005). In previous works, stability of encapsulated flavor has been defined as the release and the oxidization of actives (Soottitantawat et al., 2004, 2005).

26.3.3.3 Characterization of Flavor Release: Methods, Rates, and Mechanisms

Release of flavor continues to be the key issue in selecting an appropriate encapsulation system for various applications. The rational design of encapsulation systems requires a physicochemical understanding of the mechanisms from which components are released (Whorton, 1995; Whorton and Reineccius, 1995; Gunning et al., 1999). The well-controlled release of ingredients is one of the most important properties of encapsulates (Shahidi and Han, 1993; Baranauskiené et al., 2007). Generally, the moisture content of encapsulates, storage relative humidity, and temperature affect the release rate of flavors (Gunning et al., 1999). Some review papers or chapters relevant to flavor release have been published and more detailed information is available elsewhere (Versic et al., 1988; Shahidi and Han, 1993; Madene et al., 2006; Zhang et al., 2010).

Usually two methods are applied to characterize the flavor release of encapsulates. The first is the measurement of the headspace release of flavor volatiles. GC is the most commonly used method to quantify the release of volatile flavor components into the headspace from the encapsulated flavor systems (Kim and Morr, 1996; Gunning et al., 1999; Marcuzzo et al., 2010). Kim and Morr (1996) developed a modified dynamic headspace analysis by collecting orange oil volatiles using helium as the purge gas, followed by GC analysis. Results showed that the gum arabic-microencapsulated particles had the highest volatile release rate among all the wall materials studied. In order to obtain frequent or real-time volatile release data other types of analyses techniques must be used. DVS-fast GC-FID was developed to quantify volatile release in a constant relative humidity and temperature environment (Bohn et al., 2005a, b). Over time the humidified air containing the volatile compounds was expelled from the back of the DVS and conveyed to the fast-GC-FID for quantification via the sampling line (Figure 26.13). Besides GC, other possible flavor release quantification methods include melting enthalpy (Ponce Cevallos et al., 2010), e-nose (Rodríguez et al., 2013), liquid-gas chromatography (Fabra et al., 2012), and mass spectrometry (Mortenson and Reineccius, 2008).

Another approach to measure flavor release it to determine the residual flavor by solvent extraction of encapsulates followed by GC analysis (Soottitantawat et al., 2004, 2005; Ko et al., 2012). The corresponding experimental setup is shown in Figure 26.14; the powder is spread in a thin layer in a glass bottle and stored in a desiccator while the relative humidity is controlled. The bottles are then removed from the desiccator to extract and measure the residual amounts of d-limonene at fixed time intervals and the release rate is determined using Avrami's equation (Eq. 26.3):

$$R = \exp[-(kt)^n] \tag{26.3}$$

where R is the retention of d-limonene, t is the storage time, k is the release rate constant, and n is a parameter representing the release mechanism.

26.3.3.3.1 Release Rates

For a single microcapsule, the flavor release rates can be zero, half, or first order. Usually, zero order occurs in the reservoir type when the core is a pure flavor; half order release rate generally occurs with matrix particles; and the first order release rate is observed when the core material is a solution. For a mixture of microcapsules, the release rate will differ from zero, half, or first order due to the ensemble of microcapsules, which vary in particle size and wall thickness (Versic et al., 1988; Shahidi and Han, 1993). Zhang et al. (2010) presented the relationship between flavor release and time, as shown in Figure 26.15.

26.3.3.3.2 Mechanism of Release

The release mechanisms fit into several categories: release by physical rupture, by diffusion, by dissolution or melting, and by biodegradation, as summarized in Figure 26.16.

26.3.3.3.2.1 Release by Physical Rupture The wall of encapsulate can be fractured or broken by external forces like pressure and shearing, or by internal forces due to the selective permeability. Encapsulates made from fats or waxes are insoluble in water and the flavor could be released by physical rupture such as chewing. Also, the active core could be released by the application of a swelling agent. The physical-fractured release is complete in a relatively short time compared to other release mechanisms (Shahidi and Han, 1993).

26.3.3.3.2.2 Release by Diffusion Diffusion is the predominant mechanism in controlled release from an encapsulation system (Cussler, 1997; Madene et al., 2006). This process is driven by concentration gradients and by permeability of the active through the carrier materials (Shahidi and Han, 1993).

There are two major mechanisms of diffusion in an encapsulation system. One is molecular or static diffusion caused by the random movement of the molecules in the stagnant fluid, where the rate of molecular diffusion varies only slightly

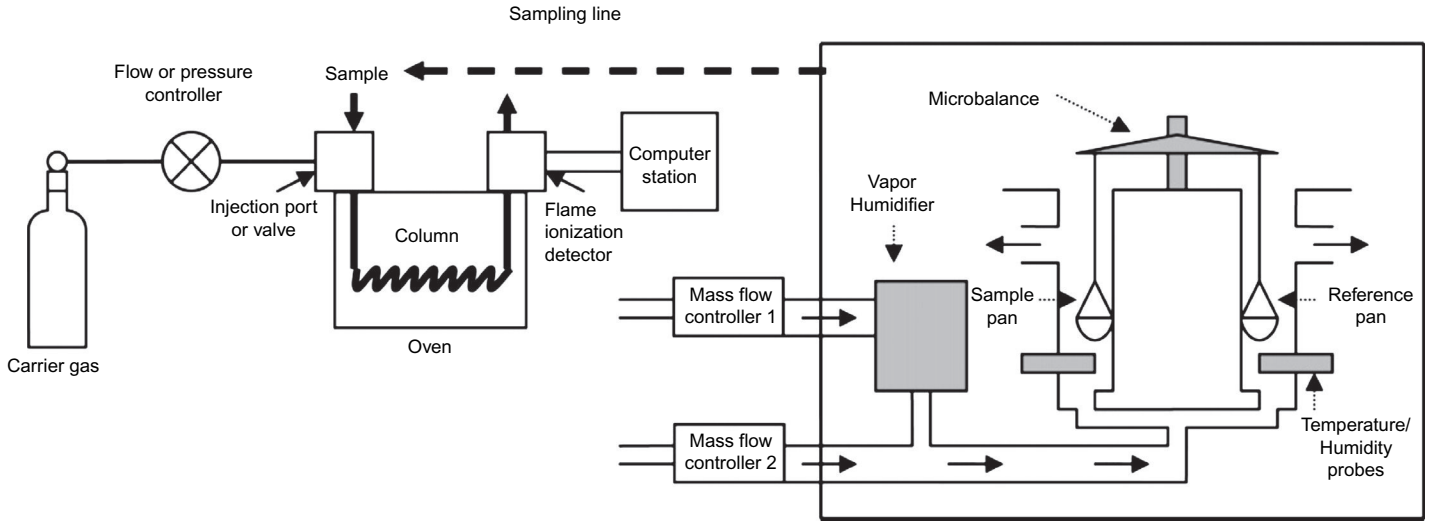


FIGURE 26.13 Schematic diagram of DVS-fast GC system. The components on the left comprise the fast-GC FID system. The components on the right comprise the DVS system. *(Source: Bohn et al., 2005b; Reproduced with permission from ACS Publications, Washington, DC.)*

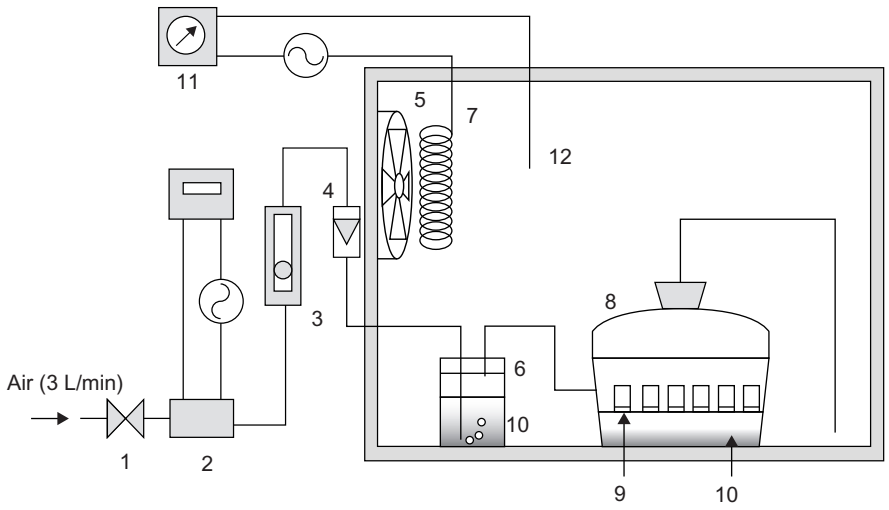


FIGURE 26.14 Experimental equipment for measuring the release and oxidation of encapsulated d-limonene and moisture sorption isotherms of the spray-dried powder. 1. Flow control valve, 2. air pump, 3. flow meter, 4. valve, 5. fan, 6. bubbling bottle, 7. heater, 8. desiccator, 9. sample powder, 10. saturated salt solution, 11. temperature regulator, 12. thermostat. *(Source: Soottitantawat et al., 2004; reproduced with permission from ACS Publications, Washington, DC.)*

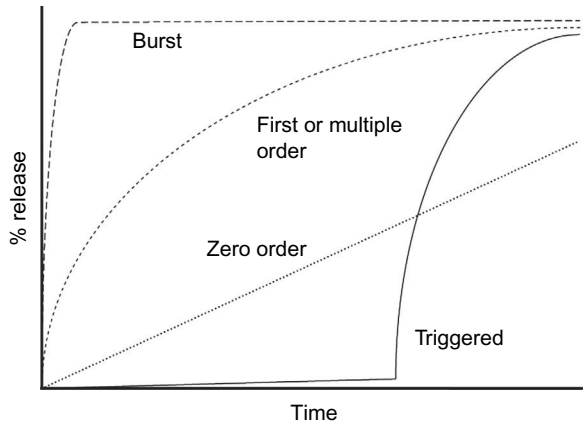


FIGURE 26.15 Release profiles from encapsulates. Burst release is typical for soluble or broken ones. Release might also be triggered after a certain period of time by, e.g., pH change or addition of enzymes. Zero-order release is obtained with oversaturated amounts of active in the core or with microcapsules where the thin shell is the rate limiting step. First- or multiple-order release is common for matrix type of encapsulates and release due to other interactions. *(Source: Zhang et al., 2010; reproduced with permission from Springer-Verlag, New York, NY.)*

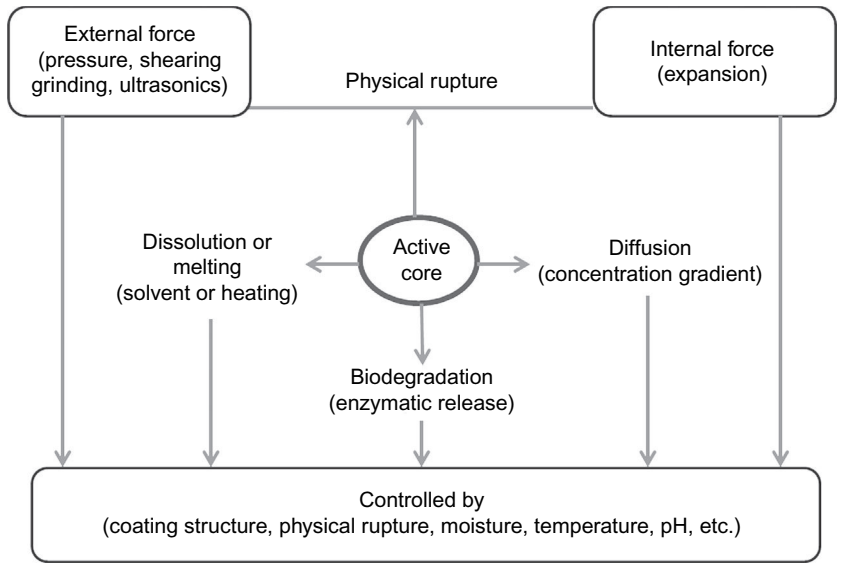


FIGURE 26.16 Release mechanisms of encapsulated flavors, modified from Shahidi and Han (1993). *(Reproduced with permission of Taylor and Francis, New York, NY.)*

with flavor type. The second mechanism is eddy or convective diffusion. The rate of eddy diffusion is usually much higher than that of static diffusion; also, it is independent of the flavor type (de Roos, 2003; Madene et al., 2006).

The diffusion also depends on the size, shape, polarity, and molecular weight of the active molecules, and the conformation and chemical composition of the coating materials, as well as attractive forces such as hydrogen bonding and van der Waals interactions, degree of cross-linking, and the amount of crystallinity (Shahidi and Han, 1993). In general, diffusion through a carrier material decreases with increasing molecular size, decreasing volatility, and increasing log *P* of the flavor compounds (Goubet et al., 1998). When an amorphous coating material is in a glassy state, molecules may have little relative mobility, and the diffusion increases when the coating reaches the rubbery state (Gunning et al., 1999; Ubbink and Schoonman, 2003; Soottitantawat et al., 2004).

26.3.3.3.2.3 Release by Dissolution or Melting The integrity of the coating can be destroyed by dissolution in an appropriate solvent or by melting with heat. Water-soluble coatings can be easily dissolved by increasing the moisture in the systems. For insoluble coatings, thermal release, e.g., baking, is a common release mechanism. A multitude of coating materials are available that could be used for these types of encapsulates, including lipids, modified lipids, or waxes. The particles are usually stored at temperatures below the melting point before subjecting them to the controlled release condition (Sparks et al., 1995; Madene et al., 2006).

26.3.3.3.2.4 Release by Biodegradation Release from microcapsules can also be accomplished by biodegradation processes (Shahidi and Han, 1993). For example, lipid coatings may be degraded by the action of lipases.

26.3.3.4 Oxidation

Many flavorings are susceptible to oxidation and readily develop off-notes during storage, such as orange oil and limonene (Soottitantawat et al., 2004, 2005; Chen et al., 2013). The oxidation stability of encapsulated flavors is important for estimating the shelf life of the product and applications in the food industry. The major determinants of oxidative stability are the carrier choice, type of volatile compounds, powder particle size, dryer inlet and outlet temperature, and water activity (Figure 26.17); more detailed information regarding oxidative stability can be found elsewhere (Reineccius, 1989). Chang et al. (1988) reported that larger particle size exhibited a more protective effect against oxidation. Anker and Reineccius (1988) and Beristain et al. (2002) showed that generally the oxidation rates decrease with an increase of water activity in the range of 0.001−0.536 and 0.108−0.628, respectively. However, Hardas et al. (2002) observed contradictory results for the effects of water activity on flavor oxidation compared with the studies by Anker and Reineccius (1988) and Beristain et al. (2002). Beristain et al. (2002) and Gunning et al. (1999) mentioned that the oxidation of the encapsulated flavors has been related to collapse and crystallization. A better understanding of the effect of storage conditions on the oxidation stability would be useful in quality control and future applications (Soottitantawat et al., 2004).

To characterize the oxidation levels, the flavor oxides are usually chosen as indicators and GC is the preferred method of analysis. Limonene oxide and carvone are commonly used indicators of oxidation in the case of citrus oils (Kim and Morr, 1996; Soottitantawat et al., 2004, 2005; Charve and Reineccius, 2009) (see Figure 26.17).

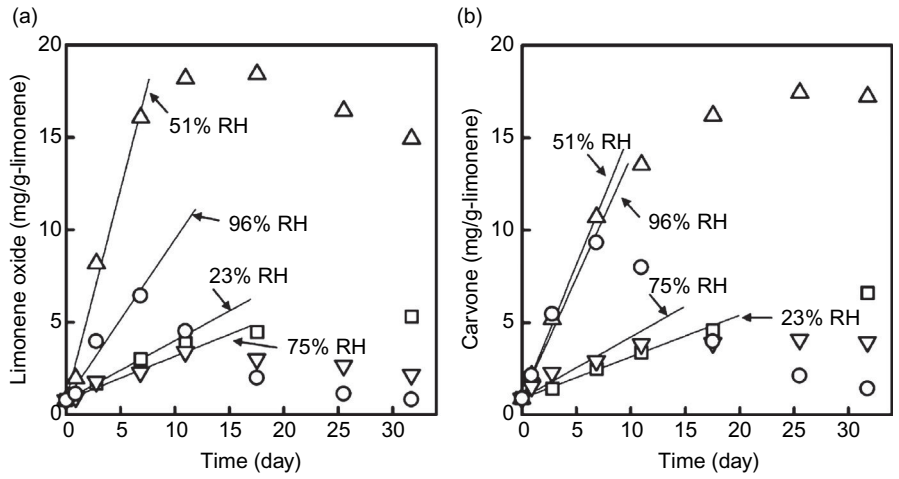


FIGURE 26.17 Formation time-course for limonene oxide and carvone in a spray-dried powder stored under various humid conditions at 50°C with HI-CAP 100 used as the wall material: (a) limonene oxide, (b) carvone. □, 23% RH; △, 51% RH; ▽, 75% RH; O, 96 % RH. *(Source: Soottitantawat et al., 2004; reproduced with permission from ACS Publications, Washington, DC.)*

26.3.4 Safety Testing

26.3.4.1 Toxicology

All food additives including encapsulated flavors must undergo a rigorous scientific safety evaluation to assure the non-toxicity before they can be approved for use (Wandrey et al., 2010). The safety of the encapsulates should be checked before use in human diet; usually, animal tests in terms of measuring changes in some clinical pathological parameters are considered to be appropriate methods to verify the safety of products.

A range of different types of information are required by the US Food and Drug Administration (FDA) for a petition for use of a new food ingredient. These include chemical data (chemical identity, purity, and other specifications), environmental effects that may result from the use of the material, estimated daily intake (EDI), acceptable daily intake (ADI), etc. (Rulis and Levitt, 2009). FDA requires considerable data to support the safety assessment of an ingredient with considerable population exposure, as shown in Table 26.7. Badee et al. (2012) conducted short-term toxicity studies with mice that revealed that feeding a diet containing encapsulated peppermint oil did not significantly affect liver function, or glucose and cholesterol levels; however, creatinine content was slightly increased after feeding. The study

TABLE 26.7 Toxicological Testing of Food Ingredients (US Food and Drug Administration)

Study Number	Toxicity Studies	Recommended Tests
1	Short-term tests for genetic toxicity	A test for gene mutations in bacteria; *in vitro* test with cytogenetic evaluation of chromosomal damage using mammalian cells; an *in vitro* mouse lymphoma thymidine kinase ± gene mutation assay; an *in vivo* test for chromosomal damage using mammalian hematopoietic cells
2	Acute oral toxicity tests	Limit tests; dose-probing tests; up-and-down tests; pyramiding tests
3	Short-term toxicity studies with rodents	Ophthalmological examination; hematology; clinical chemistry; urinalyses; neurotoxicity screening/testing; immunotoxicity
4	Short-term toxicity studies with non-rodents	Same as 3
5	Subchronic toxicity studies with rodents	Same as 3
6	Subchronic toxicity studies with non-rodents	Same as 3
7	Chronic toxicity studies with rodents	Ophthalmological examination; hematology; clinical chemistry; urinalyses
8	One-year toxicity studies with non-rodents	Same as 3
9	Carcinogenicity studies with rodents	Same as 7
10	Combined chronic toxicity/carcinogenicity studies with rodents	Consulting with the FDA before conducting a combined study
11	*In utero* exposure phase for addition to carcinogenicity studies or chronic toxicity studies with rodents	Same as 7
12	Reproduction studies	Optional neurotoxicity screening; optional immunotoxicity screening; gross necropsy and microscopic examination
13	Developmental toxicity studies	Examination of dams and fetuses; histopathology
14	Neurotoxicity studies	Characterization of the neurotoxic effects; determination of dose–response relationships
15	Others	Metabolism and pharmacokinetics; neurotoxicity; immunotoxicity

Summarized from Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook (2007), accessed via http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM222779.pdf on Feb 12, 2014.

TABLE 26.8 Analysis Procedures for Bacteria used as Food Safety Indicators

Microorganism	Analysis Procedures
Escherichia coli and coliform group	Controls
	Dry rehydrated film count (*E. coli*/coliform Petrifilm) method
Staphylococcus aureus	Plating/coagulase controls
	Direct plating for *S. aureus*
Enterobacteriaceae	Coagulase test
	Petrifilm™ controls
Lactic acid bacteria	Dry rehydratable film (Petrifilm) method
	Controls
	All-purpose Tween pour plate method

Summarized from Quantitative Analysis of Bacteria in Foods as Sanitary Indicators. In: Microbiology Laboratory Guidebook, FSIS, USDA (2011), accessed via http://www.fsis.usda.gov/wps/wcm/connect/03f8ce1e-b7e7-4257-8047-dcd215d0ae49/MLG_3_01.pdf?MOD = AJPERES on Feb 12, 2014.

by Al Qarawi et al. (2002) illustrates the effect of licorice extract consumption on the rennin−aldosterone−angiotensin system of male Wistar rats by use of a short-term study. Their results showed dose-dependent increases in plasma renin and sodium along with decreases in plasma cortisol and aldosterone levels.

26.3.4.2 Microbiology

Microbiological testing is one of the potential tools that can be used to evaluate whether a food safety risk management system is providing the level of control it was designed to deliver. Most commercial flavorings will not support the growth of microorganisms since most flavor compounds and essential oils are toxic to microorganisms. For this reason, flavor companies typically run a large number of microbiological assays. Spray-dried products are an exception in that they are sometimes subjected to microbial testing. The microbiology lab may be set up to run standard plate counts, yeasts, molds, and lactobacilli. *Salmonella* and other pathogens are generally sent out to service laboratories for analysis. Microbiological testing is a tool that could provide industry and regulatory authorities with tangible evidence of control (Todd, 2004; Van Schothorst et al., 2009). *Escherichia coli*, *Staphylococcus aureus*, Enterobacteriaceae, and lactic acid bacteria are common indicators for safety of food ingredients (Todd, 2004). The *Microbiology Laboratory Guidebook of the Food Safety and Inspection Service* (USDA, 2011) specifies many useful procedures for microbiological testing. Table 26.8 shows the analysis procedures for some common microorganisms potentially found in encapsulated flavors.

26.4 CONCLUSION

In order to achieve maximum performance of the different encapsulation technologies available today, there is a need to design flavor encapsulation through a retro-design approach where knowledge of both core and wall carrier is fundamental to the choice of a suitable encapsulation technology. Additionally, the knowledge and understanding of the physical, mechanical, chemical, and microbiological properties that flavor encapsulates develop during the process (formulation/process parameters) (Table 26.9) are important to the continuous improvement of these processes and which guarantee sustained quality to the end users and consumers. Currently, there are numerous techniques available to characterize these properties. In this chapter characterization tools used throughout an encapsulation process are reviewed. Most chemical studies regarding flavor encapsulation are limited to flavor release and/or storage stability. However, studies are limited concerning the incorporation, performance, and storage stability of flavor encapsulates in food products or other intended end uses.

TABLE 26.9 Control Points of a Flavor Encapsulation Process with Respect to Characterization of Physical, Mechanical, Chemical, and Microbiological Properties

	Control Point	Property Affected	Cause	Effect	Characterization Tools
Raw materials (core/wall)	1. Glass Transition Temperature 2. Degree of Crystallinity	Physicochemical properties	$>T_g$ Polymorphism	Caking/Flowability	DSC/TGA/X-ray Diffraction/NMR
Encapsulation process	1. Glass Transition Temperature (T_g) 2. Degree of Crystallinity	Physicochemical properties (Thermal properties)	$>T_g$	Caking/Flowability/ Polymorphisim	DSC/TGA/X-ray Diffraction/NMR
	3. Morphology – Surface – Inner structure – Particle size	Physical/Chemical properties Physical properties Physical properties	Process parameters	Fissures/porosity, environment exposure N/A Caking/Flowability	SEM/ESEM/TEM TEM/CLSM Laser diffraction
	4. Encapsulation Efficiency	Chemical	Process parameters	Flavor loss	GC-MS/FID/HPLC
	5. Flavor Retention	Chemical	Process parameters	Aroma loss (dissolution)/Stability	GC-MS/FID/HPLC
	6. Flavor Active Dispersion	Physicochemical properties	Process methods and parameters	Changes in flavor distribution	GC, GC-MS; Gravimetric methods; Absorbance measurement
Storage/handling	1. Storage Condition	Physicochemical properties	– Temperature – Relative humidity	Caking/Flowability	
	2. Mechanical Strength	Physical properties	Process parameters	Physical rupture and integrity	Resistance, compression, micropipette aspiration technique, texture analyzer, micro-manipulation technique
	3. Flavor Stability	Chemical property	Storage conditions	Aroma loss	GC, DVS fast GC-FID, enthalpy, e-nose
	4. Safety Testing	Biochemical property	Toxic ingredients, hazardous microorganisms	Food safety	Animal tests, microbiological tests

Abbreviations: T_g: Glass transition temperature; DSC: Differential scanning calorimetry; TGA: Thermogravimetric analysis; NMR: Nuclear magnetic resonance; SEM: Scanning electron microscopy; ESEM: Environmental scanning electron microscopy; TEM: Transmission electron microscopy; CLSM: Confocal laser scanning microscopy; GC: Gas chromatography; GC-MS: Gas chromatography-mass spectrometry; FID: Flame ionization detector; HPLC: High-performance liquid chromatography; GC-FID: Gas chromatography flame ionization detector; DVS fast GC-FID: Dynamic vapor sorption coupled with fast gas chromatography-flame ionization detection.

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Chapter 27

Real-Time Analysis of Oxidative Barrier Properties of Encapsulation Systems

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27.1 INTRODUCTION

Encapsulation systems are widely used in food, pharmaceutical, and cosmetic applications for delivery of diverse compounds including vitamins, flavors, bioactive compounds, hydrophobic drug molecules, proteins, and live microorganisms. In these diverse examples, encapsulation approaches are selected to improve solubility of materials in the bulk phase, control release of the encapsulated material, and in certain cases improve chemical stability and maintain functionality of the encapsulant (Shaikh et al., 2009; de Vos et al., 2010; Tikekar et al., 2013). Previous studies have shown that many of these diverse classes of encapsulants are inherently susceptible to oxidative degradation (Gohtani et al., 1999; Heinzelmann and Franke, 1999; Boon et al., 2008). Thus, the shelf-life of many products that contain encapsulated materials is often determined by the oxidative stability of these encapsulants.

Free radical-induced oxidation is one of the major pathways that lead to oxidation of encapsulated bioactives. The free radicals can be generated by diversity of conditions including enzyme−substrate reactions (such as glucose oxidase and oxygen), heating during processing operations, environmental factors such as light, and interactions of food additives such as metal ions and oxidants (Donnelly and Robinson, 1995; McClements and Decker, 2000). The free radicals generated at the interface of encapsulation systems subsequently permeate across the interface of the encapsulation systems and react with the encapsulants. These reaction steps lead to initiation of free radical-mediated oxidative chain reactions. Many of these reactions are further propagated by the presence of oxygen. In the case of nano- to micro-scale encapsulation systems, the problem is further exacerbated due to a tremendous increase in interfacial surface area as compared to the bulk materials (McClements and Decker, 2000). Therefore, research has been focused on arresting generation and transport of free radicals in these encapsulation systems. Several strategies such as addition of antioxidants in the aqueous or encapsulated phase, application of surface active materials with antioxidants and metal chelating properties, and engineering of interfacial thickness using a layer-by-layer approach have been investigated with varying degrees of success (Mei et al., 1998; Klinkesorn et al., 2005; Astete et al., 2011).

Stability of encapsulants has been conventionally evaluated by measuring the oxidation by-products of encapsulated materials as a function of formulation engineering and storage and packaging conditions. Although the conventional approach has been the basis of shelf-life testing in diverse food and chemical industries, this approach is significantly time consuming, destructive, and cannot be easily adapted for rapid screening of large numbers of formulations without significant resources. Depending on oxidative susceptibility of the encapsulant, the oxidation process can take several weeks. Since the observed oxidation rate is specific for the selected encapsulant and encapsulation matrix, the results obtained from such a study cannot be easily extrapolated to other encapsulants that may be encapsulated using the same matrix. Thus, there is a need for a rapid analytical technique to measure barrier properties of encapsulation systems independent of the encapsulant.

In this chapter, we will discuss novel and rapid techniques that are being developed for real-time and quantitative measurements of barrier properties of encapsulation systems and their applications in diverse encapsulation systems. These techniques are based on non-invasive real-time measurements of interaction of free radical and oxygen-sensitive probes

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00027-3

encapsulated within encapsulation systems with free radicals generated in the aqueous phase and the ambient oxygen. Application of these novel approaches for engineering encapsulation systems is also discussed.

27.2 RAPID METHODS TO MEASURE INTERACTION OF ENCAPSULATION SYSTEMS WITH OXIDIZING AGENTS

27.2.1 Measurement of Interactions of Encapsulation Systems With Hydroxyl radicals

Hydroxyl radicals are generated within food systems through the action of metal ions and are responsible for oxidation of diverse sensitive compounds (Choe and Min, 2006). To measure interactions of hydroxyl radicals with the encapsulation core, we have developed a non-invasive and a real-time spectroscopy and imaging approach (Tikekar et al., 2011a). This novel approach is based on an encapsulation of a hydroxyl radical-sensitive dye, carboxy-H_2DFFDA (5-(and -6) carboxy-2′,7′-difluorodihydro-fluorescein diacetate) in the lipid core of the encapsulation system. This dye is converted from non-fluorescent form to fluorescent form upon interaction with hydroxyl radicals. Thus, the rate of increase of fluorescence intensity of this fluorescent dye acts as a good indicator of measurement of hydroxyl radical transport rate across the interface. It is important to note that sensitivity of carboxy-H_2DFFDA towards hydroxyl radicals is significantly higher than that towards other free radicals such as superoxide and peroxyl radicals. Thus, the change in fluorescence intensity can be specifically attributed to transport of hydroxyl radicals across the interface. The hydroxyl radicals were generated using the well-established Fenton reaction. The reaction mechanism that generates hydroxyl radicals from Fenton's reagents is given in Eq. (27.1):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \tag{27.1}$$

The addition of Fenton's reagents as oxidizers allowed us to control the rate of hydroxyl radical generation by varying the addition of ferrous salt and hydrogen peroxide. Figure 27.1 shows a representative example of measurement of hydroxyl radical transport rate across the interface of whey protein-stabilized oil-in-water emulsion. The fluorescence intensity data obtained using fluorescence spectrophotometry were complemented with fluorescence imaging data that clearly show an increase in fluorescence intensity within individual emulsion droplets upon interaction with hydroxyl radicals (Figure 27.2). The method was found to be sensitive to the factors that change the rate of hydroxyl radical generation such as concentration of oxidants (ferrous salt and hydrogen peroxide) (Figures 27.3a and b) and addition of metal chelators (EDTA) (Figure 27.4). In order to validate that the transport of hydroxyl radicals correlated with oxidation of encapsulated oil phase, we used a similar approach to measure oxidation products of the encapsulated oil. In this case, we added a fluorescent dye, diphenyl-1-pyrenylphosphine (DPPP) to the oil phase. DPPP is specifically oxidized by lipid peroxides (generated by oxidation of oil phase by hydroxyl radicals) and is not sensitive to oxidation by aqueous hydroperoxide radicals (Gomes et al., 2005). Figure 27.5 shows increase in fluorescence intensity of DPPP as a result of reaction with lipid peroxide radicals generated due to hydroxyl radical-mediated oxidation of encapsulated lipids. These results validate that measurement of free radical transport across the interface correlated with lipid oxidation induced by these radicals.

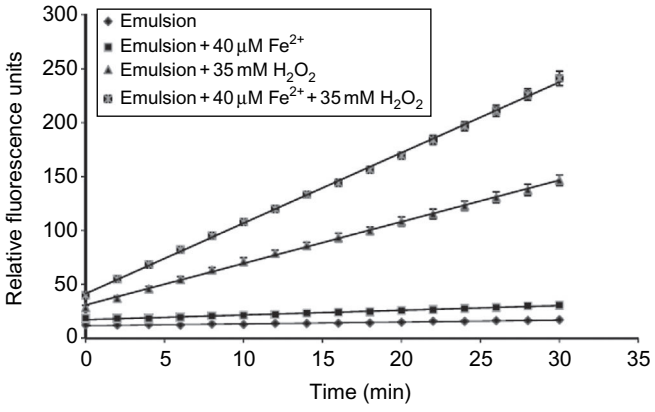


FIGURE 27.1 Increase in fluorescence of free radical-sensitive dye encapsulated in the oil phase of WPI emulsion (3% oil, 1% WPI, pH 7.0, and 50 μg/g of fluorescent dye) as a function of time after addition of Fe^{2+} (40 μM), H_2O_2 (35 mM), and 40 μM Fe^{2+} + 35 mM H_2O_2, respectively, to the aqueous phase of an emulsion. Each data point is an average of triplicate measurements ± standard deviation.

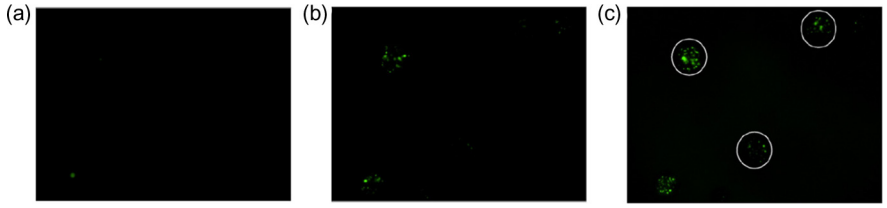
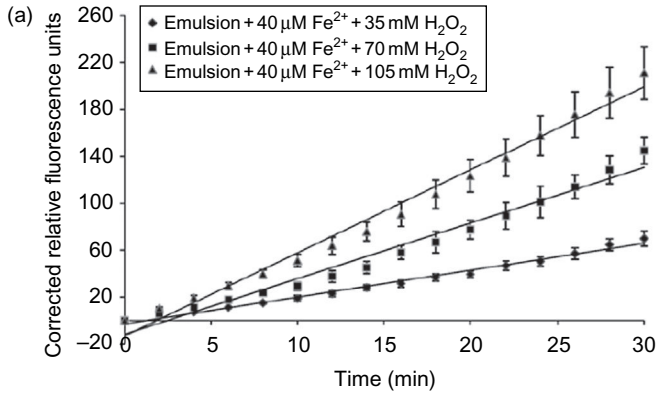


FIGURE 27.2 Time series fluorescence imaging data based on activation of free radical-sensitive dye encapsulated in the oil phase of a WPI emulsion upon exposure to Fenton's reagent (40 μM Fe^{2+} + 35 mM H_2O_2). Images were collected at discrete time points: (a) 0, (b) 30, and (c) 60 min after exposure of WPI emulsion to Fenton's reagent.



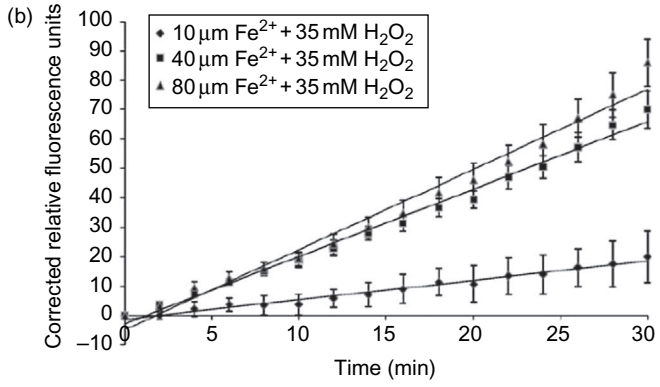


FIGURE 27.3 (a) Effect of change in concentration of hydrogen peroxide on the corrected rate of increase in fluorescence intensity of a free radical-sensitive dye encapsulated in a WPI emulsion upon exposure to Fenton's reagent (40 μM Fe^{2+} + 35 mM H_2O_2). Each data point is an average of triplicate measurements ± standard deviation. (b) Effect of change in concentration of ferrous ions on the corrected rate of increase in fluorescence intensity of a free radical-sensitive dye encapsulated in a WPI emulsion upon exposure to Fenton's reagent. Each data point is an average of triplicate measurements ± standard deviation.

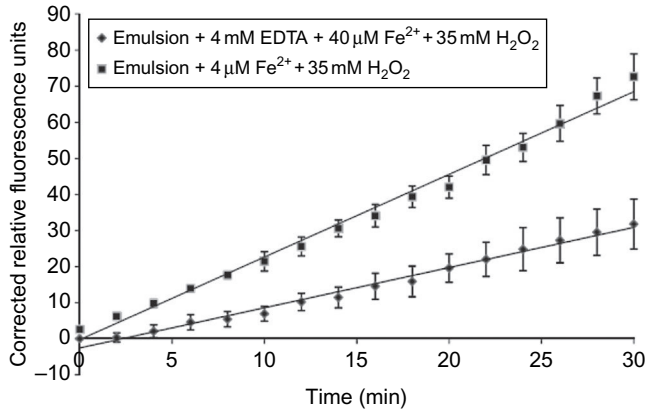


FIGURE 27.4 Effect of change in concentration of ferrous ions on the corrected rate of increase in fluorescence intensity of a free radical-sensitive dye encapsulated in a WPI emulsion upon exposure to Fenton's reagent. Each data point is an average of triplicate measurements ± standard deviation.

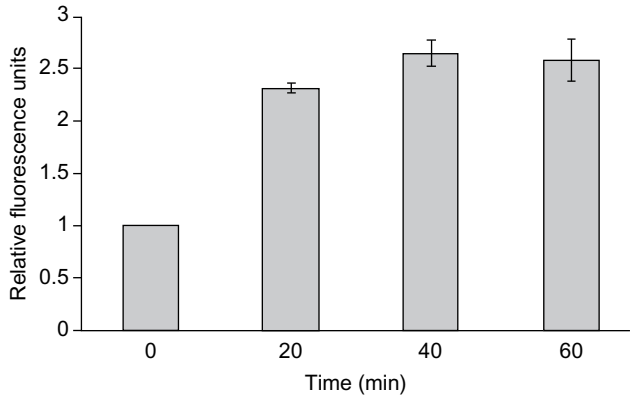


FIGURE 27.5 Relative increase in concentration of lipid peroxides measured using DPPP. Increase in lipid peroxide concentration indicates lipid oxidation induced by the hydroxyl radicals generated by Fenton's reaction system (40 μM Fe^{2+} + 35 mM H_2O_2). Each data point is an average of duplicate measurements ± standard deviation.

27.2.2 Measurement of Interactions of Encapsulation Systems With Peroxyl Radicals

The peroxyl radical is another major class of free radicals responsible for oxidation of encapsulated products. To measure interactions of the peroxyl radical generated in the aqueous phase with the lipid core of the encapsulation system, we used a similar approach to that used for measurement of hydroxyl radical interactions with the encapsulation core (Tikekar and Nitin, 2011, 2012). A fluorescent dye, BODIPY 665/676, was incorporated within the oil phase of emulsions and peroxyl radicals were generated in the aqueous phase of oil-in-water emulsion systems using 2,2′-azobis-2-methyl-propanimidamide dihydrochloride (AAPH). Due to its long half-life, use of AAPH enabled us to generate peroxyl radicals at a constant and controllable rate over an extended period of time. However, in contrast to carboxy-H_2DFFA, which showed increase in fluorescence upon interaction with hydroxyl radicals, BODIPY 665/676 showed a decrease in fluorescence intensity upon interaction with peroxyl radicals. Thus, a rate of decay in fluorescence intensity acts as a good indicator of rate of permeation of peroxyl radicals. Figure 27.6a shows a loss in relative fluorescence of BODIPY 665/676 upon interaction with peroxyl radicals in Tween-20-stabilized oil-in-water emulsion and Figure 27.6b shows fluorescence images of emulsion droplets. From Figure 27.6a, it is evident that the fluorescence signal was almost completely quenched within a few hours after incubation with AAPH, indicating that this approach is

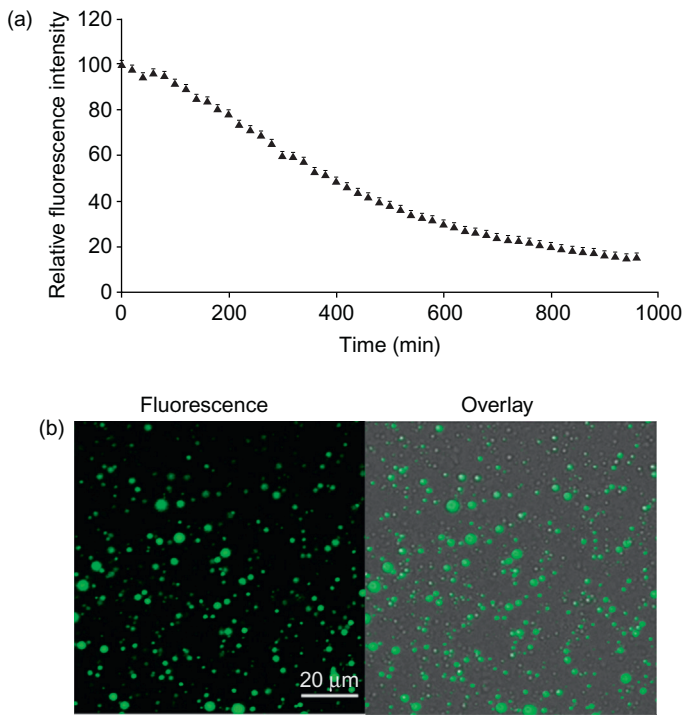


FIGURE 27.6 (a) Permeation of peroxyl radicals from the aqueous phase to the oil phase of Tween 20 emulsion along with the result of non-linear regression. Radicals were generated using 20 mM of AAPH in the aqueous phase of emulsions. The radical permeation rate was measured based on loss in fluorescence of a peroxyl radical-sensitive dye (C11-BODIPY665/676) encapsulated in the oil phase of emulsion. Each data point represents an average of three independent measurements ± standard deviation. (b) Fluorescence imaging to characterize distribution of BODIPY dye in Tween 20 emulsion droplets.

significantly faster than the traditional approach where the incubation period can extend up to weeks. Due to high hydrophobicity of the BODIPY dye, it is almost exclusively localized within the oil phase (Figure 27.6b). As a result, the loss of fluorescence intensity could be exclusively attributed to permeation of peroxyl radicals across the interface. Similar to the prior approach used for measuring interactions of hydroxyl radicals with the encapsulation core, this approach also provides precise control over the rate of generation of peroxyl radicals. This precise control is critical to develop a quantitative understanding of radical-mediated oxidation processes in encapsulation systems.

27.2.3 Measurement of Interactions of Encapsulation Systems With Oxygen

Oxygen is widely recognized as a critical component responsible for oxidation of encapsulated bioactives (McClements and Decker, 2000). Oxygen has an essential role in propagation of free radical-mediated oxidation processes. Various approaches have been developed to detect oxygen in bulk systems including food packaging systems, but these measurement approaches do not effectively translate to measurement in micro-/nano-scale systems. To measure the rate of oxygen transport to encapsulation system from the bulk phase, we have designed a novel approach for *in situ* real-time measurement of oxygen transport into the core of the encapsulation system (Tikekar et al., 2011b). This approach is based on measuring oxygen interaction with an oxygen-sensitive fluorescence dye Tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) bis(hexafluorophosphate). This dye has been extensively used for diverse applications in earlier studies, but its use for measurement of oxygen diffusion in encapsulation systems is novel. This fluorescent complex can be encapsulated in the lipid core of the encapsulation system. The interactions of this fluorescent dye with oxygen are reversible and binding of oxygen with the fluorophore results in reduction of the fluorescence intensity of the encapsulated oxygen-sensitive dye. Thus, the principle for measuring oxygen transport in encapsulation systems is similar to the approach used for measuring interactions of free radicals with the encapsulation core.

In order to measure oxygen transport, ruthenium-based dye was encapsulated in the oil phase of the emulsion systems. The emulsion was then purged with nitrogen to remove oxygen. The nitrogen purged sample was then placed in a fluorescence spectrophotometer and exposed to atmospheric oxygen. Atmospheric oxygen first dissolved in the aqueous phase and was then transported across the emulsion interface into the oil phase of emulsion. Since the solubility of oxygen in oil phase is three times higher than that in aqueous phase (Coupland and McClements, 1996), there is a significant driving force for diffusion across the interface of emulsion samples. A decrease in fluorescence intensity of ruthenium based dye was measured as a function of time. An emulsion sample not purged with nitrogen was used as a control. Figure 27.7a shows the

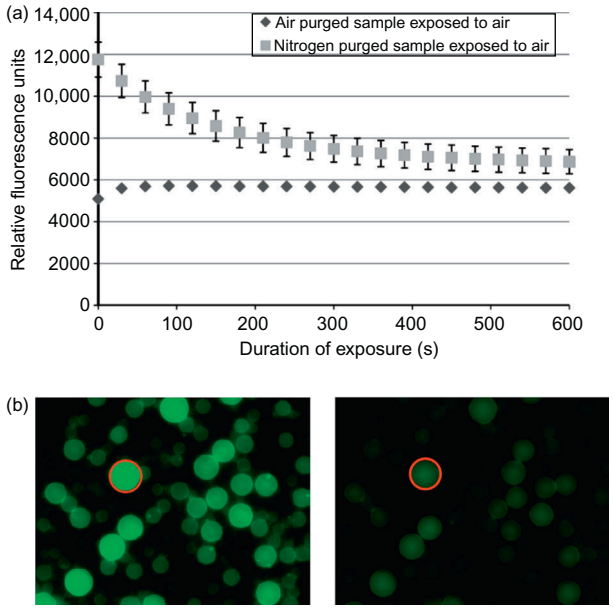


FIGURE 27.7 (a) Measurement of changes in fluorescence intensity of nitrogen-purged whey protein isolate (WPI) emulsion (2.5% oil, 1% WPI, 50 μg/g oil of the dye, pH 7.0) upon exposure to air. The fluorescence response from the nitrogen-purged WPI sample was compared with the air-purged control WPI sample at 25°C. (b) Fluorescent images of WPI emulsion when purged with nitrogen ($t = 0$ min) and (right) when exposed to oxygen for 10 min. (Magnification 200×.)

decay in relative fluorescence intensity of the dye as a function of time in whey protein-stabilized oil-in-water emulsion, while Figure 27.7b shows fluorescence micrographs of emulsion droplets before and after exposure to atmospheric oxygen. The imaging results clearly show uniform distribution of the dye within the oil droplet and a decrease in fluorescence intensity within individual droplets upon exposure to oxygen.

For quantitative characterization, the effective diffusion coefficient (including resistance to transfer from the air to well-mixed aqueous phase) for transport of oxygen in this encapsulation system can be calculated based on the following equations (Kaptan et al., 1989). Based on Fick's second law of diffusion for a spherical geometry, the ratio of concentration (C_t/C_0) is given by:

$$\frac{C_t}{C_0} = 1 + \frac{2\alpha}{\pi r}\sum_{n=1}^{\infty}\frac{(-1)^n}{n} \times \sin\frac{n\pi r}{\alpha}\exp\left(-\frac{Dn^2\pi^2 t}{\alpha^2}\right) \tag{27.2}$$

where α is the radius of sphere, D is the effective diffusion coefficient, and C_0 and C_t are concentration of oxygen at time $t = 0$ and t seconds, respectively; r corresponds to radial distance at which C is measured. Upon integration of Eq. (27.2) over volume dV, the resulting equation becomes:

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{Dn^2\pi^2 t}{\alpha^2}\right) \tag{27.3}$$

where M represents the total flux at any given time in a spherical geometry and can be calculated using the following expression:

$$M = \int CdV \tag{27.4}$$

Equation (27.3) was further simplified using a concept of $t_{1/2}$ (time required to uptake 50% of the equilibrium concentration of oxygen). The resulting equation after truncation of second-order terms simplifies to:

$$t_{1/2} = 0.0717\alpha^2/D \tag{27.5}$$

To calculate M_t/M_∞, the Stern−Volmer equation was used:

$$\frac{I_0}{I_t} - 1 = [O_2] \tag{27.6}$$

Thus, M_t and M_∞ can be obtained as:

$$M_t = \left(\frac{I_0}{I_t} - 1\right) \text{ and } M_\infty = \left(\frac{I_0}{I_\infty} - 1\right)$$

Therefore:

$$\frac{M_t}{M_\infty} = \frac{(I_0 - I_t)I_\infty}{(I_0 - I_\infty)I_t} \tag{27.7}$$

where, I_0 = fluorescence intensity under nitrogen, I_t = fluorescence intensity after t seconds of exposure to air, and I_∞ = fluorescence intensity of air purged control sample.

This ratio (Eq. 27.7) was plotted as a function of time, and $t_{1/2}$ was calculated by mathematical interpolation. An average effective diffusion coefficient was calculated for each of the emulsions based on the average $t_{1/2}$ value and the measured diameter of emulsion droplets. In this analysis, the following assumptions were made: (1) the effective diffusion coefficient is constant; (2) the aggregate data of fluorescence measurement using a plate reader represent the fluorescence quenching trend within an individual droplet—this assumption was validated based on imaging measurements of changes in fluorescence of individual emulsion droplets upon exposure to oxygen; and (3) the diffusion through a droplet is not affected by the presence of surrounding droplets, i.e., there are no interdroplet interactions due to low concentration of oil (2.5%).

27.2.4 Electron Spin Resonance-Based Methods

Thomsen et al. (1999) developed a rapid method for measuring free radicals generated in the emulsion systems using electron spin resonance (ESR) spectroscopy. In this approach, spin traps such as N-tert-butyl-a-phenylnitrone (PBN) or

2,2,6,6-tetramethylpiperidine-1-oxy (TEMPO) were dissolved in the oil phase of oil-in-water emulsion samples. These spin traps change their paramagnetic properties upon interaction with free radicals generated in the emulsion samples during extended storage, thus providing an accurate measure of extent of free radical generation and oxidation in these products (Thomsen et al., 1999). Similarly to the approaches discussed earlier, the ESR-based technique has been used to measure the barrier properties of various engineered interfaces (Berton-Carabin et al., 2012, 2013).

27.3 APPLICATIONS OF RAPID MEASUREMENT TECHNIQUES

In this section, we will discuss some of the applications of these techniques in understanding the barrier properties of encapsulation systems. Such a fundamental understanding aids in development of novel approaches to improve these barrier properties and control the oxidation and release rates within these encapsulation systems.

27.3.1 Impact of Nature of Lipid Core on Susceptibility of Encapsulated Material Towards Oxidation

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are two novel encapsulation systems that have been investigated extensively for their ability to encapsulate hydrophobic materials and control their release. The primary difference in SLNs and NLCs is that in SLNs, the lipid fraction of emulsions consists of oils that are solid at room temperature while in NLCs, usually two separate oils with different melting temperatures are chosen such that part of the oil fraction is in the solid state while the other fraction is in the liquid state. Due to these differences in the nature of lipid matrix, it has been hypothesized that the distribution of encapsulants within these two systems is significantly different. Due to crystallization of lipids, in SLNs, most of the encapsulated material is excluded from the lipid core and redistributed at the oil−water interface while in the case of NLCs, encapsulant remains trapped within liquid lipid domains surrounded by solid lipids. Due to this difference, it is expected that material encapsulated within SLNs would be more susceptible to oxidation as compared to that within NLCs. In order to test this hypothesis, we measured the rate of loss of fluorescence of BODIPY 665/676 dye encapsulated within SLN and NLC upon exposure to peroxyl radicals generated within aqueous phase of the emulsion using AAPH (Tikekar and Nitin, 2012). Figure 27.8 shows the relative loss of fluorescence within SLN and NLC samples. It is evident that rate of loss of fluorescence was much higher in SLN and NLCs, possibly due to difference in the redistribution of dye. The differences in the distribution of encapsulant were directly visualized using fluorescence imaging of SLNs and NLCs (Figure 27.9). Thus, using this approach, it was possible to prove and validate a widely accepted hypothesis on the effect of the nature of the lipid core on the susceptibility of encapsulants to oxidation. Several studies have used the ESR-based measurement approach to demonstrate these differences in the architecture of SLNs and NLCs (Jores et al., 2003; Yucel et al., 2012, 2013). The agreement between fluorescence and ESR-based measurement approaches to prove the same hypothesis further validates these measurement techniques.

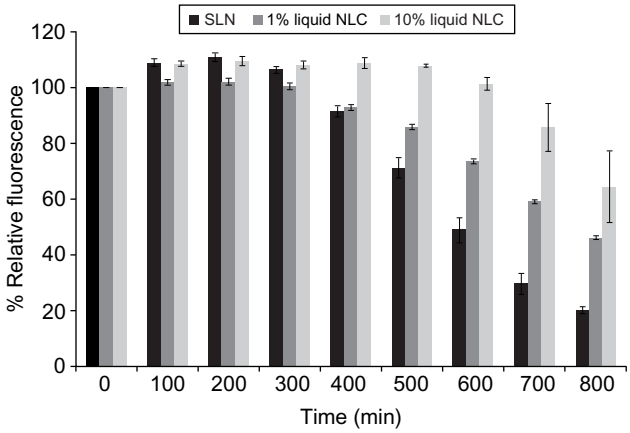


FIGURE 27.8 Susceptibility of encapsulated peroxyl radical-sensitive dye to react with peroxyl radicals generated in the aqueous phase of SLNs, 1% NLC, and 10% NLC. Results show a comparison of the fluorescence decay rate of peroxyl radical-sensitive dye encapsulated in SLNs, 1% NLC, and 10% NLC. Peroxyl radicals were generated by addition of 20 mM AAPH to the aqueous phase. All the data points are an average of triplicate measurements ± standard deviation.

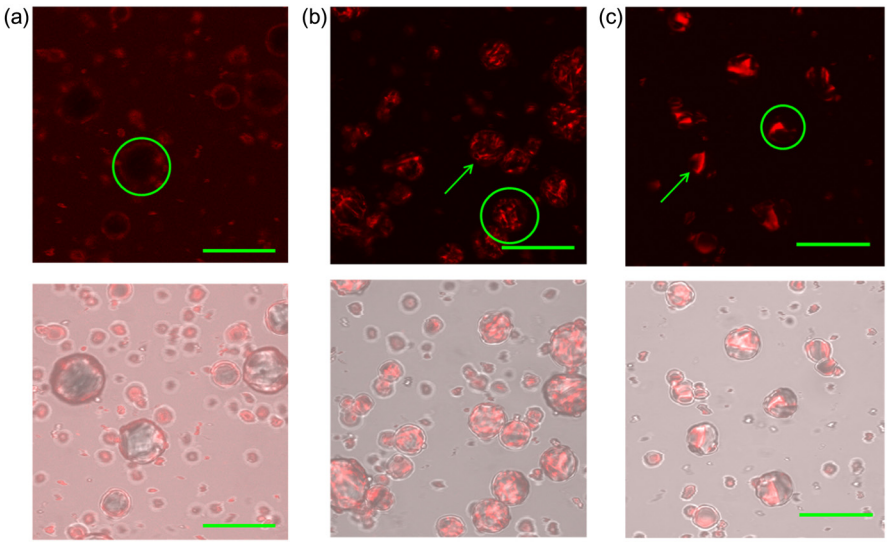


FIGURE 27.9 Confocal imaging to characterize the distribution of Nile Red within (a) SLN, (b) 1% NLC, and (c) 10% NLC.

27.3.2 Correlation Between Interfacial Mobility and Free Radical Transport

It has long been suggested that the physical properties of interfacial layers such as viscosity, melting properties, and thickness can significantly impact their barrier properties and limit the rate of transport across the interface (Opawale and Burgess, 1998; Chaiyasit et al., 2000; Waraho et al., 2011). Based on engineering of model bi-layer membranes and the novel approaches to measure interactions of free radicals with encapsulated radical-sensitive hydrophobic dye, we demonstrate that it is possible to test these hypotheses directly and *in situ* (Bricarello et al., 2012). In this study, the kinetics of the oxidation process in lipid membranes is characterized based on real-time imaging of radical transport into the lipid interface. A well-characterized azo-initiator (AAPH) is used to generate peroxyl-free radicals in the aqueous phase. As radicals permeate the supported membrane, they react with the encapsulated peroxidation-sensitive probe. As a result of this reaction, the fluorescence emission of the probe is quenched. To mimic the lipid interface of liposomes, lipoprotein, emulsions, solid lipid nanoparticles, and other structures, a planar-supported lipid membrane system was employed here.

Figure 27.10 shows the effect of interfacial mobility on susceptibility of BODPIY 665/676 dye localized within supported phospholipid bilayers. From the data it is evident that the rate of fluorescence quenching for BODPIY 665/676 was highest in the 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) bilayer followed by the DLPC bilayer incorporated with cholesterol and was the least in the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayer. Addition of cholesterol to the bilayer has been shown not only to reduce the interfacial mobility but also to straighten the phospholipids to increase the interfacial height (Filippov et al., 2003; McConnell and Radhakrishnan, 2003). Together, this can lead to enhanced barrier properties of the bilayer as demonstrated in this result. The reason behind the observed

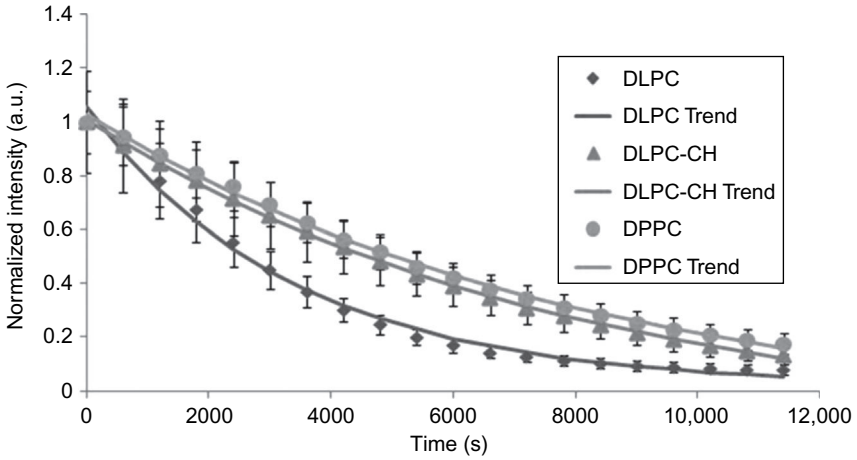


FIGURE 27.10 Physical protectants against free radical degradation. Raw data points are shown for dimyristoyl-phosphatidylcholine (DLPC), DLPC with 15 mol% cholesterol (DLPC-CH), and dipalmitoyl-phosphatidylcholine (DPPC) along with the results of the non-linear regression. All the data points are an average of triplicate measurements ± standard deviation.

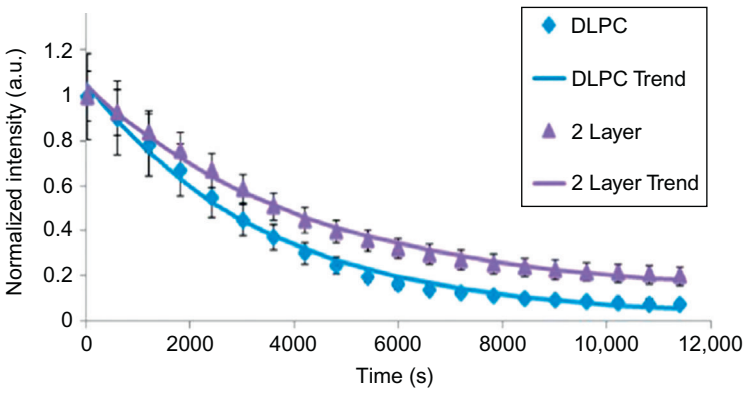


FIGURE 27.11 BODIPY fluorescence decay in the sample with an additional protective membrane (two layer) compared against the DLPC control.

difference is significantly lower lateral mobility in the DPPC bilayer as compared to the DLPC bilayer as a result of a longer carbon tail (^{16}C) as compared to DLPC (^{12}C). These results clearly demonstrate the role of interfacial mobility on transport across the interface. It also highlights the unique capability of these measurement approaches to develop fundamental understanding of effects of microscopic properties of interfacial layers on their barrier properties.

In the same study, we also demonstrated the effect increased thickness of the interface has on the rate of free radical transport. Figure 27.11 shows the rate of loss of relative fluorescence in BODIPY 665/676 in single layer DLPC bilayer and double layer DLPC bilayer. From the data it is evident that although the interfacial thickness doubled, the rate of fluorescence decay decreased only slightly. This result suggests that the layer-by-layer approach to increase the surface thickness can have only a limited effect on the rate of transport of reactive oxidants such as peroxyl radicals.

27.3.3 Effect of Antioxidant Properties of Emulsifier on Free Radical Transport

The antioxidant property of an emulsifier has long been considered as a desirable attribute to enhance the oxidative stability of encapsulated material. The underlying hypothesis behind this is that an emulsifier with antioxidant properties can quench the free radicals before they permeate into the lipid core of the emulsion. Thus, it is expected that an emulsifier with differential antioxidant activity will show significant differences in free radical permeation rates. To test the hypothesis, we compared the peroxyl radical permeation rates within emulsions stabilized by lecithin and oxidized lecithin (Pan et al., 2013). Lecithin solution was oxidized by exposure to UV light prior to a homogenization step to synthesize emulsion. Oxidation of lecithin was confirmed by changes in redox potential and thiobarbituric acid reactive substance (TBARS) assay values between native lecithin and oxidized lecithin solution. Figure 27.12 shows the rate of fluorescence quenching of peroxyl radical-sensitive BODIPY dye encapsulated within emulsions stabilized by lecithin and oxidized lecithin upon exposure to 20 mM AAPH. The results show that fluorescence decay rate was significantly

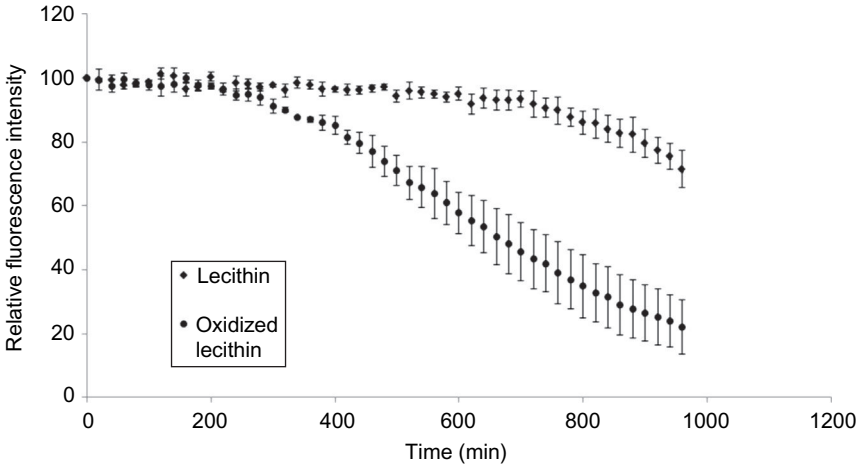


FIGURE 27.12 Permeation of peroxyl radicals from the aqeuous phase to the oil phase of lecithin and oxidized lecithin emulsions along with the result of non-linear regression. Radicals were generated using 20 mM of AAPH in the aqueous phase of emulsions. The radical permeation rate was measured based on loss in fluorescence of a peroxyl radical-sensitive dye (C11-BODIPY665/676) encapsulated in the oil phase of emulsion. Each data point represents an average of three independent measurements ± standard deviation.

higher in emulsion stabilized by oxidized lecithin compared to that stabilized by native lecithin. It should be noted that other factors, such as emulsion droplet size, pH, and temperature, remained constant in this experiment. Thus, the differential peroxyl rates could be attributed to lower antioxidant activity of oxidized lecithin compared to native lecithin. These results validate the hypothesis that antioxidant properties of an emulsifier have a significant role in limiting permeation of oxidative species across the emulsion interface.

27.3.4 Correlation Between Radical Permeation Measurements and Stability of Encapsulated Compounds

Figure 27.13 shows comparison of peroxyl radical-sensitive BODIPY dye encapsulated within emulsions stabilized by Tween 20 and lecithin upon exposure to 20 mM AAPH (Pan et al., 2013). The results clearly demonstrate that fluorescence decay rate was significantly lower in emulsion stabilized by lecithin, indicating a lower rate of permeation of peroxyl radicals. A significantly lower rate of permeation of peroxyl radicals in lecithin-stabilized emulsion was attributed to the antioxidant activity. To correlate the peroxyl radical permeation rate with oxidative stability of encapsulated bioactive, the stability of curcumin encapsulated within Tween 20 (Figure 27.14a) and lecithin emulsion (Figure 27.14b) in the presence and absence of AAPH was also evaluated. These results clearly indicate that, consistent with peroxyl radical permeation rates within these emulsions, oxidative stability of curcumin was higher in lecithin-stabilized emulsion compared to Tween 20 emulsion. These results validate that free radical permeation measurements correlate with oxidative stability of encapsulated materials and could be used as an effective tool in rational screening of encapsulation systems for enhanced stability of encapsulated material.

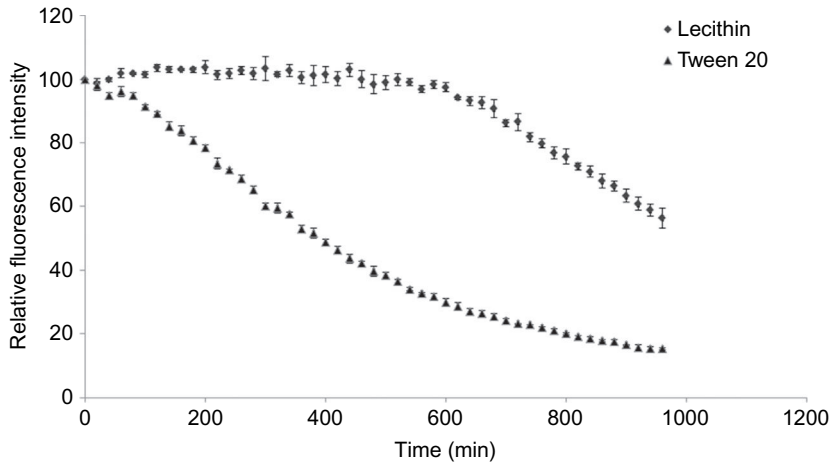


FIGURE 27.13 Permeation of peroxyl radicals from the aqeuous phase to the oil phase of Tween 20 and lecithin emulsions along with the result of non-linear regression. Radicals were generated using 20 mM of AAPH in the aqueous phase of emulsions. The radical permeation rate was measured based on loss in fluorescence of a peroxyl radical-sensitive dye (C11-BODIPY665/676) encapsulated in the oil phase of emulsion. Each data point represents an average of three independent measurements ± standard deviation.

27.3.5 Effect of Interfacial Modifications of Permeation of Oxygen Within Encapsulation Systems

Due to an established role of molecular oxygen in the catalyzing oxidative process (McClements and Decker, 2000), there is a need to limit permeation of oxygen across the encapsulation interface. In order to demonstrate the impact of crosslinking of interfacial proteins on interfacial transport of molecular oxygen, we measured the diffusion rate of oxygen across emulsion interfaces using the fluorescence spectrophotometric method discussed earlier. Figure 27.15 shows the rate of loss of oxygen-sensitive ruthenium-based dye in emulsions stabilized by whey protein isolate and crosslinked whey protein concentrate. The diffusion coefficient for oxygen was also calculated using the diffusion model through a sphere as discussed earlier. Based on the results in Figure 27.15, it is evident that crosslinking of proteins had no significant impact on the rate of loss of fluorescence of ruthenium-based dye, indicating similar diffusion rates for oxygen across these two interfaces. The effective diffusion constants were calculated to be 0.14×10^{-12} and 0.28×10^{-12} cm^2/s and were not significantly different from each other. Based on the effective diffusion coefficient values, one would expect significant barrier properties for oxygen diffusion. However, due to the submicron size of emulsion droplets, the transport of oxygen was complete within 2–3 minutes. These results highlight a significant challenge in limiting transport of oxygen across encapsulation interfaces.

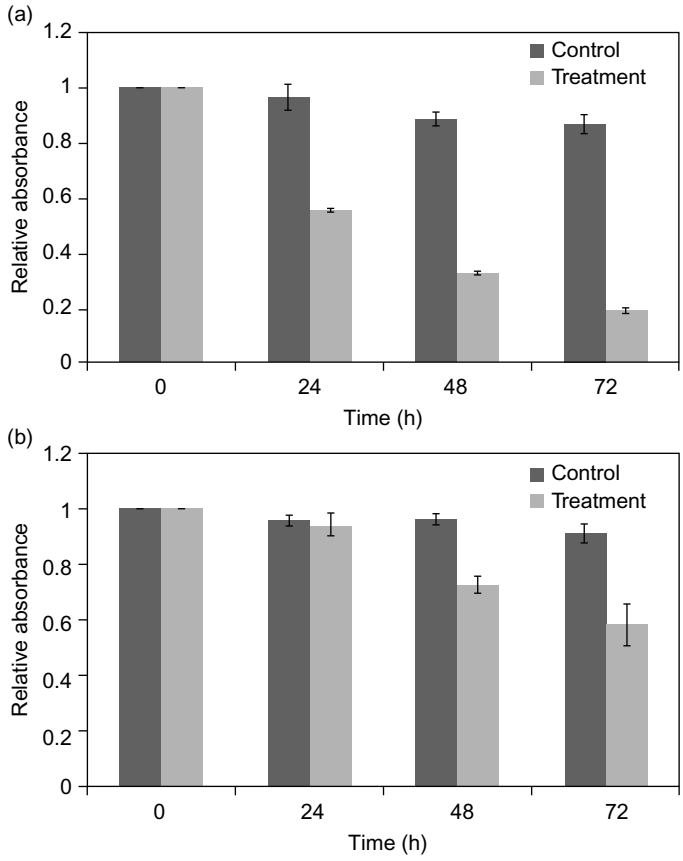


FIGURE 27.14 (a) Oxidative stability of encapsulated curcumin in Tween 20-stabilized emulsions with and without AAPH treatment. Both the control and the treatment emulsion samples were stored in the dark at room temperature for 72 hours. (b) Oxidative stability of encapsulated curcumin in lecithin-stabilized emulsions with and without AAPH treatment. Both the control and treatment emulsion samples were stored in the dark at room temperature for 72 hours.

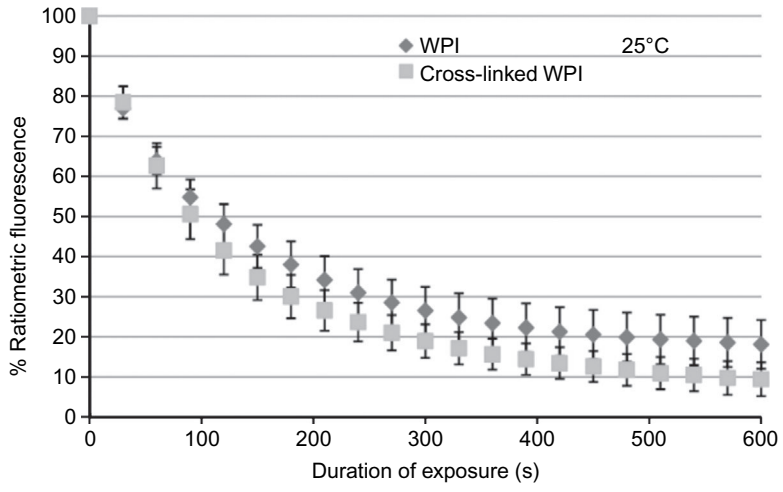


FIGURE 27.15 Comparison between ratiometric fluorescence decay of WPI and crosslinked WPI emulsions at 25°C. Each data point is an average of duplicate measurements ± standard deviation.

27.4 CONCLUSION

This chapter summarizes various novel and rapid methods developed for real-time, *in situ* measurement of transport of reactive oxygen species such as free radicals and oxygen across the interface of encapsulation systems. These methods complement the existing methods for measuring barrier properties of encapsulation systems. We also demonstrated some unique applications of these measurement techniques to evaluate engineering approaches directed at limiting the transport of reactive oxygen species across the interface. The techniques discussed in this study can also be used to

evaluate the efficacy of novel approaches designed to minimize oxidation of encapsulant such as interfacial engineering, modification of core composition, and optimized selection and localization of antioxidants. Overall these novel techniques coupled with conventional methods of analysis will aid in rationalized design of encapsulation systems with enhanced barrier properties against reactive oxygen species.

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Chapter 28

Stability Characterization and Sensory Testing in Food Products Containing Microencapsulants

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28.1 INTRODUCTION

The field of microencapsulation encompasses a wide variety of technologies, many of which are discussed in detail throughout this book. Many technologies are employed in research and the food industry for the formation of microencapsulated ingredients such as spray drying, spray chilling, extrusion coating, fluidized-bed coating, liposomes, and molecular inclusion (Shahidi and Han, 1993; Gibbs et al., 1999; Desai and Jin Park, 2005; Gharsallaoui et al., 2007, 2012). When choosing a microencapsulation technology, cost, availability of equipment, functionality, and ease of use must be considered (Jackson and Lee, 1991; Desai and Jin Park, 2005). Cost and food-grade nature of ingredients are of concern for the food industry as some technologies use non-food grade ingredients or are very expensive (Shahidi and Han, 1993; Gibbs et al., 1999; Desai and Jin Park, 2005). This chapter will focus on assessing the stability of microcapsules and their sensory impacts on food systems. The reader is encouraged to explore sources for details on specifics relating to the microencapsulation technologies discussed (Jackson and Lee, 1991; Shahidi and Han, 1993; Gibbs et al., 1999; Desai and Jin Park, 2005). Regardless of the technology used to produce microcapsules, stability assessments of microcapsules are recommended to determine if a microencapsulation process was successful. Stability can be measured by focusing on various core or wall properties. A variety of processing parameters and environmental factors dependent on the processing method used and formulation of the microcapsule can effect these stability measurements in their own way. While there is no set definition of what makes a microcapsule stable, a variety of measurements are commonly referred to in scientific literature to examine microencapsulated particle wall and core stability.

28.2 MEASURING STABILITY

No defined set of specific parameters on which to rely when evaluating the stability and integrity of a microencapsulated system has yet been identified. There is no "universal" measurement for stability. Researchers and industry professionals alike have thus employed a variety of methods to determine stability. These methods generally examine a variety of physical and chemical properties of the wall and the encapsulated core.

28.3 FACTORS AFFECTING WALL STABILITY

Understanding the structural, physical, and chemical properties of the microencapsulated wall material is important in determining the stability of the entire system.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00028-5

28.3.1 Surface Morphology and Characteristics

The microscopic surface structure of microencapsulated particles is one of the key parameters in understanding a microencapsulated system (Desai and Jin Park, 2005). The surface morphology of a microencapsulated system is influenced by the encapsulation technique and the processing variables associated with that technique (Shahidi and Han, 1993). For example, variables such as inlet temperatures, size of the drying chamber, atomization method, slurry viscosity, and drying properties of the medium all heavily influence the particle size of spray-dried microcapsules (Walton and Mumford, 1999). An ideal microencapsulation process would fully entrap a core material, leaving no residue on the outside of the particle (Shahidi and Han, 1993; Gibbs et al., 1999). For this reason, the presence of the core on the wall structure is often something experimenters aim to minimize. Monitoring the structure and composition of the wall through microscopy can help ensure a successful microencapsulation process.

28.3.1.1 Microscopy

Microscopic observations can be used to elucidate the surface and walls of the microcapsule. The ability to observe cracks, dents, surface imperfections, wall thickness, uniformity, and smoothness are very useful in understanding that various particle properties and bulk characteristics are related to morphology (Walton and Mumford, 1999). Often, scanning electron microscopy (SEM) is used to examine the structural differences of a variety of microencapsulated systems (Young et al., 1993a,b; Rosenberg and Sheu, 1996; Sankarikutty et al., 1988; Kim et al., 2002; Tonon et al., 2009; Fritzen-Freire et al., 2012; Gharsallaoui et al., 2012; Ying et al., 2012). The microscope used in SEM scatters an electron beam across the surface of a sample, producing secondary electrons that are then interpreted by the microscope to produce images. In order for this secondary electron beam to be produced, the sample needs to have a reactive surface. For this reason, food particles analyzed with SEM often need to be coated with reactive precious metals such as gold, palladium, or alloy mixtures (Rosenberg et al., 1985).

This technology allows for the investigation of surface cracks, dents, and irregularities. The presence of these characteristics is generally undesired in the microencapsulated system as they may adversely affect important parameters such as long-term stability, microencapsulation efficiency, volatile retention, or flowability (Rosenberg et al., 1985; Sheu and Rosenberg, 1998). Making a microencapsulated system using maltodextrin-produced microcapsules with a spherical shape but more cracks resulted in lower oil retention (Sankarikutty et al., 1988). Researchers observed that altering maltodextrin dextrose equivalent values increased microencapsulation efficiency while producing smooth and wrinkled surfaces. Somewhat contrary to some of these results, the smoothness of a microencapsulated particle is typically desired in some literature (Sheu and Rosenberg, 1998). Researchers have noted that a high whey protein isolate (WPI) concentration may be desirable to form smooth surfaces; those particles with dents had improved volatile retention with lower WPI concentrations (Sheu and Rosenberg, 1998). Because of observations such as these, it is important to always observe and determine the microencapsulation morphology that works best for your particular system.

28.3.1.2 Electron Spectroscopy for Chemical Analysis

Electron spectroscopy for chemical analysis (ESCA but sometimes called X-ray photoelectron spectroscopy, XPS) is a technology used to examine the major chemical components and distributions on particle surfaces. The technology allows the determination of elements on the surface (about the first 10 nm) of a particle. ESCA uses an X-ray source to provide photoelectron signals via electrons that are unique to specific atoms of interest. These signals are analyzed and it becomes possible to identify specific atoms based on their signal. Use of this method to analyze the ratios of fat, protein, and lactose on the surface of food powders was developed by a group at the Institute for Surface Chemistry. Described in detail in their publications (Fäldt et al., 1993) they have used the relative ratios of carbon, hydrogen, and nitrogen to determine the signal produced associated with surface fat. This group and other researchers have been able to use ESCA for the surface analysis of powders (Fäldt and Bergenståhl, 1996; Kim et al., 2002, 2005b, 2009).

ESCA has been applied to examine various dairy powders. Research conducted by Fäldt and Bergenståhl (1996) was able to showcase the power of ESCA in observing and quantifying surface fat content in ways that are not possible through SEM and extraction techniques. For example, these researchers were able to observe movement of fat on to particle surfaces over time due to lactose recrystallization using ESCA, which was unable to be seen in SEM images. In spray-dried whey and lactose emulsions, surface fat content can range between 40 and 60% according to ESCA. Due to this high content of surface fat, it was concluded by Fäldt and Bergenståhl that whey protein and lactose wall materials serve only as “mediocre” encapsulants. They cited similar studies that reported successful, yet contradicting results when microencapsulating using whey proteins as wall materials. On the basis of SEM and extraction techniques, it was

observed that low surface fat was achieved using whey proteins and that whey proteins were excellent wall materials (Young et al., 1993a,b). The direct comparison of these two studies is difficult due to the use of different wall material oil phases, emulsification steps, and other spray drying parameters that most likely affect results. Due to this, making a definitive conclusion on the superiority of either method is difficult. Further research may be necessary to determine the most effective way to analyze surface composition of particles.

28.3.2 Particle Size

Particle size of microcapsules is often measured in the investigation and characterization of capsules (Sankarikutty et al., 1988; Young et al., 1993b; Hogan et al., 2003; Picot and Lacroix, 2003; Turchiuli et al., 2005; Fritzen-Freire et al., 2012). Microencapsulated particles generally are classified in the size range of 0.2−5000 μm (King, 1995). Particle size can influence physical properties such as moisture content, water activity, mass transfer, dispersion, core retention, and stability of wall and core materials, just to name a few (Chang et al., 1988; Walton and Mumford, 1999; Reineccius, 2004). Due to the influence of particle size on stability, a variety of methods can be used to measure particle size including visual optical microscopy, laser diffraction, dynamic light scattering, centrifugal separation, and time of flight (O'Hagan et al., 2005). Each of these methods has their optimal size ranges and sample types they can best accommodate. While these and many more methods exist, the most popular explored methods of particle size determination are laser diffraction and optical microscopy. Laser diffraction uses static light that is passed through a dispersion of particles in order to produce a light scattering signal that is interpreted by the device (O'Hagan et al., 2005). Optical methods are often employed by investigators counting a large number of particles in several specified ranges and then averaging to determine a measurement. When particles are smaller than 5000 μm, they are considered microcapsules (Anandaraman and Reineccius, 1980).

Particle size in relation to stability of the microencapsulated system is often investigated in scientific literature. Research done by Hogan and others (2001) observed that microencapsulation efficiency of dried emulsions increased as the dextrose equivalent (DE) value (ranging from 0 to 50) and particle size increased. The same researchers observed that when only considering the wall/DE value (28, corn syrup solids), the ratio decreased, and despite particle size increasing encapsulation efficiency decreased. These conflicting conclusions led to the belief that particle size was not the only factor affecting microencapsulation efficiency. Larger particles generally take longer to dry, causing them to expand, balloon, and form surface imperfections (Chang et al., 1988). This extended drying time can also lead to loss of volatiles and/or increased exposure of sensitive encapsulated compounds to atmospheric conditions (Chang et al., 1988). While a larger particle may have a lower surface area to volume ratio that would aid in core retention, it also takes longer to dry. This increased drying time can lead to core loss, especially when encapsulating a volatile compound (Chang et al., 1988). While the definitive correlation between particle size and stability seems to vary in the literature, it should be noted that knowledge of a system's particle size has the potential to impact a variety of physical, chemical, and sensory measurements.

28.3.3 Moisture Content and Water Activity

While moisture content and water activity are both indicators of the amount of water contained within a system, they both measure different "types" of water. They are both important for the stability of dried products (Picot and Lacroix, 2004). Moisture content is the measure of total moisture contained within a system. The moisture content is expressed as a percentage ranging from 0 to 100%. Water activity is a measure of the available water in a system for chemical and microbial reactions. The water activity of a system is normally expressed as a value between 0 (no free water) to 1 (all free water). The range of moisture content and water activity that keeps the microencapsulated system stable will vary greatly depending on the glass transition temperatures (T_g) of the matrix. Preserving this glassy matrix is influential to stability preservation in dried products (Bruni and Leopold, 1991). The glassy state is effective at forming a protective environmental barrier to limit molecular mobility (Ananta et al., 2004). This limited movement helps to lessen the impact of external stressors that may affect the microencapsulated core stability such as oxidation (Crowe et al., 1998). It is important to store and dry particles in temperatures below their given T_g. Not doing so may allow the system to return to a rubbery state that does not provide the same protection (Crowe et al., 1998). Most dried microencapsulated particles fall on the lower end of both these moisture scales. A moisture content between 1 and 6% and a water activity below 0.3 are generally produced via microencapsulation (Reineccius, 2004; Reid and Fennema, 2007). Spray-dried powders and particles exhibiting water activities below 0.3 would be considered stable as at this level they are not able to participate in microbial or biochemical reactions that could lead to degradation (Reid and Fennema, 2007).

Particle size may also affect moisture content, which is important for particle stability (Reid and Fennema, 2007). In terms of moisture content, Sankarikutty and others (1988) observed that their spray-dried particles in the ranges of 1−28 μm did not exhibit free-flowing properties due to elevated moisture content of the samples. Some researchers reported that particle size did not affect moisture content (Chang et al., 1988). Perhaps these differing observations are related to increases in particle size leading to decreased hygroscopicity. This decreased hygroscopicity leads to a slower rate of moisture uptake, which can affect long-term particle stability. Techniques used to measure both water activity and moisture have been thoroughly reviewed in other books (Reid et al., 2001; Fontana and Campbell, 2004; Fontana, 2007). Examples of both water activity and/or moisture content being used as part of microencapsulation stability analysis can be found extensively in a wide variety of published literature (Chang et al., 1988; Sankarikutty et al., 1988; Rosenberg and Sheu, 1996; Sheu and Rosenberg, 1998; Hogan et al., 2001; Picot and Lacroix, 2003; Corcoran et al., 2004; Fritzen-Freire et al., 2012; Ying et al., 2012).

28.4 FACTORS AFFECTING CORE STABILITY

While the reasons for use of microencapsulating ingredients are numerous, they generally surround the protection, isolation, or controlled release of a core ingredient (Shahidi and Han, 1993; Gibbs et al., 1999). Due to this, the effective and efficient encapsulation of core ingredients in the microencapsulated system is of primary importance. Efficiently encapsulating these materials will lessen the detrimental effects of environmental factors such as light, pH, temperature, and oxygen.

28.4.1 Environmental Factors Affecting Core Stability

The way in which environmental factors affect the stability of core materials varies depending on the core material being microencapsulated. Despite this variability, the effects that these factors have on several classes of cores and how they are measured can be explored.

28.4.1.1 Light

A common reason for the microencapsulation of a variety of core materials is due to their sensitivity to light (Shahidi and Han, 1993). When exposed to light and the right environmental factors, a variety of core materials such as carotenoids (Barbosa et al., 2005; Shu et al., 2006), vitamins (Wilson and Shah, 2007; Riaz et al., 2009), polyphenols (Trela and Waterhouse, 1996), and lipids (Scrimgeour, 2005) can degrade, isomerize, and/or generate free radicals due to their unique chemical structures.

For example, the compound bixin, a carotenoid found in seeds of the annatto tree, is highly sensitive to light. When this compound is exposed to UV radiation, it can isomerize multiple times and produce isomers with decreased color intensity (Montenegro et al., 2004). Decreased intensity could be a problem for the food industry, as annatto extracts are primarily used for the coloring of a variety of liquid, solid, and semi-solid food systems (Scotter, 2009). In order to attempt to avoid this problem, Barbosa et al. (2005) microencapsulated bixin by spray drying using gum arabic or maltodextrin as wall materials. They were able to observe longer decay times with encapsulation as compared to non-encapsulated bixin. Investigation into optimal formulations for other light-sensitive carotenoids processed by spray drying to improve encapsulation yield and efficiency is also of importance to other research groups (Lee and Chen, 2002; Shu et al., 2006).

28.4.1.2 pH

The isolation of core materials from harsh environmental conditions (which can be pH) is one of the many reasons to encapsulate materials. While the stability losses due to pH are core specific, a variety of detrimental reactions can occur such as a decrease in probiotic viability (Kailasapathy and Chin, 2000; Picot and Lacroix, 2004; Champagne and Fustier, 2007), chemical structure changes (Trela and Waterhouse, 1996), and autoxidation (Kirby et al., 1991). For these reasons and others, microencapsulation technologies are utilized to increase stability.

Microencapsulation is a technology that can be used to enhance the viability of probiotic bacteria in foods during processing and during the transit through the human stomach (Ding and Shah, 2009). The pH of the stomach is extremely acidic, as low as 2, making it inhospitable for many microorganisms including the two major genera of probiotic bacteria, *Lactobacillus* and *Bifidobacterium* (Kailasapathy and Chin, 2000; Picot and Lacroix, 2004; Ding and Shah, 2009). This harsh environment is a challenge for probiotic supplementation of foods. Probiotic bacteria should be

present in high amounts ($\approx 10^6$ bacteria per gram) in the product and be able to withstand the harsh environment of the stomach to reach the large intestine to elicit their beneficial effects (Shah, 2000, 2007; Lourens-Hattingh and Viljoen, 2001). Researchers have observed that through microencapsulation of probiotic bacteria in whey protein-based microcapsules, certain strains of *Bifidobacterium* showed significantly higher viability after storage in yogurt for 28 days (Picot and Lacroix, 2004). The same strain of *Bifidobacterium* (*Bifidobacterium breve* R070) also exhibited higher rates of survival in simulated gastric conditions than free cells. However, even within this study, another strain of *Bifidobacterium breve* saw no improved viability in the yogurt-simulated gastric solution due to microencapsulation (Picot and Lacroix, 2004). It is important to realize that the ability of probiotic bacteria to survive these harsh conditions regardless of microencapsulation method employed may be genus, species, and/or strain specific (Lian et al., 2002; Picot and Lacroix, 2004; Ding and Shah, 2009).

28.4.1.3 Temperature

Elevated temperatures due to environmental conditions and processing parameters can pose problems for a variety of heat labile core materials. While microencapsulation does not always involve elevated or lowered temperatures, some methods such as spray drying, spray chilling, freeze drying, and air suspension use these temperatures to make the methods possible (Shahidi and Han, 1993; Gibbs et al., 1999). It is important to understand the sensitivity of the particular core material being microencapsulated as well as the principles behind each technique to ensure maximum stability for the microencapsulated core material is maintained.

While high temperatures are ideal for the inactivation of pathogenic microorganisms, they can also destroy beneficial bacteria. This poses an interesting challenge for researchers wishing to use microencapsulation by spray drying for the delivery of probiotic bacteria. Probiotic bacteria are most often defined as "live microorganisms, which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host" (FAO/WHO Experts' Report, 2001). Part of this definition includes the live bacteria. To achieve this, manufacturers need to protect the bacteria against the harsh conditions of the stomach and upper gastrointestinal (GI) tract (Naidu et al., 1999; Shah, 2000). This is where microencapsulation becomes important. Research has shown that despite the high temperatures employed in spray drying, survival of bacteria has increased compared to free bacteria in the model GI (Picot and Lacroix, 2004), storage (Goderska and Czarnecki, 2008), and food systems (Kailasapathy, 2006). Using moderate outlet temperatures, the temperature primarily responsible for heat-related damage to microorganisms will help to increase probiotic stability (Lian et al., 2002; Ananta et al., 2005). When outlet temperatures are kept between 80 and 90°C, probiotic viability is greater than compared to that with higher temperatures (Masters, 1985; Ananta et al., 2005). A comprehensive review regarding the microencapsulation and probiotic bacteria and ways to measure core viability can be found in the literature (Shah, 2000; Rokka and Rantamäki, 2010; Silva et al., 2011).

Additional examples of microencapsulated heat-sensitive core materials include flavor compounds. Despite this sensitivity to heat, spray drying is the most widely used method for flavor encapsulation (Reineccius, 1988, 2004; Sankarikutty et al., 1988; Desai and Jin Park, 2005; Gharsallaoui et al., 2007, 2012). Fast formation of the low moisture particle surface facilitates selective diffusion. This low moisture surface (7−23%) limits permeability of small flavor compounds but allows the diffusion of water, which lowers the particle temperature by evaporative cooling (Judson King, 1995; Reineccius, 1988, 2004). Despite this, flavor compounds are labile to heat during processing due to their volatile nature and the drying process should be carefully monitored to ensure proper encapsulation (Chang et al., 1988; Sankarikutty et al., 1988; Galmarini et al., 2008). Due to its versatility, volatile retention using spray drying can be improved by adjusting drying temperatures, infeed solids, wall materials, additives, and a variety of other variables (Reineccius, 1988, 2004; Walton and Mumford, 1999; Desai and Jin Park, 2005; Gharsallaoui et al., 2007). Inlet temperatures in the range of 160−210°C and outlet temperatures above 100°C have been cited as optimum temperature levels for flavor retention (Reineccius, 2004). High infeed solids help in the fast formation of the initial semipermeable dry layer on the particle, therefore limiting diffusion and improving volatile retention ((Reineccius, 2004). Despite this, an optimum infeed concentration will be based on the particular carrier and flavor encapsulated (Reineccius and Bangs, 1985; Reineccius, 1999). A thorough review on this topic can be found in Reineccius and Bangs (1985).

28.4.2 Oxidation Effects on Core Stability

Since one of the major goals and beneficial effects associated with microencapsulation involves the isolation of a core material from its environment (Shahidi and Han, 1993), this makes microencapsulation particularly useful for those core ingredients that are susceptible to oxidative degradation. Measuring the oxidation of the encapsulated ingredients

can provide information regarding how successful the encapsulation process was both immediately after processing and over long-term storage.

Examples of monitoring core material oxidation over both the long and short term can be found from many articles. Although processing techniques can cause lipids to initially oxidize (Gharsallaoui et al., 2012), it is important also to look at the oxidative stability of the microcapsule over time. Oxidation can affect core materials in a variety of ways. It has been shown to be responsible for microorganism death during storage (Teixeira et al., 1996). Utilizing headspace analysis coupled with gas chromatography, Moreau and Rosenberg (Moreau and Rosenberg, 1996) monitored the hexanal and oxygen uptake as markers of sample oxidation. They found that microencapsulated anhydrous milkfat (AMF) oxidized more slowly when stored in the dark as opposed to the light, exhibited no oxygen uptake, and had lowered hexanal production. Another practical application of oxidation measurements to monitor stability of microcapsules was observed in research involving microencapsulated fatty acids. Due to their ability to oxidize easily, incorporation and long-term stability of these ingredients into foods pose a challenge. Research involving the microencapsulation of fish oils and omega-3 polyunsaturated fats is looking at overcoming these challenges (Andersen, 1995; Kolanowski et al., 1999; Hogan et al., 2003; Serfert et al., 2010; de Conto et al., 2012). One group of researchers (Hogan et al., 2003) used peroxide values to monitor oxidation and looked at the effect that maltodextrins of varying lengths, core/wall ratios, and antioxidants have on oxidation. This is just one example of many using microencapsulation to reduce oxidation of core materials.

28.4.2.1 Measurement of Core Oxidation

Core oxidation not only affects lipid-based systems, but has also been shown to negatively affect the viability of microencapsulated probiotic organisms (Teixeira et al., 1996; Champagne and Fustier, 2007). Oxidation of the core material, if applicable to the microencapsulated system, can be measured in a variety of ways using sensory evaluation or chemical techniques, or a combination of both. While no singular oxidation detection methodology will identify all products of oxidation, a combination of methods can be employed to fully investigate the oxidation of a sample. Methodologies to measure oxidation include thiobarbituric reactive substances (TBARS), peroxide-oxygen value, conjugated dienes, and gas chromatography (GC). Further detailed information on how to perform these methodologies and what they identify can be found in the scientific literature (Gray, 1978; Guillén-Sans and Guzmán-Chozas, 1998; Dobarganes and Velasco, 2002).

28.4.2.2 Measurement of Surface Oxidation

When using lipid-based core materials or carriers in a microencapsulated system, their presence on the surface of a microcapsule is not only an issue from a microencapsulation efficiency standpoint, but also as regards storage and stability. Lipids on the surface of particles can decrease the flowability and dispersibility of powders (Fäldt et al., 1993; Fäldt and Bergenståhl, 1996; Kim et al., 2005b). Due to the ability of lipids to oxidize readily, the microencapsulated system may degrade prematurely if lipids on the surface of the particle are exposed to oxygen, light, heat, or any other oxidation-promoting factor (Fäldt et al., 1993; St. Angelo et al., 1996; Hardas et al., 2000; Kim et al., 2005a). It is therefore important to be able to quantify their presence on the microcapsule surface.

Quantification of surface fat can be accomplished through the use of modified extraction techniques. These techniques are described as modified due to the relatively short exposure time to an organic solvent as opposed to more traditional extraction techniques for measurement of total fat content. While methodological specifics may vary, when examining the use of surface fat of a microencapsulated ingredient the basic steps remain the same from procedure to procedure. A small amount of microencapsulated powder is exposed to a solvent (typically petroleum ether (Sankarikutty et al., 1988) for a short amount of time (under 15 minutes) with slight agitation. The solvent with extracted fat is then filtered through a membrane followed by solvent evaporation to determine the amount of fat in the solvent (Fäldt et al., 1993; Young et al., 1993b; Picot and Lacroix, 2003; GEA Niro, 2005; Kim et al., 2005a; Turchiuli et al., 2005).

Quantifying fat content on the surface of particles has helped explain the stability and functionality of microcapsules for several studies. The agglomerative nature of microencapsulates produced by Picot and Lacroix (2003) was attributed to the relatively low microencapsulation efficacy (a function of the surface or free fat) of their microcapsules. When fat is used as core ingredient, microencapsulated fat will oxidize at a slower rate than non-encapsulated fat (Turchiuli et al., 2005). Therefore, its presence on the surface is an indication of decreased microencapsulation efficiency and may lead to an ultimate decrease in the oxidative stability of the matrix (Sankarikutty et al., 1988).

28.5 SENSORY IMPACTS OF MICROENCAPSULATED INGREDIENTS IN FOODS

28.5.1 The Field of Sensory Evaluation

While sensory evaluation has been carried out on foods and consumer goods since humans first came into existence, formal testing and evaluation can be traced back to wartime efforts made in World War II with the desire to improve the quality of American soldiers' food (Dove, 1946). There was a strong desire to establish a systematic and scientific methodology to test for sensory qualities and acceptance of these foods (Foster, 1954; Stone and Sidel, 1985). The scientific discipline of sensory evaluation is most commonly described (Stone and Sidel, 1985; Lawless and Heymann, 1999b) using the definition developed by the Sensory Evaluation Division of the Institute of Food Technologists (IFT). This definition describes sensory evaluation as "a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touching and hearing" (Anonymous, 1975). While defining a field of study with a singular definition is not completely inclusive of the entire discipline, for basic purposes, this definition seeks to describe the overarching goals and focus of sensory evaluation (Stone and Sidel, 1985). Each sense addressed in the above definition could be elaborated upon in great detail, and while each is important to the overall acceptance and quality of foods, only basic knowledge surrounding each sense and the perception related to will be discussed in this chapter.

28.5.2 Sensory Attributes and Human Senses

28.5.2.1 Appearance and Vision

When we first receive a product for sensory evaluation, the first judgment is typically made on appearance, thus, the human sense of sight is extremely important. Appearance is perceived through the eye when reflected light travels through the eye and to the brain where signals are interpreted (Lawless and Heymann, 1999a). Appearance can serve as a marker for quality (e.g., ripened vegetables), safety (e.g., the presence of green mold), and completeness in processing (e.g., bread sufficently darkened), and can influence the acceptance (i.e., liking or preference) and perception (i.e., flavor and intensity) of food products (DuBose et al., 1980; Francis, 1995; Lawless and Heymann, 1999a; Zellner and Durlach, 2003; Delwiche, 2004; Shankar et al., 2010).

Color is considered a three-dimensional property consisting of hue (the observable specific colors; 10 exist), value (the lightness or darkness of a color), and chroma (the brightness of a color) (Munsell, 1905; Lawless and Heymann, 1999a). This is the primary reason why human subjects should not be asked to measure color. Asking sensory panelists to score three properties on a single scale would be like asking a panelist to rate texture, color, and flavor on the same scale. Mathematical relationships describing color exist, and are highlighted in several outside sources (Clydesdale and Ahmed, 1978; Lawless and Heymann, 1999a).

28.5.2.2 Taste and Gustation

There exist five basic flavors associated with the human perception known as gustation: sour, sweet, salty, bitter, and savory (umami). These chemical senses are perceived by taste buds located in the oral cavity and in portions of the throat and esophagus (Meilgaard et al., 1999; Roper, 2012). Contrary to popular belief, the five basic tastes are perceived in all areas of the tongue and not limited to specific areas or the popularized "tongue map" (Lindemann, 2001; Roper, 2012). The physiological and biochemical mechanisms associated with the perception of each basic taste are further described in several excellent scientific review articles and book chapters (Lindemann, 2001; Chandrashekar et al., 2006; Sugita, 2006; St. John and Boughter, 2008).

28.5.2.3 Odor and Olfaction

The perception of olfaction is the most complex and elusive of the human senses (Savic, 2001). Olfaction is highly sensitive in comparison to gustation: thousands of odors are recognized at much lower concentrations compared to some of the most potent taste compounds (Nef, 1998). Olfaction is important not only in food selection, preference, and acceptance but in biological and physiological functions (Nef, 1998; Axel, 2006; Stevenson, 2010). The senses of olfaction and gustation are combined with the perception of "chemical feeling" factors (cooling, heating, etc.) in order to produce the common sensory descriptor of flavor (Meilgaard et al., 1999).

During the process of olfaction, volatile compounds enter the nasal cavity either nasally (through the nose) or retronasally (through the nasal cavity) and bind to cilia extending from the olfactory epithelium. These cilia start a cascade

of signals eventually ending at the olfactory bulb, which serves as a relay station to the portion of the brain responsible for aroma processing (Axel, 2006). Information relating to the specific theories of olfactory transduction, signaling, and olfactory transduction are further described in other sources (Ache and Zhainazarov, 1995; Roper, 2012).

28.5.2.4 Texture and Touch

Texture is a complex form of sensory perception. The complexity of texture is clearly outlined by the multidimensional nature of the definition given by the International Standards Organization. The definition includes not only the mechanical and geometrical attributes perceived by tactile and mechanical sensations but visual and auditory responses as well (International Standard ISO 5492 and British Standard BS 5098:1992, 1992). Texture perceived via proprioception in the oral cavity corresponds to deeper pressure responses while somesthesis refers to lighter surface responses. These two perceptions can function during the process of mastication and swallowing (Szczesniak, 2002; Kilcast and Leatherhead Food International, 2004). In-depth analysis into each of these perceptive touch mechanisms can be found in the literature (Christensen, 1984).

Existing at somewhat of a dichotomy, texture is regarded as one of the primary drivers of the liking for food products (Szczesniak, 1990; Moskowitz and Krieger, 1995; Wilkinson et al., 2000) yet it often goes unremarked on by consumers unless the subject is specifically raised (Szczesniak and Kahn, 1971). The lexicon used to describe textural attributes is vast (Szczesniak, 1963; Civille and Szczesniak, 1973). Specific textural attributes listed are often associated with negative or positive connotations. Although these connotations may be linked to cultural and psychological factors, general trends have been observed (Szczesniak and Kahn, 1971). In the end, however, an acceptable or optimal texture is product and demographic specifics should be examined using sensory analysis.

28.5.3 Sensory Impacts of Microencapsulated Food Ingredients

While the food ingredients used in microencapsulation could affect a myriad of sensory properties if not encapsulated, including taste, odor, flavor, and texture, considerations for these sensory properties still need to be addressed when assessing their inclusion in food via microencapsulation. Despite the use of relatively inert wall materials in terms of taste and odor (Sanguansri and Augustin, 2010), and the attempt to completely isolate encapsulated flavor and aroma compounds from their environment, the effects of microencapsulated ingredient on sensory properties have been widely investigated (Hinton, 1970; Minifie, 1989; Boccio et al., 1996; Xia and Xu, 2005; Kailasapathy, 2006; Muthukumarasamy and Holley, 2006; Galmarini et al., 2008; Homayouni et al., 2008; Serfert et al., 2010; de Conto et al., 2012).

28.5.3.1 Textural Impacts of Microencapsulated Food Ingredients

Research in the field of sensory evaluation regarding particle size influence on food matrices has led to varying conclusions regarding their effect on texture. General conclusions on absolute size detection thresholds and acceptability are hard to reach, as results are particle size, shape, concentration, and matrix specific (Hinton, 1970; Cook, 1972; Evans et al., 1991; Tyle, 1993; Imai et al., 1995, 1997; Engelen et al., 2005). For example, in confectionery literature, it has been observed that the minimum particle size detected by the palate is 25 μm (Hinton, 1970). Therefore, incorporating a particle greater than this size into a chocolate matrix may elicit a negative textural response due to lack of smoothness. However, the limit of particle size for optimum texture is matrix specific, with values for milk (65 μm) and dark chocolate (35 μm) varying considerably (Minifie, 1989). It is also noted that particle shapes of various ingredients such as sucrose or cocoa powder also influence the textural properties of grittiness and roughness (Minifie, 1989). It is generally observed, however, that as particle size and concentration increase in a medium, so does the perceived grittiness of the sample (Tyle, 1993; Imai et al., 1995, 1997; Kilcast and Clegg, 2002; Engelen et al., 2005).

The influence of a particular particle, however, on textural attributes is not only dependent on particle size and concentration but also on the particle's surface morphology and matrix of application (Tyle, 1993). Thus, microencapsulation research that looks at the textural impact of these particles on matrices can only truly describe the particular product of application. It is hoped that these particle influences can be extrapolated to other similar food systems. Investigating the influence that alginate-encapsulated probiotics have on the hedonic, textural, flavor, and purchase intent of fermented sausages, Muthukumarasamy and others (Muthukumarasamy and Holley, 2006) used two different methods to produce microencapsulated *Lactobacillus reuteri*: extrusion and emulsion. These methods produced dramatically different-sized microcapsules. Microencapsulation by extrusion produced particles with diameters in the micrometer range while emulsion technology produced spheres in the millimeter range. Despite these differences, neither type

of microcapsule influenced the texture of the food system. The authors attribute this to the matrix, noting that the extruded capsules resemble fat and despite being visible went undetected at the usage level.

Textural impacts of microencapsulated ingredients, however, are not only a result of particle size. The ingredient in its native form may impart textural characteristics such as astringency on the food matrix (Peleg et al., 1999). An example of an ingredient that may impart taste, bitterness, textural, and astringency influences into a food matrix is grape seed extract (GSE) (Chira et al., 2009). This extract, composed of a variety of polyphenolic compounds, presents a challenge for food matrix incorporation due to its ability to easily oxidize, polymerize, and react with proteins (Chira et al., 2009; Davidov-Pardo et al., 2011). Researchers investigated the influence of these spray-dried microencapsulated antioxidants on the sensory quality and consumer perception of baked goods (Davidov-Pardo et al., 2012). Conducting a complete descriptive analysis using quantitative descriptive analysis (QDA) as well as a consumer test, they were able to both qualitatively and quantitatively describe their products. QDA revealed that while the encapsulated GSE helped to slightly reduce the color changes and improve antioxidant activity, flavor, aroma, and textural attributes were affected by the microencapsulated ingredients. The QDA panel noted changed textural attributes such as the development of a dryer mouthfeel and decreased tenderness with the inclusion of the microencapsulated GSE. Despite these changes, results of the consumer evaluation and purchase intent yielded positive results; no significant differences in mouthfeel liking existed between the cookie formulations. While the researchers were unable to eliminate the flavor and textural impacts of GSE on this food matrix, microencapsulation did aid in improving antioxidant activity. Furthermore, consumers did not notice differences in textural attributes of the product that the trained panel did. These results are promising and will hopefully lead to further investigation.

In summary, when considering the sensory impact of the microcapsule in a food system, the particle shape, concentration, size, sample matrix, and ingredient properties should be considered for each product and its influence quantified and understood through sensory analysis (Tyle, 1993).

28.5.3.2 Flavor and Odor Impacts of Microencapsulated Food Ingredients

As mentioned previously, ingredients are often microencapsulated to mask or hide unpleasant tastes and/or odors (Gibbs et al., 1999). Being able to do so may allow the application of an ingredient to a food that may otherwise negatively impact its sensory quality. Ferrous sulfate is a readily soluble and highly bioavailable form of iron, an essential micronutrient (Boccio et al., 1997). Despite these benefits, the micronutrient also has the ability to interact with other components of the matrix (lipids, vitamins, amino acids) producing metallic and oxidized off-flavors (Boccio et al., 1997; Xia and Xu, 2005). Incorporating this form of iron into food systems would be beneficial, as iron deficiency is the most common nutritional deficiency in the world (Horton and Ross, 2003). To overcome these challenges, researchers have turned to microencapsulation (Boccio et al., 1996; Xia and Xu, 2005). Through the use of liposomes, milk was successfully fortified with ferrous sulfate with high efficiency (67%) and improved stability was imparted using cholesterol and Tween 80 (Xia and Xu, 2005). Limited sensory work was conducted using a small number of panelists to determine if differences in flavor arose due to the inclusion of the encapsulated ferrous sulfate affecting the "off-flavor" of the milk. While specific data are not reported, the published article states that the fluid milk "did not differ greatly from control milk in color and off-flavor" (Xia and Xu, 2005). While these results are promising, details including testing methodology, significance levels, ratings, and statistics would help to strengthen the authors' findings. Another published article regarding the microencapsulation of ferrous sulfate using lecithin suffers from the same lack of specific details on sensory analysis (Boccio et al., 1996). While the article highlights the successful microencapsulation of ferrous sulfate, it is only stated that the sensory properties of the milk were unchanged. No analysis or specifics regarding testing methodology are given (Boccio et al., 1996). While the aforementioned papers addressing the incorporation of microencapsulated food ingredients without altering sensory properties are promising, specific details regarding their sensory methodologies and results leave many unanswered questions. Despite these instances, the results of detailed research utilizing sensory properties to observe effects of microencapsulated ingredients on flavor have been published (Kailasapathy, 2006; Muthukumarasamy and Holley, 2006; Galmarini et al., 2008).

The aromatic sensory properties of six microencapsulated orange oils spray dried in carbohydrate-based coatings were investigated using both difference testing and descriptive analysis (Galmarini et al., 2008). For the difference testing portion of the study, assessors were asked to generate attributes that characterized differences existing between the paired samples along with the triangle test. Frequencies of these generated terms for specific samples were used for the identification of control samples for the descriptive analysis portion of the study. Results of the difference tests concluded that all samples, when paired, exhibited significant differences except for one pairing. Descriptive analysis investigated 14 different aroma attributes using control samples (uniquely identified for each attribute from the

difference test), which identified several significant differences among the samples. Descriptive analysis revealed that the incorporation of trehalose and sucrose into the encapsulation matrix generated particles with different aroma compositions. Samples containing sucrose were characterized as having aromatic attributes such as "freshly squeezed," "tangerine," "candy," and pungent while trehalose-containing samples were characterized by "oody" and "marmalade" notes. While the effect of these two ingredients on individual encapsulated matrices will not be identical, it is important to note how the encapsulation matrices can alter sensory flavor properties.

28.5.3.3 The Impact on Hedonic Ratings and Consumer Perception Due to Microencapsulated Food Ingredients

While the inclusion of microencapsulated ingredients may not be something necessarily advertised by a manufacturer, the microcapsules used have the potential to modify consumer perception and acceptance. The sensory attributes that specifically contribute to overall acceptance and liking are determined by the individual consumer (Moskowitz and Krieger, 1995) but generally form the following hierarchy: flavor/taste, texture, appearance (Moskowitz and Kreiger, 1993, 1995). The impacts of microencapsulated ingredients on these attributes have been addressed in the above sections; however, their impact on specific overall liking is also of importance.

When a microencapsulated ingredient possesses sensory attributes that are in misalignment with or unfavorable to a food matrix, the goal is to provide little or no change to primary sensory qualities of the product. If sensory changes are desired, it is hoped that they occur in a manner favorable for the product. These changes and their effect on overall liking of the product can be monitored through acceptance testing. Using overall acceptance tests, many of the above studies and others found no difference (Boccio et al., 1996; Xia and Xu, 2005; Kailasapathy, 2006; Muthukumarasamy and Holley, 2006; Homayouni et al., 2008; Davidov-Pardo et al., 2012) from a control formulation in one or more of their microencapsulated included formulations. This is seen as positive, as a majority of the above microencapsulated ingredients have the potential to impart negative sensory attributes if they were not encapsulated.

With the knowledge that microencapsulated ingredients are present in a food matrix, consumer perception of the product may change. By providing added benefits, many microencapsulated ingredients incorporated into foods create products known as functional foods—foods that provide nutritional benefits beyond basic needs (Bech-Larsen and Grunert, 2003). And while some research has shown consumers are willing to compromise on taste for added health benefits (Urala and Lähteenmäki, 2004) not all are in agreement (Verbeke, 2006). Due to this disparity in opinions, it is important to understand the effect the particular food system has on consumers. The research mentioned previously utilizing GSE in cookies found several significant impacts of added antioxidants on consumer perception of their product (Davidov-Pardo et al., 2012). Their work showed that the best rated cookies had the highest purchase intent ($p < 0.01$), signifying the important influence of a good tasting product on purchase intent. Despite this significant correlation, only 25% of participants said they definitely or probably would pay more for these antioxidant cookies. This may be a result of mistrust or lack of knowledge concerning the usage and benefits of functional foods. Over 70% of participants found the statistic regarding the content of antioxidants (in as much as a 4 oz. serving) moderately or not at all believable. Educating the consumer on the encapsulated ingredients may lead to an increased interest in purchasing the enriched food, as belief in the antioxidant statement and a willingness to pay a higher price were significantly and positively correlated. Therefore, success is not only achieved from producing a good tasting product; it is also important to have a market of consumers interested and willing to pay for the new product.

28.5.4 Considerations for Sensory Testing of Microencapsulated Food Ingredients

A variety of sensory methods can be employed to measure the sensory impact of microencapsulated food ingredients on the food system. Before deciding on a sensory method, the safety and legality regarding the use of the microencapsulated ingredient must be considered. Microcapsules must be produced in a food-grade facility, utilizing only food-grade materials (Gibbs et al., 1999). This includes the core, wall, and any other additives used in the production of a microcapsule. Additionally, legal limits, if applicable, surrounding the preparation, inclusion, and administration of the particular core in relation to human subjects need to be considered and followed throughout sensory testing. In an industrial setting, a governing body to make decisions regarding the ethical nature of a sensory study may not exist. While the presence of such a body is not required, the ethical treatment of all those involved should always be considered (Morris, 1967).

The decision to run a sensory test on a microencapsulated system should, like all other aspects of a research design, be considered at the beginning of the investigation. When sensory evaluation is seen as an integral component of

research rather than an afterthought, the results produced will undoubtedly be of more value to the investigator. What the investigator wants to determine through sensory analysis will dictate the methodology of the test, the way the results are analyzed, and the conclusions they will be able to draw.

28.5.5 Choosing a Sensory Methodology for Testing

The initial choice of a particular sensory test is one of the most important decisions an investigator can make regarding sensory analysis. The selection of a specific method should be based on the experimental goals of the research. There are three major types of sensory tests: discriminatory, affective, and descriptive. Discrimination tests are employed to simply determine if the two samples are different. Commonly used discrimination tests include the triangle test, duo-trio test, tetrad test, and two- and three-alternative force choice (AFC) tests. Affective testing is used to assess the preference for or acceptance of a product using target consumers. Affective tests can be classified into qualitative (focus groups, one-on-one interviews) or quantitative (preference, acceptance) tests. Descriptive testing uses trained judges to assess a complete sensory profile of a given product by identifying qualitative and quantitative aspects. Various methodologies for descriptive analysis exist including, but not limited to, the Spectrum™ method, QDA, flavor profile, and texture profile. Best practices for the above-mentioned sensory test methodologies are outlined in various textbooks published by leaders and pioneers in the sensory field (ASTM Committee E-18 on Sensory Evaluation of Materials and Products, 1981, 1992, 1996; Stone and Sidel, 1985; Lawless and Heymann, 1999a; Meilgaard et al., 1999).

28.6 CONCLUSION

Characterizing the stability of microencapsulated food ingredients involves the determination of a variety of both chemical and physical properties. The researcher is entitled to investigate whatever parameters they see fit for their specific system, although many methods presented in this chapter are commonly used. It is suggested, however, that both the wall and core stability are considered when determining which methods to utilize. Ingredients have the ability, even when microencapsulated, to affect a myriad of sensory properties. Basic knowledge of these sensory properties and the methodologies used to measure them will help the researcher determine the effects the microencapsulated ingredient may have on their product. With the knowledge concerning the stability of an ingredient and its effect on sensory properties, food products containing microencapsulated ingredients can be produced and sold on the market. These products may have the ability to prevent disease states, and provide increased shelf-life, functionality, or stability. The possibilities are endless and the field of microencapsulation still needs further investigation and improvement.

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Part VI

Regulatory, Quality, Process Scale-Up, Packaging, and Economics

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Chapter 29

Regulatory Considerations of Encapsulation Used in the Food Industry

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29.1 INTRODUCTION

One of the biggest challenges facing product developers is balancing the desired functionality of their ingredients in the consumer product with the increasing complexity of regulations, food laws, and consumer preference. This balance plays a critical part in the cost/benefit analysis performed when selecting encapsulating ingredients. The primary regulatory considerations when using encapsulated food ingredients pertain to the type of encapsulant selected for the project: ensuring the ingredient fits the needs of the customer, the application, and the market. These regulatory considerations can be summarized under source trials, labeling requirements, and safe use.

From a regulatory standpoint, selecting an encapsulating ingredient solely based on function may not be ideal. A major consideration should be the source of the ingredient. Each source can potentially create repercussions in the labeling or safe use of an ingredient. Some of the major regulatory concerns to review when looking at encapsulant source are animal derivatives, allergens, biotechnological genetic modification, organic, natural claims, and nutritional content. While this chapter is not meant to be all encompassing, it will outline some of the major questions when considering which encapsulant to choose when developing a product. While this information is current at the time of writing, companies must ensure the data they are using is the most recent version as regulations and requirements for compliance can change. For more information the Institute for Food Technologists offers a very helpful summary page with links to a wide variety of information on food additives (Institute for Food Technologists, 2014).

29.2 ANIMAL DERIVATIVES

Animal derivatives can significantly complicate the regulatory compliance of an ingredient and put many hurdles in front of export. The list of animal-derived encapsulants can be extensive; it includes waxes (when insect derived), gelatin (often animal or fish product), animal fats, animal proteins, albumin (egg), caseins (dairy), and chitosan (crustacean shells). Animal derivatives may not meet the customer claims in some cases, such as vegetarian or vegan. Lack of formal regulatory definition regarding these claims introduces further questions to be resolved. Dairy derivatives may require additional registration before export. Meat or fish derivatives may need governmental inspection certificates or statements of health due to fears of animal handling safety or disease (such as bovine spongiform encephalopathy, BSE-TSE). Additionally, even relatively "simple" claims such as kosher or halal can be challenging when working with animal derivatives; meat or fish components are more difficult to find in kosher and halal certified forms.

It is not always obvious which ingredients are derived from animal sources and sometimes they may not be listed at all; when used as a processing aid or additive they may not be listed in the ingredient statement from the manufacturer. Care should be taken to receive manufacturer documentation to substantiate any of these classifications; ingredient statements solely should not be interpreted to support any legal claim as incidental additives are not required to be labeled per 21 CFR 101.100(a)(3) (FDA, 2013b). Many encapsulants could be considered incidental additives depending on the circumstance and the type of product in which they are found. Incidental additives are not required to be listed so long

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00027-3

as they are present at insignificant levels and have no technical or functional effect. For example, white refined sugar is sometimes filtered through bone char, creating vegan and vegetarian difficulties. So before making any claims, it is important to get formal statements from the manufacturers to legally support the language desired for the product.

29.3 ALLERGENS

Furthermore, animal derivatives can have the additional complication of containing allergens, but allergens are much further reaching than only animal-derived ingredients. In the United States, labeling of the "Big 8" allergens is required: milk, egg, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean. These are considered by the US Food and Drug Administration to be major food allergens per the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) (FDA, 2006). There are very few exceptions made to these labeling claims; one is for highly refined and deodorized oils derived from specific allergenic materials such as soybean. One should not rely on an exemption to allow the use of a selected encapsulant as most (if not all) will require allergen labeling if derived from any of the major food allergens (FDA, 2013f). Furthermore, certain sensitizers, such as sulfites, are required to be labeled in the US. Many starches can contain sulfites as a byproduct of the processing to which the ingredients are subjected. Sulfites are required to be labeled on consumer products when over 10 ppm (FDA, 2013b). Typically, the level in the consumer product is less than 10 ppm but regulatory departments do need to track the level of sulfites coming from the various starches and maltodextrins as the effect may be cumulative. Required allergen labeling has had a huge positive impact on the level of consumer choice for safe products for consumption and does continue to change as new data become available. These new data may allow for even more consumer choice.

In 2013, the US FDA published a regulation allowing the voluntary use of the "Gluten Free" claim on consumer products so long as they meet specific requirements, including a limit of 20 ppm on the unavoidable presence of gluten (FDA, 2013d). Products meeting the claim cannot intentionally contain ingredients that are wheat, rye, barley, or any crossbreed of those grains that was not processed to remove gluten. While this provides more confidence in safe gluten-free choices for the celiac consumer, it does need to be considered when selecting an encapsulant. Starches and maltodextrins especially may be derived from cereals such as wheat and may not support gluten-free claims. There are, however, some "smart" choices for encapsulants, so this market could be a good area of opportunity for product developers.

As allergen labeling is required for the major allergens in the US, an ingredient using these encapsulants may also be subject to labeling. Allergen content and labeling is not desirable in many consumer markets, so must be considered when selecting ingredients. The definition of what is defined as an allergen and how allergens are labeled internationally is not harmonized, causing complexities for regulatory departments to meet the demands of export. Unless the animal-derived or allergen-containing encapsulant delivers functional benefits above other, less controversial ingredients, companies will likely find it preferable to avoid them. Export barriers may exist in practice even if not in theory; sometimes the export documentation is very complex and very challenging to meet so avoiding those types of ingredients may be helpful. There are good alternatives.

29.4 GENETIC MODIFICATION AND ORGANIC

Carbohydrates are the most common source of encapsulation materials. While carbohydrates can be derived from marine, microbial, or animal sources they are most often refined from plant crops. In such cases, often encapsulating agents are derived from crops that have been subject to biotechnological genetic modification (GM). This can include maltodextrins, starches, and dextrins. Derivation from GM crops can create another type of trade barrier; even if accepted in theory in the countries of export or for that matter even if allowed for use in the United States. There is a growing concern over the use of GM in the US. GM ingredients currently are not required to be labeled in the US but many states are working on passing state regulations requiring that this change. This is causing many industry groups and large food companies to consider pushing the FDA to work faster on national legislation to prevent disharmonized regulations state by state. The possibility of companies needing to understand and comply with dozens of different standards of thresholds and label requirements is a valid concern.

Due to this perceived gap in the body of US regulation, there is a growing movement pushing towards third party certification standards for substantiating that an ingredient or consumer product does not contain GM-derived ingredients. Many companies and supermarkets are already putting measures in place, either requiring ingredients not containing GM or are working towards a goal of GM labeling on their products. In many countries outside the US, especially in Europe, GM ingredients are required to be labeled, can only be imported if on the approved list of strains, and even then are often banned by consumers (European Commission, 2013). GM regulations differ significantly from country to

country and are at this time controversial in the US. Depending on the customer's needs, the consumer application and the market product developers may likely find it beneficial to avoid GM ingredients. Often organic certification is viewed as one way this controversy can be avoided.

Many developers see organic certification as a way to bypass some of these trials posed by GM-derived ingredients. Traditionally, organic certification was the most conservative way to address many of the GM claims for countries in which they are in place. But in today's market, organic materials have challenges that need to be considered in the cost/benefit analysis of encapsulates. The development of third party standards for "Non-GMO" claims may cause issues where organic certified products may not always be accepted. Cost may be high. Supply may be low, inconsistent, or subject to high minimum orders. High lead time and potential harvest-to-harvest variation may also be a factor. The functionality of the ingredient may not be comparable to that of more processed alternatives. US Organic Certification is not accepted in all countries, so export may be difficult, although some countries such as Canada and Japan have worked on organic equivalency agreements. Despite these difficulties, organic carriers are an area of recommended investigation for any product developer. While cost may be higher and function not ideal, some organic ingredients, like organic certified gum acacia, can provide regulatory simplicity if they are not animal derived, not considered an allergen, and not found in GM form. Organic certification is sometimes viewed as the best legal support for additional desirable claims such as "natural" in today's regulatory environment.

29.5 "NATURAL" CLAIMS

"Natural" claims are at the heart of much controversy around the world but especially today in the US. There are dozens of lawsuits that can easily be found in any internet search. The reason for these lawsuits is a gap in US regulation: the lack of definition for "natural." "Natural" has only been regulated and defined as it pertains to "natural flavors." This reference can be found in 21 CFR 101.22(a)(3) (FDA, 2013c):

> *The term natural flavor or natural flavoring means the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional. Natural flavors include the natural essence or extractives obtained from plants listed in 182.10, 182.20, 182.40, and 182.50 and part 184 of this chapter, and the substances listed in 172.510 of this chapter.*

Two important factors in this definition to point out are source and process; process is typically where the most controversy is occurring. While this definition can legally only be applied to ingredients considered "natural flavor," it has been cautiously extrapolated by some companies to other types of food.

Companies may build an internal natural labeling policy and reference these sources of natural flavors and these processes that create natural flavors to help substantiate their perspective. Sometimes they may reference the USDA requirement for "minimal processing," which is similarly vague: "product was processed in a manner that does not fundamentally alter the product" (USDA FSIS, 2011). Since there is a lack of regulation, there still may be lawsuits when consumers' expectations disagree with company practices. Unfortunately, the types of lawsuits do not all share a common perspective; some question any processing beyond "kitchen" practices whereas others object to any product being called natural if it contains genetically modified ingredients. Some lawsuits are even questioning typical microbiological processing when the enzyme is not naturally occurring. The result is that at this time, it is relatively simple to point out what would not be considered natural, such as chemically modified starches. But the inverse is not possible beyond the definition of "natural flavors" as noted above; even a purely enzymatically processed starch may not meet consumer expectations for "natural" due to extensive processing. Again, "natural" is a claim not standardized or harmonized internationally. Different countries do have different standards on what is allowed and prohibited in regard to this controversial claim. Caution should certainly be applied when deliberating the legality of any natural claim to ensure it is truthful and not misleading. The concept of claims being truthful and not misleading is the foundation for the legality of all claims made about consumer products, whether formally part of regulation or not.

29.6 NUTRITIONAL CONTENT

In comparison, a more clear analysis can be made when reviewing encapsulants for nutritional content. Nutritional statements should be readily available from the manufacturer of any encapsulant. The consumer product being developed should have clear documentation regarding nutrition claims as regulated by the FDA. Product developers will

want to select an encapsulant that substantiates and does not conflict with the desired claims. For example, a common claim for consumer products that use encapsulated flavors may be "sugar-free." In this case, 21 CFR 101.60(c) (FDA, 2011) outlines the specific regulated requirements of a food product that meets the labeling claim, including but not limited to containing less than 0.5 g of sugar and containing no ingredient that is a sugar or is generally understood by consumers to contain sugars. Some encapsulants may even provide the benefit of contributing to the overall dietary fiber content of the consumer product. Gum acacia, for example, is a carbohydrate but has high insoluble fiber content. The encapsulant can be used without the overall calorie contribution of four calories per gram typical of most carbohydrates. It is important to reference US FDA requirements for all nutrition claims; but this challenge is less than some considerations as the regulations are already in place and the data easily requested from manufacturers of ingredients. International standards have been regulated and outlined more clearly than the more controversial claims. Additionally, flavors and many encapsulated food ingredients are used at levels that typically will not overtly impact the overall nutrition of the final consumer product. Final consumer products may be subject to nutrition content analysis to substantiate any nutrition panel or label claims, further reducing the legal responsibility of the ingredient developer.

29.7 SAFE CONSUMPTION

Potentially, the largest responsibility of the ingredient developer and the supporting regulatory department is to ensure that the encapsulant selected for the ingredient meets and complies with all food standards in place in the country of manufacture and the countries of export. While progress continues to be made on a global scale, additive regulations are not standardized or harmonized across all countries. Each country, or in some cases each association, trade union, or international standard, may have different requirements for the purity of the ingredient, the allowed uses of the ingredient, and the allowed amount of the ingredient in specified consumer applications. Legislation may apply to ingredients across all consumer food products or may be regulated by product type; meaning that a country may have regulations about what ingredients can specifically be used in hard candy but may also have additional requirements that allow specific ingredients at usage levels across all product types. Regulations may apply to ingredients specifically allowed for use in flavors, may apply to additives directly added to consumer food products, or may have carryover provisions that restrict or allow both. To make things more complex, there is no "one source" for all of this information. There is no master database or service that a company can subscribe to in order to ensure full global compliance.

In the US, additive compliance is largely a function of the Generally Recognized as Safe (GRAS) program of sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (FDA, 2014a). Any additive intentionally added to a food must be adequately shown to be safe under the conditions of its intended use. Intended use means the allowed usage rates anticipated for the specific consumer food product in which the specific ingredient has been reviewed for safety for the function it is intended to perform. In practice, this may mean that a material shown to be GRAS as an encapsulant for flavors in chewing gum at 0.2% may not be GRAS as an encapsulant for flavors in reconstituted dry beverages or may have a different allowed usage rate. This is because the two consumer products have different consumption patterns, different usage levels of the ingredients, and potentially different consumer markets. Some GRAS materials along with an outline of their specifications and intended use and restrictions (if any) can be found in 21 CFR part 184 (FDA, 2013a). More details can be found on the List of Select Committee on GRAS Substances (SCOGS) register of 373 GRAS substances (FDA, 2013e). Any material not found to be GRAS through the conventional procedure may require a GRAS Notification (FDA, 2014b) (see Figure 29.1). While possibly lengthy and expensive, this mechanism allows companies to voluntarily inform the FDA that the intended use of a substance is GRAS rather than petitioning through the formal GRAS process.

The FDA and industry are working together to determine the best mechanism to ensure safe ingredients reach the market in a realistic timeframe. Internationally, other mechanisms exist that are often referenced or recognized by countries, unions, associations, or Standards.

The most commonly referenced international standard which is helpful for global additive compliance review is the Codex Alimentarius—an international food standard developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (http://www.codexalimentarius.org/scientific-basis-for-codex/jecfa/en/). This international scientific committee administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) has evaluated more than 2500 food additives. The publicly accessible database of specifications provides synonyms, definitions, manufacturing descriptions, functional uses, and critical characteristics for these food additives. The *Codex Alimentarius* creates recommendations for global food standards that are voluntary for implementation but are viewed as best practices for countries developing their national food standards for the first time or working on modernizing their requirements. It can be useful to review and refer to the Codex standards when working

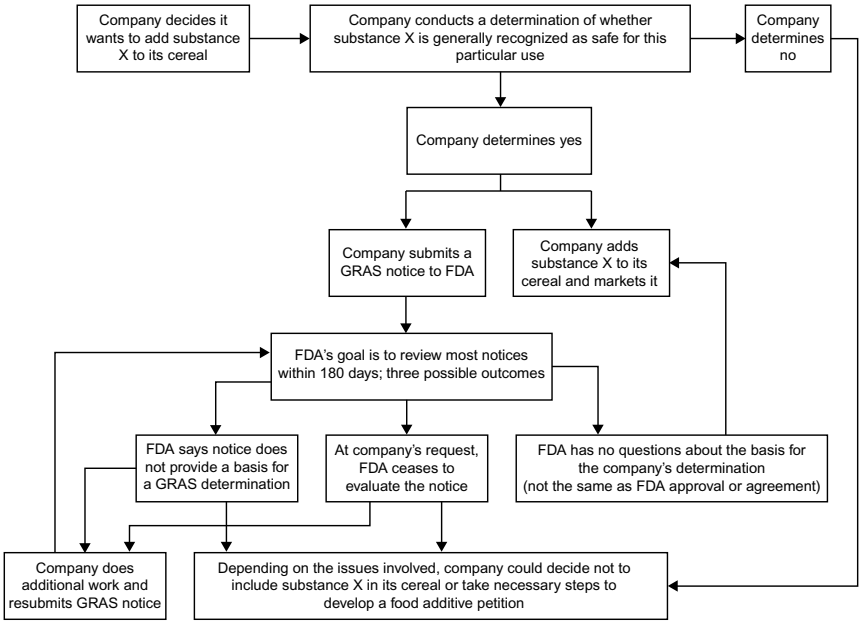

FIGURE 29.1 Options available to a company participating in the GRAS Voluntary Notification Program.
Notes: (1) This graphic shows some of the steps in FDA's voluntary notification program and options potentially available to a company; it is not intended to show all possible variations. For a more detailed description of the program, see FDA's "Guidance for industry: Frequently asked questions about GRAS" at http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredient sandPackaging/ucm061846.htm (accessed December 11, 2009). (2) At any point, a company might decide not to include the substance in its product or might proceed to market without FDA's response; if the use of a food substance is GRAS, it is not subject to premarket review and approval by FDA. (3) The analysis assumes the company is acting in accordance with the law; while participation in the notification program is voluntary, companies must comply with the law. (4) Regardless of whether the use of a substance is a food additive use or is GRAS, there must be evidence that the substance is safe under the conditions of its intended use; a GRAS substance is distinguished from a food additive on the basis of the common knowledge about the safety of the substance for its intended use. *Source: GAO analysis of FDA information.*

with partners and countries outside the US. Still, the national standards in place in the country of export always have precedence over this voluntary standard for safe consumption.

Due to the complexity of this subject, a full review of these international considerations cannot be undertaken in this chapter; it is the concept that is important to understand and take into account. Compliance reviews should be done as early in the commercialization cycle as possible and not done as an afterthought. It is critical that the encapsulant be reviewed in the context of the anticipated consumer application, the likely use rate and compliance of the encapsulant in the flavor, and the use rate and compliance of the flavor in the consumer product in addition to any desired label claims reviewed in previous discussions. Each regulatory authority has different structures and policies in regard to compliance, the US being but one example of many complex food additive safety programs found internationally.

29.8 SAFE HANDLING

Consumption is not the only safe use to consider when creating and using encapsulants and encapsulated ingredients. Encapsulants and the resulting powders may be classified as combustible dust, a serious explosive hazard. As a result of a number of such combustible dust explosions, the United States Department of Labor's Occupational Safety and Health Administration (OSHA) has issued both mandatory and voluntary standards for the management of the hazard. The recommendations name the chemical, food, and pharmaceutical industries as those specifically at risk, and name sugar and starch as ingredients contributing to the hazard. Much of the OSHA work has been informed by the National Fire Protection Association's (NFPA) 654: *Standard for the Prevention of Fire and Dust Explosions from the Manufacturing, Processing, and Handling of Combustible Particulate Solid*s (Current Edition 2013) (US Dept. of Labor, OSHA, 2014).

This standard applies to all phases of use of combustible particulate solids regardless of concentration or particle size so long as the ingredients present a fire or explosive hazard. Particle size is just one aspect of explosibility with sizes of less than 500 microns being targeted specifically as smaller particles may more easily create a cloud.

Applicable measurements such as K_{st} (a dust's explosive power) should be understood or collected from vendors of all powdered or flake materials, typically through a Safety Data Sheet (SDS). OSHA and the NFPA aim at helping companies to eliminate or mitigate the primary elements that would cause a combustible dust deflagration by a number of factors such as housekeeping (like recommendations to reduce dust buildup), ventilation (air handling and dust collector practices), explosion suppression systems, and analyzing sources of ignition. It is important to understand whether the particles, fibers, chips, or flakes used to encapsulate an ingredient are subject to these requirements, and the resulting product also requiring compliance. Safe handling of ingredients or encapsulated products should not be overlooked despite the fact that they are food ingredients.

29.9 CONCLUSION

The selection of any ingredient may involve complex analysis of desired functionality, cost, supply, and other critical components. In this analysis, the regulatory considerations cannot be overlooked. Not reviewing ingredient source can cause commercialization difficulties and geometrically increase the difficulty of exportation. Ensuring safety in use is a fundamental expectation for any consumer product, both for consumption and for safe handling in the manufacturer's plant and in the customer's plant. Meeting and legally substantiating marketing and labeling claims cannot be an afterthought in the development cycle. Regulatory departments must ensure thorough data and documentation is collected from each vendor to legally support any claims made. Product developers, marketing, and regulatory can make great partners. Together they can confirm the benefit of each type of encapsulant as it is weighed against the additional considerations to ensure the best choice is made for a successful customer solution.

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Chapter 30

Process Scale-up Considerations for Microencapsulation Processes

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30.1 DEFINITION OF SCALE-UP WITHIN THE CONTEXT OF MICROENCAPSULATION PROCESS TECHNOLOGY

This can be defined as the experiments to accomplish a production rate or product mass that is larger than previously attempted for a given process. This generally involves similar process technology but on a larger scale such that the overall throughput is substantially increased.

This is generally accomplished by first transferring a laboratory-scale process from often only grams to possibly a few kilograms in a pilot production scale by means of reasonable multipliers with a reasoned and thorough analysis of each operation. Paramount in this reasoned analysis of the production of a quality product is the notion that the process must be robust; that is, the production needs to be reproducible without excessive amounts of variability. And then from this pilot scale, one may have to scale to hundreds or thousands of kilos per batch or larger using another reasoned set of scale-up studies. Also, the process must produce a quality product as defined by meaningful specification of physical attributes and performance parameters.

During the scale-up of a microencapsulation process, one should put specific monitoring techniques in place to adequately understand that the control of the process is being maintained. This will greatly facilitate the understanding of the process, allowing one to gather sufficient information to know that the critical quality attributes of various intermediates or products are going to be achieved.

This chapter will attempt to look at ways in which a team consisting of engineers and scientists can work together to examine a microencapsulation process and plan a series of exercises and experimental protocols to accomplish a full scale-up of a laboratory process.

30.2 PHYSICAL PHENOMENA IN CONTROLLED-RELEASE PROCESS TECHNOLOGY

In any kind of process, one should look at the individual steps in the process, which might include solids or liquid additions, mixing by any number of means, dispersions, emulsification, atomization, evaporation, solidification, sieving, milling, drying, evaporation, fluidization, etc. Each of these steps needs to be examined on how that step might be scaled. In many microencapsulation processes, there may be common rules of thumb such as keeping mass ratios of air/liquid in a two fluid atomizer constant on scale-up, keeping impeller radial speeds constant to provide similar shear rates, ensuring emulsification processing produces similar drop sizes, etc. These rules of thumb are often passed along by those skilled in those processes and are not always found in the literature. It is always wise to contact those with extensive experience in those processes to learn some of these "tricks of the trade." However, much can also be learned by published equations modeling each of these process steps. The following is not meant to be exhaustive but illustrative of the analyses that might be carried out.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00030-3

30.2.1 Emulsification

This is the process of dispersing one liquid phase into another liquid phase where the first might be termed the dispersed phase or internal phase or discontinuous phase and the other phase might be termed the continuous phase or external phase. This is usually done by application of shear forces with the possible addition of chemical emulsifiers or other ingredients that would serve to stabilize the dispersed phase so as to prevent the coalescence of the drops; that is, to stabilize the system. This emulsion might be the first step in a spray drying process to make microencapsulated flavor oil or possibly to carry out a coacervation microencapsulation that would wrap the internal or dispersed phase in a shell.

In the scale-up of an emulsion, one would generally keep the same concentrations of emulsifiers but the problem may be how to scale the equipment and its operational parameters to obtain the same emulsion characteristics. In general, one is looking to operate in such a way so as to produce an equivalent dispersed phase drop size. The drop size is related to the amount of shear that is applied. The equipment suppliers should be able to provide some of the necessary information here such as the rotor speeds produced in a laboratory rotor/stator homogenizer unit and as to what rotor speeds and rotor/stator clearance or hole sizes are necessary at a particular operational speed on larger equipment that would obtain approximately the same drop size. As a part of scale-up, the engineer also should understand the impeller Reynolds number generated at the tip speed of the high shear mixer: one should keep the same Reynolds number and other dimensionless numbers such as diameter to liquid height ratio and residence time distribution. Also, care should be taken to prevent any dead spots within the mixing vessel. This can be achieved by using baffles. The temperature of the contents of the mixing vessel has to be maintained constant (Levin, 2005).

The impeller Reynolds number can be obtained by substituting the impeller diameter, D, for the pipe diameter and the angular velocity of the impeller, ND, for the fluid velocity to yield:

$$N_{Re} = \frac{ND^2\rho}{\mu}$$

where: N is the rotational speed of the impeller, D is the impeller diameter, ρ is the density of the fluid and μ is the viscosity of the fluid.

One should always go back and examine the drop size of the dispersed phase of the emulsion using good analytical methods to look for equivalence. If one needs even smaller drop sizes in an emulsion, it may be advisable to use a high pressure homogenizer that can achieve drop sizes in the submicron range. There may be a defined pumping rate through either a rotor/stator homogenizer or a high pressure homogenizer and one needs to calculate throughput and possible number of passes to achieve content uniformity in the full batch, which may be on the order of six to 10 passes theoretically through the homogenizer. This value should then be used as one moves to larger tank volumes to understand the throughput requirements of the homogenizer. Also, it is known that one can overshear certain emulsions and/or possibly generate significant heat loads, so these factors may need to be taken into account.

30.2.2 Dissolution

In the scaling of a microencapsulation process, it would be fairly common to have a dissolution step; that is, where one or more of the ingredients needs to be dissolved. There are many factors that may weigh in to the time it takes for full dissolution, including temperature of the solution, stirring rates, shear rates to dissolve higher molecular weight polymers, and solubility of the ingredient. Proceeding from a laboratory scale to a pilot scale or larger, one may determine dissolution times visually, but continuing to full production-sized batches, a short experiment to look for insoluble particles bypassing the suspension through a fine sieve should be carried out to determine the dissolution times needed. Also, if one is trying to dissolve higher molecular weight water-soluble polymers, it is well known that dispersion in a water-miscible non-solvent for the polymers such as glycerol or propylene glycol or low molecular weight polyethylene glycol may be advisable before addition to the larger vessel. This can greatly decrease the necessary time for dissolution and prevent the formation of large gelatinous "globs" of polymer that can take extended time even when higher shear rates are to be used. Also, these large gel "globs," also known as "fish eyes," have been known to find their way into stirrer bearings or other hidden recesses and discolor during subsequent process steps.

30.2.3 Agitation and/or Suspension

Many microencapsulation processes require two or more separate phases, whether it is two liquids or a solid and a liquid, and where mechanical means are used to cause them to be distributed into a uniform suspension. The solids may

stay suspended or may be dissolved, or two immiscible liquids may form a suspension or emulsion. When heating is required, agitation may be needed to increase heat transfer between the fluid and the vessel wall. One may need to scale this step from a small laboratory vessel to an intermediate pilot-scale vessel to full-scale tanks. This can often be done based on equal tip speed of the agitator, power requirement per unit volume, equal Reynolds number, or equal shaft speed. There are a number of aids that can be used in this assessment including these websites:

- Agitation Scaleup Calculation: http://checalc.com/solved/agitatorScaleup.html
- SPX Corporation's Lightnin Mixers: http://www.axflow.com/Local/Italia/LIGHTNIN%20Top%20Entry%20mixers.pdf
- Material provided by the Dalian University of Technology, China: http://ceb.dlut.edu.cn/uploads/soft/110415/7-110415154330.pdf

It is important to contact suppliers to assist the developer or process engineer in choosing or designing tanks with the proper volume, agitator dimension, tank baffles, and drain designs so that the suspension of solids is accomplished without concerns of non-homogeneity or possible dead volumes or solids buildup in a quiet area that could possibly cause drainage issues. Many of the suppliers have modeling software that can assist in these design specifications.

If one is trying to disperse one liquid into another and control the drop size, the design of the agitator and the amount of shear that it delivers are absolutely paramount. Another key element in making design decisions is the necessity for uniformity of the shear field if drop size uniformity may be advantageous.

30.2.4 Fluidization

One of the key operations in fluid bed microencapsulation is the control of the fluidization of the solids. Scaling of this process generally starts with observation of the amount of air required to fluidize the larger batch, often measured in cubic feet per minute or liters per minute. Next, a ratio of that fluidization velocity from the smaller batch that is being scaled should be taken and the ratio of the two velocities used to calculate the rate of coating to be applied, which should then be done in approximately the same proportion. However, the amount of air for fluidization is often done by experience by looking at how high the particles are moving in the up bed or how long it is taking particles to move in the down bed site glass and possibly examining the free volume contained in the bed or large air bubbles moving through the bed—again a rather inexact method especially for those without sufficient experience. A journal article describes a recent effort to identify a method that can be used to improve on this calculation (El Mafadi et al., 2003).

30.2.5 Atomization, Drying, or Solidification (Fusion)

The scaling of atomization processes, whether it be for drying as in spray drying or subsequent solidification as in spray congealing, needs to be carefully understood so that the scale-up can be accomplished with fewer experiments and in as brief a timeframe as possible. Atomization can be defined as the breakup of a liquid stream into fine particles such as a spray. Understanding this process is very important as one scales from low flow rates such as a few kilograms per hour to hundreds of kilograms per hour.

The scale-up of atomization processes depends on the type of atomizer. The majority of atomizers that are used in microencapsulation processes are one of three types: two-fluid (where air is used to shear the liquid stream into drops), pressure nozzles (pumping of liquid through an orifice, much like a squirt gun), and rotary, where liquid is fed to the center of a rotating wheel or disc rotating at sufficient speed to form the desired drop size. A discussion of each of these is beyond the scope of this chapter but is found in manufacturers' literature and in quite a few references (Masters, 1979).

30.3 BASIC QUALITY BY DESIGN PRINCIPLES

30.3.1 Introduction

"Quality by Design" or QbD was popularized in the book by Juran (1992). The Food and Drug Administration is now encouraging the use of these principles in pharmaceutical development but also the basic principles can provide basic strategies for improvement of process robustness; that is, the process as developed can routinely deliver a quality product that meets all performance specifications. Meaningful specifications would be ones that not only assure a quality product but ones that would detect small differences that might affect product performance. The QbD process helps the formulator understand how manufacturing process variables influence product quality.

30.3.2 QbD Terms

Some of the terms that you may come across in the QbD process are explained below.

1. Target Product Profile. This describes the use and efficacy of the product. Safety should play an important role here also. This can include how the microcapsule is to be delivered, how the active or key ingredient should be released, the appearance or size of the microcapsules, etc. Normally, one might also consider how much of the active or key ingredient should be contained here (potency). In general, the profile provides a statement of the intent of the development program identifying attributes that are critical to the quality of the product.
2. Target Product Quality Profile. This is closely related to the above but where the above may be a qualitative measure of the product, this is a quantitative measure of efficacy. This should be used to optimize a formulation and manufacturing process. This could be a measure of release kinetics or dissolution; that is, performance based.
3. Critical Quality Attributes. This might be described as mechanistic factors such as particle size, melting point, or hardness that determine product performance. These are physical or chemical properties that help a product meet its performance goals providing appropriate ranges or distributions to ensure product quality.
4. Critical Process Parameters. These are really process control strategies that one needs to determine so as to meet the critical quality attributes of the product. The variability in these process parameters needs to be identified and monitored and the process updated as needed to assure consistent quality.
5. Risk Analysis. In scale-up studies, it would be highly advantageous for a team bringing different skill sets to work together to develop a table or possibly what is known as an Ishikawa (1990) (fishbone) diagram. This exercise would identify all of the process variables or unit operations within the microencapsulation process that would have an impact on a particular quality attribute. The team would then rank the variables based on probability, severity, and detectability using what is known as failure mode effect analysis (FMEA) or similar tools. The experience of the team members may contribute but also experiments can be defined that would provide the necessary knowledge to fill in the diagram or table. All of this can help greatly in understanding a process and the development of effective control strategies.

   (See http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm128005.pdf).

30.4 TOOLS FOR IMPROVED SCALING OF MICROENCAPSULATION PROCESS TECHNOLOGIES

30.4.1 Knowledge of the Physics of the Processes

Physical phenomena were discussed earlier as important in understanding how one might scale up various microencapsulation processes. In performing the risk analyses as described above, one should divide the overall process into individual steps and see how these critical process parameters might need to be adjusted or ranges set in order to produce the critical attributes of the product. The following will provide some examples of how that might be done:

1. Mixing: If one is looking at forming a dispersion of a liquid phase within a continuous external phase, the agitator speed would be critical. One might calculate the tip speed of the stirrer used on the small scale and try to reproduce this on the agitator appropriate to the larger scale. The larger-scale agitator would be appropriate to the size of the vessel. A range would be set for a trial and experiments done to test either end of the limits on those ranges to determine what speed might be appropriate.
2. Atomization. If one were using a two fluid nozzle, experiments would be designed to test one or more orifices each with a range of pressures to provide a series of experiments that would best make determinations as to one or two orifices and the pressure for each that would provide the prescribed particle size distribution. If a rotary atomizer were to be used, some simple equations looking at increasing feed rates vs. velocity can be calculated to provide approximate ranges of rotary speeds to be put into the test protocols to determine the final target speed and allowed range for the final parameter.
3. Particle flow. Particle flow in fluid bed processes can exhibit fairly complex behavior; there may be bubbles, aggregates of particles, and size segregation. One may need to understand how wet microcapsules might behave during drying and how shear or added flow agents such as silica can play a role in that drying process. Also, inadequate particle flow in spray drying processes may cause blinding in restricted volumes such as rotary valves or packaging hoppers. Equipment can sometimes be modified to use air sweeps that will assist in keeping particulates flowing in the turbulent air flow.

4. Air flow/fluid flow. The dynamics of air flow in microencapsulation processes needs to be well understood during the development of increased throughput processing. This may mean holding air velocities relatively constant to assure good mixing, the understanding of laminar vs. turbulent flow in causing the proper movement of particles in the flowing stream of gases, how mass flow of air can effect drying rates in fluidized beds, but also flow velocities impacting the effect of shear on particles so as not to cause excessive particle attrition. Fluid flow velocities in concentric nozzles need to be very carefully controlled so as to provide the desired action as the drops are detached at the nozzle tips (Chigier and Beer, 1964).
5. Heat transfer. As one scales processes, heat flow should be taken into account. If evaporative processes are taking place, one must examine how heat is added to the system and whether the equipment can adequately deliver the heat in a controllable fashion as one moves to a larger scale. For heating solutions or suspensions in a tank, one should consider the effective area of the hot surface covered by the liquid to assure that sufficient heat can be transferred for the increased throughput demands. For fluidizing air systems one should think about the overall control of the process for scale-up if one chooses to simply turn up the temperature of the drying air or the air velocity, or whether the product can be overdried if these temperatures and drying air flows are not carefully controlled.

30.4.2 Experimentation

It is important when planning the scale-up of a microencapsulation process or any process to adequately plan the experiments that should be required. A protocol for the experiments should be written that will look at identifying the process parameters to produce the ranges of critical attributes. One can then produce product either for extended time periods in continuous processes or multiple batches in batch processing to confirm that the process undertaken in a controlled manner. Batch records are executed and after testing the products from the scale-up batches, a final report can be written to be turned over to plant operations at a subsequent technical transfer meeting.

30.4.3 Examination of Critical Process Parameters

During the execution of the scale-up protocol, each of the critical process parameters should be examined to see if ranges of the parameters should be tested. This might be agitator speeds, temperatures, pH ranges, etc. The protocol has to provide experiments that will adequately address each of these parameters to provide target ranges for the eventual production batch record.

30.4.4 Process Efficiency

Overall process efficiency could be defined as the weight of microcapsules with the desired properties divided by the sum of the weights of the key individual ingredients desired to make up the target product. The encapsulation efficiency of the microparticle or microsphere or microcapsule depends upon different factors; for example, in solvent evaporation microencapsulation, the efficiency of microencapsulation depends on the concentration of the polymer, solubility of polymer in solvent, rate of solvent removal, solubility of organic solvent in water, etc. (Jyothi et al., 2010). There are also losses to equipment in any process; that is, what might be coated on equipment walls, particles removed during sizing operations, removal of active that was not able to be microencapsulated, etc. Examination of the mechanisms for these losses and their root causes should be included in the scale-up and also in subsequent examination for continuous improvement projects.

30.4.5 Mass Balances

Mass balance calculations should be done as part of the examination of process efficiency. This is simply the amount of product meeting specifications added to any losses, including product sampling, spillage, product removed from process equipment before cleaning, and product separated out that does not meet specification such as removal of out-of-size specification material. This sum is then compared to the sum of the masses of materials added to the process. Any discrepancy between these sums needs to be attributed to losses in waste streams, volatile losses, or equipment losses. This exercise can also assist in looking at process economics and throughputs.

30.4.6 Developing Quality Control Tests, Specifications—the Real World

At the point of scale-up work, quality control procedures and tests are generally required. These should be related to the critical quality attributes and a realistic measure of what is required for the performance of the product. Specifications should be written that are within the bounds of what can realistically be expected from process robustness.

30.5 TROUBLESOME ASSUMPTIONS

Beware of the following notes that can affect your attempts in scaling-up processes:

1. One must be very careful that all of the important variables are completely understood.
2. The physics of each process step should be understood.
3. Geometric similarity should be maintained between the scales to ensure similar mixing characteristics. Computational fluid dynamics (CFD) can be used to ensure that the operating conditions at laboratory and commercial scale will result in similar power per unit volume (P/V) (Koganti et al., 2010).
4. There should be dynamic similarity of the two scales; that is, the ratios of all forces acting on corresponding fluids and boundary surfaces in each of the two scales are very close to constant.
5. One should understand the practical and theoretical limits of the types of equipment (pumps, mixers, atomizers, heat exchange, etc.). One should ask: Are the transfer pumps adequately sized between tanks such that batches going into a process can be made in sufficient time to keep up with microencapsulation process speeds?

30.6 WHY THERE ARE OFTEN PROBLEMS IN SCALE-UP

1. Several processes occurring simultaneously: In the rush to meet operational deadlines, experimentation may be short changed looking at multiple variables at the same time; that is, there may be drying occurring at the same time as application of coating and it becomes difficult to systematically look at changing air flows or temperatures without adjusting the coating application rates. A full design of experiment study may not be practicable within the time constraint of expected study completion. A period of "seat of the pants" experimentation based on experience may be dictated although not always advisable.
2. Materials interactions: Materials may be interacting to cause stickiness or changes in crystallinity, solubility, or volatility that can adversely affect the process. These interactions should be carefully studied before moving on to scale-up studies.
3. Good relationships with operational personnel: Something as basic as good relationships among team members of the research staff, the engineering staff in pilot facilities, and operators can be essential to successful scale-up studies.

30.7 TIME AND COST CONSTRAINTS

It has been known in a number of organizations that timelines have been shortened to the point that shortcuts are taken in the timing needed to do an adequate job of planning and executing of scale-up studies. This means reducing the number of experiments in the development of process parameters such that more than one variable is changed at a time or there is lack of testing of some variables. More often than not, this may cause later delays when there are surprises during process validation and with the multiplying of additional raw material costs and personnel time. The worst cases include violations of safety because of inadequate training of personnel or lack of caution in carrying out certain process steps. There may be induced conflicts among quality, operations, and R&D for time on the equipment to meet timelines.

30.8 CASE STUDY: SPRAY DRYING AND SPRAY CONGEALING

Without going into a great deal of detail on the process of each of the following, a few lessons learned about each of these scale-ups might be of practical use for someone reading these thoughts.

30.8.1 Emulsion System for Nutritional Supplement

The product here was a very hydrophobic, water-insoluble nutritional supplement ingredient. The formulation involved partially dissolving the active in a mixture of solvent and surfactant, which was then to be made into an emulsion for spray drying. This demonstrated lack of understanding or communication of how much shear force would be required for this operation. In the scale-up at a contract manufacturer, the contract manufacturer assured the developer that a high shear mixer was on hand for forming the emulsion. On arriving at the site, only a stirrer with a turbine blade was available. With multiple assurances, the mixture was prepared and fed to the spray dryer. Where, on a small scale, the product could be adequately atomized and the dry powder collected, the larger preparation could not be atomized and a mess was made in the dryer causing extended downtimes and an embarrassed contract manufacturer. An appropriately sized rotor/stator type of homogenizer was obtained and the emulsion was again formed and sprayed very successfully.

1. Optimization of solids content, product flowability. In trying to increase the solids content of the emulsion, which would decrease costs of drying, again the atomization was not successful on the larger scale. This should have been optimized at the smaller scale before wasting valuable time and materials.
2. Need to assure homogeneity of feed. When a heterogeneous feed system is applied in a spray drying or fluid bed process, one should always be concerned about content uniformity of the product. On scale-up, the stirrer on a feed tank was not adequately sized allowing a suspension to partially separate and cause varying assays with time in the output of the process. The stirrer was adjusted but also a pump-around loop was installed to assure that the feed suspension was uniform from top to bottom during the feed to the spray dryer. This solved the assay variations with time within individual lots of product.
3. Operational characteristics for optimization of throughput. One should always understand various operating variables and how changes might affect the quality of the product. Varying particle sizes could change bulk density or powder flow but also how the product might perform when in use. One should always move cautiously and not make too many changes too quickly, to give a system plenty of time to equilibrium. Patience is often the partner to success.

30.8.2 Spray Congealed Product

A nutritional supplement active ingredient was to be put into a hydrophobic matrix such as a hydrogenated vegetable oil. This was to control the timing of the release but also prevent some ingredient interactions. On paper, this should have been very simple to scale. Some reasonably simple equations could be used to predict atomizer speed at higher feed rates. However, there were a number of problems that had to be overcome on scale-up.

1. Melting, mixing. A contract manufacturer assured a developer that a steam jacketed tank was available. On arrival, it turned out that the geometry of the tank was not such that would allow thorough mixing of suspended solids. There were dead spots in the tank since neither the geometry of the tank nor the mixer design were adequate to assure feed uniformity.
2. Size distributions vs. feed rates. As feed rates were increased to improve the process throughput, it was known that rotary atomizer speeds should be increased to maintain average particle size distributions on the product. However, during the run, there was a change in overall breadth of the size distribution with an increase in wheel speed that affected the way the product might perform when in use. A rethink of the atomizer design had to be done to put the product back into specification.
3. Rotary atomizer design and speed. With one atomizer design, the internal method of distribution of melt flowing down an internal tubing through a series of ports to a disc was regularly blocking because of the solids in suspension. A fairly simple design change to increase the hole opening sizes greatly improved the distribution of the suspension to the disc and improved overall operation.
4. Heat tracing. Something as simple as adequately heat tracing feed lines when using molten materials delivered to atomizers, whether they be in fluid beds, spray congealing systems, or other microencapsulation equipment, is an area that is overlooked or underappreciated. Lines can simply be wrapped with copper tubing steam tracing. But without adequate heat transfer materials, lines will not necessarily be uniformly heated. Because of the larger masses of metal in valves, blockages can often occur in the valve, necessitating extended shutdowns and lengthy cleaning procedures.
5. Thermal characteristics of matrix, specific heats of solids, heat transfer. When dealing with a solid suspension in a hydrogenated oil or wax matrix, one should calculate how much heat must be removed for the particles to solidify. This heat has to be removed to accomplish solidification within the contained vessel or cone. Generally, one simply

supplies distributed ambient air flow. However, the heat transfer might not take place in an adequate time and the product can remain fairly warm or sticking to chamber walls. One may want to provide cooling of the air, which can also allow increased throughput. One should also consider how different crystal forms might give off heat exothermically long after the product is collected, which can cause lumping in the product containers that was not evident when the product was packaged. Improved cooling with chillers or inputs of cold gas can often improve throughput.

6. Continuous vs. batch mixing. The use of inline mixing for forming dispersions or even emulsions is not considered often enough. These designs can greatly reduce the number of tanks needed and although taking a little longer to set up and test, may reduce handling and improve overall process control and product uniformity.

30.9 CONCLUSION

A scale-up of controlled release processing can be a daunting challenge with many factors potentially affecting the overall outcome. Process scale-up of microencapsulation processes combines science, engineering, and experiential learning. In this chapter, a review and best practices are described for scaling up of microencapsulation processes. A team consisting of engineers and scientists can work together to examine a microencapsulation process and plan a series of exercises and experimental protocols to accomplish a full scale-up of a laboratory process. Use of Quality by Design principles during the scale-up will greatly help in reducing the time and money required to complete the process scale-up. Economics and personnel assessments are often part of the overall equation.

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Chapter 31

Microencapsulation and Packaging—Value Added Solutions to Product Development

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31.1 SMART PACKAGING: SENSORS AND HEAT MANAGEMENT MATERIALS

Conventional food packaging has been used to enable the marketing of products and to provide passive protection against environmental contaminations or influences that affect the shelf-life of foodstuffs (De Abreu et al., 2012). However, nowadays, quality assurance in food is of utmost importance because consumers demand safer, fresher, and wholesome foods, and governments impose strict regulations to ensure food safety and feed hygiene (Neethirajan and Jayas, 2011). Other important factors are greater importation and exportation of foodstuffs and that consumers are purchasing more processed food and "ready-to-eat" products compared to staple foods. Therefore, different active and intelligent packaging systems have been developed over the past few years to improve quality, safety, shelf-life, and usability of foodstuffs. Some examples of these technologies are sensors for rapid detection of food spoilage or different active systems that interact with the contents of the packaging or with the environment to increase their shelf-life. These new attributes add benefits to the food and to the consumers, and ensure that a conventional packaging becomes a smart packaging (Mahalik and Nambiar, 2010). Hence, smart packaging includes active packaging systems, which interact with the content, and intelligent packaging systems, which, apart from interacting with the content, establish a communication with the consumer. As active technologies are widely covered elsewhere, this chapter summarizes some innovative smart technologies, such as sensors and heat management materials, that already exist in the market, as well as those that are under development.

31.1.1 Sensors

An intelligent packaging system monitors the condition of packaged foods to give information about the quality of the packaged food during transport and storage (Ahvenainen, 2003; Puligundla et al., 2012). For this aim, sensors that detect alterations in foodstuffs and inform about them should be included in the packaging. The term *sensor* is defined as a device or system that responds to a physical or chemical signal to produce an output that is measurable and is proportional to the signal quantity (Lees, 2003; Neethirajan et al., 2009). The inclusion of sensors in the packaging can be used for detecting spoilage, pathogen, and contamination and even tracking and tracing the food (Mahalik, 2009). The most widely employed sensors in food packaging are based on pigments that undergo color changes triggered by various stimuli. Generally, these pigments are encapsulated or blended into carrier resins, most typically polyurethanes, the main function of which is to provide adhesion to packaging materials. The following list summarizes some of the technologies currently used for packaging sensors:

- Electronic noses and tongues: These systems consist on an array of nanoparticles that are extremely sensitive to products released by food when it spoils. Specifically, electronic noses interact with headspace volatiles, while electronic tongues react with nonvolatile compounds in a liquid. These instruments could be placed into the packaging material to give objective data for quality and safety controls (Baldwin et al., 2011). Electronic nose technology has been employed successfully for detecting fruit ripeness. There is a commercial product (ripeSense™) that consists of

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00031-5

a sensor label that reacts to the aromas released by fruit as it ripens. The sensor is initially red and graduates to orange and finally yellow. By viewing the color of the sensor, consumers choose fruit at their preferred ripeness (Kuswandi et al., 2009). With regard to electronic tongues, a wide range of applications for classification of food products can be found for wine (Wei et al., 2011), fruit juice (Ciosek et al., 2006), fat content of milk (Lawton and Pethig, 1993), or water samples.

- Time−temperature indicators (TTIs): TTIs can play a critical role in indicating the freshness and safety of a food product. These systems show irreversible changes in a physical characteristic of a product, usually color or shape, in response to temperature history. Some are designed to monitor the evolution in temperature along the distribution chain, while others are designed to be used in consumer packages. As a result, they monitor and communicate which food products are safe to consume and which are not. This becomes extremely important when foods need to be stored under specific conditions such as heat or freezing. In the case of foods that should not be frozen, a TTI would indicate whether the food had been improperly exposed to cold temperatures. Conversely, a TTI could specify whether foods sensitive to heat had been exposed to unnaturally high temperatures and even the duration of exposure. There are TTIs presently available on the market that have different working mechanisms based on chemical, physical, and biological principles. For chemical or physical response, they are based on chemical reaction or physical change to time and temperature, such as acid−base reaction, melting, polymerization, and so on. For biological response, they are based on the change in biological activity, such as microorganisms, spores, or enzymes, to time or temperature. The technology commonly used in TTIs is thermocromic ink dots (Kuswandi et al., 2009). Commercial products that use this technology are MonitorMark™ from 3M™ (www.3m.com), Timestrip® from Timestrip Plc (www.timestrip.com), FreshCheck® from Temptime (www.fresh-check.com), or Smart TTI Labels® from Vitsab (www.vitsab.com).
- Leak indicators: Modified atmosphere packaging (MAP) is an active packaging technology in which the atmosphere of the package is not air, but generally consists of a lowered level of oxygen (O_2) and a heightened level of carbon dioxide (CO_2). Therefore, a leak in MAP means a considerable increase in the O_2 concentration and a decrease in the CO_2 concentration, which could produce a quicker deterioration of the packaged food. Thus, there are leak indicators for MAPs based on O_2 and CO_2 detection. Usually, a typical visual O_2 indicator consists of a redox dye, a reducing compound, and an alkaline compound. However, there are also O_2 detection systems based on oxidative enzymes (Mattila et al., 1990; Gardiol et al., 1996). The indicator can be formulated as a tablet (Smolander et al., 1997; LeNarvor et al., 1993), a printed layer (Mattila-Sandholm et al., 1998; Davies and Garner, 1996), or laminated in a polymer film (Smolander et al., 1997). Regarding CO_2 sensors, conventional indicators such as Severinghaus type (Severinghaus and Bradley, 1958), electrochemical potentiometric CO_2 sensors, or nondistributive infrared (NDIR) sensors are usually expensive, large in volume, vulnerable to contamination and some of them require the destruction of a sealed package. Thus, on-pack, continuous CO_2 composition indicating sensors have been developed. These sensors can be classified in two types: those based on the color change of a pH indicator dye (colorimetric) and those based on the CO_2-induced fluorescence change of a luminescent dye (Puligundla et al., 2012).
- Sensors for food pathogens and contaminants: There are different systems for the detection of pathogens and contaminants. However, hardly any are integrated within the packaging, thus most of these are incorporated within devices and require the extraction of a sample to determine the presence of the target molecule. At present there are some developments in this field based on measuring changes in gas composition within the package as a result of microbial growth (Mattila et al., 1990), on reactions caused by microbial metabolites that produce color change of chromogenic substrates of enzymes produced by contaminating microbes (DeCicco and Keeven, 1995), or on the detection of the microorganism itself (Kress-Rogers, 1993).
- Sensors for identification and tracking: In modern distribution chains where raw materials may be coming from different regions to be processed in one site and finally distributed to consumers in many different regions, it is very important to identify products in case of any problems. In this field, barcodes are commonly included in the packaging; however, these are expected to be replaced by radio-frequency identification tags (RFID). RFID tags are composed of an integrated circuit that stores data and an antenna that communicates with a reader and transmits and receives information. There are many companies that manufacture RFID tags, and they have been used for years in high-value products, such as electronics or clothing. RFID tags in the packaging industry do not have an associated power source, and gain energy to transmit information from the incoming radio waves from a reader (Kuswandi et al., 2009).

31.1.2 Heat Management Materials

The active packaging interacts with the contents or the surrounding environment to extend shelf-life or to improve safety, sensory, or usability properties of foodstuffs. Different active packaging technologies have been designed over

the past few years, such as oxygen scavengers, moisture absorbers, flavor and odor absorbers/releasers, and antimicrobial systems. Nevertheless, a critical parameter that activates chemical reactions and microorganism growth is temperature. It is well known that refrigeration temperatures have preservative effects on perishable products and, thus, by controlling the temperature along the different stages of the distribution chain, a great number of deteriorative processes could be avoided and the shelf-life of products could be increased.

A possible approach to control thermal fluctuations during storage and distribution of foodstuffs, maintain a desired temperature, and thus prevent the spoilage of food is to design an active packaging system with the ability of increasing the thermal energy storage capacity of the containers. For this aim, the inclusion of temperature buffers such as phase change materials (PCMs) in the packaging matrices is a plausible solution. PCMs are materials that undergo a phase change in a narrow range of temperatures that depend on the application. In such materials, latent heat is stored during melting and released during freezing, therefore, they are able to buffer thermal variations along the cold chain. PCMs are generally classified into two categories: inorganic materials, such as salt hydrates, and organic materials, such as paraffins or fatty acids. The first ones store very high latent heat; however, they present some disadvantages due to their instability, dehydration in the process of thermal cycling, as well as a high degree of corrosiveness and supercooling (i.e., the difference between melting and crystallization temperatures). Otherwise, organic PCMs have a lower latent heat compared to salt hydrates, but they are more stable and show no corrosive behavior to the surrounding container (Aydn and Okutan, 2011). Nevertheless, thermal energy storage systems based on PCMs present some drawbacks in the food packaging area because for the refrigeration application the PCMs are typically in the liquid phase at ambient temperature and, more importantly, they need to undergo a phase change (i.e., from liquid to solid and vice versa) at the target temperature to exert the desired functionality, so their handling is complicated. A feasible solution to avoid these problems is the encapsulation of the PCM in a solid matrix (shell material). A primary purpose of encapsulation is holding the liquid and/or solid phase of the PCM and keeping it isolated from the surroundings. This ensures correct composition of the PCM that otherwise may have changed due to interaction of the PCM with the surroundings. Other advantages of encapsulation are flexibility in frequent phase change processes, an increase in heat transfer rate, and enhancement in thermal and mechanical stability of the PCM. Additionally, it can also improve the compatibility of hazardous PCMs that cannot be directly used or immersed in certain applications such as food storage (Salunkhe and Shembekar, 2012). Regarding the shell materials, common substances that have been used for PCM encapsulation are polypropylene, polyolefin, polyamide, silica, polyurea, urea-formaldehyde, copper, and aluminum (Salunkhe and Shembekar, 2012).

Based on capsule size, encapsulated PCMs can be classified as macrocapsules when capsule size is above 1 mm; microcapsules when capsule size is from 1 to 1000 μm; and nanocapsules when capsule size is from 1 to 500 nm and most typically between 1 and 100 nm. At present, macroencapsulation is the most common way of encapsulating the PCM for thermal energy storage applications. There are some companies that commercialize this kind of product, such as Climator (see www.climator.com), which produces rectangular-shaped pouches of plastic foil integrated with metallic layers, and Rubitherm (www.rubitherm.com), which uses rectangular boxes to encapsulate the PCM (Salunkhe and Shembekar, 2012).

Regarding micro- and nanoencapsulated PCMs, there are different mechanisms for encapsulation that can be classified in chemical methods, such as *in situ* polymerization, interfacial polycondensation, and complex coacervation, and physical methods, such as centrifugal, fluidized bed, spry drying, and aerodynamic and electrohydrodynamic processing (www.Fluidnatek.com). Micro- and nanoencapsulation result in higher heat transfer rates as compared to that of macroencapsulation (Khudhair and Farid, 2004; Hawlader et al., 2003). This is attributed to a substantially higher surface area to volume ratio, ability to withstand the change in volume during phase change process, and less chemical reactivity of PCM with the shell material. Among these methods, chemical techniques were found to be employed by commercial companies that manufacture microencapsulated PCMs, such as Microtek, Bioinicia, and BASF. Nevertheless, small amounts of PCM are encapsulated by most chemical methods and they render too large particle sizes for some applications (Sánchez-Silva et al., 2010).

Specifically for the food packaging application, macroencapsulated PCMs are the most used nowadays in different formats such as boxes, sheets, or sachets. However, these formats do not allow the protection of foodstuffs in the last stages of the cold chain, that is transportation from the supermarket to home and storage in home freezers. For that reason, the incorporation of PCMs in conventional packaging structures could be a plausible option for thermal protection of foods. For this aim, electrohydrodynamic high-throughput processing such as the Fluidnatek® technology could be a feasible option for the PCM encapsulation and later incorporation in the packaging. This technique uses high voltage electric fields to produce electrically charged jets from viscoelastic polymer solutions, which on drying, by the evaporation of the solvent, produce ultrathin polymeric structures (Li and Xia, 2004). This method has recently been

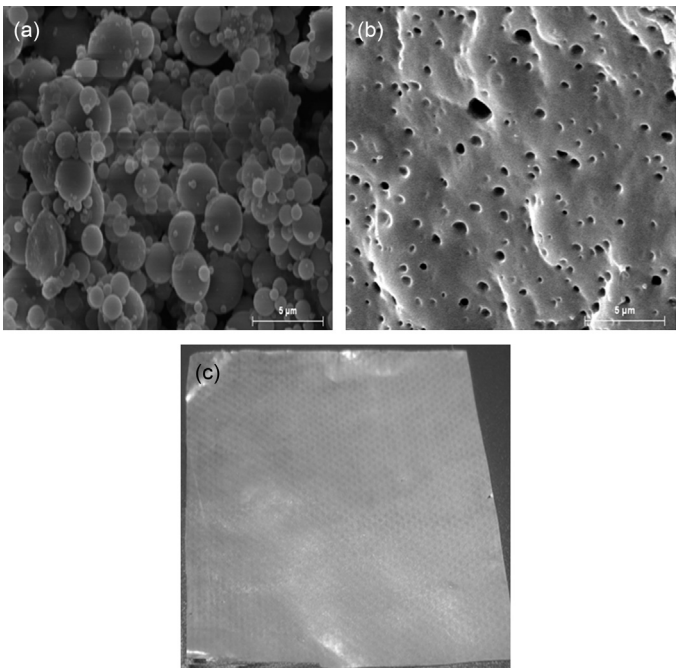

FIGURE 31.1 Polymer-PCM capsules obtained through electrohydrodynamic processing. (a) Encapsulated PCM capsules; (b) the microstructure of a film containing the submicron voids where the PCM is allocated; (c) a polymeric multilayer film containing the encapsulated PCM.

proven to encapsulate different materials, including PCMs, with significant yielding and flexibility in design, obtaining submicron-sized structures (Torres-Giner et al., 2008; Fernandez et al., 2009; Lagaron et al., 2011; Lopez-Rubio and Lagaron, 2012; Pérez-Masiá et al., 2013). This technique allows the suitable utilization of biomass-derived materials and of biodegradable polymers as encapsulating elements, with the corresponding environmental benefits associated with their use. Figure 31.1 shows some polymer-PCM capsules obtained through electrohydrodynamic processing. Figure 31.1a shows PLA-PCM spherical capsules (beads), Figure 31.1b shows the microstructure of a film containing the nanovoids where the PCM is allocated, and Figure 31.1c shows a film containing PCM.

The PCM micro- and nanocapsules obtained through the electrohydrodynamic technique can be integrated into the packaging matrices through various conventional packaging processing technologies and ideally they should not change the physical appearance and overall properties of the packaging film.

31.2 BIOACTIVE PACKAGING

As mentioned in the introduction and in the previous section, packaging materials and, specially, polymeric or biopolymeric packaging materials are increasingly playing an active role in keeping and assuring product quality and safety. Bioactive packaging is a rather novel concept of packaging that goes a step further and aims to provide the packaged product with an added functionality (Lopez-Rubio and Lagaron, 2006). It was initially conceived to be used in the food area, to overcome a number of problems related to the direct incorporation of certain bioactive or functional substances in food products. Thus, the idea was to use the packaging wall as the reservoir for the bioactive compounds that will be either immobilized or released in a controlled manner to the inner packaged food product.

Specifically in the food area, the development of novel functional foods is limited due to a number of reasons, highlighting the activity loss of the functional substance during food processing, storage, or commercialization; incompatibility of the bioactive ingredient with the food matrix; undesirable sensorial changes in the food product; and so on.

Different factors can influence or diminish the bioactivity of diverse functional substances along the shelf-life and during the gastrointestinal passage, highlighting the presence of oxygen, light, and temperature during processing of foods, enzymes, pH, or even the interaction with other nutrients. Therefore, the concept of bioactive packaging seeks to protect these substances using biomaterials, either through technologies of micro- and nanoencapsulation and/or through the incorporation of these functional ingredients within the packaging walls. The term *bioactive packaging* also includes those materials provided with enzymatic activity exerting a health-promoting benefit through transformation of specific foodborne components. Figure 31.2 shows schematic pictures of the different conceptual bioactive packaging designs.

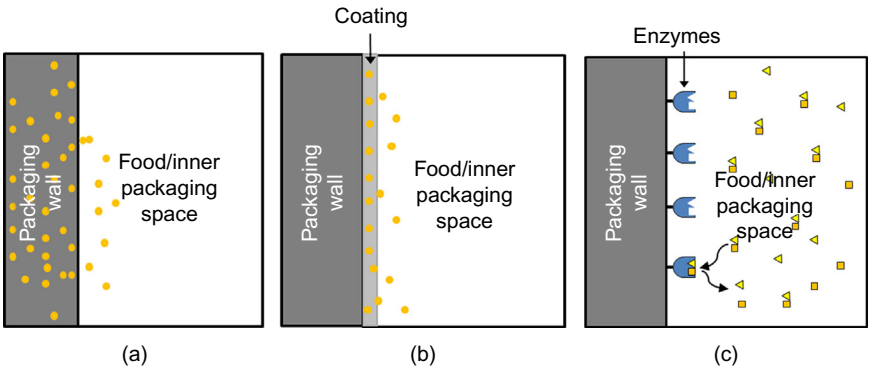

FIGURE 31.2 Schematic representation of the different conceptual bioactive packaging designs. (a) Integration of the bioactive/encapsulated bioactive substance in the packaging wall for controlled release applications; (b) incorporation of the bioactive/encapsulated bioactive substance in an inner coating of the packaging wall; (c) covalent linking of enzymes to the packaging wall for in-package processing of food.

Direct incorporation and controlled release of bioactive ingredients from the package wall can be attained through smart blending, generating the so-called controlled release packaging (CRP). CRP is one of the most innovative and challenging technologies for releasing bioactive compounds at controlled rates suitable for enhancing the quality and safety of a wide range of foods (Lacoste et al., 2005). The idea is to create different morphologies by blending various polymers under specific conditions so as to achieve controlled release of the incorporated substances. From medical and pharmaceutical research, the procedures for achieving release under various conditions are well established, but this knowledge has been of limited use in the food packaging area. Several polymeric systems have been developed for the controlled release of antimicrobials (Iconomopoulou and Voyiatzis, 2005), antioxidants (Heirlings et al., 2004), and even enzymes (Buonocore et al., 2003).

For further protection, the bioactive substances can be encapsulated before they are added to the packaging wall. In fact, although bioactive packaging is a rather novel concept at an early stage of development, micro-, submicro-, and nanoencapsulation technologies are drawing a great deal of attention and there are a number of interesting systems with potential commercial application. Cyclodextrins containing bioactives, for instance, have been incorporated as additives for controlled release into polylactide-co-polycaprolactone films (Plackett et al., 2007). Specifically, α-cyclodextrins were found suitable for long-term controlled release of an antimicrobial substance from polymer films.

Finally, enzymatic packaging was devised some time ago as an efficient method to have a "processing plant" within the same package but, in this specific case, a covalent linking of the enzymes to the packaging wall is necessary.

Regarding the materials used for bioactive packaging applications, polymeric sustainable and/or biodegradable biomaterials, such as chitosan, polycaprolactone, and zein, are the preferred vehicles for the delivery of the bioactive substances. However, there are several commercial developments for canned beverages. One example is FreshCan® Wedge technology, a patented delivery system jointly developed by Ball Packaging Europe and Degussa FreshTech Beverages, which enables dry sensitive ingredients, such as vitamins, to be dispensed into a canned beverage only when the can is opened (Mohan, 2006). But even in this type of technology, the active ingredient is contained within a polymeric container, the so-called "wedge," which is a polypropylene device containing 10 ml of the dry active ingredient. This plastic container is air- and watertight, and when the can is opened, the internal pressure decreases, causing the wedge end to spring open. Thus, sensitive substances are not dissolved in the beverage until it is consumed (O'Sullivan and Kerry, 2008).

Various nanotechnologies, such as the use of nanoclays and electrospun or electrosprayed active or bioactive nanostructured materials, are offering invisibility within the package (i.e., transparency and required mechanical properties) and will also become excellent carrier and protective systems in which the active principal can be better dispersed and, therefore, be more effective (Lagaron et al., 2006). These novel nanotechnologies will also enhance the plastic or bioplastic properties and will act as functional barriers against unintended migration or as scavengers of potential toxic by-products, increasing, therefore, the quality and safety of food products during commercialization.

In this section, some examples of these novel technologies are presented, divided according to the bioactive packaging concepts described in Figure 31.2.

31.2.1 Incorporation of Bioactive/Encapsulated Bioactive Substances in the Packaging Wall

Some packages have been developed that add a specific nutrient that cannot be preserved in the food product. Among the ingredients or nutrients that may be lost during storage or cannot be added directly to the food due to matrix incompatibility, vitamins, phytochemicals, marine oils, and flavonoids are the most interesting ones.

For instance, the precursors of vitamin A (i.e., carotenoids) have been associated with lower incidence of several types of cancer, including lung, stomach, and skin (Mayne et al., 1994). An increasing trend in the food industry is toward replacing synthetic additives with natural products. However, creating suitable water dispersible forms of carotenoids is difficult because of the limited solubility of pure carotene crystals. Encapsulation is a potential approach to transform liquids into stable and free-flowing powders, which are easy to handle and incorporate into dry food systems. Alternatively, either the carotenoid powders or the encapsulated carotenoids can be mixed with the polymer to develop packaging structures. As an example, the different stability of β-carotene incorporated in biopolyester-based (Lopez-Rubio and Lagaron, 2010) and hydrocolloid-based (Lopez-Rubio and Lagaron, 2011) packaging structures have been studied. In the case of biopolyesters, a plasticizing effect of the carotenoid was observed, thus modifying to a great extent the physical properties of the packaging material. Moreover, biopolyesters were not able to keep the antioxidant activity of the carotenoid and, thus, these bioactive molecules rapidly degraded when exposed to ultraviolet light, acting as natural antioxidants and preventing the oxidation of the polymeric matrices (Lopez-Rubio and Lagaron, 2010). However, incorporating the carotenoid as a glycerol solution to hydrocolloid-based matrices resulted in a great stabilization of the molecule (Lopez-Rubio and Lagaron, 2011).

Phenolic compounds have been also incorporated into biopolymeric films to develop materials with antioxidant and/or antimicrobial activity. For instance, zein films were developed containing different phenolic acids or flavonoids, both showing antioxidant activity and, specifically, the films containing the phenolic soluble compound gallic acid also showed antimicrobial activity against *Listeria monocytogenes* and *Campilobacter jejuni* (Arcan and Yemenicioğlu, 2011).

Antioxidant polymeric films have also been developed through the incorporation of nanoencapsulated curcumin (a phenolic compound derived from the root of turmeric) and ascorbyl dipalmitate (ADP), which is a fatty ester derivative of ascorbic acid (Sonkaew et al., 2012). The nanoparticles were developed using a rather novel encapsulation technique based on supercritical fluid technology, and were subsequently incorporated into the polymeric solutions to form the antioxidant packaging films through a casting methodology (Sonkaew et al., 2012).

Finally, in the market, there are several commercial developments of polymeric materials with antimicrobial properties containing diverse bactericidal molecules such as silver (Agion® compounds) or triclosan (Microban products, U.K.). Both compounds are compatible with a range of polymers and are mainly elaborated by adding the antimicrobial compound to the extruder when the film or coextrusion is produced (Coma, 2008).

31.2.2 Incorporation of the Bioactive/Encapsulated Bioactive Substance in an Inner Coating of the Packaging Wall

An alternative to the direct incorporation of the bioactive compounds to the packaging wall is to apply them as a coating. This has the advantage of placing the specific additive in a controlled manner without subjecting it to high temperature or shearing forces. In addition, the coating can be applied at a later step, minimizing the exposure of the product to contamination.

There are several examples in the literature in which bacteriocins have been applied as coatings in polymer surfaces. For instance, nisin/methylcellulose in polyethylene (PE) or nisin coatings in PE, ethylene vinyl acetate (EVA), polypropylene (PP), polyamide (PA), polyester, acrylics, and polyvinyl chloride (Chen and Williams, 2005). These developments are also aimed at keeping high concentrations of the antimicrobial substances at the food surface, through the release of the compounds from the coating material.

Coatings of second generation may contain nutrients or other bioactive compounds that have a positive effect on health, especially due to the application of new microencapsulation or nanoencapsulation techniques. In this way, coating materials would act as carriers of these bioactive compounds to be transported to target sites such as the intestine without losing its activity, being within a matrix during its passage through the gastrointestinal tract (Korhonen, 2005). In this sense, the electrospinning technique used as a nanoencapsulation technology has a great potential for coating applications. In electrospinning, polymer nanofibers may be obtained by the application of a strong electrical field between a grounded target and a polymer solution that is pumped from a container through a needle. Specifically for coating applications, the electrospun fibers can be directly deposited onto the packaging film surface and, given their high specific surface, a good interfacial adhesion is normally observed (Fabra et al., 2013). One of the advantages of the technique is that it does not require the use of temperature and, thus, heat-sensitive bioactive molecules may be encapsulated in the fibers and directly applied as inner coatings in packaging materials. Recently, encapsulation and stabilization of several bioactive compounds (including antioxidants and probiotics) has been achieved by means of electrospinning (Fernandez et al., 2009; Lopez-Rubio et al., 2009; Lopez-Rubio et al., 2012). These electrospun

nanostructures exhibit a range of unique features and properties that make them ideal carriers for the protection and stabilization of sensitive added-value food components (Lopez-Rubio and Lagaron, 2012). It is foreseen that the application of such structures as coating materials in food packages for the controlled release of bioactive molecules has a great potential to generate novel bioactive packaging structures.

31.2.3 Enzymatic Packaging

Incorporation of enzymes to the package wall can facilitate in-package processing (Brody and Budny, 1995). Unlike the current situation in which most foods deteriorate in quality during storage, products exposed to enzymes bound to packaging materials might improve during storage (Soares and Hotchkiss, 1998). Many enzymes are currently being used in several food transformation processes; however, more recently there have been a number of trials reported in which these enzymes are also immobilized in packaging materials. The choice of the immobilization method (adsorption, ionic binding, covalent attachment, crosslinking, or entrapment/encapsulation) and of the biomaterial support for the manufacturing of enzymatic packages depends on the nature of the biocatalyst (e.g., whole cells or purified enzymes, from fungal or bacterial origin, either native or genetically modified), the envisaged storage conditions, the type of food to be packed, and the specific application of the biocatalyst (Lopez-Rubio et al., 2006).

Enzymatic packaging is mainly designed for liquid foods, as the packaging materials, where the enzymes are immobilized, need to be in direct contact with the food product. Two promising concepts deal with milk. The first one is based on the ability of the enzyme cholesterol reductase to transform the cholesterol in coprosterol, which is not absorbed in the intestine. However, to the best of our knowledge, the previous enzyme has not been successfully immobilized to date, but by using such a technology, untreated milk could be packaged and in the time taken to transport the package to the consumer, it conceivably could become free of cholesterol (Brody and Budny, 1995). The second concept is in-package production of lactose-free milk through the attachment of the enzyme lactase to produce milk for lactose intolerant people. Goddard et al. (2007) used a yeast-derived β-galactosidase that was covalently attached to a surface-modified polyethylene film, and sustained enzyme activity over a range of temperature and pH similar to that of free lactase enzyme. However, the method of surface modification for covalent attachment involves the use of toxic chemicals, such as glutaraldehyde, which is usually unsuited for food applications.

To avoid the use of toxic chemicals for enzyme immobilization, mineral supports could be added to the biopolymer materials. Addition of tiny mineral particles, usually referred to as nanoclays, apart from improving the mechanical and barrier properties of the package walls, have proved to be very efficient in enzyme binding. Within this area there are also some ventures to develop novel technologies based on the use of specific inorganic nanocarriers for the production of bioactive enzymatic (β-galactosidases) films (Lagaron, 2005a,b). Several research studies have been carried out concerning the attachment of enzymes in diverse clay mineral supports. There are several mechanisms by which enzymes can be immobilized on clay minerals. What remains essential in all of these novel technologies is to maintain the enzyme functionality, and here is where current research efforts are being focused. For instance, Barish and Goddard (2011) have developed a method based on free radical graft polymerization, in which a biocompatible polymer (polyethylene glycol, PEG) is grafted from the surface of ozone-treated low-density polyethylene (LDPE) resulting in a surface-functionalized polyethylene to which a range of amine-terminated bioactive molecules (such as peptides, enzymes, and some antimicrobials) can be immobilized. However, this is an emerging field where there is still a lot of room for improvement.

31.3 INNOVATIVE PACKAGING TECHNOLOGIES: PRINTING, PRINTED ELECTRONICS, AND SCRATCH AND SNIFF

There is a revolution brewing just over the horizon in the world of printing. New developments in conductive ink and nanotechnology are leading a new era of electronic printing that integrates new functions into printed surfaces themselves. This advanced technology provides a versatile method of creating beverages including coffee, juices, teas, soft drinks, alcoholic drinks, and many others by means of a unique application of a microencapsulated delivery system. Nowadays, Bemis Company Inc. (Neenah, Wisc.), a producer of films and packaging for the food and healthcare industries, is working with printed electronics pioneer Thin Film Electronics ASA (Oslo, Norway) to develop a flexible sensing film that can wirelessly communicate data. Concretely, they are working on printed temperature sensors and these printed sensors are designed to monitor the temperature of perishable foods and also pharmaceuticals.

Apart from electronic printing, other companies such as Additive Advantage LLC (www.theadditiveadvantage.com) are focused on the development of clusters of microencapsulated liquids, solids, powders, or suspended particles

capable of being printed onto a substrate thereby facilitating the latent release of the core material when predetermined external conditions are introduced into the system. These microcapsule clusters are printed on the interior surface of a cup. After hydration with water, juice, and so on, the microcapsules dissolve and the encapsulated ingredients are released in the correct proportion, for instance, a juice cup with the capability of delivering multivitamins, having each vitamin discretely encapsulated and affixed to the container wall thus preventing the constituents from mixing until it is ready to be consumed.

Another innovative packaging technology is based on scratch and sniff. This refers to a product that has aromatic droplets encapsulated in it and is activated through touch. The scratch-and-sniff label is not a new technology and the concept has been around for more than 30 years. However, as demand for the technology is growing, there is increasing competition among olfactory sample suppliers and as a result there is now a variety of sample systems available. This technology is perfect for food and beverages where aroma plays an active role (i.e., coffee), and eye-catching packaging that releases a pleasant fragrance takes multisensory experience to another level for the consumer.

31.4 CONCLUSION AND OUTLOOK

This chapter has shown that innovative strategies, where protective encapsulation provides a means to generate new or more efficient functionalities, are being implemented in the packaging sector. Encapsulation is a broad term that aims to protect active and bioactive substances from leaking and from the environment, and additionally allows conveying technologies by either confining or allowing controlled release of these substances. Applications such as sensors, temperature buffers, novel functional foods design, bioactives protection, aroma release, and so on. can take advantage of encapsulation processes, especially when combined with nanotechnology. The most significant growth area is currently the encapsulation of aromas for brand recognition and of functional costly ingredients such as probiotics, but also for marine oils and antioxidants. The future will definitely see all of these technologies being more and more extensively trialed, scaled up, and/or further implemented by industries to become commodities rather than niche applications. Of particular future interest are the further development of temperature buffers, particularly as they become more cost effective, nanosensor applications, and the implementation of high-throughput electrohydrodynamic processing technologies as a means for size- and morphology-controlled encapsulation, which is moving quickly from the lab to pilot plants within research and industrial facilities.

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Chapter 32

The Economics of Microencapsulation in the Food Industry

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32.1 INTRODUCTION

The food industry continues to show an increasing interest in the use of microencapsulation. It is considered by many to be a successful delivery system; it allows manufacturers and others to tap into new consumer/health trends; and innovative, new food and beverage microencapsulation methodologies are constantly under development.

There are, however, numerous factors that must be considered when determining the economic viability of using microencapsulation in a particular application. Fortunately, this process has become a science (Chandrasekaran, 1977) in itself—a science that deals with the analysis of these factors to determine the feasibility, practicality, and cost of both the materials and/or the services (Risch and Reineccius, 1987, 1995).

Microencapsulation is frequently identified as a service, rather than a product that has been developed for sale. For example, the encapsulation of fragrance oils for print advertising uses proprietary fragrance oils, which are supplied by the printer or the advertising agency. This is a service because the fragrance oil itself is apparently unchanged. If the microcapsules are further formulated with water, binders, or even colorants, then a material product is made and sold. Whatever the case, the following analyses are used for both materials and services.

32.2 THE PROCESS

In order to determine the feasibility, practicality, and cost of a particular microencapsulation process, consider the following influencing factors:

- Appropriate microencapsulation technology or method for achieving controlled release (Nixon, 1976)
- Coating material, if not already specified by the customer
- Complexity of the microencapsulation project
- Feasibility of producing the final material/service to meet the customer's specifications
- Cost to produce/manufacture the final microencapsulated material/service.

The step-by-step analytical process follows:

1. Identify the core/active that is to be microencapsulated. Determine the following factors/variables:
   - Core material/compounds to be used—identified by a CAS number/numbers
   - Changes that may occur in the physical nature of the core material (at established processing temperatures)
   - Solid or liquid (liquids are more difficult to handle)
   - Density of the core (encapsulation is a volume-based packaging process)
   - Solubility and volatility of the core (fragrance oils frequently have a highly volatile top note)
   - Stability of the core (there are reports of peroxide explosions and configurations)

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00032-7

- Properties of the core, particularly those related to the chosen method of controlled release
- Complexity of the core (single molecule, solution, mixture)
- Customer- or vendor-supplied core (a custom microencapsulation company (Versic, 2013) is an expert in certain microencapsulation technologies, but not in all of the possible cores or applications).

2. Identify the intended use of the microcapsules (Benita, 1996). Determine the following factors/variables:
   - Size distribution of final microcapsules (narrower size distribution typically produces a lower yield)
   - Particle shape (acicular and platelet morphologies present special problems in technologies where the wall is applied as a liquid)
   - Percent core loading/percent deliverable active (spray drying may deliver 20% of active while a reservoir capsule may deliver 80% active)
   - Processing aids (these add cost when added and subsequently removed)
   - Processing parameters need to meet the specified requirements (additional processing, often called secondary operations, adds cost)
   - Additional factors/variables—final processing (washing, addition of other formulation materials); final formulation/collection of the product; and slurry, dried and isolated to a free-flowing powder, then sieved to a specific size range.
3. Determine the appropriate process and wall material based on the previous requirements. Determine the following factors/variables:
   - Selection (Hsieh, 1988) of an appropriate technique which is the most economical (more than one microencapsulation technique could be utilized to achieve the specified requirements)
   - Batch or continuous process (not all batch processes can be continuous)
   - Startups and downtimes
   - Wall material
     - Type and complexity of wall
     - Amount of wall usually expressed as a volume percent of the core volume
     - Number of walls to be applied.
4. Identify all general and administrative costs/other factors. Determine the following factors/variables:
   - Quality control
   - Recovery and disposal of waste materials
   - Packaging and storage
   - Shipping and handling (DOT)
   - General and administrative costs (Versic, 2006)
   - Regulatory considerations (FDA, EPA, OSHA).
5. Determine guiding principles for volume production (Duncan and Seymour, 1989) (factors that can cause substantially lower prices with increased volume production). Determine the following factors/variables:
   - Lower raw material prices
     - Purchase larger quantities to realize volume discounts
     - Select just-in-time suppliers to enable the maintenance of smaller inventories
   - Dedication of manufacturing equipment
   - Improved utilization of labor
   - Lower overhead, general, and administrative rates (Versic, 2002)
   - Increased manufacturing efficiencies
     - Fewer startups
     - Less downtime
     - Continual inspections and QC
   - More favorable shipping costs.

32.3 CRITERIA

The price sheets presented in this chapter are based on, but not limited to, the following criteria:

- Core materials and capital costs have been omitted so that processing costs can be easily seen
- Pricing is determined with a simplistic, straightforward method, and does not show every possible variation, e.g., different overhead rates (O/H) may apply to different operators or equipment
- Nominally, each example is based on a density of 1
- Pricing has been calculated using arbitrary numbers which may not represent actual costs.

Thus, the following information cannot be used to compare the cost of one technology to the cost of another because:

- Descriptive pricing has been omitted due to its proprietary nature
- The cost information presented should not be associated with or tied to a particular service provider or technology.

32.4 PROCESSING COSTS

In this chapter, processing costs are shown for the following encapsulation technologies (Roseman and Manadorf, 1983):

- Spray drying and spray chilling
- Coextrusion
- Fluid bed (Wurster) coating
- Coacervation
- Liposome encapsulation
- Parylene encapsulation.

Figure 32.1 shows the relationship between costs and the operating conditions in spray drying and spray chilling. Consider the following:

- Process efficiencies are directly related to operating conditions
- Water content in the initial feed mixture can significantly affect the cost because the cost of water removal is the main contributor to overall processing costs

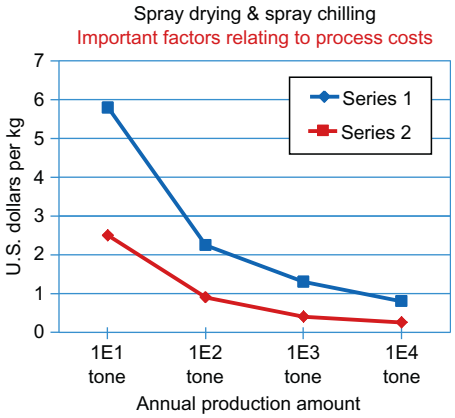

FIGURE 32.1 Relationship between cost and the operating conditions in spray drying and spray chilling.

TABLE 32.1 Different Costs Based on the Final Step in the Process (Capsule Collection), i.e., Obtaining the Capsules as a Dry Free-flowing Powder
Coextrusion
Coating Materials Comparison

	Chemical Hardening	Hot Melts	Starch Collection
Collection System Utilized	**Chem. X-link**	**Congealing**	**Powder Col.**
Total production rate per yr	45,455 kg	432,900 kg	220,960 kg
Total production rate per yr	~29 kg	~170 kg	~45 kg
Number of nozzles per unit	32	50	50
Capsule production rate hr/nozzle	~0.9 kg	~3.4 kg	~0.9 kg
Labor (Including OH & GA) 2 employees × 1–6 hr shift	$450,000		
9 Employees × 1–7.7 hr shift		$2,815,313	
3 employees × 1–8 hr shift			$765,000
Total Cost per kg	**$9.90**	**$6.50**	**$3.46**

- The upper line with squares shows the higher costs associated with higher water content
- The lower line with diamonds shows the relative impact of costs with improved operating conditions.

Essentially, the costs relating to higher water content are double those relating to improved operating conditions.

TABLE 32.2 Differences in Cost between Products in the Pharmaceutical Industry and other Types of Manufacturing
Fluid Bed (Wurster) Coating
Pharmaceutical Production vs. Industrial Production

	Enteric Coating w/a size range of 700–1150 μm		
	Pharma.	**Industrial**	**Industrial**
Amount of core to be processed	300,000 kg	300,000 kg	2.1 mil. kg
Batch size per Wurster Coater unit size	60 kg in 18″	60 kg in 18″	420 kg in 46″
Spray rate	350 g/min	350 g/min	2.45 kg/min
Raw Materials (including cost & GA)			
Wall materials Pharma. latex	$1,341,000		
Wall materials Industrial latex		$80,426	$562,981
Total raw material costs per kg/Core	**$4.47**	**$0.27**	**$0.27**
Labor (Including OH & GA)			
2 employee × 3 shifts	$276,000		$210,000
1 employee × 3 shifts		$120,000	
Total Labor Costs per kg/Core	**$0.92**	**$0.62**	**$0.27**
Total Cost per kg/Core	**$5.39**	**$0.89**	**$0.54**

TABLE 32.3 Comparison of the Cost of Different Coating Materials—the Differences Among Walls Applied Out of Solvent, such as Latex or as a Hot Melt
Fluid Bed (Wurster) Coating
Coating Materials Comparison

	Size range of 150–500 μm		
	Cellulosic In Solvent	**Cellulosic Latex**	**Wax Hot Melt**
Amount of core to be processed per yr	57,600 kg	164,571 kg	230,400 kg
Batch Size per Wurster Coater unit size	50 kg in 18″	50 kg in 18″	40 kg in 18″
Coating required per batch	110 kg	37 kg	17 kg
% Coating level/% solids content	18%/10%	18%/30%	30%/100%
Spray rate kg per min./Cycle time in hr.	0.4/5	0.4/1.75	0.4/1
Raw Materials (including cost & GA)			
Wall materials	$444,998	$2,275,875	$345,600
Total raw material costs per kg/Core	**$7.73**	**$13.83**	**$1.50**
Labor (including OH & GA)			
2 employee × 3 shifts	$290,000	$276,000	$276,000
Total Labor Costs per kg/Core	**$4.12**	**$1.38**	**$0.84**
Total Cost per kg/Core	**$11.85**	**$15.21**	**$2.34**

TABLE 32.4 Effect of the Core Size on Cost
Fluid Bed (Wurster) Coating
Particle Materials Comparison

	Organic solvent based cellulosic coating for pharmaceutical applications		
Size range in microns	**1000 μm**	**150–500 μm**	**<150 μm**
Amount of core to be processed per yr	216,000 kg	57,600 kg	18,925 kg
Batch Size per Wurster Coater unit size	60 kg in 18″	50 kg in 18″	40 kg in 18″
Coating Level (% coating @ 10 microns)	6%	18%	35%
Coating required per batch @ 10% solids	38.3 kg	110 kg	161 kg
Spray rate kg per min	0.45 kg/min	0.4 kg/min	0.3 kg/min
Cycle time in hr	1.7 hr	5 hr	9 hr
Raw Materials (including cost & GA)			
Wall materials	$479,788	$439,315	$290,000
Total raw material costs per kg/Core	**$3.56**	**$12.66**	**$34.06**
Labor (Including OH & GA)			
2 employee × 3 shifts	$290,000	$290,000	$290,000
Total Labor Costs per kg/Core	**$1.34**	**$5.03**	**$15.32**
Total Cost per kg/Core	**$3.56**	**$12.66**	**$34.06**

TABLE 32.5 Relationship between Batch Size and Cost
Coacervation
Volume Price Comparison

Gelatin wall with 90% core loading delivered as a 50% capsule slurry		
Amount of core to be processed	5.00 kg	550.00 kg
Amount of deliverable product	11.00 L	1,235.00 L
Raw Materials (including cost & GA)		
Wall materials	$21.25	$2,337.50
Processing aids		3.50
Drying aids		
Total raw material costs	**$21.25**	**$2,341.00**
Labor (including OH & GA)		
1 employee, processing cost	$1,500.00	$1,480.00
1 employee, formulation cost	750.00	555.00
1 employee, sieving & drying cost		
Total Labor Costs	**$2,250.00**	**$2,035.00**
Total Cost	**$2,271.25**	**$4,376.00**
Cost per kg of processed core	$456.00	$7.96
Cost per liter of final product	$207.27	$3.54

TABLE 32.6 Additional Cost of Isolation, i.e., the Conversion of Wet Capsules into a Dried or Isolated Form
Coacervation
Slurry Versus Dried Material Price Comparison

	Gelatin wall with 90% core loading delivered as dried material	
	Slurry	**Dried**
Amount of core to be processed	550.00 kg	550.00 kg
Amount of deliverable product	1,235.00 L	617.50 kg
Raw Materials (including cost & GA)		
Wall materials	$2,337.50	$2,337.50
Processing aids	3.50	3.50
Drying aids		
Total raw material costs	**$2,341.00**	**$2,391.00**
Labor (including OH & GA)		
1 employee, processing cost	$1,480.00	$1,480.00
1 employee, formulation cost	550.00	370.00
1 employee, sieving & drying cost		$1,480.00
Total Labor Costs	**$2,035.00**	**$3,330.00**
Total Cost	**$4,376.00**	**$5,721.00**
Cost per kg of processed core	$ 7.96	$ 10.40
Cost per liter of final product	$ 3.54	$ 9.27

TABLE 32.7 Volume Price Comparison
Coacervation
Volume Price Comparison

	Poly (methyleneurea) (UF) with 70% core loading delivered as a 50% capsule slurry	
	Slurry	**Dried**
Amount of core to be processed	5.00 kg	550.00 kg
Amount of deliverable product	14.50 L	1,595.00 L
Raw Materials (including cost & GA)		
Wall materials	$9.30	$1,023.00
Processing aids		3.50
Drying aids		
Total raw material costs	**$9.30**	**$1,026.50**
Labor (including OH & GA)		
1 employee, processing cost	$1,500.00	$1,480.00
1 employee, formulation cost	500.00	740.00
1 employee, sieving & drying cost		
Total Labor Costs	**$2,000.00**	**$2,220.00**
Total Cost	**$2,009.30**	**$3,246.50**
Cost per kg of processed core	$401.86	$5.90
Cost per liter of final product	$138.57	$2.04

TABLE 32.8 Additional Cost Associated with a Secondary Operation
Coacervation
Slurry Versus Dried Material Price Comparison

	Poly (methyleneurea) (UF) with 70% Core Loading Delivered as a Dried Material	
	Slurry	**Dried**
Amount of core to be processed	550.00 kg	550.00 kg
Amount of deliverable product	1,595.00 L	795.00 kg
Raw Materials (including cost & GA)		
Wall materials	$1,023.00	$1,023.00
Processing aids	3.50	3.50
Drying aids		
Total raw material costs	**$1,026.50**	**$1,026.50**
Labor (including OH & GA)		
1 employee, processing cost	$1,480.00	$1,480.00
1 employee, formulation cost	740.00	370.00
1 employee, sieving & drying cost		1,110.00
Total Labor Costs	**$2,200.00**	**$2,960.00**
Total Cost	**$3,246.50**	**$3,986.50**
Cost per kg of processed core	$5.90	$7.25
Cost per liter of final product	$2.04	$5.01

TABLE 32.9 Differences in Cost Due to the Use of Two Different Processes, i.e., Urea-formaldehyde (also known as PMU and UF) and Gelatin
Coacervation
Technology Comparison

	Delivered as a Dried Material	
	PMU	**Gelatin**
Amount of core to be processed	550.00 kg	550.00 kg
Amount of deliverable product	795.00 kg	617.50 kg
Raw Materials (including cost & GA)		
Wall materials	$1,023.00	$2,337.50
Processing aids	3.50	3.50
Drying aids		50.00
Total raw material costs	**$1,026.50**	**$2,391.00**
Labor (including OH & GA)		
1 employee, processing cost	$1,480.00	$1,480.00
1 employee, formulation cost	370.00	370.00
1 employee, sieving & drying cost	1,110.00	1,480.00
Total Labor Costs	**$2,960.00**	**$3,330.00**
Total Cost	**$3,986.50**	**$5,721.00**
Cost per kg of processed core	$7.25	$10.40
Cost per liter of final product	$5.01	$9.27

Table 32.1 illustrates the different costs based on the final step in the process (capsule collection), i.e., obtaining the capsules as a dry free-flowing powder. Table 32.2 demonstrates the differences in cost between products in the pharmaceutical industry and other types of manufacturing. Table 32.3 compares the cost of different coating materials. In this case, the chart shows the differences among walls applied out of solvent, such as latex, or as a hot melt. Table 32.4 shows the effect of the core size on cost. As core size goes up, the cost goes up. The primary cause of this cost difference is machine time. Machine time goes up by a factor of 5. In Table 32.5, the relationship between batch size and cost is presented. A factor of 100× increase in batch size produces a final product cost factor difference of 60×. Table 32.6 illustrates the additional cost of isolation, i.e., the conversion of wet capsules into a dried or isolated form.

TABLE 32.10 Minimal Cost in Preparing a "Proof of Concept" Sample vs. One that Meets cGMP (Current Good Manufacturing Practice) for Actual Pharmaceutical
Liposome Encapsulation
Proof of Concept vs. Pharmaceutical (aseptic area)

		Proof of Concept	Pharmaceutical
Qualification of Raw Materials:			X
Manufacturing in aseptic area:			X
Documentation:			X
QC of final product:	⟶	minimal	**X**
Testing of final product:			X
Release of final product:			X
Security of aseptic area:			X

TABLE 32.11 Effect of the Wall Material Percentage on Final Cost
Parylene Encapsulation
Coating Weight Comparison

Amount of core to be processed	100.00 kg	100.00 kg	100.00 kg
Amount of coating to be applied by weight	0.03%	0.25%	1.00%
Amount of deliverable product	100.03 kg	100.25 kg	101.00 kg
Raw Materials (including cost & GA)			
Wall materials	$73.80	$615.00	$2,460.00
Processing aids			
Drying aids			
Total raw material costs	**$73.80**	**$615.00**	**$2,460.00**
Labor (Including OH & GA)			
1 employee, processing cost	$1,580.00	$1,580.00	$1,580.00
1 employee, formulation cost			
1 employee, sieving & drying cost			
Total Labor Costs	**$1,580.00**	**$1,580.00**	**$1,580.00**
Total Cost	**$1,653.80**	**$2,195.00**	**$4,040.00**
Cost per kg of processed core	$16.54	$21.95	$40.40
Cost per liter of final product	$16.53	$21.90	$40.00

In Table 32.7, it is clear that there is a highly magnified effect when using a 550 kg dried batch of capsules over 5 kg of slurry. Note that urea-formaldehyde (UF) capsules are exceptionally easy to isolate. Table 32.8 illustrates the additional cost associated with a secondary operation. Table 32.9 shows the differences in cost due to the use of two different processes, i.e., urea-formaldehyde (also known as PMU and UF) and gelatin. Table 32.10 shows the minimal cost in preparing a "Proof of Concept" sample vs. one that meets cGMP (current good manufacturing practice) for actual pharmaceutical use. In some countries, cGMP practices are legislated. The focus in both concepts, however, is on production guidelines that safeguard the health of the patient and result in the production of good quality medicine, medical devices, or active pharmaceutical products. The effect of the wall material percentage on final cost is presented in Table 32.11. Due to the high cost of the raw material for the wall (parylene dimer), the percentage of wall has a significant effect on the cost per kg/liter of the final product.

32.5 CONCLUSION

Today, manufacturers and others in the food industry are looking for newer and better ways to enhance the value of their products as well as differentiate themselves in the marketplace. Microencapsulation is being seen by many as an effective technology for achieving these goals.

The microencapsulation process, although complex and efficient, cannot be described as good or bad—only as appropriate or inappropriate to meet the specific requirements of each application. Further, the suitability of microencapsulation cannot be determined without taking a close, careful look at the viability of the economics of using this leading-edge process.

By analyzing and considering all of the economic factors involved with microencapsulation, today's food makers can put themselves in a position to clearly determine if this technology will work for them. If the economics are viable, this could dramatically improve their ability to add new ingredients, develop more novel food properties, provide more protection for process food products, penetrate new market segments, and achieve other important objectives in this ever-changing, dynamic marketplace.

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Part VII

Microencapsulation Applications

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Chapter 33

Novel Concepts and Challenges of Flavor Microencapsulation and Taste Modification

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33.1 INTRODUCTION

The advent of flavor science and the need for application-specific flavor delivery have led the introduction of the microencapsulation field into the food and beverage industries. This chapter provides a brief historical review of flavor encapsulation, the contemporary review of flavor encapsulation, and many of the formulation and processing challenges associated with the microencapsulation of flavors. The chapter addresses the chemical and physical interactions that take place within the flavor phase, along with interactions that occur at the interface of the flavor and encapsulation substrate or matrix. A detailed look at process-oriented matrix effects and post-processing chemistry is also examined, as well as how to optimize flavor stability and release. While this chapter reviews the various ingredients used to encapsulate flavorings and common encapsulation techniques, the focus is directed to specific examples of encapsulated flavors. This chapter concludes by exploring various case studies and patented art surrounding aspects of encapsulated preparations.

The discovery of the encapsulation of flavor is well documented and arose from the efforts in the late nineteenth century regarding the dehydration of common foods, including dairy, vegetables, and fruit items to reduce weight for ease of transportation (Masters, 1979). The first direct example of retention of volatile organic compounds was observed in 1937 when A. Boake Roberts and Company used acetone to preserve the color and flavor of a tomato purée by means of a spray drying process. After spray drying the purée, residual acetone was observed within the tomato powder (Risch and Reineccius, 1995). More contemporary examples of encapsulation development include hot melt injection (Beck, 1972) and hot melt extrusion (Fulger and Popplewell, 1999). Additional encapsulation techniques used within the food industry include: spray congealing, complex coacervation, molecular inclusion complexation, lipid encapsulation and coating, co-crystallization, and other processes in order of decreasing prevalence; an outline of their prevalence is provided in Table 33.1 (Porzio, 2004). Each of these techniques, and their associated benefits, is well documented in scientific journals, and innovations in this space frequently appear in patent filings. Spray drying is still the most widely used technique within the flavor industry (Nedovic et al., 2011). In recent years, additional techniques have been developed and commercialized, including spinning disk atomization systems, coacervation, and liposome entrapment (Gouin, 2004). The current flavor encapsulation field is populated with a variety of exciting techniques to meet the challenges of product development and manufacturing requirements (yields, stability, efficiency, particle size, cost, or solubility).

Various techniques proposed for encapsulation have been employed to encapsulate flavorings in a variety of food and beverage applications. There are usually two formats, mechanical microsphere or chemical microcapsule formations, which the encapsulation scientist uses when manufacturing microencapsulates. However, in certain circumstances, control over the external influences does not inhibit internal chemistries from taking place. A common example of internal chemistries at work can be seen with the formation of acetals from non-stabilized flavorings in some solvents. For

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00033-9

TABLE 33.1 Common Food Industry Encapsulation Techniques and Attributes

Technology	Morphology	Approx. Use in Industry (%)	Load (%)	Particle Size (μm)
Spray drying	Matrix	85–95	5–50	10–400
Fluid bed coating	Reservoir	2–4	5–50	5–5000
Spray chilling/cooling	Matrix	3–9	10–20	2–200
Melt injection	Matrix	≈1	5–20	200–2000
Melt extrusion	Matrix	≈1	5–40	300–5000
Emulsification	Matrix	≈1	1–100	0.2–5000
Inclusion complexation	Molecular inclusion	<1	5–15	0.001–0.01
Liposome entrapment	Various	<1	5–50	10–10,000
Rapid expansion of supercritical fluid	Matrix	<1	20–50	10–400
Freeze drying	Matrix	<1	Various	20–5000
Coacervation	Reservoir	<1	40–90	10–800
Coextrusion	Matrix	<1	70–90	150–8000

Adapted from Porzio (2012) and Zuidam and Shimoni (2010)

instance, the interaction of propylene glycol (solvent) with benzaldehyde (flavor component) results in the formation of benzaldehyde propylene glycol acetal (Heydanek and Min, 1976). From an organoleptic standpoint, a microencapsulated cherry flavoring that contains both benzaldehyde and propylene glycol will acquire a medicinal taste over time due to acetal formation.

Microencapsulation techniques are used for a wide range of functionality across each of the food and beverage market segments. The most common purpose for microencapsulation within the industries involves the protection of sensitive ingredients from moisture, oxygen, or light (Nedovic et al., 2011). As an example, orange oils have been encapsulated by lactose and caseinate to prevent orange oil deterioration caused by heat, humidity, light, and oxygen (Edris and Bergnstahl, 2001). Cinnamon oil and garlic oil have been encapsulated using β-cyclodextrin to extend release time and shelf-life (Ayala-Zavala et al., 2007). Additionally, microencapsulation is frequently used to convert liquids into free-flowing powders for ease of handling, to prolong shelf-life of a consumable, to prevent premature ingredient release, to reduce product flammability, to provide controlled ingredient release, and to mask undesirable taste, odor, or color attributes (Barbosa-Cánovas et al., 2005; Desai and Park, 2005; Poncelet, 2006; Lakkis, 2008b). Taste modification or taste masking by microencapsulation is a topic covered later in this chapter and represents an exciting new frontier in food science for health and wellness ingredients. There are many challenges surrounding flavor microencapsulation, including the chemical and physical properties of the flavor systems that are to be encapsulated, and the need for a well-established thermodynamically favorable phase equilibrium. This topic is also covered more extensively later in this chapter with specific examples.

Flavorings are composed of many different volatile components that share equilibrium when applied to food and beverage products. The perception of flavor is a central driver that determines the sensory behavior of food products. It is an overall experience triggered by the combination of aromatic compounds (perceived by olfactory epithelium), gustatory components (perceived by taste receptor cells on the tongue, such as sweet, sour, salty, bitter, and umami), chemical heat principals (irritation sensation perceived by vanilloid receptors on filiform taste buds), and physical properties of foods (texture and temperature) (Delwiche, 2004). The perception of flavor is highly complex and incompletely defined as a biochemical process that begins in the oral and nasal cavities and terminates within the limbic brain structures, precisely where memories and emotions are concurrently integrated and stored (Shepherd, 2012). This explains why the perception of flavors is described as being an emotional experience. An exhaustive overview of the known

neural circuitry and biochemical cascades involved in flavor perception is not a focus of this chapter. However, flavor does play a significant role in determining the acceptability and preference of a food item among different consumers. For this reason, many of the aspects of flavor delivery, including physical form, release mechanisms, particle size, and morphology constitute active areas of investigation for food scientists (Shahidi and Han, 1993; Lakkis, 2008b).

The exposure to flavor and its influence on the senses leads to consumer choice and consumer preference. In many cases, consumers build their preferences on well-established flavor profiles. However, many flavor components are susceptible to external environmental effects and influences such as air, light, moisture, pH variation, and ingredient−ingredient interactions, which lead to change in flavor profiles over the course of a product's shelf-life and may not meet the consumer's expectation after an extended period of storage. Therefore, it is important to find technologies that will sustain a consistent flavor profile over the life of the product. With increasing consumer expectation within the food industry, controlled release techniques are required to deliver flavor at prescribed time points during processing or consumption.

33.2 CHALLENGES OF FLAVOR ENCAPSULATION

33.2.1 Typical Flavor Composition

The chemical and physical properties of a flavor system are important considerations in making an appropriate microencapsulation technique selection. In many regards, the microencapsulation process is the central step between flavor formulation and finished product application. The complexity of a flavor system and the finished application can make the microencapsulation process an arduous task. A flavor system includes numerous volatile organic compounds—a mixture of pure chemicals and natural extracts. As shown in Table 33.2, a typical pineapple flavor includes nine pure flavor chemicals combined with an additional seven essential oils at differing levels of concentration. The essential oils described in Table 33.2 may contain hundreds of different chemical components, resulting in a flavor containing thousands of different chemical compounds. Each of these chemical components introduces a unique chemistry that influences the overall chemical and physical properties of the flavor system.

Chemical and physical properties found within flavor mixtures depart from the theoretical realm and become an empirical exercise of interpretation for the flavor chemist and encapsulation scientist alike. Thermodynamic and organoleptic factors are also explored in order to assist with the creation of a composite model to predict the overall flavor performance and behavior. These factors include: volatility, taste and aroma thresholds, solubility parameters and free energy relationships, and lastly the identification of components that heavily influence phase equilibrium (i.e., various flavor solvents or diluents). These different factors establish the ground work and definition of complex mixture phase equilibrium, and also provide an understanding of the overall chemistries involved within a flavor to assist in selecting the appropriate microencapsulation technique.

TABLE 33.2 Typical Pineapple Flavor Formulation

Pure Compounds	(wt %)	Essential Oils	(wt %)
Allyl caproate	5	Oil of sweet birch	1
Isopentyl acetate	3	Oil of spruce	2
Ethyl acetate	15	Balsam Peru	4
Ethyl butyrate	22	Volatile mustard oil	1
Terpinyl propionate	3	Oil cognac	5
Ethyl crotonate	5	Concentrated orange oil	4
Caproic acid	8	Distilled oil of lime	2
Butyric acid	12		
Acetic acid	5		

Source: Pavia et al., 1999.

33.2.2 Characterization of Flavor Phase Equilibrium Through the Use of Vapor Pressure, Molecular Size, Solubility, Taste and Aroma Threshold, and Free Energy Relationships

As previously stated, flavors are multi-component chemical systems that exhibit an overall composite chemistry. This section describes the thermodynamic properties involved with phase equilibrium that can be utilized to formulate microencapsulated flavors. Successful selection of encapsulation materials is important in order to retain the authenticity of the flavor profile. Incremental perturbations in formulation and processing parameters can lead to an optimized consumer preferred flavor.

33.2.2.1 Vapor Pressure

Flavor systems exhibit a dynamic combination of partial pressures owing to their volatile organic compound composition. Each flavor chemical's partial pressure contributes to the overall volatility of the flavor. There is enormous complexity in predicting volatility as a flavor develops beyond a tertiary component system. One method of characterizing a flavor system is to examine the vapor pressure–temperature relationship for each component within the system. This technique offers utility for liquid flavor systems along with understanding the influence of temperature during microencapsulation processing. It can become a predictive tool for flavor chemists and microencapsulation scientists who continually seek new approaches to better understand the effects of formulation and microencapsulation techniques to minimize process-related flavor loss.

Vapor pressure plays an important role when exploring how a flavor chemical's volatility will react to a change in temperature. A simplified view of volatility can be demonstrated by either boiling points or flash points for discrete chemicals. This provides flavor chemists and microencapsulation scientists with a brief perspective on the volatility drivers of a flavor system, and how to potentially minimize microencapsulation process-oriented flavor loss. Both of these intrinsic properties provide important information about the volatile nature of a flavor system and its individual components. For a model flavor containing isoamyl acetate, ethyl butyrate, and limonene, the boiling points and flash points are listed in Table 33.3. From this table it is evident that limonene is less volatile than isoamyl acetate, ethyl butyrate, or water. It would be expected in an open system that the compounds with lower boiling point would transition into the gas phase more rapidly than the components with higher boiling points. These two factors can provide information to the formulator about which chemicals may pose certain volatility-loss risks that need to be accounted for during microencapsulation processing. Flavor chemists usually need to rebalance or reformulate a flavor system to account for losses during the development of a microencapsulated flavor.

Another approach to understanding volatility is through evaluating the relationship between temperature and pressure over a temperature range as opposed to building interpretations off single fixed values as in the case of boiling points or flash points. This relationship is commonly called the Antoine equation and was developed out of the Clausius–Clapeyron equation to demonstrate phase transformation curves (Fermi, 1966). The Antoine relationship is an expression that utilizes viral coefficients that can easily be obtained from the literature. The Antoine equation relates temperature-dependent viral coefficients to the temperature range of interest, and generates a pressure curve; one embodiment of this equation is shown in Eq. (33.1):

$$\log_{10} P = A - \frac{B}{C + T} \tag{33.1}$$

TABLE 33.3 Boiling Point and Flash Point of d-Limonene, Isoamyl Acetate, and Ethyl Butyrate at 760 mm Hg (Reineccius, 1999)

Compound	Boiling Point (°C)	Flash Point (°C)
d-Limonene	176–178	48
Isoamyl acetate	143	25
Ethyl butyrate	120	20
Water	100	n/a

From this equation, pressure (P, bar) can be related to temperature (T, kelvin). The coefficients A, B, and C are pure chemical-specific coefficients.

Through the use of the Antoine relation, vapor–temperature phase transition curves can be generated as a means for understanding volatility for pure compounds over a range of temperatures. This information assists with formulation and processing parameter optimization of various microencapsulation techniques to ensure that flavor loss is minimized during microencapsulation. Sobel et al. (2010) demonstrated an example of volatility control through vapor pressure–temperature relationships for pure chemicals in spray dry processing and modification of drying temperature parameters (Sobel et al., 2010). The research described a series of pure flavor chemicals (d-limonene, benzaldehyde, ethyl butyrate) that were microencapsulated by means of spray drying. The authors demonstrated that improvement in flavor chemical retention operates as a function of spray dryer inlet temperature. This work demonstrated that benzaldehyde did not experience appreciable loss as a function of drying temperature. This observation was attributed to the relatively low vapor pressure of benzaldehyde under the temperature range as shown in Figure 33.1. A 2009 study conducted by the University of Illinois at Urbana-Champaign's Department of Industrial Engineering demonstrated similar results for spray drying of menthol at different drying temperatures (Carnahan et al., 2009).

Figure 33.1 demonstrates that water has a greater vapor pressure relative to benzaldehyde at temperatures above 35°C. For the spray drying inlet drying temperatures that were explored (130, 160, 190°C), the reported data demonstrated similar flavor chemical retention over all thermal processing schemes as shown in Figure 33.2.

As mentioned in previous chapters, spray drying is a popular method used to create microencapsulated flavors that involves the mixing of heated air with a cloud of hydrated flavor and matrix material. To prepare an encapsulation with the above flavor chemicals using the spray drying technique, it is important to consider the vapor pressure of the individual components relative to water. Using a simplified flavor system containing benzaldehyde, ethyl butyrate, and d-limonene, it is possible to construct a vapor pressure chart containing the relationship between individual vapor pressures for each component to their boiling points by way of the Antoine equation (Eq. (33.1)). The unitless Antoine coefficients can be used to model the phase transition curves for pure chemicals, making it possible to compare relative vapor pressures over broad temperature ranges (Table 33.4). With the constructed Antoine graph, it becomes evident that components are more likely to pose challenges in terms of volatility under specific thermal conditions. In specific, by using the vapor pressure chart as shown in Figure 33.1, it is apparent that water has higher vapor pressure than benzaldehyde, ethyl butyrate, and limonene at temperatures above 80°C. Therefore, spray drying at temperatures above 80°C cause water to evaporate more rapidly than the flavor chemicals, which favors the retention of flavor within the encapsulation system upon dehydration. At temperatures below 80°C, the vapor pressure of ethyl butyrate is greater than that of water. Consequently, spray drying schemes at temperatures below 80°C will result in significant losses of ethyl butyrate upon dehydration due to the flavor chemical's preferential volatilization.

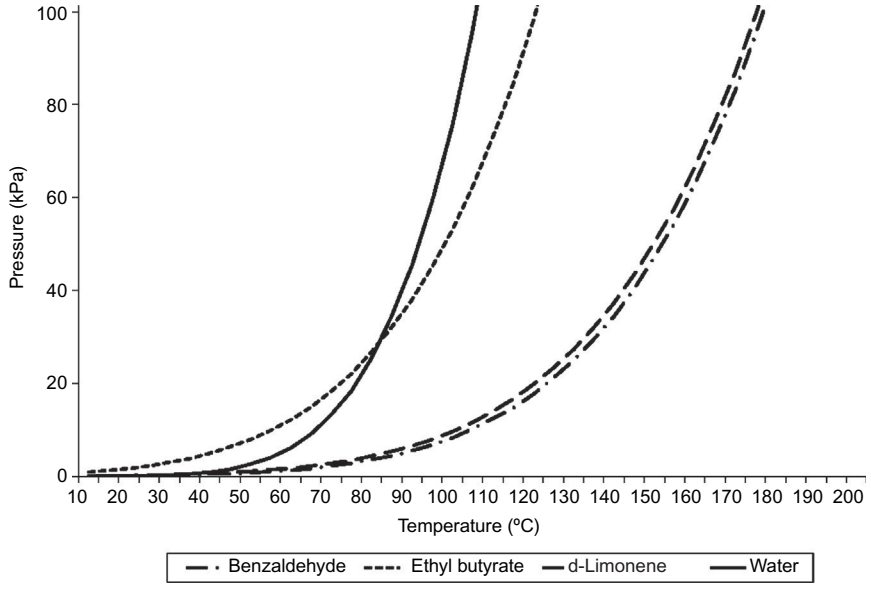


FIGURE 33.1 Vapor pressure–boiling point chart of isoamyl acetate, ethyl butyrate, d-limonene, and water.

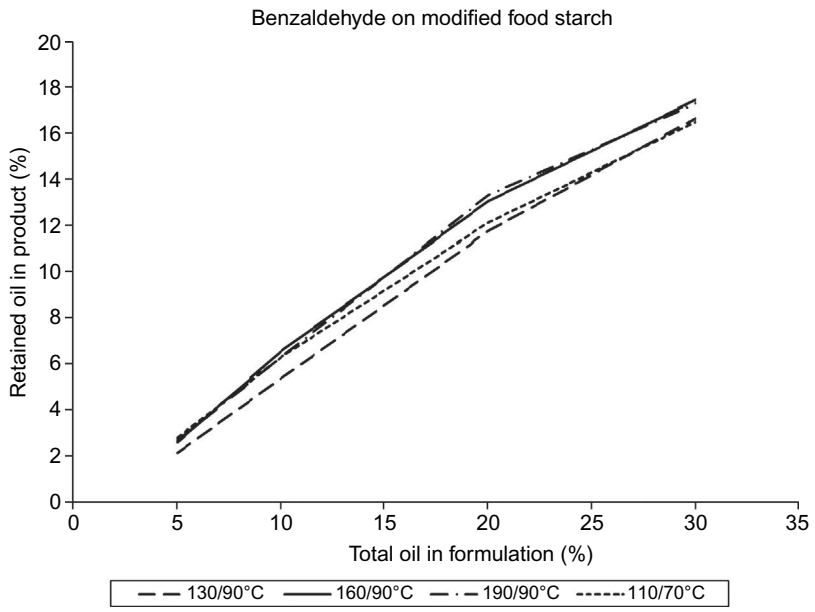


FIGURE 33.2 Retention of benzaldehyde as a function of spray drying inlet temperature.

TABLE 33.4 Antoine Coefficients for d-Limonene, Benzaldehyde and Ethyl Butyrate at Specific Temperature Ranges

	A	*B*	*C*	Temp. Range (K)	Temp. Range (°C)
d-Limonene	6.75946	2040.295	−19.639	290–450	17–177
Ethyl butyrate	4.33187	1509.443	−45.284	254.7–394.0	−19–121
Benzaldehyde	3.87652	1380.729	−94.98	299.4–452	26–179
Water	3.55959	643.748	−198.043	n/a	n/a

33.2.2.2 Molecular Size and Transport

Molecular size is an intrinsic property that influences the mobility of a molecule through a shell or matrix material during and after the microencapsulation process. In general, higher molecular weight compounds demonstrate slower diffusivity compared to lower molecular compounds. This fact plays a critical role during and after the microencapsulation processes used in the food industry, as flavor diffusivity greatly influences flavor retention and finished product performance. A primary example of molecular size and transport of flavor compounds is demonstrated by the theory of selective diffusion as it relates to spray drying.

Thijssen and Rulkens (1968) proposed the theory of selective diffusion (Figure 33.3). This theory states that, during spray dry microencapsulation, a thermally induced phase separation occurs at the surface of the emulsion droplet. The formation of solid film at the surface significantly decreases the molecular transport because the diffusion rate through a solid is slower than through a liquid. Selective diffusion theory supports the notion that low molecular weight compounds have a greater diffusivity than heavier molecular weight compounds. Thijssen proposed the following drying scheme for selective diffusion theory. The newly developed surface film allows water to diffuse preferentially, but reduces the mobility of common flavor chemicals (Thijssen and Rulkens, 1968).

The onset of film formation operates as a function of thermal processing parameters, and so, as input temperatures increase, the rate of film formation occurs more rapidly. The capacity of the drying rate of the system is directly influenced by the air flow, inlet temperature, and outlet temperature (Eq. (33.2)) in the following manner:

$$Rate_{drying} \propto Rate_{air\ flow}\ (T_{inlet} - T_{outlet}) \tag{33.2}$$

Thijssen and Rulkens (1968) and Menting et al. (1970) demonstrated a strong dependency of the diffusion coefficients of volatile organic compounds in aqueous solutions on the water concentration, and also that decreasing water

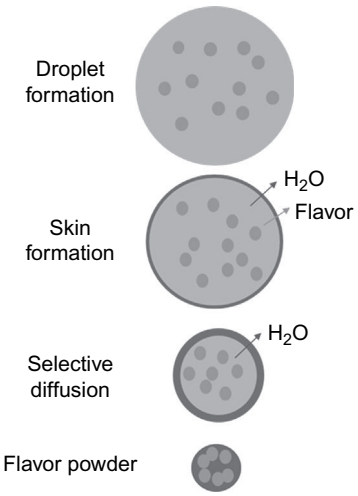


FIGURE 33.3 Selective diffusion theory (Thijssen and Rulkens, 1968).

concentrations are correlated with strong decreases in diffusion coefficients for water and other volatile organic compounds in maltodextrin. It has been demonstrated that, as the water concentration at the surface of the forming encapsulated particle decreases to a sufficiently low point, termed the critical concentration, the diffusion coefficient ratio of volatile aromatics to water becomes sufficiently low to consider the system impermeable to the volatile aromatics (Menting, 1969). Regarding the spray drying process itself, there exists internal and external resistance to mass transfer in the matrix material and in the drying gas, respectively. Therefore, if the water concentration at the matrix surface is rapidly lowered below this critical concentration during processing, volatile flavor compound retention may be substantially increased. This effect is demonstrated in the earlier examination of ethyl butyrate and the relatively high retention values observed in combination with higher (i.e., 190°C) spray drying temperatures (Sobel et al., 2010). A more detailed review of mass transfer and related concepts is covered in Chapter 3 of this book.

33.2.2.3 Phase Equilibrium

Prior to microencapsulation processing, ideal flavor systems are composed of a single phase whereby all the components share similar chemistry. Thermodynamic factors controlling the flavor phase equilibrium include flavor concentration, diluent selection, partial molar free energy or chemical potential, and intermolecular forces (orientation forces, hydrogen bonding, induction forces, and dispersion forces). Phase equilibrium and solubility represent central considerations in the selection of microencapsulation technique and vice versa.

33.2.2.3.1 Chemical Potential and Intermolecular Forces

Chemical potential is a measure of partial molar free energy and describes the tendency of molecules to transition from a region of higher chemical potential to a region of lower chemical potential. From the perspective of chemical potential, phase equilibrium is defined as the minimization of free energy for the total sum of chemical potentials, and is described by the fundamental equation of thermodynamics in Eq. (33.3), and the Gibbs free energy function is defined by Eq. (33.4) (Carter and Styer, 2000):

$$\sum_{j=1}^{m}(\mu_j dn_j) = U - TS + PV \tag{33.3}$$

$$G = \sum_{j=1}^{m}(\mu_j dn_j) \tag{33.4}$$

The volatility of a system can be defined by various intermolecular forces applied and accepted by a population of molecules as they interact with each other. The factors controlling the equilibrium of these interactions in a liquid flavor system can be described specifically by the chemical potential, whereby each component's chemical potential is a measure of the driving force between components. In an ideal case, a flavor system would have a single phase whereby all the components share a common solubility. In this regard, flavor compounds must be paired with an appropriate solvent to achieve the desired equilibrium.

33.2.2.3.2 Solubility, Linear Solvation Energy Relationships (LSER), and Flavor Delivery

Solvent characterization, expressed as free energy relationships, and more specifically as linear solvation energy relationships, is an approach that predicts the solvent and solute interactions of simple and complex flavor−matrix systems. This experimental approach can be used to characterize the matrix for encapsulation along with selecting an appropriate solvent to create a single phase equilibrium for the flavor to be encapsulated. In addition to describing the range of intermolecular forces, this technique also examines solvent cohesion, alternatively referred to as cavitation, and the capacity for electrostatic stabilization and hydrogen bonding interactions. The original form of the LSER equation (Eq. (33.5)) made use of a series of coefficients to describe the relationship between the solute's partition coefficient (K), excess molar refractivity (R_2), polarizability (π_2^H), hydrogen bond donor acidity (a_2^H), hydrogen bond acceptor basicity (b_2^H), and gas−liquid partition coefficient into hexadecane (L^{16}). Those LSER coefficients having subscript 1 (i.e., X_1) represent the ability of the solvent phase to engage in complementary interactions with the solute:

$$\log\ K = c + r_1 R_2 + s_1 \pi_2^H + a_1 \sum \alpha_2^H + b \sum \beta_2^H + l_1\ \log L^{16} \tag{33.5}$$

This equation and its theoretical and practical uses have been covered in extensive detail (Vitha and Carr, 2006).

$$SP = c + Aa + Bb + Ee + Vv \tag{33.6}$$

The former equation (Eq. (33.5)), which has been revised in the form of the latter for ease of reference (Abraham et al., 2004), relates solute partitioning (SP) to the following solute attributes: capacity for hydrogen bond acceptance (A), hydrogen bond donation (B), polarizability (E), cavity formation energy (V), and a system constant (c), which behaves independently of the probe solutes (Eq. (33.6)). LSER values are obtained by multiple linear least squares regression. Sobel et al. (2010) presented an investigation of common encapsulation matrix materials used to envelop flavor compounds in spray drying processes. In this presentation, LSER values were calculated for modified food starch as well as gum acacia (Table 33.5) using hexanal as the flavor compound. These data were used in partial justification for specific flavor retention behaviors observed in finished spray-dried products.

Sobel et al. (2010) demonstrated that the LSER values derived for modified food starch with corn syrup solids had more similar values compared to the published values for the flavor compounds used in the spray drying experiments. This similarity in LSER values results in favorable interactions, or superior retention of hexanal in modified food starch systems compared to that using gum acacia. This behavior was observed in the case of hexanal; however, Sobel et al. (2010) stated that specific interactions may drive the retention of other flavor components in a range of different encapsulation substrates (e.g., benzaldehyde heavily retained by gum acacia due to electrostatic stabilization of aromatic ring). The application of the LSER method is a valuable indicator for matrix material selection and a predictor for phase equilibrium.

Figure 33.4a,b demonstrates the manifestation of the LSER value agreement between the flavor compound and the encapsulation material. Using the LSER model and the values calculated for the encapsulation materials, it is possible to identify which types of interactions are driving the retention of flavor compounds and also the magnitude at which the interactions are occurring. This information can be used by the encapsulation scientist and the flavor chemist to determine which encapsulation materials are suited for particular flavor systems.

Hildebrand and Hansen solubility parameters provide the opportunity to use computational modeling to offer a unique perspective on the tendency of flavor chemicals, solvents, and matrix materials to engage in a range of intermolecular interactions, including energy contributed by dispersion forces, dipolar intermolecular forces, and hydrogen

TABLE 33.5 Experimentally Derived LSER Values for Modified Food Starch and Gum Acacia

	R	π	α	L^{16}	B
MFS	0.038	0.413	1.467	0.757	0.816
(p-value)	0.923	0.260	0.023	2.62e-12	2.017e-8
GA	−0.736	1.070	2.698	0.892	0.936
(p-value)	0.033	0.0004	1.25e-5	1.54e-13	1.29e-11

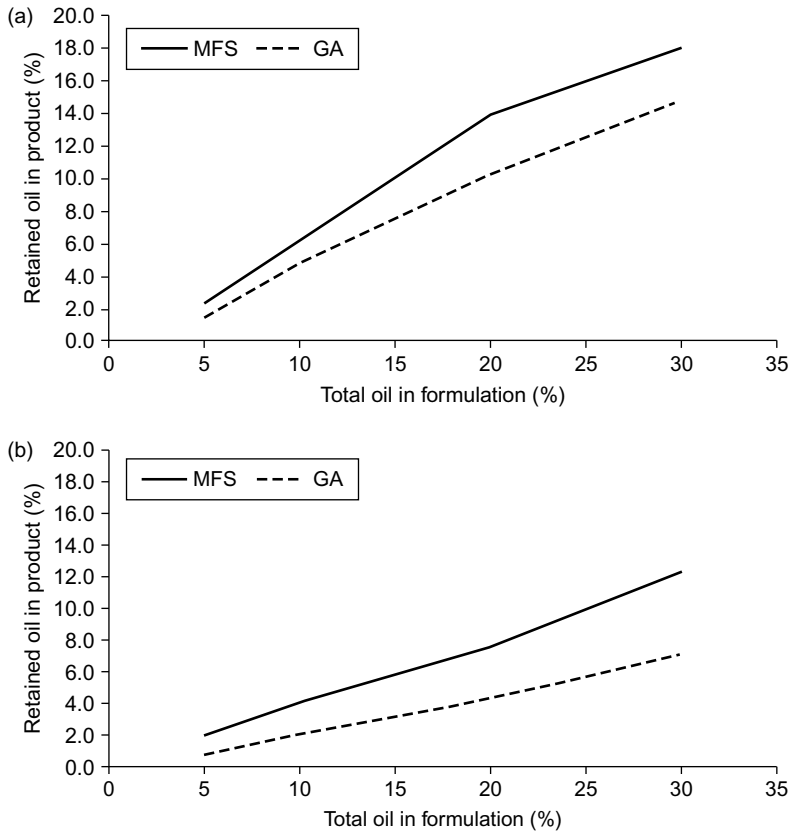


FIGURE 33.4 Retention of hexanal (a) and hexanol (b) spray dried at inlet temperature 160°C on modified food starch (MFS) and gum acacia (GA).

bonding forces. Applied knowledge of Hildebrand and Hansen solubility parameters can also provide insight into the potential of a particular probe solute to dissolve into a specified solvent or matrix substrate. Using the Hansen solubility model, it is possible to compare the energies contributed by hydrogen bonding (δ_h), dispersion interactions (δ_d), and intermolecular forces (δ_p) to yield theoretical predictions regarding the extent that a chemical, or composite chemical population, will dissolve into a specific solvent or encapsulation material. Characteristics of some solvents used in the flavor industry are given in Table 33.6.

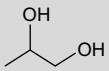
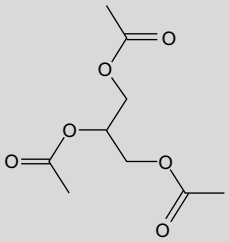
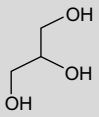
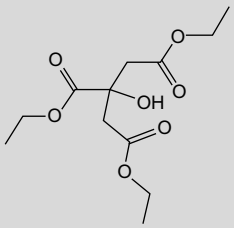
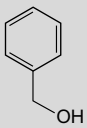
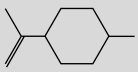
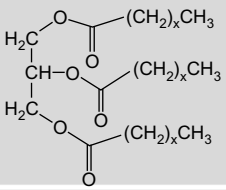
33.3 SUMMARY OF COMMON FLAVOR MICROENCAPSULATION TECHNIQUES

It is important to pair flavor systems with appropriate materials and techniques to achieve the desired finished product functionality. There are two general categories of microencapsulation used within the food science industry: mechanical microspheres and chemical microcapsules. Mechanical microspheres are typically prepared by physical encapsulation techniques including spray drying, fluidized bed spray coating, spray cooling or chilling, melt extrusion, liposome entrapment, or molecular inclusion complexation. Chemical microcapsules are typically prepared by means of coacervation or ion crosslinking processes. A variety of common encapsulation techniques and related examples are briefly discussed in the following sections.

As outlined in Table 33.7, different encapsulation techniques may be more efficacious for certain flavor systems due to the affinity of the flavor components for certain encapsulation operations or materials. Therefore, the interfacial properties of the flavor delivery system are typically modified to suit the requirements of the encapsulation process.

Most flavor systems may utilize a variety of solvents or diluents (Table 33.7) as a basis for defining the solubility of the system. For example, water is a solvent commonly used in spray drying to deliver a non-polar flavor/carrier emulsion feedstock into the spray dryer for forming the encapsulation. It is desirable to use solvents or diluents that prevent the flavor fraction from partitioning into the solvent phase to prevent flavor loss during the selective evaporation of the solvent.

TABLE 33.6 Structure and Characteristics of Some of the Solvents Used in Flavor Delivery

Flavor Delivery Solvent	Structure	Properties	Log P	Reference
Propylene glycol		Miscible in water, susceptible to acetal/ketal formation, odorless/tasteless, poor solvent for non-polars	−0.92	UNEP (2001)
Ethanol		Stronger solvent for non-polar compounds than propylene glycol, acetal/ketal formation can be suppressed by the addition of water, heat and vapor pressure issues	−0.14	Reineccius and Heath (2006)
Triacetin		Slightly soluble in water, acetic acid odor, flavor at high levels, strong solvent for non-polar compounds, last resort delivery system	0.21	UNEP (2002)
Glycerin		Weak solvent for non-polar compounds, typically used in concert with ethanol for heat stability	−1.76	Hansch et al. (1995)
Triethyl citrate		Very strong non-polar solvent, odorless accompanied by bitter taste, insoluble in water, good heat stability, typically used with other solvents	0.19	Chemicalize.org (2013)
Benzyl alcohol		Water insoluble, characteristic floral and benzaldehyde notes, used mainly for concentrated flavor systems	1.1	Hansch et al. (1995)
Limonene/Terpenes		Oxidative stability concerns, strong non-polar solvent, used together with citrus flavor (isoprene based)	4.83 (Limonene)	Reineccius and Heath (2006)
Medium chain triglycerides		Prone to moisture, oxidation, difficulty with solubility of polar compounds	–	–

Emulsifiers are also widely utilized to modify flavor systems to create certain interfacial properties that will suit specific preferred encapsulation techniques and materials. In general, emulsifiers are used to create balance between flavor and encapsulation materials. If the target flavor system has lower polarity overall, a higher polarity (water-soluble) emulsifier may be used to create a stable and homogenized oil−water emulsion with encapsulation materials. As an example, the addition of a common emulsifier, polyglycerol ester, into an emulsion containing gum arabic and d-limonene has been observed to improve emulsion stability, thus increasing flavor retention and decreasing the presence of surface oil post spray drying (Paramita et al., 2010).

TABLE 33.7 Technique and General Polarity Requirements of the Delivery System

Encapsulation Techniques	Preferred Flavor Delivery System
Spray drying	Non-polar; polar
Melt injection/extrusion	Polar medium preferred
Spray cooling/chilling	Non-polar; polar
Molecular inclusion	Dispersion/cavitation, non-polar
Coextrusion	Typically non-polar

The art of modifying the interfacial properties of flavor system to suit encapsulation materials and techniques is also common in melt injection, melt extrusion, and coacervation processes. The amorphous matrix materials used in melt extrusion and melt injection are typically polar in nature (i.e., modified food starches, gums, maltodextrins, sucrose, dextrose, or polyols). In order to make a lipophilic flavor system disperse in these amorphous materials, emulsifiers are typically added into either the lipophilic flavor fraction or the amorphous matrix. As disclosed by Porzio and Zasypkin (2009), the emulsifier polysorbate 60 was added to several different amorphous matrix materials to achieve the required interfacial properties between the flavor fraction and the encapsulation materials.

In coacervation processes, the addition of surfactants is commonly used to alter the surface affinity between oil droplets and the coacervate phase to create stable oil-in-water (O/W) emulsion that leads to uptake of greater percentages of oil droplets within the coacervates. For example, Rabiskova et al. (1994) reported an optimal hydrophilic−lipophilic balance (HLB) range from 2.5 to 6 for encapsulation of oil having HLB:4 in gelatin−gum arabic complex coacervates to provide overall superior oil uptake and desirable encapsulation efficiency. Addition of surfactants beyond this range will modify the surface properties of the oil droplets in such a manner so as to prevent the desired encapsulation behavior. In most cases, the end product application defines the encapsulation strategy which subsequently dictates the solubility requirements of the flavor.

33.3.1 Spray Drying

Spray drying is an encapsulation technique used abundantly within the food and beverage industry to convert liquid flavors or other ingredients into dry form. As described in Section 33.2.2, spray drying is based on selective diffusion theory, whereby an emulsion becomes dispersed into smaller droplets within a heated environment; a thermally induced phase separation occurs where the encapsulation matrix materials form a crust layer on the droplet surface reducing the mobility and diffusion of flavor compounds. The following is an example of a spray drying process directed at preparing a simplified flavor system. Reineccius et al. (1995) prepared encapsulated orange oils and investigated the effects of oxidation as a function of different encapsulation materials (gum acacia, modified food starch). In one example, these investigators prepared an emulsion consisting of 65% water, 28% gum acacia, and 7% orange oil, whereby the carrier substrate (gum acacia) was permitted to hydrate overnight. The orange oil was incorporated into the hydrated carrier and then homogenized just prior to spray drying at inlet temperature 200°C and exit air temperature 100°C. In another example, these investigators prepared an emulsion containing 38.6% water, 31.5% Maltrin-100, 17.5% gum acacia, and 12.4% orange oil to examine the effects of a combination of carrier substrates; this emulsion was processed in the same manner as for the previous example.

33.3.2 Spray Chilling

Spray chilling, or spray cooling, is based on a concept similar to that of spray drying with the exception of particle dehydration; a molten, flavored emulsion becomes atomized, cooled, and solidified. An important consideration in spray chilling is the selection of suitable encapsulation substrates that can undergo solidification in response to the relatively low temperatures of cold air or a quenching bath. It is necessary to maintain a chilling chamber temperature sufficiently below the melting temperature of the encapsulation substrate. In spray chilling, typical shell substrates include stearin, waxes, polyols (melting point 45−122°C), and mono- or diacylglycerols (melting point 45−65°C). In spray chilling,

typical shell substrates include fractionated or hydrogenated vegetable oils (melting point 32−42°C) (Risch et al., 1995; Abbas et al., 2012; Sillick and Gregson, 2012). In one example, garlic oil and ester compounds were encapsulated using hydrogenated cottonseed oil for a bakery application. Despite the conclusion that this encapsulation did not provide improvement in flavor retention, Finney and Reineccius (2009) suggested that it may provide controlled flavor release in response to a thermal trigger. In another example, erythritol was used in a spray chilling technique for flavor encapsulation in a chewing gum application (Gregson and Sillick, 2011). During this process, mint flavors were encapsulated using erythritol by means of spraying the molten emulsion into a chilled limonene bath. It was observed that this spray-chilled mint flavor provided significantly stronger perception of flavor intensity compared to a conventional spray-dried (control) sample in a chewing gum application. In a similar study, Sillick and Gregson (2012) also explored the encapsulation of limonene, nicotine, methyl salicylate, and cinnamic aldehyde.

33.3.3 Melt Injection

Melt injection is an encapsulation process that was first patented by Swisher in 1957. In this process, Swisher used orange peel oil, emulsifiers, and antioxidants to prepare an emulsion to be blended with molten carbohydrates (corn syrup, 42 DE) in a sealed, pressurized tank with nitrogen. These molten contents were violently agitated and then extruded through die into a cold isopropanol bath under agitation to induce solidification (Swisher, 1957, 1962). In this design, solid extrudate was fractionated into smaller pieces as a result of agitation; the residual water and flavor oils deposited on the product surface were removed via the isopropanol bath (Swisher, 1962). This batch-type process has commercial scalability limitations, and the solvent used during this process presents a substantial cost consideration (Porzio, 2008).

33.3.4 Melt Extrusion

Melt extrusion is an encapsulation process that evolved from melt injection, and it is based on similar art. In melt extrusion, the matrix materials (i.e., corn syrup solids, dextran, modified starch) are melted at temperatures above their glass transition temperature (T_g) in a twin-screw extruder, allowing for the dispersion and mixing of core materials (i.e., liquid or dry flavors) within. The matrix is continuously homogenized and pushed forward via directional conveying screw segments through an exit die. As the product exits the extruder into the external environment where temperatures are much lower than the T_g, the molten extrudate forms a glassy or amorphous solid. The glassy solid can then be pulverized to a desirable particle size and washed to remove residual surface oil before final drying (Risch Sara, 1988; Zasypkin and Porzio, 2004; Lakkis, 2008b; Porzio, 2008). Chapter 8 in this book provides more information on extrusion microencapsulation processes.

Compared to melt injection, melt extrusion demonstrates superior production efficiency and commercial scalability owing to its continuous process (Porzio, 2008). Fulger and Popplewell (1999) disclosed a process whereby diacetyl, vanilla extract, and beef flavors were encapsulated with matrix materials consisting of saccharide and maltodextrin. During this process, the investigators explored the addition of flavor into the pre-melted matrix materials prior to extrusion, and also direct addition of flavors into molten encapsulation matrix materials already present in the extruder. Porzio and Zasypkin (2009) disclosed a similar process exploring additional matrix materials consisting of carbohydrates, cellulosics, proteins, gums, sugar, polyols, and mono and disaccharides. The encapsulated flavors examined included butter, cinnamon, and lemonade flavors.

33.3.5 Molecular Inclusion Complexation

Molecular inclusion is a technique that takes advantage of materials containing structural cavities used to entrap "guest" molecules. In the food and beverage industry, cyclodextrin is commonly used as a "host" material in this technique (Hedges et al., 1995). There are several methods used to initiate complexation between "guest" molecules and cyclodextrin materials. Typically, "guest" molecules are incorporated into hydrated cyclodextrin solutions. This process encourages precipitation of cyclodextrin upon inclusion of "guest" molecules; precipitates can be separated via filtration and then dried. "Guest" molecules may also be blended with dry or solid cyclodextrins with minimal water to form a paste-like complex. Although seldom used in the food and beverage industry, an additional method involves forcing gas through cyclodextrin solution to initiate "guest−host" complexation (Shahidi and Han, 1993; Zuidam and Shimoni, 2010). Cyclodextrins may also be processed by means of spray drying for convenience (Reineccius et al., 2002). Such encapsulation methods using cyclodextrin have been used in the food industry to provide flavor stability and also to mask

undesirable tastes and aromas. One example of a bakery application involved the encapsulation of lemon oil via cyclodextrin. In this case the encapsulated lemon oil provided a stronger perception of flavor intensity despite lower usage levels (Qi and Hedges, 1995). Other applications making use of cyclodextrin involve flavor masking of fishy notes (Hedges et al., 1995) and masking of bitter taste associated with caffeine (Bohannon, 2008) and stevioside sweetener (Szejtli and Szente, 2005). More details on microencapsulation using cyclodextrins are given in Chapter 23 of this book.

In addition to cyclodextrins, hydrophobic modified starches have also been investigated for their ability to entrap polyphenols (curcurmin) through hydrophobic interactions and hydrogen bonding (Yu and Huang, 2010).

33.3.6 Coacervation

Coacervation is a technique that produces encapsulates by liquid−liquid phase separation of hydrocolloids from an initial solvent. The driving forces that cause separation include temperature changes, pH modifications, or addition of other reagents having a preferential affinity to the solvent over the colloidal materials (Versic, 1988; Lakkis, 2008b). During the phase separation process, the continuous shell material component begins to envelop the dispersed core materials thereby forming liquid capsule-configuration particles. These liquid capsules can undergo solidification by means of thermal input, desolvation, or crosslinking to form solid particles. In the food industry, most coacervates are prepared via complex coacervation (Fang and Bhandari, 2012). In typical complex coacervation processes, encapsulation materials generally consist of two polyelectrolytes with complex molecular structures having opposing electrostatic charge upon coacervation (Thies, 2007). Gelatin and gum arabic are materials abundantly used for complex coacervation. Wampler and Soper (2000) disclosed a method to create heat-stable flavorings via complex coacervation, demonstrating stability in heated food products including deep fried foods, microwaved foods, and baked foods. Refer to Chapter 12 for details related to the coacervation process.

33.3.7 Novel Techniques

33.3.7.1 Evaporation-Induced Self-Assembly (EISA)

An area of active investigation within the flavor industry involves the exploration of different approaches to encapsulation that do not require heat and that can provide superior particle size regulation. EISA is a technique that has been suggested to improve encapsulation product quality. The process of EISA is mediated by an amphiphilic encapsulation material and a volatile solvent. During the encapsulation process, the polarity in the solvent phase becomes increasingly hydrophilic during solvent evaporation, inducing self-assembly of amphiphilic materials by means of hydrophobic attraction. Amphiphilic materials can form spherical micelles that incorporate hydrophobic compounds internally. These micelles attach their hydrophobic components with other hydrophobic materials and position their hydrophilic ends toward the water phase. As water evaporation occurs, a dry powder of encapsulated particles is produced. Wang et al. (2013) demonstrated a method to encapsulate citrus flavors via EISA using zein (maize protein) as the encapsulation material in an ethanol−water system. The encapsulated product demonstrated core−shell morphology with the citrus flavor enclosed.

33.4 SUMMARY OF FLAVOR MICROENCAPSULATION MATERIALS

In addition to selection of encapsulation technique, the consideration of appropriate encapsulation materials is also important in optimizing encapsulation performance. In Table 33.8, common encapsulation materials are shown paired with well-suited encapsulation techniques. As previously described, the selection of appropriate materials is usually determined by the encapsulation objectives including controlled release, physical modification, flavor masking, or ingredient protection. Additional considerations regarding matrix material may include the limitation of particle size or specific product labeling including kosher, natural, non-caloric, or non-cariogenic.

In general, the most common encapsulation matrix materials (Table 33.8) include carbohydrates, waxes, lipids, proteins, gums, cellulosics, food grade polymers, and derivatives thereof. Encapsulation materials should have the following characteristics: GRAS (generally recognized as safe) status, film-forming capacity, dispersibility in a GRAS solvent, ability to form a stable emulsion with core materials, taste neutral (especially for flavorings), non-reactive with core materials and solvents during processing and storage, and cost conscious (Shahidi and Han, 1993; Barbosa-Cánovas et al., 2005; Zuidam and Heinrich, 2010).

TABLE 33.8 Common Encapsulation Matrix Materials, Methods, and Common Use Purposes

Materials Types	Examples[a]	Potential Encapsulation Methods	Main Interests for Encapsulation	References
Lipids	Beeswax, candelilla and carnauba waxes, paraffin, tristearic acid, diglycerides, monoglycerides, wax emulsions, liposome, glycerol distearate, and natural or modified fats	Spray chilling, fluidized bed spray coating	Controlled release: triggered release by thermally or mechanically delayed flavor release Protection: provide good barrier against moisture	Cherukuri et al. (1991, 1994); Finney and Reineccius (2009); Lenzi et al. (2011); Milanovic et al. (2010); Popplewell and Porzio (2001)
Proteins	Gelatins, soy proteins, whey proteins, zein, casein, albumin, hemoglobin, peptides, and gluten	Spray drying, coacervation, hot melt injection/extrusion, cool congealing coacervation	Controlled release: triggered release upon hydration and slow flavor release through dissolution, diffusion, and erosion Protection: provide good barrier against moisture and water vapor Physical modification: transfer liquid material to stable free-flowing powder	Baranauskiené et al. (2006); Castro and Johnson (2013); Nesterenko et al. (2013); Sengupta et al. (2013); Wang et al. (2013)
Carbohydrates	Starches, maltodextrins, chitosan, sucrose, glucose, lactose, dextran, corn syrup, and sucrose cyclodextrins	Spray drying, hot melt injection/extrusion, molecular inclusion	Controlled release: triggered release upon hydration or mechanically upon erosion Protection: provide good barrier against oxygen Physical modification: transfer liquid material to stable free-flowing powder Cost effective	Eraso and Herrera (2013); Porzio and Zasypkin (2009); Sosa et al. (2013)
Cellulose	Carboxymethylcellulose, methylcellulose, ethylcellulose, nitrocellulose, acetylcellulose, cellulose acetate-phthalate, and cellulose acetate-butylate-phthalate	Spray drying, hot melt injection/extrusion, coacervation	Controlled release: triggered release upon hydration and slow flavor release through dissolution, diffusion, or erosion Protection: provide certain level of heat stability Physical modification: transfer liquid material to stable free-flowing powder	Lenzi et al. (2011); Lou and Popplewell (2003); Ma et al. (2013)
Gums	Gum acacia, agar, sodium alginate, and carrageenan	Spray drying, hot melt injection/extrusion, crosslinking, coacervation	Controlled release: triggered release upon hydration and slow flavor release through dissolution, diffusion, or erosion Protection: provide good barrier against oxygen and certain level of heat stability Physical modification: transfer liquid material to stable free-flowing powder	Castro and Johnson (2007); Lenzi et al. (2011); Petzold et al. (2013); Porzio and Zasypkin (2009); Zasypkin (2011)
Food grade polymers	Polypropylene, polyvinylacetate, polystyrene, and polybutadiene	Hot melt injection/ extrusion	Controlled release: extended flavor release for chewing gum application	Lenzi et al. (2011)

[a]*General material list adapted from Shahidi and Han (1993) and Lakkis (2008b).*

The ratio of flavor to encapsulation matrix material has also been identified as an important factor influencing encapsulation efficiency and particle size. In general, the ratio of flavor to matrix material is approximately 1:4 (w/w). Greater ratios of matrix material generally result in improved retention during spray drying, which can be explained by an increased diffusion path length to the surface of the particle provided by the increased matrix concentration (Charve and Reineccius, 2009; Zuidam and Heinrich, 2010). However, there is usually an optimum concentration of matrix materials to maximize encapsulation efficiency depending on the formulation. In an example exploring the encapsulation of ethyl acetate with modified food starch via spray drying, the optimum in-feeds solids content was observed to be 50%. Increasing or decreasing the solids content to flavor resulted in lowering retention of ethyl acetate (Reineccius and Bangs, 1985). This observation may be explained by the effect of emulsion viscosity, which is positively correlated to the concentration of encapsulation material (Reineccius, 2004). While positive impact on flavor retention was observed with increasing emulsion viscosity (wall material concentration), higher viscosities may inhibit the formation of discrete particles resulting in flavor loss.

Different encapsulation matrix materials have different encapsulation attributes. Accordingly, matrix material selection, and the establishment of combinations of matrix materials that work well with a target flavor system, typically require experimental investigation. For example, Charve and Reineccius (2009) evaluated the encapsulation of d-limonene, (E)-2-hexenal, (E)-cinnamaldehyde, and citral using hydrocolloid-based (gum acacia, modified starch) and protein-based (sodium caseinate and whey and soy protein isolates) encapsulation materials, citing the effects of flavor degradation during storage and subsequent flavor retention.

These researchers reported that gum acacia provided superior protection for aldehyde compounds during 28 days of storage at 40°C. However, gum acacia's protection against limonene oxidation was less effective compared to the protection provided by protein-based wall materials. Non-enzymatic browning presented in all protein-based materials containing aldehyde compounds, a consequence of a chemical reaction between amino acids and carbonyl compounds; browning was not observed with carbohydrate-based matrix materials. Results similar to those observed by Charve and Reineccius (2009) were reported by Kim et al. (1996) during the preparation of an emulsion containing orange oil blended with protein materials and gum acacia.

Differences in encapsulation performance were also observed in experiments involving the encapsulation of rosemary oil using maltodextrin and gum arabic. It was observed that gum arabic provided higher encapsulation efficiency and smaller particle size compared to the case when maltodextrin was used (Janiszewska and Witrowa-Rajchert, 2009). In some cases, superior protection of flavor compounds may be achieved through the use of specific combinations of encapsulation matrix materials. As an example, Lee et al. (2005) found that mixtures of maltodextrin and gum arabic provided improved protection of pine flavor compared to the use of maltodextrin alone for encapsulation. However, it is worth noting that, although using a combination of sucrose and maltodextrin as matrix materials for flavor encapsulation demonstrated substantial improvement in the retention of strawberry and orange flavors, the stability of the encapsulation decreased as a result of the lower glass transition temperature of the encapsulated product (Carolina et al., 2007).

33.5 APPLICATIONS OF MICROENCAPSULATED FLAVOR

As stated in the introduction of this chapter, flavor encapsulation techniques and the application thereof have been widely used within the food and beverage industries; the use of encapsulation is a trend that is predicted to continue growing (Gouin, 2004). Flavor ingredient encapsulation has been used for the purpose of controlled release, protection of ingredients, flavor masking, and flavor enhancement. This section will describe examples of microencapsulated flavors as they are extended into finished product applications.

33.5.1 Controlled Release

In a variety of food and beverage applications it is desirable to regulate the release of flavor during specific phases of the consumer experience. Controlled release may specify the release of flavor during a baking cycle, for example, or at a particular point during consumption. Flavor release can be modified to improve or create unique product experiences such as extended flavor release or flavor changing products for the confectionery market. There are a number of controllable factors and triggers surrounding the release of flavor described in previous chapters. Factors influencing the controlled release of flavor share common release mechanisms including dissolution, diffusion, melting, mechanical fracturing, or changes in pH (Lakkis, 2008b). Different encapsulation particle morphologies, including matrix type and reservoir type (core−shell), provide different release characteristics, which usually influence the selection of encapsulation technique.

Lakkis suggested that microencapsulates with core−shell morphology provide superior controlled release attributes with zero-order diffusion. Encapsulates with this particle morphology, however, are susceptible to the immediate release of flavor in the presence of shell imperfections, resulting in reduced control of flavor release in cases where the shell has become compromised. Hybridization of matrix-type and core-shell encapsulates exist in order to provide certain flavor release kinetics (Lakkis, 2008b). The following subsections will highlight areas within the food and beverage industry that make use of encapsulated flavor. Examples of products using a controlled flavor release system have been seen in the confectionery, bakery, dairy, and beverage markets.

33.5.1.1 Chewing Gum

Chewing gum is a complex food system that has unique chemical and physical properties defined by a predominantly hydrophobic gum base substrate. The hydrophobicity of the gum base interacts favorably with many polar flavor ingredients resulting in the binding of flavor components to the gum base, in turn resulting in slower release of flavor and reduced flavor intensity (Cherukuri et al., 1995). In many cases, the addition of more flavor to overcome the lack of perceived intensity is not effective because greater concentrations of flavor result in deleterious textural issues. That is to say, greater flavor levels can plasticize the gum base, or create inappropriate organoleptic features such as unbalanced flavor or bitterness (Mansukhani and Cherukuri, 1988). To minimize these negative interactions, de Roos (2000) suggested that the encapsulation barrier of spray-dried flavorings inhibits the binding interaction between flavor and gum base. More recently, other encapsulation techniques apart from spray drying have been employed to control and sustain unique modes of flavor release. Flavor release in chewing gum is the subject matter of Chapter 34 in this book.

33.5.1.1.1 Upfront Flavor Release

Rapid and uniform flavor release in a chewing gum application was reported by de Roos (2000) using flavor systems encapsulated with water-soluble carbohydrates. Flavors were reported to be released instantly upon the dissolution of the water-soluble encapsulation materials. Other water-soluble materials including hydrocolloids, polysaccharides, polyols, and combinations thereof have also been disclosed in numerous patent applications within the confections market, and more specifically within the chewing gum space (Cherukuri et al., 1995; Lenzi et al., 2011). In one example, peppermint flavor was encapsulated within gum arabic via spray drying, and the resulting encapsulates were then coated with an additional layer consisting of gum arabic and acesulfame potassium via fluidized bed spray coating. Different forms of encapsulation offer the protection for flavors against the relatively extreme chewing gum manufacturing processes, and also reduce to some extent the flavor−gum base interactions allowing flavors to be released instantly by dissolution in the saliva during mastication (Cherukuri et al., 1995).

33.5.1.1.2 Sustained Flavor Release

Sustained flavor release in chewing gum presents a challenge to formulators because of the aforementioned features of the chewing gum base. Several patents have disclosed art that addresses sustained flavor release and controlled flavor release rates in chewing gum through the use of various encapsulation materials (Lenzi et al., 2011; Castro and Johnson, 2013). Such art involves the use of different encapsulation matrix materials that are either polar or non-polar in origin. These subtle changes in shell solubility enable amplification or attenuation of flavor release. The inventors also suggest the application of multiple coatings on microencapsulated flavorings to further control flavor release kinetics (Lenzi et al., 2011; Castro and Johnson, 2013). This concept resulted in the creation of a chewing gum that demonstrated different, discrete stages of flavor release.

Encapsulation materials that are relatively hydrophilic are preferred for early onset flavor release in the process of gum mastication, as these systems will dissolve out of the gum in tandem with the sweeteners. Moderately hydrophobic encapsulation materials are preferred for secondary flavor be released, and highly hydrophobic encapsulation materials may be used to delay flavor release even further. The release of flavor from gum is also influenced by the hydrophobicity of the flavor oil itself. Generally, water-soluble flavor will be released from gum more quickly than oil-soluble flavors during mastication. A delayed flavor release system was elaborated by Castro and Johnson (2013), in which these investigators used encapsulation materials consisting of gum acacia, corn syrup solids, carrageenan, and pectin in a spray drying process, followed by addition of a vinyl polymer coating via extrusion. The resulting encapsulation system demonstrated slower dissolution, and was applied in chewing gum to deliver flavor at a later stage in the mastication process.

33.5.1.1.3 Flavor Changing Chewing Gum

Chewing gum that provides a flavor changing experience may be prepared by delivering, or releasing, two contrasting flavors at different points in time. As previously mentioned, a desirable flavor release profile can be achieved by selecting encapsulation materials that have different dissolution attributes. For example, Carroll et al. (1984) have disclosed a method of using one liquid flavor and one encapsulated flavor to prepare a flavor-changing chewing gum. The encapsulated flavor may be prepared with water-insoluble encapsulation materials including vinyl polymers, polyesters, waxes, gums, proteins, or their derivatives. During the gum mastication process, the liquid flavor partitions out of the gum matrix into the saliva, providing the initial flavor perception. This event is followed by a relatively slower release rate of flavor from the water-insoluble encapsulation, providing the perception of a secondary flavor.

33.5.1.2 Flavor Changing Ice Cream

Encapsulated flavors have also been used in ice cream applications. Fenner (2011) prepared a lipid encapsulated flavor designed to melt in response to temperatures typical of the consumer's mouth. The flavor profile of ice cream may be modified by incorporating a liquid flavor to deliver the initial flavor perception, and also incorporation a microencapsulated flavor to provide the perception of a secondary flavor.

33.5.1.3 Encapsulated Flavor in a Beverage Straw

Another novel product containing microencapsulated flavorings is Sipahh® Straws. The straw design makes use of two layers in which encapsulated flavors and sweeteners reside. When using the straw, a dairy beverage will be flavored and sweetened as the beverage-soluble encapsulated flavor system slowly diffuses into the beverage stream to impart its sensory properties.

33.5.2 Protections

Encapsulated flavors have been used in food products for the purposes of protecting flavor from oxidation, loss, heat-abusive environment, and undesirable interactions within the food system, thus improving products' shelf-life and quality. A number of examples are illustrated in confections and bakery products.

33.5.2.1 Chewing Gum

As previously mentioned, chewing gum base typically demonstrates strong binding to hydrophobic flavors resulting in diminished flavor release (Cherukuri et al., 1995). Certain flavor compounds, including cinnamaldehyde, have also been found to degrade dipeptide sugarless sweeteners, such as aspartame (Harvey, 2010). The chewing gum manufacturing process consists of heating and shearing, which can result in significant losses of low-boiling point flavors (i.e., fruit esters), as well as cause deviations from the original flavor profile in finished products (Castro and Johnson, 2013). Numerous patents have detailed the use of encapsulated flavors to overcome the challenges presented by chewing gum systems.

In general, encapsulated flavors have been demonstrated to improve flavor release by reducing the interaction between flavor compounds and chewing gum base materials. Polyols, whose melting points are above those temperatures typical of chewing gum manufacturing processes, have been used as wall materials to provide heat-stable encapsulation for flavors (Sillick et al., 2011). The application of a secondary coating onto the previously described encapsulation form has also been investigated. Acesulfame potassium was included in gum acacia solution for using as a second coating on flavor powders to increase its heat stability (Cherukuri et al., 1995). Castro and Johnson (2013) also disclosed a method to improve the retention of low boiling point flavors through the process and also provide extended duration flavor release. These inventors describe an encapsulated flavor system whose operative design is based on sequential layering. Initially, flavors were encapsulated using a variety of materials, including acacia gum, corn syrup solids, hydrogenated starch hydrolysates, carrageenan, or pectin, which was then followed by a secondary coating of vinyl polymer.

33.5.2.2 Hard Candy

The manufacture of hard candy involves the preparation of a molten amorphous saccharide into which a flavor system becomes incorporated. Various forms of encapsulations have been used to improve flavor performance and dispersion during the heat-intensive manufacturing step. Heat responsive polymers, such as hydroxypropyl cellulose (HPC), have

been shown to provide heat stability to temperature-sensitive flavors, thus reducing process-oriented flavor loss and improving the dispersion of flavor during the manufacturing step (Lou and Popplewell, 2003). Another investigation explored the use of coacervation using hydrocolloid materials (i.e., agar−agar, gum arabic, carboxymethylcellulose, and gelatin) to improve the heat stability of flavors during the manufacture of hard candy (1−5 minutes at 137−140°C) (Wampler and Soper, 2000).

33.5.2.3 Bakery Products

Bakery products also involve thermal processing steps to obtain desirable texture, color, and aromas. However, flavor loss is a common result of heating processes. As disclosed by Wampler and Soper (2000), flavors were encapsulated using coacervation methods to offer heat stability during the baking process and controlled release while chewing in the mouth.

In certain cases, flavors must be encapsulated to prevent or reduce their interaction with yeast. Cinnamon, clove, allspice, and nutmeg flavors have been found to inhibit the ability of yeast to act as a leavening agent (Black et al., 1998; Lakkis, 2008a). Conversely, apple flavors delivered in yeast-fermented bread are known to be consumed as nutrients by yeast, resulting in a distorted flavor profile (Black et al., 1998). This is a brief illustration of ways encapsulation is used to provide protection for flavors and for active ingredients in specific applications.

33.5.2.4 Dry mix Beverage

Dry mix beverages provide flexibility and convenience for the consumer by alleviating the transport and space constraints of the ready-to-drink counter (Sanker et al., 1997). However, this type of product is not suited to support the retention of volatile components, including coffee aromas, esters, acetaldehyde, essential oils, and sulfur compounds, which reduces the experience of freshness (Boskovic et al., 1992). As a result, the flavor industry employed microencapsulation as a solution to improve flavor performance within these applications. The requirements for dry mix beverages are that all ingredients, including flavor microencapsulates, rapidly dissolve upon hydration. Encapsulation materials used for these applications commonly involve hydrating hydrocolloids such as modified starch, maltodextrin, gum acacia, and polyols. These encapsulation materials have proven successful in meeting this segment's needs through addressing the flavor ingredient stability requirements (Boskovic et al., 1992; Sanker et al., 1997; Borse et al., 2011).

Ceriali et al. (2008) and Zeller et al. (2003, 2005) described a microencapsulation technology that was used to enhance coffee aroma release in dry mix hot coffee. The inventor's patent art describes a process for incorporating an encapsulated flavor delivery system into a dry mix hot coffee to improve upfront aroma release. In this embodiment, the inventors developed a flavor encapsulate that, upon hydration, would float to the beverage surface and release flavor/aroma compounds in the head space. Results of this research are detailed in Chapter 35 of this book.

33.5.3 Taste Masking

In the food industry, it is challenging to prepare certain food items without some of their original flavor attributes, or to incorporate traditionally unpalatable nutritional ingredients (Barrier et al., 2010). These flavor attributes may appear in foods naturally, by microbial or enzymatic degradation, by heat treatment, by oxidation of lipids, or by the addition of certain functional ingredients including vitamins, minerals, polyphenols, or antioxidants (Galindo-Cuspinera, 2011). Masking undesirable taste and aroma attributes is a common objective in many different applications. To reduce the perception of these undesirable attributes, it is necessary to deliver additional flavor components in an effort to mask the offending components. As an example, sugar is commonly used to suppress sourness or bitterness perception in items like lemonade or yogurt (Galindo-Cuspinera, 2011). However, in certain cases, the use of flavor to mask offending ingredients cannot achieve the desired sensory effect. Therefore, encapsulation techniques have been utilized to create barriers around the source of the offending ingredient to reduce the initial perception of the undesirable attributes.

33.5.3.1 Masking of Fish Odor

Aquatic food products emit fishy odors, and it is often desirable to reduce the perception of these aromas to improve palatability among consumers. Cyclodextrins, materials commonly used to encapsulate aroma compounds via molecular inclusion, have been used in canned fish products to encapsulate such offending aroma compounds. In one investigation, the sulfide compounds in canned fish products were decreased from 137.7 to 3.3 μg % in the presence of cyclodextrins (Qi and Hedges, 1995). Chapter 23 of this book deals with microencapsulation using cyclodextrins.

Fish oil, which contains high levels of polyunsaturated omega-3 fatty acids, have been added to a variety of food products to reduce the risk of heart disease (Harris, 2004). However, fish oil's inherent odor presents the challenge of using it in foods or beverages. Therefore, fish oil is commonly delivered into the consumer market in an encapsulated form. Several encapsulation materials have been used to encapsulate fish oil, including gelatin (Cho et al., 2003), whey protein and cyclodextrin (Na et al., 2011), zein protein (Zhong et al., 2009), chitosan (Klaypradit and Huang, 2008), and ethyl cellulose (Kantor et al., 1990) to reduce the fishy taste and smell perception and prevent oxidation. Fish oil encapsulated with ethyl cellulose has been applied in several food products including flavored gelatin, orange juice, flavored agar gel, yogurt, and peanut butter without report of detecting undesirable notes through a period of 3 weeks (Kantor et al., 1990).

33.5.3.2 Caffeine

Caffeine, derived from coffee leaves, beans, or teas, is routinely used to stimulate the central nervous system, reduce drowsiness, and restore concentration. However, the bitter taste associated with caffeine does not align with certain food applications, as in the case of sweet or savory items. Therefore, caffeine is usually delivered in these food products in an encapsulated form to reduce the perception of bitterness. Bohannon (2008) has disclosed a method to deliver caffeine, encapsulated with mono- and diglycerides, polymeric materials, lipids, and oils, in several foods products including icing, breakfast bars, and other food additives without bitterness detection.

33.5.3.3 Flavor Masking by Molecular Inclusion

Cyclodextrins have unique structural designs that allow for entrapment of "guest" molecules (refer to Section 33.3.5). Cyclodextrins have been used to mask undesirable tastes and aromas. Several examples of the use of cyclodextrin to reduce the perception of undesirable aromas and tastes in current food products address grassy components from soybean, off-flavors in stale rice, bitter components in orange juice or grape fruit juice (limonin and naringin), and bitterness associated with natural sweeteners (i.e., rebaudioside A) (Szejtli and Szente, 2005). While cyclodextrin has been approved in the USA as a GRAS food material, its usage is limited in the EU. In Europe, the use of β-cyclodextrin is limited in certain food products including chewing gum, potato, cereal, flour, or starch-based snacks, as well as in flavored beverages.

Currently, other cyclodextrins (α- and γ-) are only recognized as novel food ingredients under regulatory review (Jafari et al., 2008). Alternative materials have been investigated for their ability to encapsulate unpalatable food materials. Sporopollenin exine is a new encapsulation material extracted from *Lycopodium clavatum* spores (Brooks and Shaw, 1978; Barrier et al., 2010). "Guest" material such as fish oil can be loaded into the exine's structure via simple mixing under vacuum. This encapsulation material has demonstrated the ability to encapsulate fish oil at a 1:1 ratio without the detection of fish notes (Barrier et al., 2010). A detailed account of the use of exine as microencapsulating agent is given in Chapter 24 of this book.

33.6 CONCLUSION

Flavor plays a central role in consumer food and beverage products. As the diversity of food and beverage applications continues to grow, so does the need for new methods of flavor delivery. One way to address flavor delivery is through the field of microencapsulation. The fields of flavor microencapsulation and taste modification are highly diverse sciences and arts consisting of an equally diverse collection of techniques and materials. There are many regulatory, processing, and sensory benefits of microencapsulation. The benefits of microencapsulation do not come without challenges, and so continuous efforts are directed at the identification of encapsulation materials and techniques appropriate for specific flavor innovations.

ACKNOWLEDGMENTS

The authors would like to express gratitude to FONA International, Inc. for its support in the composition of this chapter. Additionally, the authors would like to express gratitude for the guidance and collaborative efforts of Dr. Anilkumar Gaonkar.

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Chapter 34

Flavor Release and Application in Chewing Gum and Confections

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34.1 INTRODUCTION

Microencapsulation of flavors in chewing gum and other confections to control the sensory performance has long been a key technology as evidenced by the large number of chewing gum patents over the last 30 years. It has been in practice since microencapsulation technology developed for industrial and pharmaceutical industries was translated for use in food applications. Initially, the cost of microencapsulation technology was limited to practical use in high value, high cost materials that can absorb the additional processing costs. Flavor microencapsulation in chewing gum was one of the first applications in the food industry. The cost per pound for higher-priced flavors allows for a smaller incremental cost increase as compared to commodity food ingredients using this technology.

Flavor release in chewing gum is paramount to providing flavor enhancement, innovation, and competitive advantages in chewing gum marketing. Slow release of ingredients such as flavors, sweeteners, and acidulants plays a role in the overall sensory perception, and in the number one challenge in marketing gum—longer-lasting taste. Chewing gum normally has good taste for the first 2−5 minutes of chewing. Slow release of flavor, sweetness, sourness, and their combinations will prolong consumer perception of longer-lasting taste and flavor and in improving the marketability of chewing gum products. This is key to the success in a competitive market where flavor enhancement adds value. Microencapsulation of flavors, high intensity sweeteners such as aspartame, acesulfame-K, surcralose, and stevia in slow-release delivery formats will provide for flavor enhancement and flavor retention. Microencapsulated citric, malic, and other acidulants allow for slow release of sour flavors, especially in fruit-flavored gums and flavor-changing gums.

Today, microencapsulation technology has been applied to a number of active materials with flavor microencapsulation focused on providing unique delayed, controlled, and sequential release profiles to give gum and confectionery manufacturers distinct advantages over their competition. Handling, safety, and extended shelf-life protection of raw flavor materials has also driven additional consideration of microencapsulated flavors outside of sensory performance. This chapter will discuss the most common microencapsulation technologies used mainly for chewing gum, and a few examples for other confections. It will also discuss the expected sensory performance over chew time when using microencapsulation technology (see also Meyers, 2008, 2011a,b, 2012a,b).

34.2 WHY MICROENCAPSULATE FLAVORS?

Flavor microencapsulation enables product developers to control the behavior and performance of flavor systems. Marketing of chewing gum and other confections with unique flavor-changing performance, longer-lasting flavor/sweetness/sourness, and other unique consumer experiential traits (i.e., tingling, heating/cooling, other sensates) has increasingly been desired by consumers in recent years.

Microencapsulation of flavors protects flavor profiles by isolating raw materials over their shelf-life, and in the finished chewing gum and confectionery products. Interactions between the ingredients of the chewing gum base and flavors, presence of oxygen and pro-oxidant ingredients, and environmental changes (temperature, pH, etc.) all can be slowed down by a protective layer (coating) around the active flavor and/or by matrix encapsulation.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00034-0

Finally, microencapsulated flavors provide a format that is easy to handle in manufacturing environments. It is much easier and safer to handle a stable dry powder as against volatile liquids. It is easier in production environments to weigh ingredients rather than meter them in liquid form. More information on microencapsulation of flavors can be found in Chapter 33.

34.3 MICROENCAPSULATION FORMS

There are various microencapsulation technologies used by the flavor industry and by chewing gum and confectionery manufacturers. The main technologies are discussed in other chapters of this book (Part 3) and a short overview of the key ones used in flavor delivery in confections is presented below and summarized in Table 34.1A:

1. **Spray drying:** Spray drying is the traditional first technological approach and most used where a powdered form of flavor is desired.
2. **Compaction:** Compaction granulation compresses spray-dried powdered materials into large granulations. They are then broken, in a second process, into larger particles (compared to the fine spray-dried powders) with varying size distributions. The granulation mixtures may be enhanced with fruit purees, acids, etc. to make the profile more complex.
3. **Agglomeration:** Agglomeration also achieves increased particle size similar to compaction—but a more gentle process is used, leading to slower flavor release rates in chewing gum and other confections.
4. **Spray chilling:** Spray chilling is utilized in applications where release by heat is desired; or the product needs protection from moisture during shelf-life storage in ingredient or final product confection form.
5. **Biopolymer films:** Biopolymer films are flat sheets that have entrapped liquids in a dried film. Most microencapsulation technologies utilized spherical or three-dimensional structures for improved diffusion characteristics. So, films have not been typically used except for their marketing appeal in recent years. These films have been used in stand-alone breath strips or cut into confetti pieces and applied for visual appeal in gum, candy-coated confections, and other applications. Typically, a cellulosic polymer (pullulan, HPMC, etc.) is the base film material in which liquid flavor is immobilized.
6. **Molecular inclusion:** Molecular inclusion works well with some fruit flavors—but it can be comparatively expensive with low loading levels and it is typically a batch operation. It is mostly used by flavor companies in combination with spray-dried flavors to balance flavor and key notes lost during the spray drying process.
7. **Glass microencapsulation:** Glassy matrix microencapsulation has grown in use by flavor and gum companies in the last few decades because of very long shelf-life ($>3-4$ years) and is most suitable for oxidative-sensitive flavor oils such as citrus.
8. **Fluid bed spray granulation**: Fluid bed technology is typically considered for controlled, delayed, or sequential release and is the next level up from spray drying for most chewing gum and confectionary applications. Flavor is incorporated by plating or spray drying onto a carrier followed by a coating process that applies a succession of layers in a fluidized bed coating unit.

Microencapsulated flavors and actives can be minimally coated or highly coated depending on desired release and protection needed. Coating materials are usually immiscible with the active material (many flavors are non-polar,

TABLE 34.1A Properties of Flavor Microencapsulates Obtained from Different Methods

Encapsulation	Particle Size (microns)	Load (%)	Flavor Effect in Chewing Gum*
Spray dry	20–100	20–50	U
Compaction	1000–3000	5–20	B, T, V
Agglomeration	500–3000	5–20	U, V
Fluidized spray dry	200–400	20–50	B, T, V, L**
Glass extrusion	200–2000	5–8	V, T, S

* Key: U = Upfront flavor release; B = Flavor burst; T = Texture effects; V = Visual effects; S = Flavor stability/extended shelf-life; L = Long-lasting flavor (**hot-melt fluidized bed).
Source: Courtesy of Symrise, Inc. and Rosa, 2006.

so the coating materials typically are polar—gums, proteins, etc.). Coating materials can be solid fats applied as hot-melt coatings that solidify at room temperature. Release mechanisms include heat, physical forces (chewing), or slow release over time in saliva if emulsifiers are used in the coating. Other types of coating materials include lactose polymers, cellulosics, modified starches, polyvinyl pyrrolidone (PVP), and other materials approved for chewing gum applications. Some of these materials are not approved for regular food—but can be used in chewing gum, since it is not intended to be swallowed.

9. **Beadlet microencapsulation (microcapsules):** Beadlet microencapsulation, also referred to as microcapsules, is a technology that entraps liquid flavor in the center of a spherical capsule. The technology is good for novel "burst"-type applications. Release occurs by chewing and breaking the microcapsule to release the flavor. Beads can be colored and visible on the surface of confections as part of marketing the uniqueness of the technology. The coating can be gelatin or other material that can harden over time and is immiscible with liquid oil (so it will not be solubilized by the flavor).

34.4 MICROENCAPSULATION FORMS—OTHER TYPES

New technologies are continually being developed to improve on existing designs and performance. Many chewing gum and flavor manufacturers have internally developed, niche (and proprietary) technologies. Many of these are covered by other authors in this book. The more popular niche technologies are presented below and summarized in Table 34.1B:

- Spinning disk
- Coacervation
- Submerged nozzle (alginates in calcium bath)
- Liposomes using emulsion technology
- Nanotechnology, which is also being investigated for microemulsions, etc.
- Other niche methods discussed in this book.

TABLE 34.1B Properties of Flavor Microencapsulates Obtained from Less Utilized Methods

Encapsulation	Particle Size (microns)	Load (%)	Flavor Effect in Chewing Gum*
Coacervation	20–800	40–50	B, T, L, V
Submerged nozzle	800–5000	70–90	U, B, T, L, V
Alginate/gelatin caps	1000–3000	50–70	B, T, L, V
Liposome	0.02–0.10	5–10	U, L, S
Cyclodextrin	20–100	equimolar	U, S, L
Spray cooling/chilling	20–100	15–20	B, T, V, L, S
Biopolymer film	250–2500	5–20	V, B, T

* Key: U = Upfront flavor release; B = Flavor burst; T = Texture effects; V = Visual effects; S = Flavor stability/extended shelf-life; L = Long-lasting flavor.
Source: Courtesy of Symrise, Inc. and Rosa, 2006.

34.5 CHEWING GUM APPLICATIONS—DESIGNING FOR CUSTOMIZED PERFORMANCE

Microencapsulation type and particle size distribution will provide for special effects. The use of larger particles will allow for increased flavor impact due to uneven distribution of flavor in chewing gum. Liquid flavor is mixed throughout the chewing gum and provides a constant level of flavor perception when consumed. From a sensory standpoint, the longer the chew time, the less the flavor perception with constant flavor release due to background noise levels rising with chew time. Encapsulated flavor is concentrated in localized areas of the gum mixture and released during chewing to give a "flavor burst" effect, and the sensory perception is higher flavor levels over chew time.

Microencapsulated flavors will deliver a greater impact than the same flavor level in liquid form. Customization and blending liquid flavor with microencapsulated flavors of various particle size and type will provide unique sensory

performance over chew time. For example, upfront impact is obtained from liquid flavor, followed by bursting of flavor and sustained or sequential release over chew time with various microencapsulated forms. Flavor concentration and loading has to be controlled to prevent "hot spots" in order to provide strong flavor perception without flavor burning or harshness from too high a flavor load.

Flavor boosting via microencapsulation is important for a number of reasons. There may be dosage constraints due to the price of the liquid flavor raw material or the microencapsulated form. Some cooling compounds, such as menthol, have limitations as there is an increased bitterness at higher use levels, making the overall gum and confection flavor too bitter. In some cases, there are regulatory concerns that limit the amount of a flavor material intake (e.g., the World Health Organization has an Acceptable Daily Intake (ADI) for menthol of 0.2 mg/kg of body weight/day). The final constraint is the production environment and worker safety. Microencapsulated forms prevent dusting and irritating flavor/cooling/heating compounds from coming in contact with sensitive membranes in the eyes and respiratory system.

The benefits of microencapsulated flavors can be demonstrated analytically as well as from sensory performance. Figure 34.1 shows results of analytical testing of a glassy matrix microencapsulation of menthol crystals. The data are provided courtesy of Firmenich, Inc. using a proprietary analytical tool called AFFIRM® (Analysis of Flavors and Fragrances In Real tiMe) allowing the monitoring of volatiles exhaled by a panelist while chewing. Nine-fold increase in the concentration of menthol was demonstrated with the encapsulated menthol. Figure 34.2 shows the same chewing gum sample with results from a sensory testing.

Results show significant analytical and sensory differences between the microencapsulated menthol and the equal quantity in unmicroencapsulated, raw (neat) form. The analytical testing tool is more sensitive and objective than the

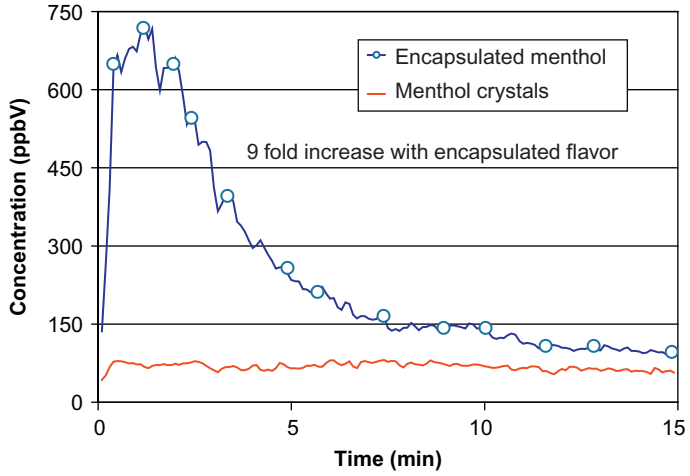

FIGURE 34.1 Boosting of menthol (measured analytically) in sugar-free chewing gum—effect of microencapsulation of menthol. *(Courtesy of Firmenich, Inc.)*

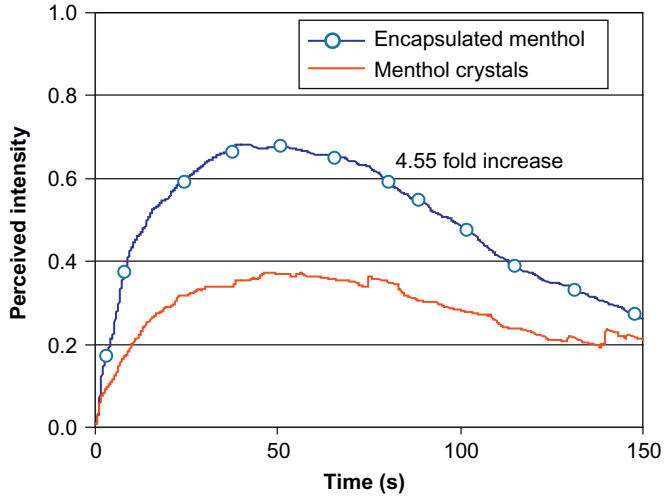

FIGURE 34.2 Boosting of menthol (measured by sensory test) in sugar-free chewing gum—effect of microencapsulation of menthol. *(Courtesy of Firmenich, Inc.)*

sensory (subjective) testing—but the perception by consumers showing perceived four-fold increase in flavor/cooling perception with the microencapsulated form over the neat form at equal flavor levels is the key benefit of the technology.

Figures 34.3 and 34.4 show similar analytical and sensory data for liquid peppermint flavor components in chewing gum, with similar results to those for microencapsulated menthol crystals. Figure 34.3 shows the analytical results for microencapsulated menthol and menthone flavor materials in a glassy matrix. Figure 34.4 shows the sensory results for these microencapsulated materials in a finished peppermint flavor versus the liquid flavor. Sensory results confirm a significant boosting effect over the first 5 minutes of chewing of sugar-free gum with microencapsulated flavors. Many liquid flavor molecules are hydrophobic and can be bound more tightly with the hydrophobic gum base materials. Microencapsulated actives are not bound to the gum base and can release more flavor and have a higher sensory impact during chewing.

Figure 34.5 shows sensory results of two different microencapsulation technologies—spray-dried versus glassy matrix extrusion, used for "hot mix" flavors added with liquid peppermint flavor in sugar-free chewing gum. The results were obtained using 20 trained panelists and the same level of hot mix ingredients. As seen, the more sophisticated glassy matrix microencapsulation statistically outperforms the spray-dried microencapsulated flavor. The higher impact is due to the ability of the glassy matrix having a lower affinity for the gum base over the spray-dried flavor. The flavor is not as bound to the hydrophobic gum base, where similar flavors partition towards the gum base and are not as readily released in the saliva.

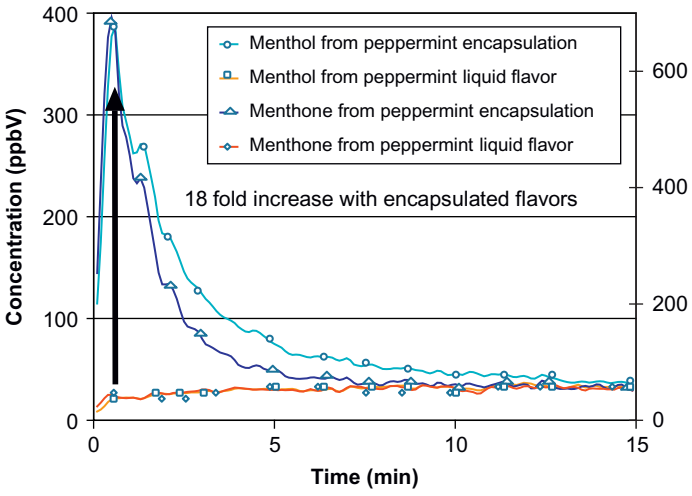

FIGURE 34.3 Boosting of peppermint flavor (measured by sensory test) in sugar-free chewing gum—effect of microencapsulation. *(Courtesy of Firmenich, Inc.)*

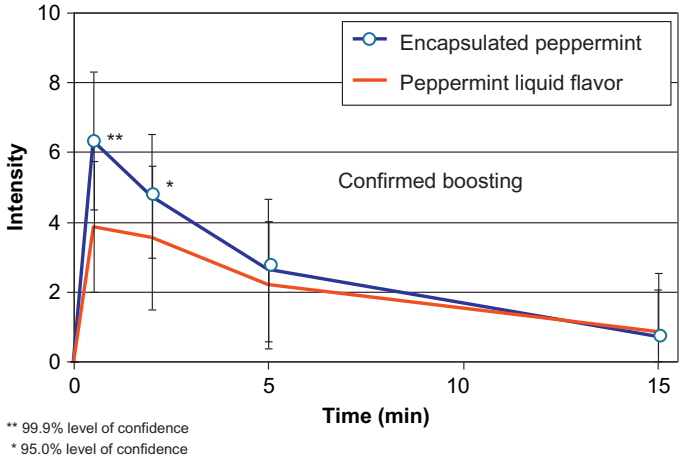

FIGURE 34.4 Boosting of peppermint flavor (measured by sensory test) in sugar-free chewing gum—effect of microencapsulation. *(Courtesy of Firmenich, Inc.)*

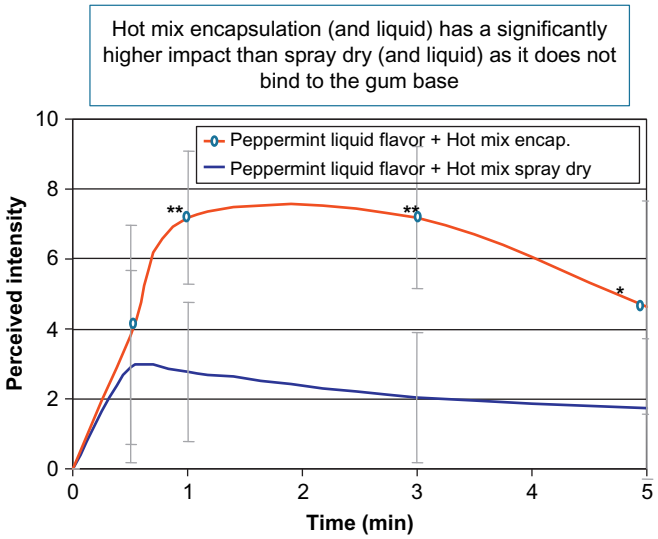

FIGURE 34.5 Comparison of two microencapsulation technologies—"hot mix" performance system of Firmenich proprietary microencapsulation in chewing gum. Differences are statistically significant at 99.9% (**) and 95% (*) level of confidence. *(Courtesy of Firmenich, Inc.)*

TABLE 34.2 Cost (USD)/kg of Microencapsulation Forms in Chewing Gum at Equivalent Peppermint Flavor Level in Gum

Flavor Format	Cost/kg of Chewing Gum (USD)
Liquid alone @ 1.4%	0.81
Liquid @ 1.2% + Spray dried flavor @ 1.0%	1.03
Liquid @ 1.35% + Glass extruded @ 1.0%	1.04
Liquid @ 1.2% + Fluidized bed @ 1.25%	1.25
Liquid @ 1.2% + Gelatin capsule @ 0.3%	1.07

Note: Pricing circa 2006.
Source: Courtesy of Symrise, Inc. and Rosa, 2006.

The desirable performances shown in Figure 34.5 are achieved with technology that adds processing cost. This is one of the key factors in deciding if use of the microencapsulation technology is economically feasible. Table 34.2 shows relative costs of using microencapsulated flavors in chewing gum (price per kilo of finished chewing gum).

Cost is always a significant factor in deciding whether to use microencapsulated flavors. A review of the cost-in-use is key to determining if microencapsulation is cost prohibitive or a viable option. Decisions should not be based on price/kg alone. The value of having a longer flavor shelf-life for raw materials or finished confections has value. The ability to offer unique release profiles such as controlled, delayed, and sequential release has value to differentiate the product from competition. Most evaluations are based on shelf-life and the upfront, middle, and long-lasting flavor profiles that one can achieve with various microencapsulation technologies. Table 34.3 shows the cost-in-use benefits of various microencapsulated flavors.

34.6 MICROENCAPSULATED FLAVORS—WHEN TO USE THEM?

As encapsulation adds significant cost, it is sometimes better to consider adding higher levels of liquid and/or lower cost spray-dried flavors as against the use of more sophisticated (and costly) microencapsulates. The use of higher liquid (neat) flavors may have limitations in chewing gum and confections. Too much flavor will act as a solvent or plasticizer for the gum base and make the finished gum too soft or sticky. High speed gum wrapping operations can be compromised if the sheets of gum do not maintain desired stiffness. Too much flavor, which affects release of other components (sweeteners) or the flavor, will be too harsh, giving a "chemical" or "burning" sensation in the mouth.

TABLE 34.3 Cost of Microencapsulated Flavors vs. Cost−In-Use

Encapsulation Form	Shelf Life (months)	Upfront Release	Middle Release	Long Lasting	Typical dosage without other flavor	Price Range $ per kg	Cost-In-Use $ per kg Finished
Liquid flavor	6−12	X			0.2−1.0%	10−90	0.02−0.90
Submerged Nozzle	24	X	X	X	0.4−0.8%	90−150	0.36−1.20
Fluidized Bed	24	X	X		0.5−1.0%	25−40	0.13−0.40
Extruded Glass	24−48	X	X		1.0−2.0%	15−25	0.15−0.50
Compacted	12	X	X		1.0−3.0%	10−30	0.10−0.90

Note—pricing circa 2006.
Source: Courtesy of Symrise, Inc. and Rosa, 2006.

Microencapsulation should be used for its special effects or to help solve a processing problem. There are benefits and drawbacks to all microencapsulation systems.

Matching a microencapsulate's properties with the desired outcome is important. One size does not fit all needs; and most microencapsulates are customized for specific performance and application. For example, if the objective is to get a "bursting" flavor effect in a chewing gum application, a gelatin microcapsule with a high flavor load could be the best option. A glass-extruded microencapsulate may not have enough flavor load to provide the desired effect—but may be the best option if gelatin capsules cannot withstand the high shear of gum processing equipment. In some cases, high humidity or high water activity in the final product and processing and storage conditions may prohibit use of some materials such as carbohydrates and gelatins. Finally, there can be other non-technical limitations. For example, kosher and vegetarian products cannot use certain or any gelatin ingredients respectively. Sugar-free products cannot use certain carbohydrates present in glass extrusions, etc.

34.7 TO BE EFFECTIVE, MICROENCAPSULATED FLAVORS ALSO REQUIRE SUSTAINED AND LONG-LASTING SWEETNESS AND SOURNESS

Delivery of sweeteners and acids are just as important as that of the flavor, because they increase perceived flavor levels in gum and confectionery applications. Because of their water-soluble nature, sweeteners and acids are depleted first as one begins to chew the gum. It is estimated that about more than half of the added flavor still remains in the chewing gum when the bolus (the gum mass left over after a consumer stops chewing) is discarded. It is interesting to note that addition of a small amount of sweetener and acid to the bolus and further chewing can bring out more flavor perception. Hence, prolonged release of sweetener and acid form is part of the solution to obtain a long-lasting flavor release in chewing gum. Several patents can be found on microencapsulation of sweeteners and acids.

Sweetness "brings back to life" flavor that is trapped in the gum base and only perceived with increased sweetness delivery over chew time. Use of sweet notes in flavors as well as building high intensity sweeteners into the flavor microencapsulation system may be required so sweetness is delivered at the same time as the flavor. Sucralose's lingering effect can provide better longer-lasting flavor as well as complement the flavor profile of the chewing gum—but may be undesirable in other confectionery applications where sweetness is perceived long after the confection has been consumed. Some sustained- and controlled-release microencapsulation methods used for flavors can be applied separately for sweeteners and acids.

34.8 WHERE IS MICROENCAPSULATED FLAVOR APPLIED IN CHEWING GUM APPLICATIONS?

Typically, it is homogeneously mixed into the gum base near the end of mixing to limit flavor loss and mechanical stress on the microencapsulates. It should be mixed into the gum completely before other liquids (solvents) are added, such as liquid flavors that could compromise the microencapsulated coating. Besides addition in the gum matrix, encapsulated flavors are mixed with "rolling" or "dusting" compounds and applied on the surface of stick and tab gums before being compressed into place.

Surface addition of spray-dried flavor provides upfront impact of flavor along with the sweetness of the rolling compound (sugar, polyols, high intensity sweeteners). Microencapsulation shells can be brightly colored so they are visible as capsules or specks on the surface of stick/tab gum and sugar pan-coated shells of pellet gums (giving consumers a visual cue to the gum's unique properties). Pan-coated pellet gums can have microencapsulated flavors added during dusting of the gum center (with hydrocolloids) or during successive application of layers of the hard shell coating (sugar/polyol solutions).

34.9 CHALLENGES IN MICROENCAPSULATING FLAVORS

Volatile flavors in liquid as well as microencapsulated forms (especially fruit flavors) may be lost, or the perceived flavor profile changes in the gum over its shelf-life. Understanding how the flavor profile may change can help developers with formulating flavor chemicals in the final custom flavor. Flavor companies have expertise in analyzing and customizing flavors to limit changes or compensate for changes during finished chewing gum's shelf-life. Restrictions in cost/benefit, regulatory, and sensory performance are the key deciding factors for microencapsulated flavor application in chewing gum.

34.10 OTHER CONFECTIONERY APPLICATIONS

Besides chewing gum which has been covered extensively in this chapter, flavor microencapsulation is also used in other confectionery items such as compressed tablets or mints, hard candies, liquid-filled confections, granola bars, and as visual specks in all the above applications. Fluidized bed and extruded glass microencapsulation technologies provide stability for citrus oils in compressed tablets. In granola bars, extruded glass microcapsules and compacted granulated flavors are added to the surface after the harsh processing conditions to maintain higher flavor levels that would be lost due to high shear processing. For hard candy, such as chewing gum, upfront impact and flavor release is accomplished by dusting spray-dried flavor powders on the surface. In liquid centers, nanoemulsions can be created to improve flavor delivery and impact.

34.11 CHEWING GUM PATENT REVIEW—MAIN COMPANIES: WRIGLEY, WARNER–LAMBERT, CADBURY–ADAMS/KRAFT FOODS GLOBAL, NABISCO/HERSHEY CONFECTIONERY COMPANIES (1990–2013)

Microencapsulation patents related to chewing gum and confections are listed at the end of this chapter in Appendix 1 and divided by the main active players in chewing gum encapsulation technology over the last 20 + years. For a more thorough review, patents should be evaluated as far back as the late 1970s through the 1980s when competitive patent positions were very active between the key players. This review was completed searching the US Patent and Trade Office (USPTO).

34.12 CONCLUSION

Flavor companies and large multinational chewing gum manufacturers have an extensive range of microencapsulation technologies that give product developers many options and control of flavor performance over chew time. Limitations with respect to cost, regulatory aspects, and sensory impact must be considered in selecting one technology or a combination of microencapsulation technologies. Microencapsulates must solve a problem or add unique special effects to justify their added cost. These special effects include isolation and protection of flavor and flavor burst to obtain controlled, sustained, and sequential release profiles for competitive advantages.

APPENDIX

Chewing Gum Patent Review

Patent activities (US patent numbers and patent titles) on use of microencapsulated flavors and other actives in chewing gum and confectionery applications by Wrigley, Warner–Lambert, Cadbury–Adams/Kraft Foods Global, and Nabisco/Hershey companies from 1990–2013 are given below:

Wm. Wrigley Jr. Company

8,617,623 Chewing gum with delayed-interaction modifier
8,414,939 Food product with an encapsulated lecithin material
8,337,923 Encapsulated antimicrobial material
7,416,751 Method of controlling release of N-substituted derivatives of aspartame in chewing gum and gum produced thereby
7,364,761 Chewing gum containing physiological cooling agents and method of preparing
7,244,454 Chewing gum product including encapsulated aspartame and sodium bicarbonate and process of preparing
7,022,352 Encapsulated flavors and chewing gum using same
6,955,827 Method of controlling release of antimicrobial agents in chewing gum
6,770,308 Chewing gum including encapsulated acid mixtures
6,759,066 Chewing gum formulations including encapsulated aspartame and sodium pyrophosphate
6,692,778 Method of controlling release of N-substituted derivatives of aspartame in chewing gum
6,592,912 Method of controlling release of antimicrobial agents from chewing gum and gum produced thereby
6,586,023 Process for controlling release of active agents from a chewing gum coating and product thereof
6,472,000 Method of controlling release of bitterness inhibitors in chewing gum and gum produced thereby
6,455,080 Chewing gum containing controlled release acyclic carboxamide and method of making
6,428,827 Long flavor duration releasing structures for chewing gum and method of making
6,165,516 Method of controlling release of caffeine in chewing gum
5,501,864 Method of making sugar-containing chewing gum with prolonged sweetness intensity
5,447,565 Apparatus and method for coating particles
5,415,880 Fruit flavored chewing gum with prolonged flavor intensity
5,227,182 Method of controlling release of sucralose in chewing gum using cellulose derivatives and gum produced thereby
5,217,735 Method of making chewing gum with delayed release ingredients
5,198,251 Gradual release structures for chewing gum
5,192,563 Strongly mint-flavored chewing gums with reduced bitterness and harshness
5,169,658 Polyvinyl acetate encapsulation of crystalline sucralose for use in chewing gum
5,169,657 Polyvinyl acetate encapsulation of sucralose from solutions for use in chewing gum
5,165,944 Gradual release structures for chewing gum
5,164,210 Zein/shellac encapsulation of high intensity sweeteners in chewing gum
5,154,939 Use of salt to improve extrusion encapsulation of chewing gum ingredients
5,154,938 Gum composition having dispersed porous beads containing plasticizers
5,154,927 Gum composition containing dispersed porous beads containing active chewing gum ingredients and method
5,153,011 Chewing gum flavor ingredient
5,139,798 Polyvinyl acetate encapsulation of codried sucralose for use in chewing gum
5,139,794 Use of encapsulated salts in chewing gum
5,139,787 Gum composition containing dispersed porous beads containing active chewing gum ingredients and method
5,124,160 Granulation of active ingredients using polyvinyl acetate and alcohol
5,112,625 Aqueous zein coated sweeteners and other ingredients for chewing gum
5,108,762 Gradual release structures for chewing gum
4,997,659 Alitame stability in chewing gum by encapsulation
4,986,991 Chewing gum having an extended sweetness
4,978,537 Gradual release structures for chewing gum
4,963,369 Gum composition containing dispersed porous beads containing active chewing gum ingredients and method
4,931,295 Chewing gum containing high-potency sweetener particles with modified zein coating
4,919,941 Chewing gum containing delayed release protein sweetener and method

Warner–Lambert

6,479,071 Chewing gum and confectionery compositions with encapsulated stain removing agent compositions, and methods of making and using the same

6,361,298 Methods and apparatus for making seamless capsules
6,238,690 Food products containing seamless capsules and methods of making the same
6,174,466 Methods for making seamless capsules
5,888,538 Methods and apparatus for making seamless capsules
5,795,588 Encapsulated product
5,679,389 Chewing gum compositions having increased flavor and sweetness and methods for preparing same
5,595,757 Seamless capsules
5,266,335 Microencapsulated flavoring agents and methods for preparing same
5,126,151 Encapsulation matrix
5,108,763 Microencapsulated high intensity sweetening agents having prolonged sweetness release and methods for preparing same
5,082,671 Low moisture sucralose sweetened chewing gum
5,077,051 Sustained release of active agents from bioadhesive microcapsules
5,064,658 Encapsulated synergistic sweetening agent compositions comprising aspartame and acesulfame-K and methods for preparing same
5,059,416 Zinc compound delivery system with improved taste and texture
5,057,328 Food acid delivery systems containing polyvinyl acetate
5,043,169 Stabilized sweetener composition
5,009,893 Breath-freshening edible compositions of menthol and a carboxamide
5,004,595 Multiple encapsulated flavor delivery system and method of preparation
4,983,404 Controlled release flavor system and method of preparation
4,981,698 Multiple encapsulated sweetener delivery system and method of preparation
4,971,797 Stabilized sucralose complex
4,954,353 Anhydrous chewing gum with improved stability
4,933,190 Multiple encapsulated sweetener delivery system
4,931,293 Food acid delivery systems containing polyvinyl acetate
4,929,447 Encapsulation composition for use with chewing gum and edible products
4,915,958 High-base gum composition with extended flavor release
4,911,934 Chewing gum composition with encapsulated sweetener having extended flavor release

Cadbury Adams/Kraft Foods Global (formerly Pfizer/Warner–Lambert and currently Mondelēz International)

8,597,703 Delivery system for active components as part of an edible composition including a ratio of encapsulating material and active component
8,591,974 Delivery system for two or more active components as part of an edible composition
8,591,973 Delivery system for active components and a material having preselected hydrophobicity as part of an edible composition
8,591,972 Delivery system for coated active components as part of an edible composition
8,591,968 Edible composition including a delivery system for active components
8,389,032 Delivery system for active components as part of an edible composition having selected particle size
7,879,376 Taste potentiator compositions and edible confectionery and chewing gum products containing same
7,851,000 Taste potentiator compositions and edible confectionery and chewing gum products containing same
7,727,565 Liquid-filled chewing gum composition
7,041,277 Chewing gum and confectionery compositions with encapsulated stain removing agent compositions, and methods of making and using the same

Nabisco (currently Hershey gum business)

5,532,004 Chewing gum containing hydrophobic flavorant encapsulated in a hydrophilic shell
5,458,891 Chewing gum containing flavorant adsorbed in cross-linked elastomeric polymer
5,338,809 Chewing gum or confection containing flavorant adsorbed on silica
5,087,461 Double-encapsulated compositions containing volatile and/or labile components, and processes for preparation and use thereof

5,015,483 Liposome composition for the stabilization of oxidizable substances
5,009,900 Glassy matrices containing volatile and/or labile components, and processes for preparation and use thereof
4,975,270 Elastomer encased active ingredients

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Chapter 35

Novel Microencapsulation System to Improve Controlled Delivery of Cup Aroma During Preparation of Hot Instant Coffee Beverages

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35.1 INTRODUCTION

Instant coffee products inherently lack the intense aroma characteristic of ground roast coffees. Consequently, reconstituting instant coffee powders in hot water deprives consumers of the full sensory experience that brewing roast coffee affords. Substantial differences exist in the level, composition, and sensory perception of aromas released from filtered coffee brews versus those from prepared instant coffee beverages (Pollien et al., 1997; Sanz et al., 2002). Aromatic essential oil pressed from roast coffee beans is commonly plated onto spray-dried or freeze-dried coffees to provide package headspace aroma to convey freshness upon jar opening (Mishkin et al., 1964). Methods exist to even replenish headspace between openings by plating onto microporous coffee particles (Saleeb and Hudak, 1981; Dwyer, 1982; Hudak and Saleeb, 1984). Numerous methods are known for controlling composition, strength, and sensory character of aromatized coffee oils, but a review is outside the scope of this chapter. Oil plating typically does not noticeably enhance perception or consumer acceptance of beverage aroma, but can increase acceptance of cup flavor (Oliveira et al., 2009). Conversely, it is widely known that increasing oil to much higher levels than typically used for jar aromatization can noticeably strengthen beverage aroma, but may produce visible oil slicks and turbidity and also promote extraction of flavors that negatively impact cup quality and balance. Accordingly, the present authors found the amount of aromatized coffee oil could not be sufficiently increased in instant coffee to match beverage aroma impact provided by a novel oil-free liquid coffee essence without adversely affecting beverage appearance and flavor quality (Ceriali et al., 2005; Zeller et al., 2008a). Our work leading up to that discovery formed the basis for this chapter.

Numerous methods to incorporate aromatized coffee oils into instant coffee powders to enhance beverage flavor and cup aroma have been published or patented over the last several decades. Molecular-inclusion complexation of coffee aromas in β-cyclodextrin (Szente and Szejtli, 1986) was proposed to increase beverage aroma impact. However, preferred delivery methods would encapsulate coffee aromas to minimize oxidation and loss of labile constituents in storage until instant coffee products comprising capsules are dissolved in hot water. More advanced methods encapsulated aroma in pressurized particles that burst upon contact with hot water or in floating capsules that dissolve on the beverage surface to release aroma above the cup. Early methods encapsulated coffee oil or other aroma dispersed in a comminuted expanded glassy matrix by grinding solidified gas-injected viscoelastic evaporated coffee extracts (Arora et al., 1991) or extruded melts (Arora and Rankowitz, 1992). Melts may be solidified under pressure to reduce aroma loss (Fulger and Popplewell, 1996). Another route involved co-extruding a coffee oil core containing dissolved gas into a molten coffee shell and cutting the extrudate into capsules upon exiting the die before hardening; when added to hot water, dissolved gas expands to rupture the capsules and oil is spread across the beverage surface to release an aroma

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00035-2

burst (Garwood et al., 1995). Another method entrapped pressurized gas and dispersed coffee oil in an extruded coffee melt that was solidified under pressure and ground to provide particles that release a burst of cup aroma (Cheng et al., 1998). However, all these methods can produce beverage oil slicks when coffee oil is used. Methods reported to prevent slicks utilized aromatized hydrolyzed coffee oils mixed with (Chmiel et al., 1996), or encapsulated within (Rushmore et al., 1996), instant coffee particles; when such particles are dissolved in hot water, the hydrolyzed oil spontaneously forms an oil-in-water microemulsion and simultaneously releases an aroma burst.

A notable advance in aroma delivery was the development of aerated coffee capsules that float and dissolve more rapidly in water than particles or capsules formed from pressurized coffee melts. The first reported method emulsified aromatized coffee oil into aerated aqueous coffee solution and desiccated small droplets in soluble coffee powder to create floating aromatized capsules (Tuot, 1985). The present authors modified that method by replacing coffee oil with aromatized volatile liquids and freezing droplets in liquid nitrogen before desiccating (Zeller et al., 2002a,b, 2004); the volatile liquids increased cup aroma intensity and prevented oil slicks, while freezing helped reduce aroma loss and improve control of capsule shape. The present authors are unaware of any coffee cup aroma delivery systems shown to be more effective than our novel methods summarized in this chapter (Zeller et al., 2002a,b,c, 2004, 2006a, 2008a; Ceriali et al., 2005). These methods not only effectively deliver volatile liquids to the beverage surface, but drive evaporation to increase aroma release rate and intensity while maximizing limited supplies of natural coffee aroma and minimizing impact on flavor and appearance.

Application of aroma enhancement methods to coffees or mixes whitened with milks or creamers is more complicated because aroma components may associate with dissolved milk proteins or partition into fat droplets in brewed (Steinhart and Bücking, 1999; Bücking and Steinhart, 2002; Parat-Wilhelms et al., 2005; Denker et al., 2006) or instant (Kim et al., 1995) coffees to reduce impact. One study of instant coffees formulated with model additives found added protein had no measurable impact on aroma upon dissolution in hot water, but lipid or carbohydrate additives reduced headspace volatile concentration despite accelerating delivery to the headspace (Fisk et al., 2012b). Another study demonstrated that adding sugars to coffee beverages affects partitioning of model key aroma compounds between liquid and vapor phases to different extents depending on sugar and coffee composition and aroma polarity (Piccone et al., 2012). Methods to increase cup aroma impact from coffee beverage mixes formulated with fatted milk or creamer powders are known and include limiting fat content or delaying dissolution of fatted powders relative to coffee powder (Zeller et al., 2008b), or reconstituting coffee powder before fatted powders using two-step beverage preparation schemes (Zeller et al., 2008c).

Numerous methods have been reported to improve delivery of coffee or other aromas by utilizing food particles containing pressurized gases entrapped in closed pores. One method provides an aromatizing agent by agglomerating particles or droplets of flavor with a foaming agent (Witschi and Maccinnes, 2002) such as a powder comprising carbohydrate, protein, and pressurized gas (Bisperink et al., 2001). Water addition releases gas bubbles from the foaming agent that carry volatile flavors to the headspace, and optional use of fat as agglomerating agent may help direct the aromatizing agent to the surface. Other known pressurized foaming agents are comprised mainly, or exclusively, of carbohydrate (Darbyshire et al., 2003; Zeller et al., 2006b) or protein (Holtus et al., 2005; Zeller et al., 2006c). A related method entraps volatile flavors directly in closed-pore powders, along with pressurized gases, to provide an aroma burst upon dissolution in water (Poortinga et al., 2009). Foaming coffees also are known that contain pressurized gases (Zeller et al., 2006c, 2010; Imison, 2009; Fisk et al., 2012a) or supercritical fluids (Imison et al., 2008) within spray-dried or freeze-dried particle voids. Pressurized coffees dissolve in water to provide foam similar in appearance to brewed espresso crema and it was recently found that gas liberation increases both the rate and amount of inherent aromas released above the cup relative to conventional instant coffees (Yu et al., 2012). It was also recently shown that presence of crema on brewed espresso beverages enhances above-cup aroma release (Barron et al., 2012).

35.2 NOVEL MICROENCAPSULATION SYSTEM DEVELOPMENT

This novel system, demonstrated to enhance aroma delivery from instant coffee during beverage preparation while avoiding problems related to coffee oil, evolved in stages. Water-immiscible organic volatile carrier liquids (VCLs) were separately aromatized with volatile model coffee flavors and shown to increase, to different extents, the rate of aroma delivery from hot water when compared to a similarly aromatized non-volatile oil reference. Aromatized VCLs, and reference, were then separately entrapped in low-density coffee capsules by emulsifying liquid droplets into aerated viscous aqueous coffee solutions containing dispersed small gas bubbles, dripping into liquid nitrogen to freeze, and then desiccating in a bed of milled soluble coffee powder. When added to hot water, these capsules dissolved while floating to release aromatized liquids onto the beverage surface. The VCL capsules ruptured faster than reference

capsules to release entrapped droplets that rapidly evaporated to provide stronger aroma with less impact on beverage flavor. Only reference capsules produced an enduring slick. VCLs having relatively low boiling point and water solubility increased rates of capsule dissolution and aroma release. In the next stage, model aromas were replaced by aromatizing carriers with natural coffee volatiles, with similarly beneficial results when entrapped in floating capsules. Finally, the use of VCLs was eliminated by removing the most water-soluble and least-volatile components from natural coffee aromas. These water-immiscible, carrier-free, oil-free coffee essences were similarly dispersed as liquid droplets in aerated solutions and entrapped in coffee capsules. After blending with instant coffee and adding hot water, capsules dissolved on the beverage surface to release floating droplets that rapidly evaporated to provide an intense burst of roast coffee aroma without causing unsightly appearance or unpleasant flavor.

35.3 GUIDE TO RELATED PUBLICATIONS BY THE AUTHORS

VCLs as a new class of volatile flavor carrier was patented for use in coffee beverages (Zeller et al., 2002a) and dehydrated food systems (Zeller et al., 2002b). Application of VCL technology to increase model aroma impact from instant coffee beverages was presented at the 20th ASIC International Scientific Colloquium on Coffee and a technical manuscript was published in the proceedings (Zeller et al., 2004). Methods to aromatize VCLs with natural coffee aromas were patented (Zeller et al., 2002a) and presented at the 21st ASIC, followed by publication of a manuscript in proceedings (Zeller et al., 2006a). In addition, methods to create volatile carrier-free natural coffee essences were patented (Ceriali et al., 2005) and presented at the 22nd ASIC, followed by publication of a manuscript in proceedings (Zeller et al., 2008a). Since all content that follows in this chapter, with the exception of microscopy images, was previously disclosed in various forms among those publications, and considerable overlaps exist, further citation will not be provided in order to improve readability. Methods used to create and encapsulate aromas, and to analyze release, are only generally described below, but details can be found in published manuscripts and patent applications. Any details not captured among those publications can be assumed to have been disclosed in ASIC meeting oral presentation materials. Patent numbers of these inventions noted in the reference section correspond to first grants (all US). Applications subsequently granted in other countries within those patent families may contain different claims and provide different scopes of protection.

35.4 VOLATILE CARRIER LIQUIDS

Replacing coffee oils with VCLs was found to substantially increase cup aroma intensity and delivery rate from floating coffee capsules. This invention stemmed from a physical chemistry consideration of factors affecting evaporation of volatile liquids from hot water. It was realized that fixing coffee aroma in oil stabilizes labile components, but also reduces inherent vapor pressure and ability to evaporate. Optimal VCLs would be water immiscible, have compatible odor, have low density and boiling point to rapidly evaporate from the surface of hot water to prevent slicks, and serve to improve evaporation of any weakly volatile aroma components. As shown in Figure 35.1, partial vapor pressures of

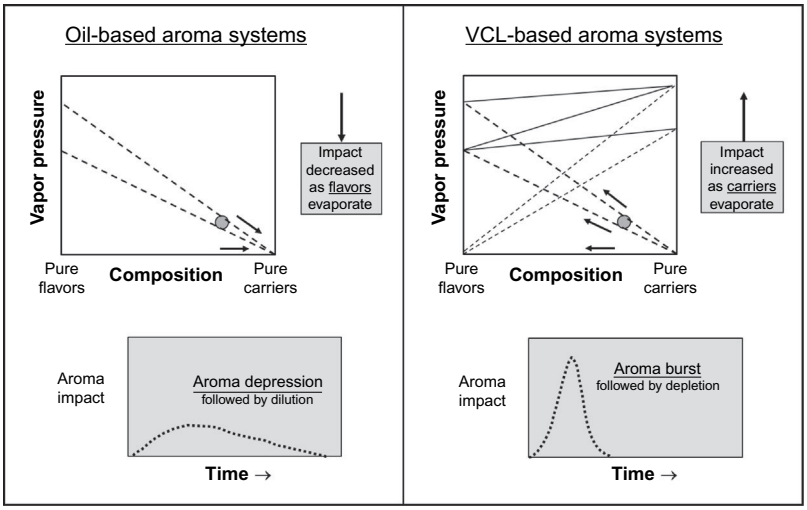

FIGURE 35.1 Hypothesized aroma release from oil-based versus VCL-based systems.

volatile flavors fixed in non-volatile oils decrease as evaporation progresses and components are diluted. In aromatized VCLs, carrier and flavor evaporate together and any aroma components less volatile than carrier are concentrated to increase partial vapor pressure. Accordingly, it was hypothesized that replacing coffee oil with VCLs should produce more rapid evaporation of volatile flavors to provide more intense aroma bursts and this improvement was later experimentally confirmed.

35.5 MODEL COFFEE AROMA SYSTEMS

Table 35.1 outlines key physical properties of conventional and novel carrier liquids and particular suitability of VCLs in this work. Table 35.2 summarizes behavior of selected carriers in hot water and the ability of d-limonene droplets to rapidly evaporate from hot water despite relatively high boiling point. The other VCLs, 2-ethylfuran and ethyl acetate, also performed well compared to conventional carriers, which dissolved, sank, or did not evaporate. Table 35.3 describes a model aroma comprising six physically and chemically diverse volatile liquid flavors compounded into different carriers to assess evaporation under various conditions. Table 35.4 highlights the superior 2-minute aroma recovery from 2-ethylfuran in gas-swept heated dry or wet (water-containing) jars. Time-sequenced headspace analysis capability was later added to study aroma component release rates. Tables 35.5 and 35.6 compare component and total release from floating coffee capsules containing emulsified droplets of model-aromatized carriers made using the syringe drip method depicted in Figure 35.2. Figure 35.3 plots time-segment normalized total aroma release from capsules versus time, and Figure 35.4 normalizes release to soybean oil, used to mimic release that would be expected from unavailable model-aromatized coffee oil devoid of inherent aroma.

Use of VCLs greatly enhanced evaporation of model aroma relative to soybean oil. Best overall performance was obtained from 2-ethylfuran and d-limonene. Initial aroma release was fastest from more volatile VCLs (2-ethylfuran and ethyl acetate), but the most aroma was released from d-limonene; the least aroma was released from more soluble carriers (ethanol and ethyl acetate). Initial release was slowest from soybean oil, likely from slower capsule rupture and evaporation. Highest peak intensity was obtained from 2-ethylfuran and d-limonene, at nearly twice the level from

TABLE 35.1 Physical Properties of Conventional Flavor Carriers and Novel VCLs

Conventional Flavor Carriers	Density (g/cc)	Water Solubility	Vapor Pressure (mmHg)	Boiling Point (°C)	Freezing Point (°C)	Suitable as Volatile Carrier
ethanol	0.79	miscible	59	78	−114	no
soybean oil	0.92	insoluble	0	N/A	−10	no
isopropanol	0.78	miscible	45	82	−89	no
propylene glycol	1.04	miscible	0.15	187	−60	no
triacetin	1.16	slight (7%)	<0.01	259	3	no
benzyl alcohol	1.04	slight (4%)	≈0.05	205	−15	no
acetoin	1.00	miscible	≈5	148	15	no
triethyl citrate	1.14	insoluble	<0.01	294	>10	no
glycerol	1.26	miscible	<0.01	290	18	no
water	1.00	miscible	24	100	0	no
Novel VCLs						
d-limonene	0.84	insoluble	2.1	175	−74	yes
2-ethylfuran	0.91	insoluble	≈50	92	< −70	yes
ethyl acetate	0.90	slight (9%)	94	77	−84	yes

Includes available literature values and estimates; water solubility data are %/wt; all data correspond to 25°C.

TABLE 35.2 Evaporation of Carrier Liquid Droplets from Hot Water

		Evaporation Time (Seconds) versus Water Temperature			
Carrier	**Volume (μl)**	**55°C**	**65°C**	**75°C**	**95°C**
d-limonene	5	160	80	35	25
	10	180	115	55	40
	15	200	130	65	50
	20	225	135	70	55
coffee oil and soybean oil	5–20	did not evaporate; produced unsightly oil slick			
2-ethylfuran and ethyl acetate	5–20	droplets rapidly evaporated from surface			
triacetin and benzyl alcohol	5–20	droplets sank to bottom of beaker			
ethanol and propylene glycol	5–20	droplets dissolved in water			

TABLE 35.3 Physical Properties of Model Aroma Components

Aroma Component	Density (g/cc)	Boiling Point (°C)	Freezing Point (°C)	Water Solubility
2-methylpropanal	0.79	64	−66	low (10%)
diacetyl	0.99	88	−2	moderate (20%)
2-ethylfuran	0.91	92	< -70	insoluble
isobutyl acetate	0.87	118	−99	very low
4-ethylguaiacol	1.06	235	15	very low
eugenol	1.07	255	−9	insoluble

Includes available literature values and estimates.

TABLE 35.4 Model Aroma Recovery from Gas-swept Heated Jars (Dry vs. Wet)

System	% Model Flavor Evaporated (within 2 minutes after addition to jar)				
	Soybean Oil	**Ethanol**	**2-Ethylfuran**	**d-Limonene**	**Ethyl Acetate**
Dry jar	62	72	81	78	48
Wet jar	40	23	41	33	29

Data are averages of duplicate analyses at 85°C; total does not include response for 2-ethylfuran. All aroma components were compounded into each carrier at 5%/wt to create model aromas.

soybean oil. Rapid initial aroma release rate and high peak intensity are both important to maximize consumer perception of cup aroma. Soybean oil produced the most prolonged release, with highest 1–2 minute aroma intensity, and was the only carrier that produced a residual slick. Poor performance from ethanol was attributed to rapid miscibility with water after release from capsules. All data are averages of duplicate experiments and model aroma totals do not include 2-ethylfuran contribution since this aroma component was also used as an aroma carrier.

TABLE 35.5 Model Aroma Component Release from Coffee Capsules

	Aroma Released (1×10^6 GC counts) during 0–10 Seconds				
Aroma Component	**Soybean Oil**	**Ethanol**	**2-Ethylfuran**	**d-Limonene**	**Ethyl Acetate**
2-methylpropanal	5.6	7.8	61.3	15.8	27.5
diacetyl	3.0	1.3	18.5	5.1	20.2
2-ethylfuran	10.1	26.5	–	38.0	59.6
isobutyl acetate	7.9	33.7	119	27.9	63.3
4-ethylguaiacol	2.3	9.6	16.9	4.2	6.0
eugenol	2.4	8.4	24.4	3.8	4.0
Total aroma	21.2	60.8	240.1	56.8	121.0
Vs soybean oil	1.00 ×	2.87 ×	11.33 ×	2.68 ×	5.72 ×
	Aroma Released (1×10^6 GC counts) during 0–30 Seconds				
2-methylpropanal	184.0	118.4	404.3	411.8	310.5
diacetyl	67.8	25.9	188.2	129.4	194.3
2-ethylfuran	521.1	384.5	–	997.0	710.6
isobutyl acetate	458.9	513.7	1278	1070.9	891.3
4-ethylguaiacol	63.7	40.7	117.1	63.2	53.0
eugenol	15.3	41.6	117.1	52.3	38.8
Total aroma	789.7	740.3	2104.7	1727.6	1487.9
Vs soybean oil	1.00 ×	0.94 ×	2.67 ×	2.19 ×	1.88 ×
	Aroma Released (1×10^6 GC counts) during 0–120 Seconds				
2-methylpropanal	752.0	358.8	724.9	1038.5	544.6
diacetyl	347.0	129.6	420.8	357.6	426.1
2-ethylfuran	2291.1	1096.3	–	2452.0	1623.6
isobutyl acetate	2443.9	1515.9	2457.2	3047.9	2066.3
4-ethylguaiacol	346.2	244.8	459.3	381.2	251.9
eugenol	211.8	233.9	539.1	369.3	207.5
Total aroma	4100.9	2483.0	4601.3	5194.5	3496.4
Vs soybean oil	1.00 ×	0.61 ×	1.12 ×	1.27 ×	0.85 ×

7 g aromatized carriers emulsified into 42 g 50%/wt aerated 5°C aqueous coffee solutions before desiccation.
0.1 g capsules were dropped onto 200 ml 85°C water in 250 ml jars swept with N_2 gas at 300 ml/minute.
Aroma fractions were desorbed from Tenax traps and analyzed using GC-MS to quantify components. Data are averages of duplicate analyses; totals do not include response for 2-ethylfuran.

35.6 COFFEE MICROCAPSULE PROPERTIES

Capsules created via the syringe drip method were generally spherical and had 1–3 mm diameter, placing them at the upper limit of the broadest common definitions of microcapsules and beyond limits of definitions requiring a size smaller than 1 or 0.1 mm. In contrast to more common microcapsules like spray-dried particles, individual coffee capsules are large enough to be picked up and counted by hand. Comparable size makes them easy to blend with granular freeze-dried or agglomerated spray-dried coffees. Since they are fairly large and earlier publications on these and predecessor capsules called them capsules, the terms capsule and encapsulation are used more than microcapsule and microencapsulation in this chapter for consistency and readability. Much smaller capsules could be made by spraying emulsions from atomizing

TABLE 35.6 Total Model Aroma Release from Coffee Capsules

	Aroma Released (1×10^6 GC counts)				
Time Segment	**Soybean Oil**	**Ethanol**	**2-Ethylfuran**	**d-Limonene**	**Ethyl Acetate**
0–10 s	21.2	60.8	240.1	56.8	121.0
10–20 s	192.9	220.7	784.9	611.9	694.5
20–30 s	575.6	458.8	1079.7	1058.9	672.4
0–30 s	789.7	740.3	2104.7	1727.6	1487.9
30–60 s	1575.4	1006.4	1663.0	2219.0	1109.7
60–90 s	994.8	463.7	518.2	829.6	600.4
90–120 s	741.0	272.6	315.4	418.3	298.4
0–120 s	4100.9	2483.0	4601.3	5194.5	3496.4

Data are averages of duplicate analyses; totals do not include response for 2-ethylfuran.

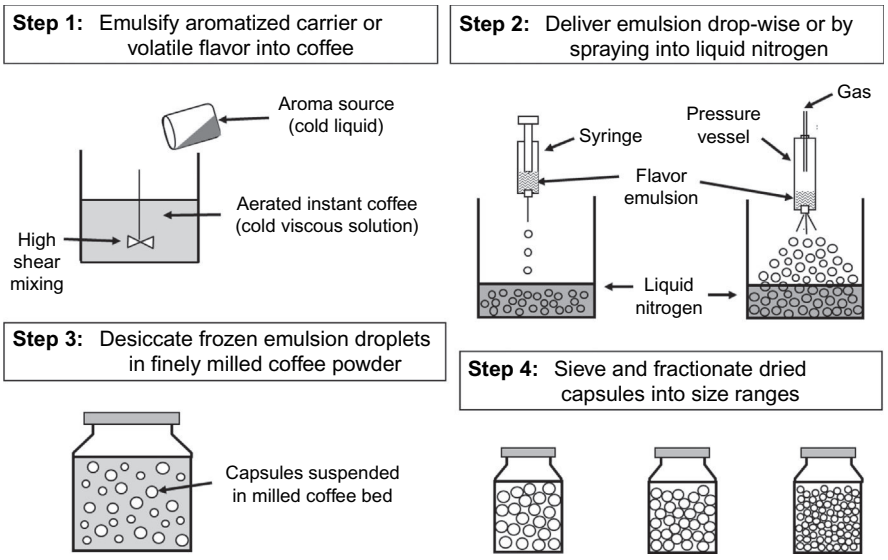

FIGURE 35.2 Method to make floating aromatized coffee capsules.

nozzles, but may dissolve too rapidly to float in hot water. Capsules made in this work contain liquid droplets and gas dispersed in a matrix surrounded by a shell formed by desiccation, incorporating elements of both matrix and core–shell encapsulation. Bulk and apparent density were typically 0.3–0.7 and 0.4–0.9 ml/g, respectively, as collectively determined by coffee solution and flavor emulsion, and gas bubble concentrations, processing conditions, and capsule size distribution.

35.7 COFFEE-AROMATIZED CARRIERS

Early exploration focused on d-limonene due to availability and low cost. It was deodorized by eluting through silica gel to remove polar impurities and minimize inherent odor before using as an aroma carrier. After demonstrating effectiveness as a model carrier, d-limonene was directly aromatized with coffee aroma frosts obtained as products of roast coffee grinding or percolation. d-Limonene was later replaced with 2-ethylfuran due to its occurrence in coffee, more compatible odor, and lower boiling point that produced faster capsule rupture and aroma release. Droplets of frost-aromatized VCLs were encapsulated in floating coffee capsules using disclosed methods. Some properties of frost-aromatized d-limonene and coffee oil are compared in Table 35.7, which shows that lower VCL freezing point enables

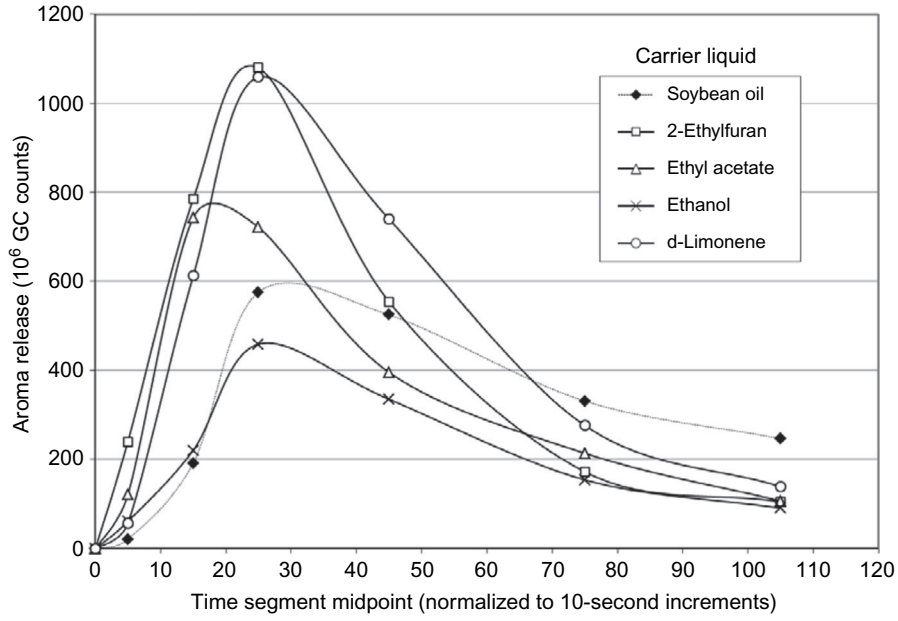

FIGURE 35.3 Model aroma release from coffee capsules in hot water.

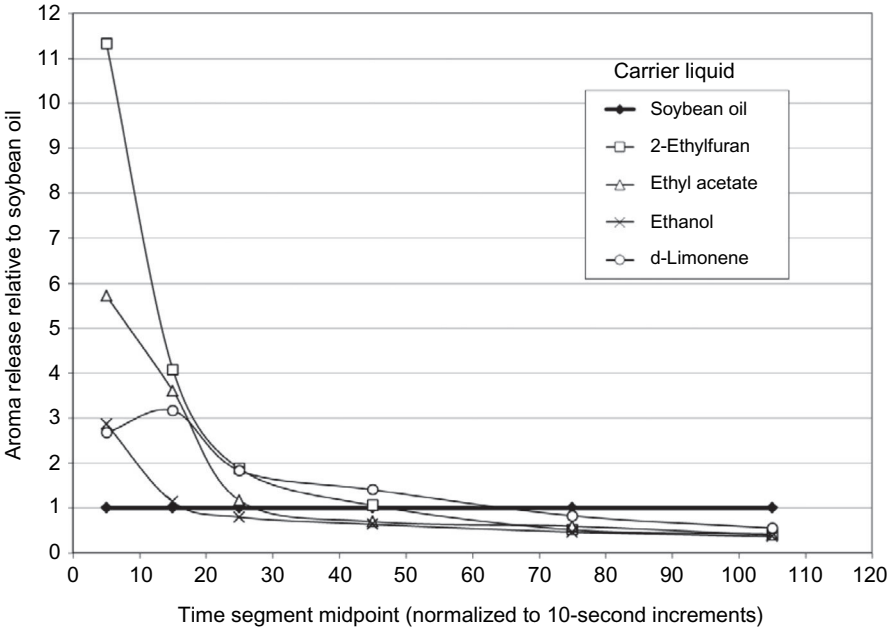

FIGURE 35.4 Model aroma release from coffee capsules normalized to soybean oil.

TABLE 35.7 Properties of Some Coffee Frost-aromatized Carrier Liquids

Carrier Liquid	Frost:Carrier Ratio (weight)	Fixation Time (hours)	Aroma Content (GC counts)	Aroma Recovery (%)	Moisture Content (%)
d-limonene	10:1	2	2100	90	0.1
d-limonene	12:1	4	2300	90	0.1
coffee oil	1.6:1	8	300	80	1.5

Additional details of methods and materials can be found in US 6699518.

greater aroma transfer from frosts. Structural elements of frost-aromatized VCL capsules are clearly visible in light and scanning-electron microscopy images, as shown in Figures 35.5 and 35.6. These capsules delivered intense bursts of roast coffee cup aroma when blended at low levels with instant coffee and added to hot water.

35.8 CARRIER-FREE COFFEE ESSENCES

Learnings were applied to make coffee capsules containing droplets of carrier-free liquid aromas. This was accomplished by removing the most water-soluble and least-volatile components from roast coffee aromas and freezing to create depleted coffee essence frosts used to aromatize coffee extracts and make capsules using the methods depicted in Figures 35.7–35.9. Table 35.8 summarizes test results that confirmed that essence capsules contained discrete liquid aroma droplets, dissolved faster, and provided stronger aroma, improved appearance, and cleaner flavor than capsules made using aromatized coffee oil. Table 35.9 provides evidence that the proportion of oil capsules used in the hot instant coffee beverage could not be increased to achieve the aroma strength provided by a much lower proportion of essence capsules without adversely affecting appearance and flavor. Results are illustrated in Figure 35.10. These novel essence capsules released a strong burst of fresh roast coffee aroma from hot water and can be used to replace coffee oils and avoid use of VCLs. They can be packaged without declaration since all components are naturally obtained from coffee.

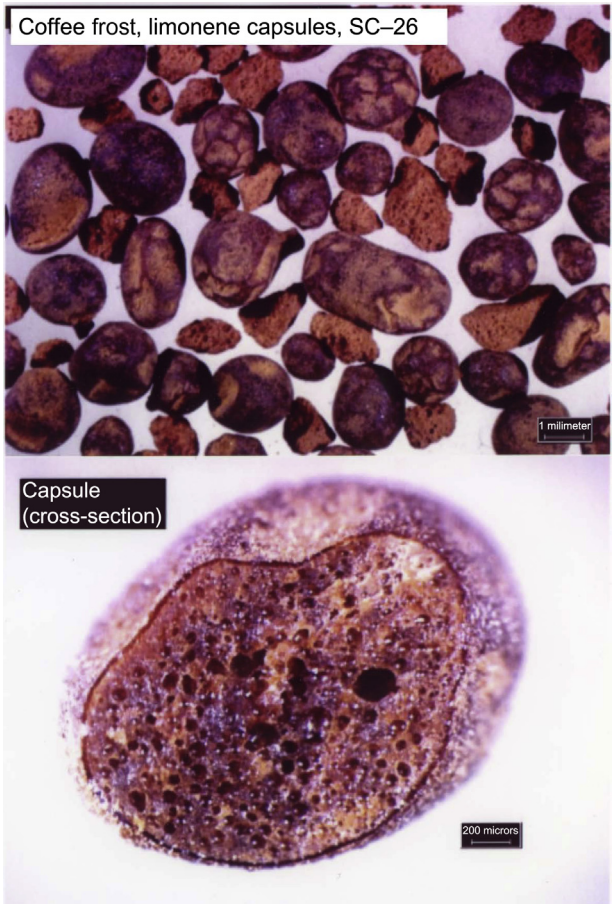

FIGURE 35.5 Coffee capsules containing coffee frost-aromatized d-limonene.

35.9 DISCUSSION

Sensory perception of aroma enhances enjoyment of coffee beverages and increasing its intensity can convey freshness and quality. Application of this novel system provides consumers with two experiences—an intense burst of preparation aroma from capsules followed by weaker sustained aroma from the instant coffee. This system overcomes the problems of

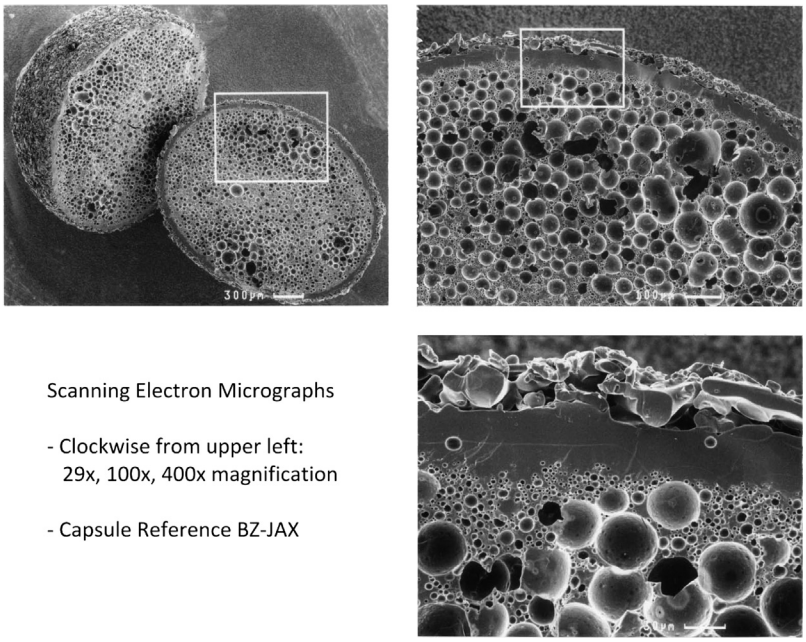

FIGURE 35.6 Coffee capsules containing coffee frost-aromatized 2-ethylfuran.

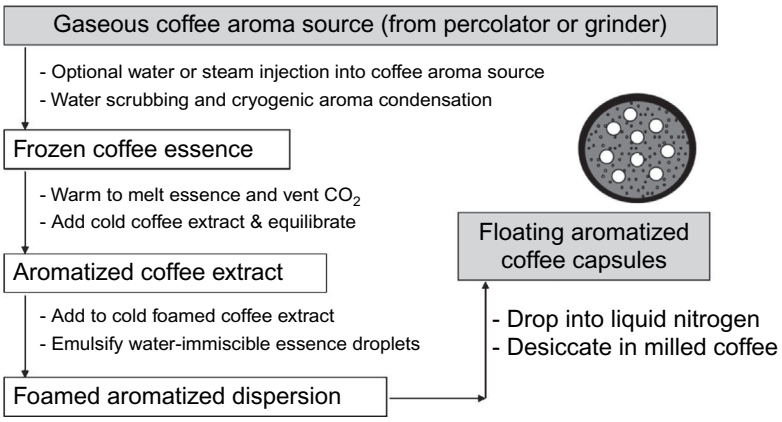

FIGURE 35.7 Method to make floating coffee essence capsules.

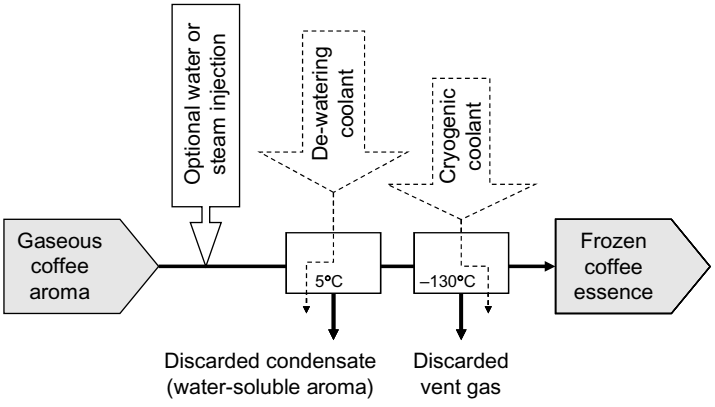

FIGURE 35.8 Method to make depleted coffee essence frosts.

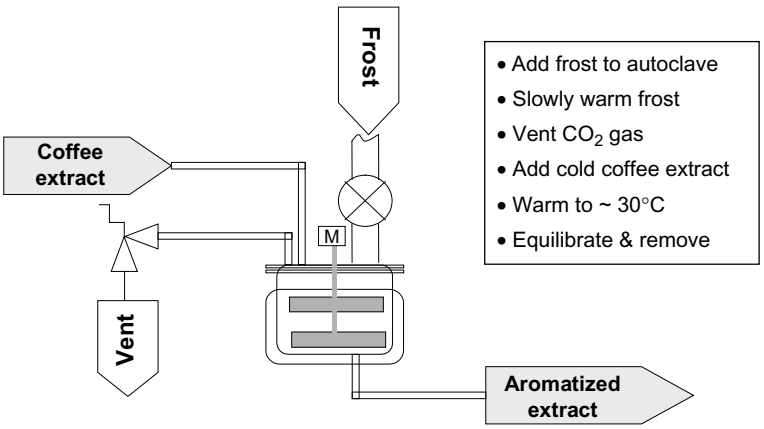

FIGURE 35.9 Method to aromatize soluble coffee extract with coffee aroma frost.

TABLE 35.8 Comparison of Coffee Essence Capsules to Coffee Oil Capsules

Capsule Type	Capsule Volatile Coffee Aroma Content
oil capsules	100 μg/g
essence capsules	2120 μg/g

	Number of Floating Capsules Visible in Hot Water		
(10 Capsules Added Per Cup)	After 10 Seconds	After 30 Seconds	After 2 Minutes
oil capsules	10	10	10
essence capsules	2	0	0

	Evaluation of Hot or Cold Instant Coffee Beverages		
	Aroma	Appearance after 30 Seconds	
	Hot Water	Hot Water	Cold Water
oil capsules	weak	floating capsules + oil	floating capsules + oil
essence capsules	strong	uniform solution	floating yellow droplets

	Evaluation of Crushed Coffee Capsules	
	Relative Aroma Strength	Filter Paper Disk Wetting
oil capsules	no noticeable aroma	visible liquid
essence capsules	noticeable aroma	visible liquid

Capsules were crushed with a spoon for 5 sec and aroma evaluated by sniffing from 10 cm distance.
Additional details of methods and materials can be found in US 7470443.

conventional carriers that dissolve in the beverage or produce a residual slick, since the novel aroma sources are biphasic with water and have temporal existence. Accordingly, this system can enhance evaporation of aroma components having low volatility, since their vapor pressure will increase in proportion to increasing concentration as more volatile components evaporate from floating droplets. VCLs have much lower freezing point and viscosity than coffee oil, which allows them to be aromatized by direct contact with frosts. Aromatization at low temperature can reduce loss of highly volatile flavors by evaporation and loss of labile flavors by thermal or oxidative degradation. Traditional carriers having lower freezing point than coffee oil, such as ethanol, benzyl alcohol, or propylene glycol, either have high water solubility or density greater than water. VCLs may also be used as solvents to extract volatile flavors directly from coffee sources, since they are easily distilled and condensed to facilitate concentration or fractionation of volatile flavors.

TABLE 35.9 Further Comparison of Capsules in Hot Instant Coffee Beverages

Ingredient Weights (g)	Evaluation of Hot Instant Coffee Beverages		
Oil or Essence Capsules + Instant Coffee (1.7 Total)	**Aroma Difference**	**Flavor Difference**	**Visible Oil Slick/Capsules**
1.7 instant (control beverage)	baseline	baseline	baseline
0.1 oil capsules + 1.6 instant	none	none	yes/no
0.3 oil capsules + 1.4 instant	none	slight	yes/yes
0.5 oil capsules + 1.2 instant	none	great	yes/yes
1.0 oil capsules + 0.7 instant	slight	great	yes/yes
1.7 oil capsules + 0.0 instant	great	overwhelming	yes/yes
0.1 essence capsules + 1.6 instant	great	similar to baseline	similar to baseline

Aroma was evaluated from 20 cm distance 10 sec after hot water addition to capsule/instant mixture.
Appearance and flavor were evaluated 1 and 2 minutes, respectively, after hot water addition.

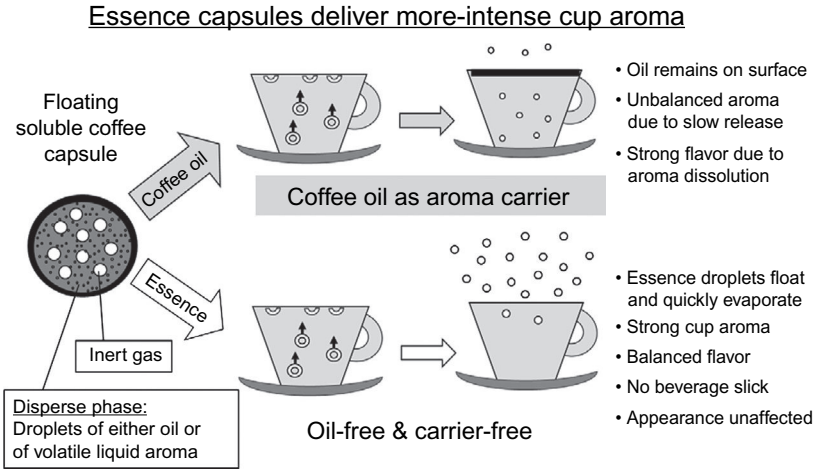

FIGURE 35.10 Comparison of coffee oil capsules to coffee essence capsules.

This work demonstrated an ability to greatly increase above-cup aroma strength by replacing oil with more functional liquids in floating coffee capsules. A range of aromatized volatile carriers or carrier-free aromas can be used to improve cup aroma delivery from hot coffee beverages. Time-release data and sensory evaluations confirmed the superiority of this novel technology over oil aromatization. Collecting such data provided strong evidence of increased aroma release rate and peak intensity. However, the full magnitude of improvement is not depicted in plots due to limitations of the analytical method and because aroma detection is not instantaneous. Initial slopes of aroma release curves are more indicative of real release rates since gas sweeping during analysis dilutes jar headspace and increases the time lag between aroma release and capture. Delivering cup aroma as a burst increased peak intensity in this closed system and maximizes probability and impact of sensory perception when aroma released in open systems must diffuse from the cup through a larger volume of air to reach the consumer. In addition to systems using homogeneous capsules, volatile flavors may be encapsulated using different carrier or capsule compositions to control rate, duration, and character of aroma released from instant beverages, beverage mixes, or other hot food preparations—for example, white capsules made by replacing coffee with maltodextrin, and coffee essence with orange peel oil, floated in cappuccino and latte beverages to release aroma without coloring froth. A wide range of other capsules were made to release diverse aromas from prepared hot instant tea, cocoa, soup, cereal, and sauce mixes.

ACKNOWLEDGMENTS

The authors gratefully acknowledge contributions of Orlando Herrera-Gomez and James White in providing headspace analyses and of Walter Popp in providing microscopy images.

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Chapter 36

Protection and Delivery of Probiotics for Use in Foods

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36.1 INTRODUCTION

Probiotic foods and dietary supplements are increasing in popularity in the marketplace, as their health promotion and disease prevention benefits are gaining more recognition from health professionals and consumers. Dairy products, such as yogurts, kefir, culture drinks, and cheeses, are the most commonly used food products for probiotic delivery, while dietary supplements, traditionally marketed in the form of capsules, tablets, and freeze-dried powders, represent a small but rapidly growing niche market. Other emerging non-dairy food applications include breakfast cereal, infant formula, nutrition bars, and powder drinks. Overall, global sales of probiotic foods, supplements, and ingredients amounted to $18.9 billion in 2009 and are projected to reach $31.1 billion in 2015 (Favaro-Trindade and Grosso, 2002; Agheyisi, 2011). Even with the numerous probiotic products that are available in the marketplace today, manufacturing processes, product shelf-life, and effective delivery remain the core challenges facing the probiotic industry, particularly in non-dairy foods and dietary supplements.

The term "probiotic" is relatively new and means "for life." It is used to classify those non-pathogenic and non-toxigenic bacteria associated with beneficial effects on the host. Probiotic microorganisms (referred to herein as "probiotics") have been defined by the United Nations' Food and Agriculture Organization (FAO) and World Health Organization (WHO) as "live microorganisms that when administered in adequate amounts confer health benefits to the host" (Jorgen, 2001). In order to exert their beneficial effects on the host, probiotics must remain viable and reach the intestine in large numbers (Favaro-Trindade and Grosso, 2002). They must retain viability during storage, and survive passage through the stomach and small intestine before reaching their place of colonization. Generally, a probiotic product must contain at least 10 million colony-forming units (CFUs) per gram in order to effectively provide any claimed health benefit (Jorgen, 2001).

The most important probiotics are lactic acid-producing bacteria, primarily from the *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* genera. Various nutritional and therapeutic benefits have been ascribed to those lactic acid-producing bacteria, including modulating immune response, lowering serum cholesterol concentrations, improving lactose intolerance symptoms, increasing the resistance to infectious intestinal diseases, decreasing the duration of diarrhea, reducing blood pressure, helping to prevent colon cancer, improving or preventing constipation, and modulating blood lipids and the *in situ* production of vitamins (Kailasapathy and Chin, 2000; Andersson et al., 2001; Isolauri et al., 2001; Marteau et al., 2001; Perdigon et al., 2001; Sleator and Hill, 2008; de Moreno de Leblanc et al., 2011; Kotzampassi and Giamarellos-Bourboulis, 2012). In domesticated and aquatic animals, probiotics can improve growth, survival, and stress resistance associated with diseases and unfavorable culture conditions (Balcazar et al., 2006). However, some controversy continues to persist concerning the insufficiency of evidence to support certain health claims in probiotic products.

Products with reduced probiotic viability have been frequently uncovered in the market, which has contributed to a perception that probiotics offer poor value compared with other health supplements (Hamilton-Miller et al., 1999). In one study on dairy products, three out of six samples tested contained no traces of live microorganisms and two contained very low concentrations (Shah, 2000). Similar results have also been reported with regard to products containing dry probiotic bacteria such as powders, capsules, and tablets (Hughes and Hillier, 1990). To compensate for

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00036-4

such loss, manufacturers usually enclose the probiotic product in special packaging and require specific storage conditions. Often, they also include an excessive quantity of probiotics in the product in anticipation that only a portion will survive by the time of consumption. In addition to questionable shelf-life viability for these products, such practices are certainly not cost-effective.

36.2 MICROENCAPSULATION AND DELIVERY CONCEPTS FOR PROBIOTICS

In a broad sense, preservation or encapsulation technologies can be used to protect the probiotic in a variety of adverse conditions, including stabilizing the cells in wet or dry form, protecting the cells during the manufacturing process, extending the storage shelf-life, and providing gastric protection and sustained or controlled release along the intestinal tract. The encapsulation generally involves various processing and drying techniques that are widely practiced in the food industry, such as freeze drying, spray drying, spray chilling, coextrusion, gel-matrix entrapment, and fluid bed agglomeration or coating, as well as enclosing the probiotic in protective packaging and oxygen-impermeable containers (Gismondo et al., 1999; Doleyres and Lacroix, 2005). Considering the fragility of the live probiotic cells, the challenge in developing a robust encapsulation technology is to select suitable food grade materials and an appropriate process that provide acceptable viability upon storage and gastric protection, especially if the cells are stored at ambient temperature and humidity.

36.2.1 Entrapment in Polymer Matrix

Among the most frequently used materials for probiotic encapsulation are polymeric materials capable of forming a strong gel matrix. Such a matrix ideally retains the bacteria along with other preservation compounds even after being sliced into small pieces or formed into thin threads or particles. Additionally, the polymeric matrix may provide some type of controlled release mechanism that protects the bacteria in the stomach but releases them at their site of colonization along the intestine. Several naturally occurring polysaccharides exhibit these requirements. For example, high amylose starch is a polysaccharide capable of forming a firm gel after the starch granules have been hydrated in boiling water and dispersed with the aid of a high-shear mixer. The controlled release mechanism occurs because the high amylose starch is not digestible by the host digestive system but readily available to the gut microflora as a source of energy. Pectin is another suitable polysaccharide that performs very similarly to high amylose starch, but it has the additional advantage of forming a matrix structure in the presence of divalent cations such as Ca^{++} that form bridges between carboxyl groups of sugar polymers (Cook et al., 2012). More specific examples include the encapsulation in alginate matrices that are capable of providing significant protection against short-term heat exposure during processing and against low gastric pH (Muthukumarasamy et al., 2006; Ding and Shah, 2007, 2009), and the encapsulation in a mixture of κ-carrageenan and locust bean gum that enhances the probiotic survival in liquid dairy products such as yogurt (Adhikari et al., 2003). In certain cases, the inclusion of oligosaccharides or fructo-oligosaccharides (FOS) within these polymeric matrices has greatly enhanced the viability of the probiotic, particularly in liquid dairy products (Anjani et al., 2004).

Alginate microspheres have been extensively used to encapsulate probiotics due to the relatively mild preparation and crosslinking conditions. Alginate is naturally occurring and non-toxic to both the bacteria and the host. It is a biodegradable polysaccharide composed of 1,4-linked-β-D-mannuronic acid and α-L-guluronic acid residues that can form a firm gel matrix by crosslinking with divalent cations such as Ca^{++}. The gastric protection and controlled release trigger is also fulfilled since alginate matrices remain firm in the acidic environment of the stomach, thereby protecting the bacteria, but they quickly disintegrate in the higher pH and phosphate-rich environment of the intestine. This results in the release of the preserved probiotic bacteria along the intestine.

Conventional methods to prepare alginate-based microspheres consist of extruding thin threads or strings, or spray atomizing alginate slurry into a solution containing calcium chloride. The alginate strings or droplets harden instantly and form particles upon interaction with Ca^{++} ions. This method has technical limitations in controlling the size and uniformity of the microspheres and difficulties in scaling up and maintaining a clean and hygienic environment. An alternative approach is to harden the alginate solution and form a firm gel matrix by internally crosslinking the alginate polysaccharide instead of dropping it into a Ca^{++} solution. The firm gel can be sliced into small pieces, dehydrated or freeze dried, and milled into powder while the bacteria and any additional preservation compounds are fully retained within the small particles (Figure 36.1). Another alternative method of forming alginate microspheres is by emulsification and internal crosslinking. This method allows for better control of particle size, but the shape of the particles is less uniform compared with atomized particles. In all those internal crosslinking methods, a pH-sensitive calcium salt

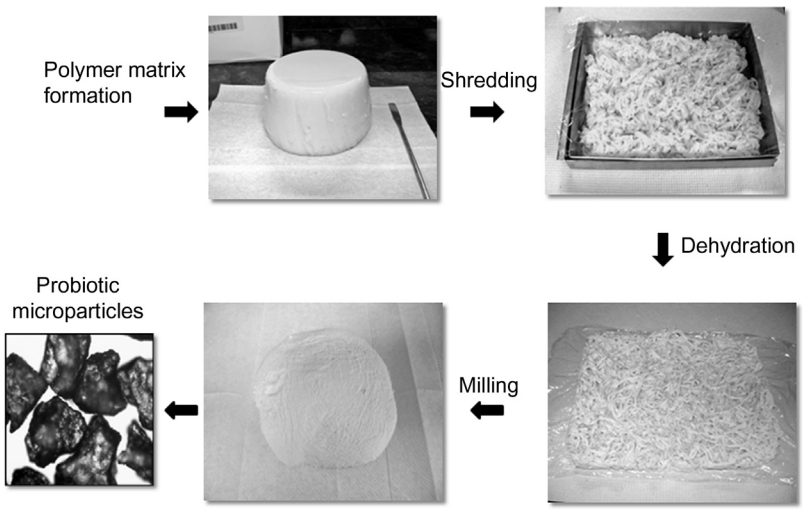

FIGURE 36.1 Production of dry probiotic microparticles: entrapment in a polymer matrix gel and drying processes. (Courtesy Advanced BioNutrition Corp, Columbia, MD, USA.)

such as calcium carbonate is typically included in the alginate slurry, and hardening it to a firm gel is achieved by acidification with a mild acid such as gluconodelta-lactone (GDL), which liberates the calcium ions.

A typical example of an alginate encapsulation technology can be found in a recent disclosure by Fang et al. (2011), which describes a thermal and gastric acid-stable encapsulation system where the probiotics are entrapped in a mixture of alginate and denatured whey protein isolate. Particles were formed in a calcium chloride bath and freeze dried for 72 hours at 25°C. It was demonstrated that the encapsulated bacteria were significantly better protected both for 2 hours in simulated gastric juice (pH 2) and for 20 minutes to heat exposure up to 60°C. Other polymer entrapping systems are discussed in more detail by Champagne and Kailasapathy (2008). Another probiotic entrapment technique, with almost no loss in viability, is provided in WIPO Patent Application WO 2004/035,885 (de Kruif and Driehuis, 2003). The technique involves the entrapment of probiotics in caseinate fibers. A concentrated suspension of lactic acid bacteria in sodium caseinate solution is added to a feed vessel and extruded through a 0.5-mm diameter needle into a coagulation solution containing lactic acid and sodium sulfate, adjusted to pH 4.2. Caseinate fibers are instantly formed in the coagulation solution, entrapping 100% of the bacteria. However, industrial scale-up of the various polymer entrapment techniques is very difficult, and processing costs are very high. Moreover, most of the conventionally produced microcapsules (e.g., calcium alginate beads or microspheres) tend to be very porous, which allows for moisture to diffuse freely in and out of the particle. This particular shortfall necessitates enclosing the encapsulated probiotic product in special packaging and storing under refrigerated conditions.

36.2.2 Fat and Polymer Coating

To overcome the lack of effective shell protection in polymeric matrix entrapment technology, a more classical core−shell encapsulation technology was developed. In the first step, the probiotic is usually entrapped in a polymeric matrix or agglomerated as described above, followed by a coating or shell-forming step. For example, Simmons et al. (2003) described a process in which lyophilized bacteria are granulated with a polymer such as microcrystalline cellulose, a stabilizer such as skim milk or short-chain sugar polymers, and a disintegrant such as starch or alginic acid. The granulated semi-dry bacteria are then desiccated at 40−70°C to reduce the residual moisture level to less than 2%. This was followed by coating with a synthetic enteric agent and plasticizer (over 25% of additional weight). This multistep process results in relatively large particles (over 425 microns) and very little protection (up to 1.5 log loss of viability). An inherent disadvantage to the coating procedure is that the relative proportion of the coating material to the active agent goes up by a cubic function of the particle size, making the process less usable for the production of particles of sizes less than 300 microns.

An interesting coating technology that takes place while lyophilizing the probiotic is described in European Patent Application 1,514,553 (Chung et al., 2003). The probiotic is first cultured in enzymatically treated aqueous protein solution, then separated from the protein precipitate by high-speed centrifugal separator and remixed with the same protein precipitate (a first coating step). The protein−probiotic mixture is added to a solution containing a polysaccharides mixture of xanthan gum, carboxymethyl cellulose, and levan (polyfructose) and a cryoprotectants mixture of trehalose,

maltodextrin or mannitol, and amino acids (a second coating step). The final probiotic mixture is then lyophilized in a freeze dryer. It was discovered that probiotic clusters with highly compacted structures are formed during lyophilization. The dry clusters are then milled to a free-flowing powder. The double-coated probiotic, which is available commercially under the trademark Duolac™, demonstrated an improved heat, acid, and bile resistance but questionable long-term storage stability under ambient storage conditions. In a study with rats, animals fed dual-coated probiotic meal at 1×10^9 CFU per day for 4 weeks have demonstrated an improved intestinal microbial balance and enhanced systemic immunity (Cha et al., 2011).

In another variation of the encapsulation-by-coating technology, Cavadini et al. (1997) described a cereal product comprising a probiotic coat or filling. In this process, spray-dried probiotics are mixed with a carrier substrate, which may be fat or a protein digest solution. The mixture is then sprayed onto the cereal product, and the whole product is dried again. Although this approach seems simple and scalable, the need to rehydrate already-dried bacteria and the additional coating and drying processes are costly and damaging to the bacteria.

Even more complex multilayer-coating technology of core probiotic particles is described in US Patent Application 2005/0,266,069 (Simmons et al., 2003). The disclosure describes several probiotic formulations comprising a core of one or more species of probiotic bacterium, a cellulosic excipient (for example, microcrystalline cellulose), and one or more additives such as disintegrants (starch, alginic acid) and stabilizers (glycerol, ascorbic acid). The probiotic core is first coated with non-enteric polymers (for example, polyvinyl alcohol, hydroxypropyl methylcellulose, hydroxypropyl cellulose) and then further coated with enteric polymers (for example, methacrylic acid-ethyl acrylate copolymer, cellulose acetate phthalate). The resulting double-coated probiotic particles have a diameter of 100−1000 microns and comprise a relatively low percentage of probiotic bacteria (1−10%). A similar double-layer coating process for stabilizing lactic acid bacteria that require an organic solution is presented in US Patent 6,365,148 (Kim et al., 2000). The microgranules containing lactic acid bacteria were enteric coated in a fluidized bed granulator with sodium alginate solution and optionally given a second coat of zein, shellac, or hydroxypropylmethyl cellulose (HPMC) in an ethanolic solution. This additional coat exhibited a superior survival rate in artificial gastric juice and disintegrated rapidly in the intestine. According to the invention, the second alcoholic coat did not cause a significant bacterial loss.

A technique for double-layer encapsulation of probiotics, suitable particularly for liquid food products that undergo short heat treatment (pasteurization at 70°C for 5 min), is provided in WIPO Patent Application 2012/020,403 (Penhasi, 2011). *Bifidobacterium lactis* granules were formed in a mixture of maltodextrin, trehalose, and HPMC using an InnojetVentilus® coater machine (Innojet Herbert Hüttlin, Steinen, Germany). The resulting granules were then coated by an inner layer comprising a mixture of HPMC and polyethylene glycol (PEG 2000) solution to obtain 20% weight gain. The resulting coated granules were then coated again with an outer coating mixture comprising hydroxypropyl cellulose (HPC) and Poloxamer-124 solution to obtain from 20 to 70% additional weight gain. The enhanced survival of the encapsulated probiotic during heat treatment allows for the pasteurization of liquid probiotic drinks. However, the use of these polymers in food products is restricted.

A similar probiotic coating technology but with three layers, which is remarkably suitable for baked food products, is described in US Patent Application 2010/0,303,962 (Penhasi et al., 2010). An aqueous-based suspension of 15% *Lactobacillus acidophilus* and *Bifidobacterium* and 15% maltodextrin and trehalose is absorbed on the microcrystalline cellulose (MCC) core substrate at a ratio of 38:62, respectively. The absorption process is carried out in a fluidized bed coater at an outlet temperature below 35°C. The first coating layer is carried out based on a hot-melt spray method using hydrogenated vegetable oil to obtain a 40% weight gain. A second enteric polymer coating layer is applied with a 6% solution of ethylcellulose E100 and sodium alginate mixture at a ratio of 85:15, respectively, in ethanol, thus obtaining an additional 20% weight gain. A third heat-resistant coating layer of calcium alginate is applied by alternately spraying aqueous solutions of 3% sodium alginate and 5% calcium chloride or a solution of 4% chitosan until an additional weight gain of 20% is obtained. The resulting multilayer encapsulated probiotics were mixed in biscuit dough and baked at 80°C for 45 minutes. The probiotic survival in the biscuit product was between 50 and 80%. In a full commercial bread-baking preparation, the multilayer encapsulated probiotics demonstrated a remarkable resistance and successfully withstood all stages of baked product preparation, including the shear force of kneading, relatively high humidity in the dough, and the heat of baking.

Although polymeric-matrix entrapping or coating of core particles demonstrated significant heat and gastric protection, the long-term storage stability of such probiotic-containing products over the required shelf-life is not sufficient. This is because the polymeric-matrix structure becomes porous after drying, and moisture is freely diffused in and out of the particles, while protective solid fat barriers tend to develop cracks and lose their integrity over time (Figure 36.2). A system aimed at providing such a robust manufacturing and shelf-storage stability is disclosed in US Patent Application 2012/0,142,531 (Mazeaud et al., 2010). A dispersion containing *L. acidophilus* is sprayed on core sucrose particles in a fluidized

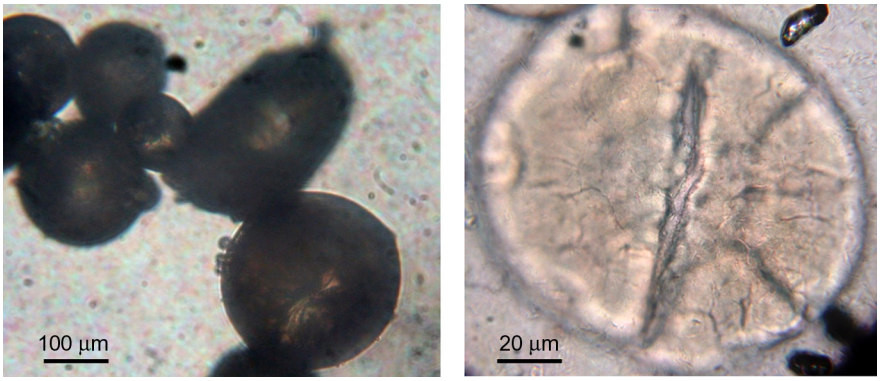

FIGURE 36.2 Development of cracks in the molten fat coat of probiotic microparticles. Left—newly coated microparticles; right—coated microparticle after 30 days storage at 37°C and 60% RH. (Courtesy Advanced BioNutrition Corp, Columbia, MD, USA.)

bed dryer. The probiotic particles was further coated with a preparation containing dissolved sucrose and potassium phosphate salts and dispersed talc (as an anti-agglomeration agent) in water. The dry particle composition formed a semi-crystalline structure, whereby the probiotics retained most of their activity. The encapsulated probiotics also retained a significant amount of activity in various products during and after the manufacturing process and during long-term storage involving a combination of high-temperature and high-moisture exposure.

As noted above, coating the probiotics with multiple moisture-protecting layers is a very slow process, and generally several layers must be added to fully protect against moisture entering the particles. In addition, those multi-coating steps are difficult to execute under aseptic conditions and economically disadvantageous because of the costly coating agents, numerous processing steps, and the excessive consumption of materials.

36.2.3 Extrusion-Spheronization

One noteworthy encapsulation process that was successfully adapted for probiotics involves the use of extruding equipment followed by spheronization. To date, extrusion-spheronization has been almost exclusively associated with drug manufacturing, where the technology has been used in an effort to improve drugs' clinical performance through controlled or delayed release action. The process typically begins with a granulation step, whereby probiotic bacteria, stabilizing agents, and other ingredients are mixed with water to form a thick paste. The wet mass is extruded through a die to form strands of uniform length and diameter. The strands are then made into spheres by first chopping them into small pellets before rounding them into spherical shapes in a spheronizer or by rotation in a conventional coating pan. The final step involves drying the spheres under forced-air conditions or in a freeze dryer or fluidized bed or tray dryer.

Kim et al. (1988) applied the conventional extrusion-spheronization process and encapsulated *L. plantarum* using cellulose powder as a carrier substrate. The procedure consists of mixing 50 parts dry bacteria with 50 parts cellulose. The mixture is combined with 54 parts distilled water and one part glycerol. The wet mass is then extruded through a die (1.2 mm orifices) and the extruded filaments are spheronized on a rotating plate. The resulting spheres are dried in a fluidized bed dryer and coated with sodium alginate and carboxymethyl cellulose. The extrusion, spheronization, and drying processes were not detrimental to the viability of the probiotic cultures. However, the encapsulated probiotics lost more than 4 logs after only 14 days at 37°C.

US Patent 6,060,050 (Brown et al., 1997) provided a modified extrusion technology that entraps probiotics in high amylose starches without using any water. The dry probiotic is separately fed into a mixing chamber and mixed with molten solid fat and starch at a temperature slightly above the melting point of the fat. The resulting molten mass is then forced through a chilled screw-type extruder die fitted with 2-mm diameter orifices and stored under refrigeration in order to maintain the shape until use. However, this formulation proved to have only limited shelf-life upon storage at ambient temperature. Another variation of this technology that involves wetting is disclosed in US Patent 6,500,463 (Van Lengerich, 1999). A homogeneous dry blend consisting of gluten, non-gelatinized wheat starch, molten solid fat, and *L. acidophilus* powder along with water are separately fed to a mixing chamber and extruded through a twin screw extruder but at a temperature just below the fat melting temperature. The extruded strands are cut into pellets approximately 0.5−1.5 mm in length and then vacuum or CO_2 dried for about 30 min to obtain stable probiotic pellets. Although this formulation demonstrated a better protection than the above formulation, it was still not sufficiently robust to preserve the probiotic viability in non-refrigerated products.

36.3 DRYING METHODS

Encapsulated probiotic bacteria have been dried with varying degree of success by several drying techniques such as spray drying (Fu and Etzel, 1995; Desmond et al., 2002; Lian et al., 2002; Ananta et al., 2005; Chavez and Ledeboer, 2007), fluidized bed drying (Kets et al., 1996; Strasser et al., 2007), and vacuum or freeze drying (Conrad et al., 2000). A detailed review of the various drying methods suitable for probiotic bacteria including their advantages and disadvantages is available in Menshutina et al. (2010) and Santivarangkna et al. (2007). This section is focused on issues associated primarily with vacuum and freeze drying, since they are the most favorable methods for drying probiotic bacteria and because of the favorably low temperature exposure during drying and better control over contamination concerns. Although freeze drying is probably the most capital expensive and energy consuming technique, once accounting for the bacterial loss in the cost analysis of different drying methods, it appears to be an advantageous and economically competitive technique (Roser, 1991). An additional critical factor to consider when drying probiotics is the water activity (A_w) of the finished dry product. Compared to temperature, pH, or other factors that can influence the bacteria, A_w has the greatest effect on determining the product's storage life. Similar to relative humidity (RH), A_w is defined as the vapor pressure of a solution divided by that of pure water at the same temperature. Water activity ranges from $A_w = 0$ for a completely dry material to $A_w = 1$ (or 100% RH) for pure distilled water. In a food matrix, A_w is generally considered the amount of "free" water that is not bound to other substances in the food product.

36.3.1 Freeze Drying

Freeze drying has traditionally been the most common method of preserving sensitive biological substances such as live bacteria and proteins, where other methods such as spray drying, fluidized spray drying, and desiccation are generally not suitable. The high drying temperatures used in those methods over an extended period of time result in significant damage to the bacteria. In addition, the probiotic material may not be sufficiently dry for the specific water-activity requirements for product stability, and thus an additional drying step by other means may be required. A conventional freeze drying process typically involves freezing the liquid containing the bacteria, and lyophilizing under vacuum while it remains frozen.

The problems associated with a typical freeze drying process, which may result in a significant loss of viability and damage to the bacteria, are due to the formation of ice crystals during the slow drying process and the freezing step itself, if not done correctly. Difficulties also result from the temperature of the frozen product in the freeze dryer (typically between −30 and −80°C), which is below the freezing point of water but well above the glass transition temperature (T_g) of a typical frozen solution. Drying within this temperature range will therefore result in the undesirable crystallization of the water molecules (Figure 36.3).

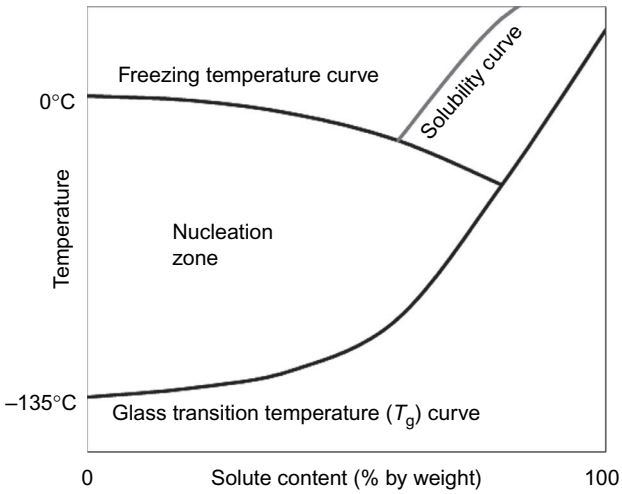

FIGURE 36.3 A typical equilibrium phase diagram for solutes (such as sugar solution) (depicted from Best, 1990). Solutes can solidify either by crystallization or by glass formation. If a solute is cooled rapidly enough, it will vitrify (form a glass, pure water vitrifies at about −135°C) at the glass transition temperature (represented by the T_g curve in the phase diagram). If cooling occurs in the presence of even a small amount of nucleating agents, ice crystals are formed, leaving a more concentrated unfrozen solute and a greater chance for nucleation to occur (represented by the area between the freezing temperature and T_g curves in the phase diagram). At a given temperature, the sugar becomes insoluble above a certain concentration (represented by the freezing temperature curve in the phase diagram).

Depressing the freezing point of the bacterial liquid is one way to minimize the damage from ice formation. The freezing point of water can be lowered by adding antifreeze agents, known as cryoprotectants (e.g., glycols, sugars, salts), that lower the vapor pressure of water. At sufficiently high concentrations (typically 50% or more), conventional antifreeze agents can allow aqueous solutions to be cooled to temperatures well below 0°C without freezing. However, careful attention must be paid when selecting appropriate cryoprotectants, as they must be able to permeate through the bacteria cell wall and also have a food grade status to be allowed for use in consumable probiotic products.

Various cryoprotective agents have been used in the literature with a varying degree of success. These include sugars, sugar alcohols, amino acids, proteins, certain synthetic polymers, skim milk, polysaccharides, and oligosaccharides. Disaccharide sugars, such as sucrose and trehalose, are particularly attractive cryoprotectants for probiotics because they help the microorganism to remain in a state of suspended animation when dried (Zayed and Roos, 2004; Otero et al., 2007; Jalali et al., 2012). There are some drawbacks to using sugars as the sole cryoprotectant. For example, large amounts of sugars (often greater than 60% by weight) must be used to effectively preserve the biological materials during the drying process. This is costly. But more serious problems include inadequate intracellular permeation, sugars' readiness to form crystals when the material is dried below its freezing point, and the low glass transition temperature, which causes instability of the preserved biological materials at high temperatures and/or in humid environments (Foerst et al., 2012). Further, a high concentration of sugars reduces the solubility of other essential compounds in the system and at the same time renders the system extremely difficult to dry.

An ideal cryoprotective solution contains a combination of non-toxic solutes that form a dry amorphous glassy phase with a glass transition temperature (T_g) well above the ambient temperature for the specific water activity of the dry product. Trehalose, due to its relatively high T_g value and its ability to replace water molecules and stabilize dry membranes, has been shown to be an effective cryoprotectant for a variety of biological materials during both ambient air drying and freeze drying (Crowe et al., 1998). However, trehalose alone is not always sufficient to stabilize bacteria, even at ambient temperature and humidity conditions. A more suitable mixture was found to be a combination of trehalose and sugar alcohols or certain proteins that provide a synergetic effect with better protection and improved cell viability over extended periods of shelf storage (Harel and Bennett, 2007; Harel and Kohavi-beck, 2012).

36.3.2 Drying by Glass Formation

The T_g of a dry substance depends, among other factors, on its chemical composition (sugars, proteins, salts), and it is directly affected by the A_w and temperature of the substance (Figure 36.3), with moisture acting as a plasticizer that further depresses the T_g (Crowe et al., 1998; Santivarangkna et al., 2008, 2011). A substance in a glass or amorphous glassy state refers to a liquid phase at such high viscosity and low water content that all chemical reactions are slowed to a near standstill, and the bacteria cells become quiescent. A crystalline form, on the other hand, refers to the formation of solid crystals from homogeneous solution, which is essentially a solid−liquid separation. Glass formation, also called vitrification, occurs naturally in some plants, including a number of mosses and ferns known as resurrection plants. Those plants can undergo severe desiccation and survive for many years in a quiescent metabolic state, only to revive upon the return of water to the environment. In most cases, the adaptation characteristic of the organism is to increase internal concentrations of certain saccharides, such as sucrose in plant cells and trehalose in living organism cells, to a level at which they form glassy states. In cryoprotective systems, the glassy amorphous solid state is typically obtained by controlled desiccation of a concentrated solution containing various sugars. The advantage of the glassy phase in achieving long-term stability results from the fact that diffusion in glassy (vitrified) materials occurs at extremely low rates (e.g., microns/year). Glassy materials normally appear as homogeneous, transparent, brittle solids.

A protective composition containing sugar (trehalose) that is partly in an amorphous glassy phase and partly in a crystalline hydrate phase has been proposed by Franks et al. (2000). The trehalose in the crystalline hydrate phase serves as an agent to dehydrate the trehalose in the amorphous glassy phase, thereby enhancing the glass transition temperature of the amorphous glassy state. This composition has been shown to stabilize single molecules such as proteins or nucleotides but can be used also to stabilize probiotics. However, if the glass transition temperature (T_g) is exceeded, either by exposure to heat or as a consequence of moisture migration into the product, the amorphous glassy state may become liable to irreversible phase separation by crystallization. US Patent 7,381,425 (Truong-le, 2006) describes a freeze drying process suitable for membranous bioactives, such as liposomes or live cells. The drying process starts by cooling the formulation to about the same temperature as that of phase transition of the cell membranes and applying a vacuum, which reduces the pressure on the formulation and allows for stable foam to develop, then freezing the foam, and finally sublimating the water from the frozen foam to produce a lyophilized dry foam composition in an amorphous

glassy phase. This delicate process is suitable mostly for drying small quantities of liposomes or other high value pharmaceutical products but impractical to apply at the industrial scale of a typical probiotic food product.

36.3.3 Drying by Foam Formation

Since sugars are inherently hygroscopic, the removal of water from a highly concentrated sugar solution and the final drying of the supersaturated syrup become extremely difficult. This drawback was first addressed by Annear (1962), who developed a formulation containing bacteria in a solution of sugars and amino acids and a vacuum drying process that involves the step of concentrating the liquid solution by boiling under reduced pressure and then forming foamy, porous concentrated syrup that is relatively easy to dry. US Patent 6,964,771 (Roser and Gribbon, 1997) describes a similar concept of drying by foam formation; it also includes a concentration step by evaporating the bulk of the water by boiling and foaming the sugary syrup under vacuum. To mitigate the damage that can occur to the bacteria during the boiling step, US Patents 5,766,520 and 7,153,472 (Bronshtein, 1997, 2000) introduced an improved protective formula, containing a mixture of carbohydrates and surfactants. In this case, drying the protective solution also involves using a stepwise process of concentration under a moderate vacuum before applying a stronger vacuum to cause frothy boiling mass with limited expansion to form stable foam. To further circumvent the boiling step, US Patent 6,534,087 (Busson and Schroeder, 2001) introduced a drying process for a probiotic solution using a vacuum oven under very mild vacuum (above 30 Torr). After achieving a certain level of drying in a very thin layer without excessive boiling of the solution, heat is applied at above 20°C and the dried product is harvested after only a few hours. The main disadvantage of the drying by foam process is the need to boil and foam the solution, which results in the solution being splattered on the walls of the drying chamber. Because of this limitation, the process is mostly suitable for drying small amounts of material.

36.3.4 Controlled Low-Temperature Vacuum Dehydration

The controlled low-temperature vacuum hydration (CLTVD) technology for drying probiotics was developed to mainly avoid the freezing issues associated with freeze drying. The technique basically involves the drying of non-frozen solution by evaporation at reduced temperature under vacuum. This technology takes advantage of the fact that the partial pressure of aqueous vapor over a liquid phase is greatly dependent on the temperature of the liquid itself. This also means that the temperature of the liquid material during the drying step can be controlled by the applied vacuum (Figure 36.4).

For example, the drying of sugary syrup can be carried out at a temperature of about −5°C, without freezing the syrup, by applying reduced pressure (i.e., vacuum) of about 3000 mTorr. When compared with a standard freeze drying process, this process has demonstrated about a 30% reduction in drying time and about a 40% reduction in running cost (King and Lin, 1995; King et al., 1998). A variation of this method was adopted for bacterial preservation in dry glassy

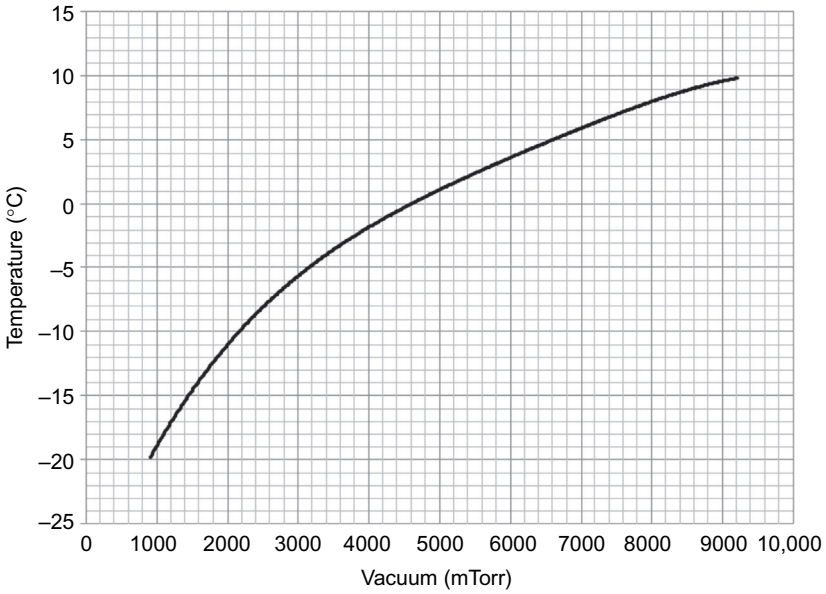

FIGURE 36.4 Vapor pressure of pure water below 10°C (depicted from Haynes, 2012–2013).

form and has been described in the patents from Bronshtein (2000) and Roser et al. (2002). The alternative method utilizes the foam formation technique described above to enhance the evaporation rate while eliminating the formation of ice crystals. This method also requires high concentrations of various sugars (typically, a combination of mono-, di- and oligosaccharides and sugar alcohols) and the addition of some foam-forming compounds and stabilizers in the liquid media. The drying process can take place in a vacuum dryer or a freeze dryer that is equipped with a vacuum-controlling system and heating source. Generally, the drying is initiated by concentrating the sugary probiotic solution to form supersaturated syrup. Further foaming and water removal progressively solidifies the syrup, which eventually turns into a solid amorphous sugar glass with very low residual water content.

This type of a drying process, in which the probiotic solution is maintained in a super-cold liquid state during the entire drying process, has the advantage of faster drying due to the evaporation of the liquid under vacuum and the increased surface area presented by the foaming surface. However, in spite of some advantages to achieving longer shelf-life stability, the process is difficult and costly to scale up because the foam requires, by definition, large volumes of space under reduced atmospheric pressure (i.e., vacuum) for the production of very little mass. In addition, the highly porous material is very sensitive to humidity and the product will readily take up moisture. But the most significant disadvantage of this process is still the inability to control and limit the expansion of the foam within the vessel, tray, or vial. The eruption and foaming nature during the boiling step results in a portion of the material being splattered on the walls of the vessel. The uncontrollable eruption and often excessive foam formation makes it practically impossible to develop an industrial-scale process. To contain the eruption during boiling, US Patents 6,306,345 and 6,884,866 (Bronshtein et al., 1999, 2002) proposed special chambers and a protocol for controlling the temperature and pressure to reduce overboiling and expansion to an acceptable level. Another approach to containing the eruption and excessive foaming is described in US Patent Application 2008/0,229,609 (Bronshtein, 2005), in which the bioactive solution is enclosed in a container or a bag covered with a breathable membrane.

An industrially scalable technology that takes advantage of both CLTVD and drying-by-glass-formation technologies, while exposing the probiotics to a minimal amount of boiling or foaming, is presented in European Patent Application 2,435,554 (Harel et al., 2010) and WIPO Patent Application 2011/094,469 (Harel et al., 2011). The technology involves a composition containing probiotics, crosslinked polymers, and glass-forming agents. The drying process is started at a vacuum pressure of about 2000 mTorr, to establish a product temperature just above freezing (Figure 36.4), while applying heat to accelerate the dehydration process.

Typically, this drying process preserves over 50% of the probiotic viability, and the expected storage shelf-life of the dry material in ambient temperature (about 25−30°C and 33% RH) is over 18 months (Figure 36.5). An additional advantage of the encapsulation composition is the significant gastric protection, as demonstrated in various lactic acid probiotic bacteria (Figure 36.6).

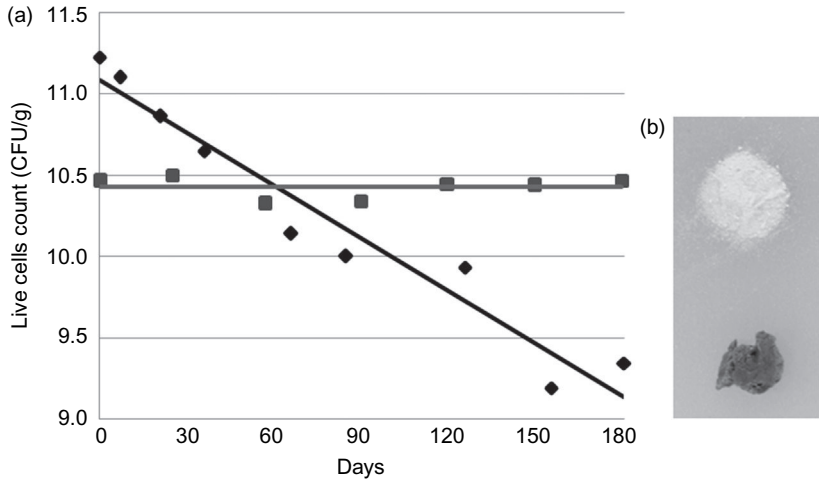

FIGURE 36.5 Storage stability of encapsulated and unencapsulated *Lactobacillus acidophilus* sp. at 25°C and 33% RH. (a) Storage stability, ♦ unencapsulated, ■ encapsulated. (b) Physical appearance of the powder containing encapsulated (top) and unencapsulated (bottom) probiotic bacteria after 180 days in a stability test.

36.3.5 Perspective on Drying Methods

Probiotics in dry form have the advantage of being more convenient and economical to transport than transporting liquids or frozen cultures. Fine particles can easily be mixed in other dry products and stored or distributed unfrozen or even

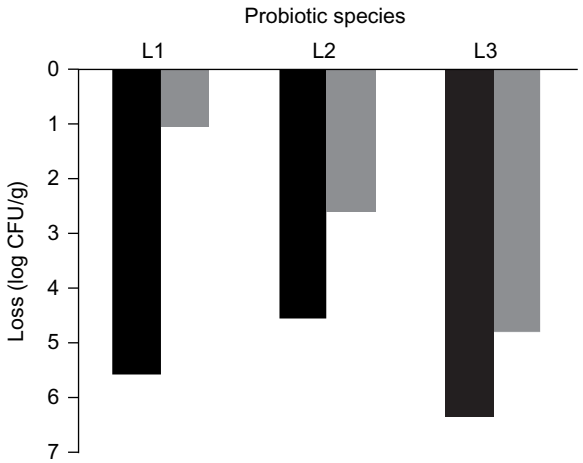

FIGURE 36.6 Gastric stability of three different probiotic species (encapsulated or unencapsulated). The probiotic species were incubated for 2 hours in simulated gastric juice at 37°C and pH 1.2 (black bar, unencapsulated; gray bar, encapsulated).

non-refrigerated (provided the bacteria are properly stabilized). However, most of the industrial drying techniques are damaging to the bacteria because of the lengthy exposure to high temperatures typically used in those methods. Although freeze drying has been the method of choice for drying probiotics, this method involves a freezing step of the probiotic culture, where most of the cell damage and inactivation usually occur (To and Etzel, 1997). Among the various drying methods described above, drying by evaporation under partial vacuum (the CLTVD method) seems to be very promising. This drying method permits high yield of live probiotic in dry form (potentially with less than 0.5 log of drying loss) and exceptional long-term stability even under non-refrigerated storage conditions. To successfully apply the method, it is critical to supplement the culture with a suitable mixture and quantity of glass-forming compounds and to maintain the drying temperature of the probiotic culture just above its freezing point. This can be achieved by controlling the partial pressure in the drying chamber and the heat supply to the probiotic culture throughout the entire drying period.

36.4 DELIVERY FORMS

36.4.1 Tablets

Probiotic supplements are available in the market in various delivery forms, including tablets, capsules, and sachets containing dry powder. Probiotic tablets are probably the most commonly available delivery form in the market. Tablets are usually produced by pressing a powdery material in a mold to form a suitable shape in a so-called tablet press machine. Sensitive biological materials, such as probiotics, are very sensitive to compression forces and heat that develop when tablets are formed, causing a severe reduction in viability. For conventional equipment, it is common for the probiotic viability to be reduced by several logs in the course of tableting. This problem cannot be avoided by simply applying lower pressure to the conventional tablet material, since the tablet has to be subjected to a minimum level of pressure in order for the shape to be maintainable.

Several solutions to this problem are proposed in the literature. For example, US Patent Application 2003/0,157,164 (Cedgard, 1996) and US Patent 7,022,338 (Cedgard, 2000) rely on a mixture of oligosaccharides, such as inulin; a gum forming agent, such as xanthan or gum arabic; and probiotics. Due to the stickiness of the gum, the pressing can consequently be applied at considerably lower pressure, resulting in reduced friction and heat generation that do not harm the bacteria. US Patent 8,007,777 (Borek et al., 2002) proposed a controlled release formulation suitable for tableting probiotics. The disclosed formulation contains a hydrophilic agent, such as hydroxypropyl methylcellulose, and an electrolytic agent, such as sodium or calcium carbonate. These components are mixed with lyophilized bacteria and compressed to form a controlled release monolithic tablet without any further need for an enteric coat.

Another approach in which stabilizing compounds are added before drying and tableting the bacteria is presented in WIPO Patent Application 2005/060,937 (Nielsen et al., 2004). The formulation includes several stabilizing compounds, such as an antioxidant; a bulking agent, such as a saccharide or sugar alcohol; and a gelatinizer, such as xanthan or alginate—each of which is introduced into the concentrated aqueous suspension of the live cells and lyophilized. The resultant stable formulation is milled and formed into tablets in a tablet press with very minimal loss.

While probiotics might be stable when kept by themselves in the tablet formulations described above, they may become highly unstable when mixed with other active ingredients, such as vitamins, minerals, enzymes, plant extracts, and trace elements. After even brief storage, the viability of the bacteria in such mixed formulations can be extremely poor. Tablet technologies attempting to address this problem were developed by forming multilayer tablets in which the probiotics were contained in a separate layer that was free from other nutritionally active materials (Fusca and Farber, 1998; Henriksen et al., 2004). However, this tableting process is complex and costly, as the ingredients must be segregated into separate compartments while losses due to compression and heat sensitivity must still be overcome.

36.4.2 Soft Gel Capsule

Delivering the probiotics within a soft gel capsule offers many advantages over other dosage forms, such as compressed solid tablets or bulk dry powder preparations. Soft capsules provide a shell protection and a dosage form that is easy to swallow and need not be flavored. The capsule also provides a good oxygen barrier due to low oxygen permeability through its shell. An interesting capsule formulation for protecting probiotic bacteria during passage through the stomach while permitting their release in the intestine is provided in US Patent Application 2004/0,175,389 (Porubcan, 2006). The capsule includes a water-free mixture of probiotic bacteria with monovalent alginate salts in gelatin or cellulose capsules. Upon contacting with the acidic environment in the stomach, the outer shell of the capsule and the alginate salts turn into a whole gel, thus providing a protective barrier against proton influx into the capsule core. The formulation's water activity is low, and consequently it has a long shelf-life.

Several probiotic products are commercially available in soft gelatin capsules, including Ultra-Dophilus® and Probiotics Plus® (both are made by Nature's Plus, Melville, NY). Small, 1-mm size minicapsules can be found in the Bifa-15® product (Eden Foods, Inc., Clinton, MI), where the bacteria are encapsulated in a seamless minuscule capsule delivery system and then admixed with oligosaccharides, sweeteners, and flavors and presented in individually wrapped, single-dose aluminum tube packages. The small seamless capsule protects the bacteria from air and stomach acid and provides additional protection from heat and light. The minicapsules are also designed to slowly release and deliver the bacteria safely and alive into the large intestine. Generally, soft gel-encapsulated probiotics require no refrigeration; however, there is no guaranteed minimum of viable cells due in part to the high water activity (free water) associated with the capsule shell.

36.4.3 Oil Carrier

Often, dry probiotic powder has been suspended in oil to increase the viability and shelf-life and to facilitate the application of probiotics to extruded food. Certain edible oils such as fish oil, olive oil, rice-bran oil, and soybean oil demonstrated enhanced storage protection and a "prebiotic" effect better than commonly used stabilizing ingredients such as proteins and sugars. For instance, the patents and patent applications of Naidu and Baksh (2003), Kirejevas and Kazarjan (2010) and Lefkowitz (2011) disclose compositions in which probiotic bacteria are suspended in edible oil and, optionally, encapsulated in a two-piece hard shell capsule. Giffard and Kendall (2002) describe a probiotic foodstuff in the form of a dried or semi-moist ready-to-eat kibble. The probiotic is first coated with a polysaccharide, fat, starch, protein, or sugar using standard encapsulation techniques. The negative effects of the moisture are mitigated by embedding the probiotic particles in fat or oil, prior to being added to the foodstuff. Other delivery forms include the incorporation of encapsulated probiotics in moist food products. For instance, US Patent Application 2005/0,153,018 (Ubbink et al., 2003) describes moist confectionery foods containing preserved lactic acid bacteria. The bacteria are spray dried and mixed in a composition comprising fats, fermented milk powder, and saccharides. The mixture is then used as the filling of a confectionery product. The problem is that barriers of solid oils and fats provide only short-term protection from humidity, since water vapor from the surrounding environment will over time diffuse through the oil and seep into the dry probiotic material.

36.5 METHODS FOR ESTIMATING PROCESS LOSS AND PRODUCT SHELF-LIFE

Encapsulation of probiotics typically involves a multistep process where the live bacteria are exposed to various stressful conditions and temperature extremes for an extended period of time. It is fairly common, for example, to lose over a log (e.g., 90% of the active biomass) of live probiotics by the time the probiotics are encapsulated in a dry state and packaged in their final delivery form. Knowing which step in the process is detrimental to the probiotics and how to avoid it is critical to the overall success of the encapsulation process. With traditional culture methods and lengthy

plating assays, one can observe the bacterial loss only in retrospect and with limited ability to assess the loss at each step of the process. There are, however, several useful viability indicators that can provide an instantaneous assessment at the single-cell level without the need for plating and cell culturing. These indicators are based mostly on fluorescent molecules, which can be detected with epifluorescence microscopy, solid-state cytometry, or flow cytometry. Each indicator is based on criteria that reflect different levels of cellular integrity or functionality.

36.5.1 Epifluorescence Microscopic Assessment

Epifluorescence microscopic assessment is one assay that stands out among others because of its simplicity and commercial availability. The assessment is usually simplified to either green-labeled live or red-labeled dead cells. The assay consists of two stains, propidium iodide (PI) and SYTO9, both of which stain nucleic acids. Green-fluorescing SYTO9 is able to enter all cells and is used for assessing total cell counts, whereas red-fluorescing PI enters only those cells with damaged cytoplasmic membranes. The stained cells can be detected with an epifluorescence microscope, or a flow cytometer. Sampling the probiotics at the beginning and at each desirable step along the entire encapsulation process, and subjecting the samples to this assay, will instantly provide an assessment with a reasonable accuracy on the effect of each step on the probiotic viability.

36.5.2 Estimating Storage Shelf-Life

Even if the bacteria survive the encapsulation and drying processes well, they can still rapidly lose viability during storage at ambient conditions. If kept protected at low temperatures and very low moisture levels, dried bacteria will most likely remain active for long periods of time (Champagne et al., 1991). But when introduced in non-refrigerated products, such as infant formula, breakfast cereal, or energy bars, the probiotics may rapidly lose their viability. As discussed above, temperature and humidity are important factors affecting the storage stability; and, therefore, encapsulation and a controlled storage environment that excludes moisture, heat, and oxidation may be needed for the long-term stability of bacteria (Carvalho et al., 2004; Santivarangkna et al., 2008). However, the challenge is how to accurately predict the shelf-life of a probiotic product.

There is a potential testing tool to significantly expedite the generation of thermal degradation data to within several hours instead of weeks or months: the prediction is then made by studying the kinetics of the bacteria's thermal degradation at much higher temperatures than those of the storage conditions. This accelerated temperature test deals mainly with drugs or vitamins, but its accuracy has also been remarkable when evaluating the shelf-life of probiotic formulations (Mitic and Otenhajmer, 1974; King et al., 1998). Classic test models of a chemical or drug are based on thermal degradation using the Arrhenius equation:

$$\log\left(\frac{k2}{k1}\right) = \left(-\frac{Ea}{2.303R}\right)\left(\frac{1}{T2} - \frac{1}{T1}\right)$$

where $k2$ and $k1$ are rate constants at temperature $T2$ and $T1$, respectively, Ea is the activation energy and R is the gas constant. Temperature is in kelvin. This general equation describes the relationship between the storage temperature and the degradation rate, which if constructed properly allows for the projection of storage shelf-life at a given temperature based on the degradation rates observed at much higher temperatures. There is some debate about whether accelerating the death of living cells by raising the temperature above their biological limitations is an accurate method to assess viability or survival at lower temperature conditions. However, within certain temperature limits, and in the absence of other good alternative methods, this method may be useful to predict storage stability many months forward. Assuming that the thermal death of the bacteria, within a certain range of temperatures, conforms to first-order kinetics, it can be approximately represented by the following equation (Toledo, 2007):

$$k = \frac{1}{t}(\log\ N_0 - \log\ N_t)$$

where N_0 is the initial CFU of the bacteria, N_t is the CFU number after a certain period (t) and k is the death rate constant expressed in $\log_{10}$ units. The best estimate of the death rate constants (k) at a given temperature could be determined by performing a least-squares analysis of the log losses of bacteria plotted over time. To plot the Arrhenius curve, the logarithms of the predetermined k-values are plotted against the reciprocals of their absolute temperature. By extrapolating the Arrhenius curve, the death rate at any desirable temperature can be predicted along the slope of the curve. Schematic charts describing the process in more details are provided in Figure 36.7.

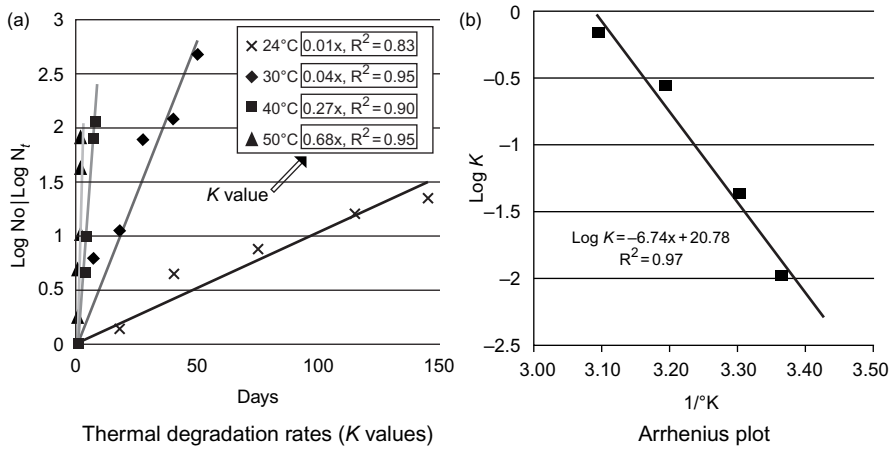

FIGURE 36.7 Arrhenius plots of (a) log CFU versus time (days) at different temperatures to obtain specific thermal degradation rates (*K* values) and (b) log *K* versus 1/K for *Bifidobacterium* sp. at 43% RH.

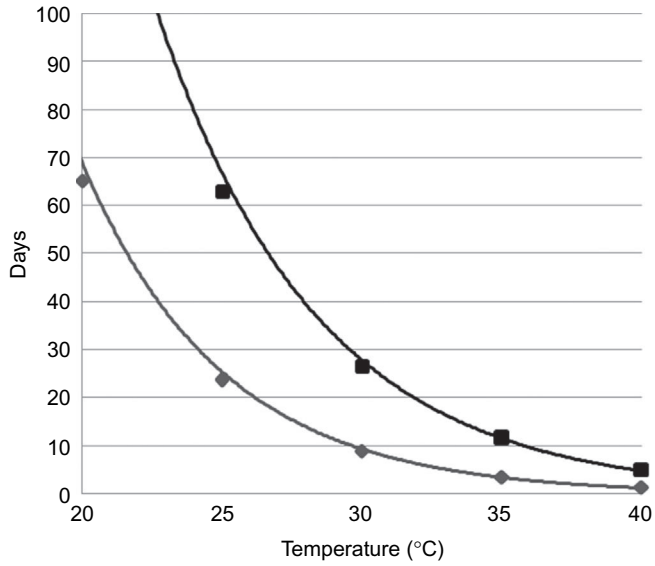

FIGURE 36.8 Shelf-life projection (days to lose 1 log) of encapsulated and unencapsulated *Bifidobacterium* sp. at various temperatures and constant exposure to 43% RH (♦, unencapsulated; ■, encapsulated).

Using a similar thermal-degradation testing program, Mitic and Otenhajmer (1974) and King et al. (1998) accurately predicted the storage stability of *L. acidophilus* many months in advance. Bacterial suspensions in skim milk were distributed in ampoules, freeze dried or vacuum dried, and the ampoules sealed under vacuum. The ampoules were then submerged in thermostatically controlled water baths maintained at 50, 60, and 70°C. At predetermined time intervals, samples were removed and analyzed for bacterial viability. The thermal death rates (*k*) were obtained from the slopes resulting from the linear plots of $\log_{10}$ number of organisms remaining versus time. The logarithms of the resulting rate constants were then plotted against the reciprocal of the absolute temperatures. Extrapolation along the Arrhenius curve allowed the investigators to predict the log loss values of samples laid aside at 4 and 20°C at various monthly intervals. After 19 months at 20°C, the actual log loss values for the remaining probiotics was 1.30, almost exactly as predicted by the thermal degradation program (1.25 log loss). Exploiting the principles of this method, we have constructed a curve that predicts the shelf-life (time to 1 log loss) of a probiotic product in various temperatures ranging from 20 to 40°C and 43% RH (Figure 36.8). The information provided by such curves in various RHs can be useful, for example, in determining storage guidelines for probiotic products.

36.6 CONCLUSION

The applications of probiotics in dairy products and in functional foods have been rapidly expanding, as their consumption is becoming increasingly popular because of heightened awareness of consumers for healthier diets. Recently, the

probiotics industry has been encouraged by various food regulatory agencies, particularly in Europe, to clinically substantiate or to remove health claims that appear on probiotic products. Sound and scientifically proven clinical evidence of probiotics on health-promoting activity is dependent on several factors, including a robust probiotic strain and quality products that retain their effective probiotic activity at the time of consumption and during the passage through the digestive tract of the host.

An effective microencapsulation system for probiotics must be gentle enough so as not to damage the live cells, and to offer extended shelf storage life and good protection from acid. Another prerequisite is that the materials used must be naturally occurring and non-toxic, as well as non-antimicrobial, to ensure that the bacteria, as well as the host, are not harmed. Microencapsulation should also impart a degree of controlled or targeted release across the small and large intestine. Typically, a choice of naturally occurring polysaccharides and proteins is suitable, as they are often nontoxic and safely biodegraded. The production of microcapsules containing probiotics is typically progressed in two major steps: first forming the particles by extrusion, atomization, or emulsion and then drying by spray drying, air drying, fluidized bed drying, or freeze drying. Most encapsulation systems are based on the crosslinking or gelling of polymer solutions after either spraying or dropping the particles into crosslinking solution or emulsifying in oil. Spray systems that have been utilized include vibrating nozzles, air-atomizing nozzles, and spinning-disk atomization. Other production methods, such as spray drying, air drying, or fluidized bed drying directly produce dry particles, though these are less common and cause a large amount of cell damage and death.

The drying of probiotics is an attractive area for further development, as most of the damage occurs during this step. Drying should efficiently remove water, but it should also be gentle enough so as not to harm the bacterial cells. With the addition of appropriate cryoprotectants, freeze drying has the benefit of being a process that most bacteria can survive well. However, freeze drying is a capital expensive and time- and energy-consuming process. An additional suitable method is drying by evaporation under partial vacuum where the probiotic culture is kept above its freezing temperature during the drying process. Another area that requires further research is the probiotic survival and retention in the digestive tract of the host. The nature of probiotics' release is of particular importance, since the percentage of the live bacteria delivered may be considerably less than that administered. As summarized above, product quality and potency need to be vigorously examined if we wish to create consistency and bring high-quality probiotic products to the market.

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Chapter 37

Protection and Masking of Omega-3 and -6 Oils via Microencapsulation

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37.1 INTRODUCTION

Oils rich in omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs) are increasingly becoming recognized for their role in reducing the risk of diseases and improving the health and wellbeing of consumers. However, their widespread use in food, feed, and supplement products has been hindered because of their poor compatibility in aqueous-based products and susceptibility to degradative and oxidative reactions, which can lead to off-flavors, off-odors, and loss in bioavailability. Microencapsulation technology offers a means to circumvent these issues, as well as to provide a means for controlled and targeted dose release depending on the design and material used. In simplest terms, the microencapsulation process involves entrapping or coating a sensitive core material (i.e., PUFAs) using a biopolymer material(s) such as proteins or polysaccharides. Due to the labile nature of omega-3 and -6 PUFAs, particular attention is needed during encapsulation to wall formulations and process designs in order to obtain the ideal structure, functionality, and performance of the microcapsules within particular food applications. Already, there have been a number of review articles and book chapters in this area; however, they have not been written from an industrial perspective (Gouin, 2004; Jin et al., 2007; Lee and Ying, 2008; Drusch and Mannino, 2009; Beindorff and Zuidam, 2010; Matalanis et al., 2011b). This chapter focuses on the practical aspects of microencapsulation technologies applied to the food industry for developing omega-3 and -6 PUFA-rich powders. Although food applications are targeted specifically, the knowledge given could easily extend to pharmaceutical, supplement, or feed applications.

37.1.1 Omega-3 and -6 Oils and their Health Benefits

Omega-3- and -6-rich PUFAs are generally recognized for their ability to reduce the risk of cardiovascular disease, along with other health benefits associated with: reducing the effects of depression; protecting an aging population from cognitive decline and dementia; visual development; and for brain development in infants (GISSI-Prevenzione Investigators, 1999; Cole et al., 2005; Richardson and Montgomery, 2005; Wang et al., 2006; Lin and Su, 2007; Bays, 2008; Dyall and Michael-Titus, 2008; Barrow et al., 2009; Larsen et al., 2011). Omega-3 and -6 PUFAs are also precursors to anti-inflammatory mediators, which have demonstrated beneficial effects towards allergies, diabetes, Alzheimer's, and other related neurodegenerative diseases (Kralovec et al., 2012). Over the last few decades there has been an increasing number of studies surrounding their efficacy, recommend doses, and allowable health claims, stemming from *in vivo* and *in vitro* experiments to larger clinical trials, a trend especially true for the longer chain PUFAs of marine origin, particularly eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). In 2010, Pottala and coworkers. released a study following the mortality of 956 patients over 5.9 years in the USA with stable coronary heart disease, and its correlation to baseline blood EPA and DHA levels (Pottala et al., 2010). The authors reported an inverse relation between blood EPA/DPA levels and total mortality, which was independent of standard and emerging risk factors (e.g., demographics, socioeconomic status, health behaviors, and inflammatory markers). Based on the building body of evidence, various organizations (e.g., American Heath Association, Canadian Heart and Stroke Foundation, UK

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00037-6

Department of Health, National Health and Medical Research Council (Australia), US Dietary Guidelines for Americans, Joint FAO/WHO Global Strategy on Diet, Physical Activity and Health, National Institutes of Health, USA; Japan Ministry of Health and Welfare, International Society for the Study of Fatty Acids and Lipids Policy Statement have made formal recommendations to their respective governments and to the public associated with the importance of consuming omega-3- and -6-rich oils in their diet (Garg et al., 2006; Barrow et al., 2009). For instance, the American Heath Association recommends a daily intake of omega-3 PUFAs of 400−500 mg EPA and DHA (Larsen et al., 2011). However, depending on the lobbying organization, levels typically range from 160 mg to 1.6 g per day (Meyer et al., 2003). Despite this, in North America, Europe, and Australia, governmental recommended daily intake is closer to ≈100−150 mg EPA and DPA per day. Since the consumption of oily fish (e.g., cod liver oil) is not widely favored by consumers, industries are left with finding alternatives (e.g., capsule supplements or encapsulated oils) and with challenges for delivering omega-3 and -6 PUFAs in foods (Holub and Holub, 2004; Garg et al., 2006; Kolanowski et al., 2007; Jacobsen, 2010, 2011).

Barrow et al. (2007) reported a comparative human bioavailability study of microencapsulated omega-3 fish oil with standard fish oil soft-gel capsules. In this study, microcapsules were prepared by complex coacervation using gelatin-based formulations to entrap long-chain omega-3 containing oils (Barrow et al., 2007). Phospholipid levels of long-chain omega-3 PUFAs were reported to increase equivalently in both subject groups, whereas triacylglycerol levels were reduced. Their findings suggest that oils delivered by microencapsulation or within soft-gel capsules have similar bioavailability. Other research works have demonstrated that omega-3 PUFAs in food matrices are bioavailable both in oil and microencapsulated forms (Wallace et al., 2000; Maki et al., 2003; Arterburn et al., 2007). Sophisticated encapsulation technologies, such as those formed using a layer-by-layer approach or multiple shell-forming approaches, may alter the bioavailability of the encapsulated omega-3 PUFAs; however, these forms may also offer the possibilities of targeted release-modified bioavailability (Mun et al., 2006; Head et al., 2007; Chung et al., 2008; Windhab and Zimmermann, 2008).

37.1.2 The Sources of Omega-3 and -6 PUFAs

Overall, the Western diet is oversupplied by omega-6 PUFAs while being deficient in omega-3 PUFAs (Garg et al., 2006). Marine oils, such as from oily fish (e.g., anchovy and sardine), represent the most widely used source of omega-3 and -6 PUFAs for nutritional supplementation and food fortification, although algal oil is the primary source of DHA for infant formulas in North America. In contrast, α-linolenic acid (ALA; 18:3n-3) is abundant in widely consumed vegetable sources such as flaxseed oil, soybeans, canola and nuts, and can be broken down in the presence of the enzyme Δ6-desaturase to form EPA and DHA (Larsen et al., 2011). However, the enzyme conversion of ALA is insufficient to yield significant quantities to influence blood EPA and DHA levels (Larsen et al., 2011), at 5−10% and 1−5%, respectively. Agri-food industries are also employing genetic manipulation of plant sources to yield higher levels of omega-3 PUFAs. In recent years, Monsanto has developed high stearidonic acid (SDA; 18:4n-3) soybean oil from genetically modified soy beans (Vazquez and Akoh, 2012), which can be more readily converted to EPA by Δ6-desaturase (Larsen et al., 2011). The presence of SDA can lead to increased levels of EPA in the blood, but had little effect on DHA levels (Larsen et al., 2011). Various microbial oils derived from algae strain manipulations and fermentation processes can also serve as producers of PUFAs, such as DHA and arachidonic acid (AA; 20:4n-6). Other areas of focus include microbes, plants, various genetically modified organisms, zooplankton (i.e., krill), and algae.

Natural oils, for instance from fish, contain 15−22% EPA and 9−15% DHA; however, levels of 50−90% with controlled EPA:DHA ratios are typically needed for fortification and supplementation requirements. Various strategies have been employed to raise levels to produce omega-3 and -6 PUFA concentrate products, which then can be encapsulated. Kahveci and Xu (2011) used repeated hydrolysis of salmon oil by *Candida rugosa* lipase, followed by short path distillation to concentrated omega-3 PUFA levels from 16 to 51%. The use of lipases is highly selective towards saturated and monosaturated fatty acids, leaving only the PUFAs attached to the glyceride backbone (Perez et al., 2011).

37.1.3 Sensitivity of Omega-3 and -6 PUFAs to Oxidation

Regardless of the source of omega-3 and -6 PUFAs for our diet, PUFAs are highly unstable at higher temperatures and oxidizing environments resulting in poor product quality due to unpleasant off-flavors and odors (Garg et al., 2006; Jacobsen, 2010, 2011; Tehrany et al., 2012). Omega-3 PUFAs are also much more unstable to oxidation than omega-6 PUFAs, such as ALA, leading to tremendous challenges for the food industry, not only to ensure a product is of high quality but also to retain bioavailability. The most widely accepted mechanism for lipid oxidation is the free radical

theory, which consists of three phases: initiation, propagation, and termination (Frankel, 2005; Lagarde, 2010; Indrasena and Barrow, 2011). During initiation, reactive oxygen species, such as $^{-}$OH and HOO•, combine with unsaturated lipids to form a water molecule and a fatty acid free radical. The latter reacts readily with oxygen to form a peroxyl fatty acid radical, which then forms another fatty acid radical and lipid peroxide. The reaction continues via free radical transfer from one unsaturated lipid to the next, in a process called "autooxidation" (propagation phase), and it persists until the radical specie concentrations are high enough so they react with themselves to form a non-radical specie (termination phase). Primary oxidative products, such as peroxides and hydroperoxides, are highly reactive and readily decompose or polymerize to produce intermediate and secondary oxidative products, such as aldehydes, epoxy, and ketones, which are released into the food environment. Transition metals naturally present in the food can also act as catalysts to promote lipid oxidation (Nuchi et al., 2001). These oxidative reactions involving PUFAs lead to unacceptable sensory issues for consumers and loss in nutritional value, and may sometimes cause health disorders (Tehrany et al., 2012). Although encapsulation provides a physical barrier to oxidation, antioxidants are typically formulated into the oil to ensure product quality and safety (Koleva et al., 2003; Frankel, 2005; Jacobsen, 2010). Some antioxidants may include tocopherols in combination with ascorbic acid or lecithin, or natural antioxidants such as rosemary extract, quercetin, chlorogenic acid, caffeic acid, or catechin (Huber et al., 2009; Martin et al., 2012). Antioxidants act by scavenging free radicals or oxygen, and metal chelation via protein-based or metal-based chelators (Decker et al., 2005; Frankel, 2005; Jacobsen, 2010).

37.2 ENCAPSULATION TECHNOLOGIES USED FOR OMEGA-3 AND -6 POLYUNSATURATED FATTY ACIDS

Industrial strategies for fortifying food products with omega-3 and -6 PUFAs focus on designing protective technologies, such as microencapsulation along with various antioxidant formulations (Ubbink and Krüger 2006; Barrow et al., 2007; Drusch, 2012). Commercially, microcapsules have been produced for entrapping oil using a range of techniques and materials, which will be further reviewed.

37.2.1 Spray Drying

Currently, spray drying dominates the industrial encapsulation of omega-3 and -6 PUFAs due to its ease of processing and low operating cost (Depypere et al., 2003; Gouin, 2004; Reineccius, 2004; Westergaard, 2004; Gharsallaoui et al., 2007). Commercial spray dryers are typically designed based on the feed material used; however, they all have nozzles or atomizers of varying sizes to disperse a liquid slurry rapidly into hot gas (150−220°C) for short times (15−30 seconds in a well-designed system) to give typically micron-sized (10−50 μm for very fine powder and up to 2−3 mm for large particles) particles suitable for a wide range of food applications (Depypere et al., 2003; Gharsallaoui et al., 2007). As the atomized emulsion droplets enter the spray dryer chamber, water quickly is driven off through a wet bulb temperature drying stage and a dry bulb temperature drying stage inside the drying chamber, leaving the biopolymers to form a hardened shell around the omega-3-rich oils (Masters, 1991; Reineccius, 2004). The slurry is typically comprised of a biopolymer-stabilized oil-in-water (O/W) emulsion, involving biopolymers that either act at the oil−water interface, such as proteins because of their amphiphilic nature, or increase the viscosity of the continuous phase (thus, increase the thickness of the boundary layers around oil droplets) to inhibit droplet coalescence. For formulation purposes, other functional ingredients may be included in the emulsions, such as film formers, fillers, antioxidants, and chelators (Gharsallaoui et al., 2007; Tonon et al., 2011). Regardless of the material selected, simple spray drying typically leaves some surface oil remaining ($\approx$1% of the total oil), which could adversely affect shelf-life of the resulting powder (Taneja and Singh, 2012). Formed powders have oil droplets dispersed within a biopolymer matrix and tend to have low payloads of $\approx$30%. The porous nature of the powders leaves the oil susceptible to oxidation; however, this can be offset by the incorporation of antioxidants and proper packaging (Taneja and Sing, 2012). Drusch and Berg (2008) conducted a thorough study on the localization of the extractable oil (including surface oil and other localized extractable oils) of spray-dried fish oil microcapsules and their impact on oxidation during storage. Fish oil oxidation was determined with the measurements of hydroperoxide content and propanal content in samples prepared using different processing conditions. Based on their study, Drusch and Berg (2008) conclude that the extractable oil itself cannot be used to predict shelf-life of the encapsulated fish oil. Vega and Roos (2006) summarized the critical influences of emulsion compositions on the physical properties of the spray-dried microcapsules and their stabilities. One of the focusing points of the review article is about surface

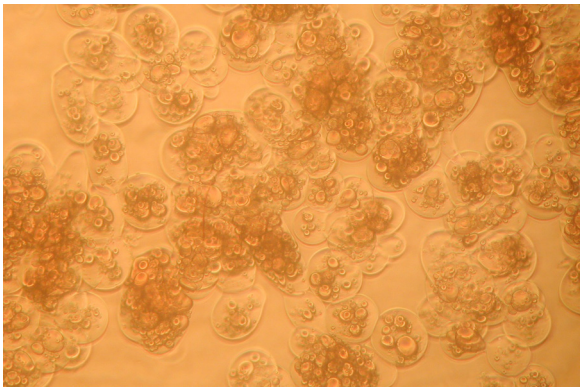

FIGURE 37.1 Gelatin−gum arabic coacervate stabilized flaxseed oil emulsion as imaged by light microscopy (Liu et al., 2010).

fat compositions, determined by elemental surface chemical analysis, and its relation with the wettability and storage stability of these spray-dried emulsion compositions.

Formulation of a stable emulsion is of the upmost importance in order to increase entrapment efficiency and payload, and to minimize surface oil. Biopolymers should encase oil droplets, as visualized under microscopy with few to no free oil droplets existing. Figure 37.1 gives an example of a gelatin−gum arabic coacervate-stabilized flaxseed oil emulsion as imaged by light microscopy (Liu et al., 2010). The multi-nuclear nature of the entrapped oil droplets enables prolonged release profiles to be achieved. In contrast, burst release occurs in mono-nuclear capsules (Yeo et al., 2005). Droplet size and emulsion stability can be altered based on biopolymer characteristics (type, concentration, molecular weight, and reactive groups present), solvent properties (pH, salts, and temperature), core-to-wall ratio, and homogenization conditions (shear, duration of shear, and type of homogenizer/fixture used) (Depypere et al., 2003; Reineccius, 2004; McClements, 2005; Jiminez et al., 2006; Vega and Roos, 2006; Gharsallaoui et al., 2007; Jafari et al., 2008). Maltodextrin or other low molecular weight carbohydrates, especially reducing sugars, are typically added at concentrations not only as dispersing aids, but as fillers to facilitate the formation of a glassy matrix for reducing oxygen permeability in the final powdered product (Auweter, 2004; Ubbink and Krüger, 2006; Drusch and Manniono, 2009). Serfert et al. (2009a) reported that free oxygen within the emulsion itself has a greater effect on lipid oxidation than the drying process. This observation by Serfert et al. (2009a) seems to be in agreement with the findings of Keogh et al. (2001). Keogh et al. (2001) investigated spray drying compositions using milk ingredients in relation to their functionalities in enhancing the oxidative stability of the so-formed microcapsules, and pointed out the importance of eliminating the entrapped air (reducing vacuole volume) inside the microcapsules.

There is a wide range of wall formations used in the literature and in industry for the entrapment of omega-3 and -6 PUFAs. For instance, Carneiro et al. (2012) entrapped flaxseed oil using various biopolymers (e.g., gum arabic, whey protein concentrate, and two modified starches (Hi-Cap™ 100 and Capsul TA)) in combination with maltodextrin, followed by spray drying. The authors reported the highest entrapment efficiencies and emulsion stabilities occurred using a Hi-Cap 100 starch−maltodextrin mixture. In contrast, the whey protein−maltodextrin mixture showed the worst entrapment properties but afforded the best protection against oxidation, highlighting the complexity of the encapsulation process. Augustin et al. (2006) entrapped fish oil by spray drying using Maillard reaction products of various protein (Na^+-caseinate, whey protein isolate, soy protein isolate, or skim milk powder) and reducing sugars (glucose, dried glucose syrup, or oligosaccharides) combinations, and heated to varyous temperatures (60−100°C) and time intervals (30−90 min). Resulting powders produced from the biopolymer formulations subjected to the Maillard reaction showed increased entrapment efficiencies and oxidative protection. Drusch et al. (2009a) investigated the role of glycated caseinate and Maillard reaction products in the stabilization of microencapsulated fish oil. The authors indicated that it was the molecular weight of the carrier matrix, rather than the antioxidative effect of Maillard reaction products, that played the central role in promoting oxidative stability of the microencapsulated oils.

Clover Corp. and its subsidiary Nu-Mega in Australia commercialized the Maillard reaction technologies (Augustin and Sanguansri, 2008) for manufacturing their microencapsulated fish oil products (Driphorm and ThermoMAX brands). Another example of utilizing Maillard reaction products to protect omega-3 oil from oxidation is the thermo-crosslinking process of spray congealed beadlets (a product of DSM) consisting of, for example gelatin and fructose at a temperature range from 90 to 140°C for a period of time from 3 to 7 minutes (Diguet et al., 2004). The beadlets

formed were a few hundred microns in diameter and produced using a modified spray drying process for a caseinate-based formulation and spray cooling process for a gelatin-based formulation (Vilstrup et al., 1998; Diguet et al., 2004; Vilstrup, 2004). Typical encapsulation loads for spray drying ranged between 30 and 50% of total solid content using biopolymers that were formulated together, but non-interacting.

Friesland Brands BV (and its subsidiary Kievit) also developed a few spray drying formulations and processes that can be used for protecting omega-3 oils in powder form. In 2004, van Seeventer and coworkers. invented a powdered formulation consisting of caseinate and sugar mixtures. Among the sugars tested, mannitol showed a unique property for inhibition of oxidation of the powder at accelerated storage conditions of 30°C under air for 16 weeks, as indicated by the peroxide value and anisidine value, as well as the sensory evaluation of the smell of the powders, and taste of the powders in milk (van Seeventer et al., 2004). In 2008, van Seewenter and Steenvelle invented an allergen-free LCPUFA-powdered composition containing modified starch as emulsifier and film former, and glucose syrup and some antioxidants (van Seeventer and Steenwelle, 2008). A FilterMAT spray dryer (GEA Niro, Denmark) was used for drying the composition into powder form. Some of these technologies have been licensed to omega-3 companies for the total manufacturing of powder products. Chen et al. (2005) developed a spray drying formulation consisting of modified starch as emulsifier and film former and a mixture of low molecular weight carbohydrates was used to form a glassy matrix. The core materials included long-chain PUFAs, such as AA, DHA, and EPA. Wu and Chen (2008) from InnoBio Ltd. revealed spray drying formulations consisting of a modified starch and a mixture of mono-, di-, and oligosaccharides for the microencapsulated omega-3-rich oils and carotenoids, for example. Cold water dispersibility and emulsion stability of the spray-dried powders are significantly enhanced by the inclusion of a small molecule surfactant in the formulation.

37.2.2 Spray Cooling/Chilling

Spray cooling/chilling (or prilling) is a process whereby a melt of lipophilic material (e.g., wax) is often used to add additional coating to a solid particle and then dispersed into a stream of cold gas to induce solidification of the micron-sized droplets (Depypere et al., 2003). In spray cooling, an ambient temperature air stream is utilized to cool a molten wax having a melting point $>45°C$, while in spray chilling, refrigerated air is typically applied for the waxes having a melting point $<45°C$ (Depypere et al., 2003). The process can be used for the double encapsulation of sensitive core materials. For instance, Subramanian and co-workers (Subramanian et al., 2012) initially entrapped algal oil containing DHA within a soy protein isolate-reducing sugar mixture (Maillard reaction product) by spray drying to yield particle sizes of 78 μm, followed by spray chilling using hydrogenated canola wax to yield a particle size of 157 μm. As mentioned in Section 37.2.1, Vilstrup (2004) described a spray cooling/chilling process for solidifying a gel-forming shell/matrix.

37.2.3 Fluidized Bed Coating

Fluidized bed coating technology is often combined with other microencapsulation technology, such as spray drying (Depypere et al., 2003). One of the advantages of fluid bed technology is its large selection of coating materials, as they can be either hydrophilic or hydrophobic in nature, solvent-mediated or non-solvent formulations. Coating thickness can also be easily controlled to create the desired wall structure. Fluidized bed technology provides another option for double encapsulation technology. For instance, Barrier and Rousseau (1997) used a fluidized bed coater to apply a gum arabic coat to spray-dried capsules prepared from modified starch, maltodextrin, or cyclodextrin aimed at inhibiting oxidation. Gautam et al. (2007) utilized this approach to coat solid lipids such as carnauba wax, paraffin or bees wax, and stearic acid onto the surfaces of milk protein-based microcapsules with entrapped oil, in order to add a moisture barrier. Van Seeventer et al. (2006) coated hydrogenated soybean oil onto microencapsulated fish oil powders using a fluidized bed coater to facilitate flowability and a neutral taste.

37.2.4 Freeze Drying

Freeze drying is not considered an economically feasible process for the encapsulation of omega-3 and -6 PUFAs due to its extremely high cost ($\approx 30-50$ times that of spray drying) (Gharsallaoui et al., 2007). Freeze drying is a process whereby ice is sublimed from the material directly to a gas, under pressure and with a small amount of heat. In general, freeze drying is mainly used to produce a tiny quantity of dehydrated porous powder with the aid of a physical grounding procedure, useful for laboratory trials and for various analytical purposes.

37.2.5 Complex Coacervation

Complex coacervation is a proven technology for microencapsulation of lipophilic core materials, and a subject of many excellent reviews (Gouin, 2004; Jin et al., 2007; Lee and Ying, 2008; Drusch and Mannino, 2009; Matalanis et al., 2011b; Schmitt and Turgeon, 2011; Thies, 2007, 2012). Chapter 12 of this book provides a general coverage of this technology. Therefore, the focus in this chapter is on its technical application in microencapsulation of omega-3- and -6-rich oils.

Complex coacervation is a phase separation process involving biopolymers of opposing net charges which interact through electrostatic attractive interactions depending on the biopolymer, solvent, and mixing conditions to form tiny colloidal-sized particles, known as coacervates (Ducel et al., 2004; Thies, 2007). Coacervation can be used in two ways for the entrapment of oils. First, a coacervate can form initially in solution, and then be used to coat the oil−water interface; or second, one biopolymer can align at the oil−water interface entrapping the oil, followed by the addition of a second biopolymer that interacts via a layer-by-layer design (de Kruif et al., 2004; McClements and Decker, 2007; Dickinson, 2008). The latter approach has been used by McClements and Decker (2007) for the entrapment of tuna oil using lecithin−chitosan in sequence to form the two layers. However, over the last decade, complex coacervation has been used as a means to achieve much higher pay loads (40−90%) and lower surface oils ($\approx$0.2% of the total oil) (Taneja and Singh, 2012). Complex coacervation involving gelatin and acacia gum has been well studied in the literature (Green and Schleicher 1957; Burgess, 1990; de Kruif et al., 2004; Thies, 2007); however, several other protein (such as whey proteins, soy proteins, and pea proteins) and polysaccharide (low and high methoxy pectins, gellan gum, and xanthan gum) mixtures are now being explored (Ducel et al., 2004; Weinbrek et al., 2004a; Benichou et al., 2007; Bédié et al., 2008; Yan et al., 2008; Jones et al., 2010). Weinbreck et al. (2003, 2004b) studied coacervation between whey protein with a number of negatively charged polysaccharides under different biopolymer and solvent conditions such as pH, ionic strength, and biopolymer mixing ratio, type, and total concentration, and in the presence of salts.

Yan and Jin (Yan, 2005; Yan and Jin, 2011) patented a novel approach for preparing multiple shell microcapsules by complex coacervation. Figures 37.2 and 37.3 give a full image (taken by light microscopy) and cross-sections

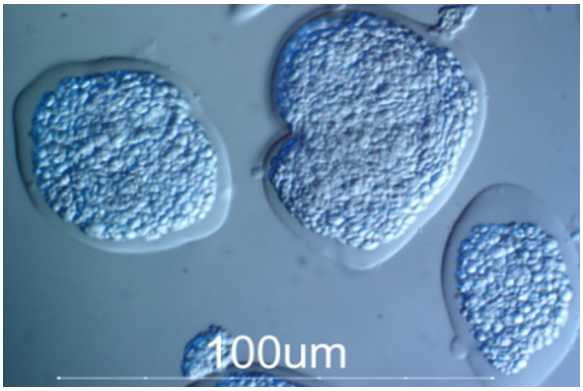

FIGURE 37.2 Light microscopic image of the multiple shell microcapsules produced by Ocean Nutrition Canada Ltd.

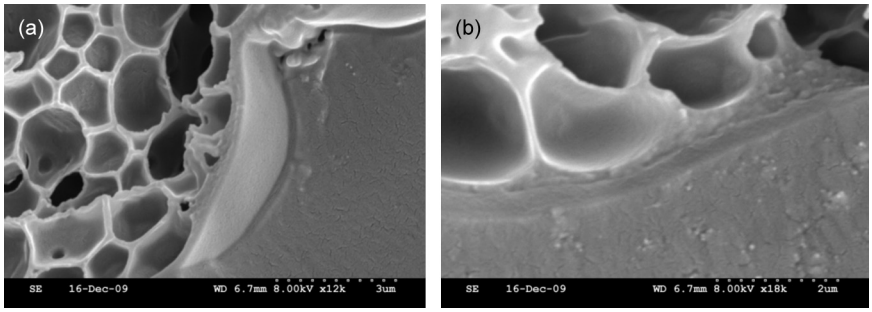

FIGURE 37.3 Scanning electron microscopy image of a cross-section of the multiple shell microcapsules produced by Ocean Nutrition Canada Ltd.

(taken by scanning electron microscopy), respectively, of a typical multiple shell microcapsule produced by Ocean Nutrition Canada Ltd. The shell materials used in the patent of Yan and Jin (2011) include the positively charged gelatin type-A and chitosan and the negatively charged gelatin type-B, polyphosphate, gum arabic, alginate, carrageenan, pectin, etc. Enzyme crosslinking with transglutaminase may be an option in the process. Shell materials other than hydrocolloids, such as solid lipid waxes, have been described for the construction of the non-coacervation shell.

In some cases, capsule integrity can be improved with the addition of crosslinking agents. The most common agents used are: (1) transglutaminse, which is an enzyme that forms covalent linkages between neighboring amino groups on the protein [gelatin (Soper and Thomas, 2000; Leuenberger et al., 2002) or beta-lactoglobulin/caseinate (Mellema, 2006)]; and (2) calcium salts to form ionic electrostatic bridges between anionic charged reactive sites on a protein and/or polysaccharide [e.g., denatured whey protein (Subirade et al., 2002) or alginate (Blatt et al., 2003)]. In recent years, other food grade crosslinkers have been investigated, and examples include tannic acid (Xing et al., 2004, 2005), plant phenolics (Strauss and Gibson, 2004), and quercetin glycosides (Huber et al., 2009; Rupasinghe et al., 2010). Recently, da Silva and Pinto (2012) reviewed physical crosslinkers that can be used to improve the mechanical properties of fish gelatin and may be utilized in the construction of microcapsules.

As a reflection of the commercialization efforts with complex coacervation technology, a few patents are worth mentioning. Vasishtha et al. (2004) in the Southwest Research Institute incorporated nanoclay into the coacervates matrix of gelatin and polyphosphate microcapsules in an attempt to reduce oxygen transfer rate in both dry and humid environments. Lumsdon et al. (2005, DuPont) invented a novel composition consisting of three polymer components, protein such as gelatin or beta lactoglobulin, negatively charged polysaccharide such as gum arabic, and a starch. Dardelle and Normand (2007, Firmenich SA) described a coacervation process in which the emulsification of lipophilic component-like fish oil takes place after cooling the two biopolymer solutions below the critical gelling temperature of the coacervation phase. Another Firmenich patent, invented by Subramaniam and Reilly (2009), disclosed a coacervation process using type B gelatin, e.g., beef gelatin, as a "Halal" product, and it would not be difficult to turn such a product into "kosher" as well. Kohane et al. (2009) from PepsiCo, Inc. novelized the coacervation process into a venue to produce tiny coacervates enclosed shell (preferred particle diameter $<0.5\ \mu m$) to encapsulate lipophilic nutrients for beverage applications.

37.2.6 Extrusion Technology

Extrusion technology has been investigated as a promising process for microencapsulation of omega-3 and -6 PUFAs due to its unique processing parameters, where high pressure, high temperature, and high shear rates are applied inside the extruder to produce a glassy matrix. In contrast to the spray drying process, extrusion is able to provide a denser amorphous carbohydrate matrix (Saleeb and Arora, 1999). Typically, low molecular weight carbohydrates are extruded at temperatures above their glass transition temperature (i.e., a critical temperature where a non-crystalline material changes from behaving as a rubbery (elastic and flexible—high permeability) material to one that is a glassy (hard and brittle—low permeability) material). As the material is extruded through the die, a rotating blade cuts the strips into small particles (submillimeters to millimeters), which are washed with a food grade solvent such as 2-propanal or limonene to remove surface oil. Most of these applications rely on a twin screw extruder to produce the extrudates.

Since the severe processing conditions in extrusion are detrimental to the quality of omega-3 and -6 PUFAs, van Lengerich (2002) from General Mills Inc. invented an approach to add the core material at a later stage of the screw-extrusion process in order to limit the exposure time. To further improve the stability, van Lengerich et al. (2004) applied an "acidic antioxidant" (e.g., citric acid, ascorbic acid, or erythorbic acid) to the matrix and claimed that the scavenging effect of these antioxidants helps remove the oxygen from the extrudates and prevents it from reaching the oil core. Other approaches have been developed to improve the stability of omega-3 and -6 PUFAs during the extrusion process over the years, through formulation considerations. For instance, Bouquerand et al. (2007) from Firmenich SA embedded coacervated omega-3 powder in a mixture of maltodextrin and modified starch. Van Lengerich et al. (2007) from General Mills Inc. applied wheat protein and sodium caseinate mixed with durum flour and glycerol (plasticizer) to form their matrix system. Prior to extrusion, 15% of Marinal C38 oil containing 38% omega-3 fatty acids was mixed into the biopolymer formulation. Saleeb and Arora (1999) from Kraft Foods Inc. invented an extruded glassy substrate as the encapsulation matrix composed of a minor component of a high molecular weight polymeric carbohydrate and a major component of a low molecular weight carbohydrate. Moore and Solorio (2009) from Solae LLC used a mixture of two soy protein isolates and tapioca starch in the extrudates. The loading levels of omega-3 fatty acids and key ingredients in some commercial products are summarized in Table 37.1.

TABLE 37.1 Omega-3 and -6 PUFA-rich Microencapsulated Extrusion Products

Manufacturer	Key Ingredients	Payloads (%)	Patent Number(s)
Firmenich	Gum arabic, sucrose, maltodextrin (DE 18)	15	US 2006/0134180
Firmenich	Starch, maltodextrin (DE 18)	25	WO 2007/026307
General Mills	Durum flour, wheat protein, Na-caseinate, glycerol	15	WO 2007/055815
Kraft Foods	HMCSS, maltodextrin	11	US 5,972,395
Solae	Tapioca flour, Supro 8000/Supro 600	10	WO 2009/076131

Despite significant research efforts, commercial launches of omega-3 and -6 PUFA-enriched cereal products are rare, possibly attributable to difficulties encountered in the encapsulation processes and effectiveness in protecting these oxidation-prone oils for extended shelf-life.

37.2.7 Other Delivery-Type Systems

Other emulsion-based technologies are also being explored for their potential for delivering omega-3 oils in foods, such as via nanoemulsions, microemulsions, and double emulsions (Sagalowicz and Leser, 2010). Nanoemulsions are similar to conventional emulsions in terms of their thermodynamic instability, except for droplet sizes with a radii ranging between 10 and 100 nm (Tadros et al., 2011; McClements, 2012). Nanoemulsions are advantageous relative to conventional emulsions as delivery systems because (1) they have greater stability against coalescence and gravitational separation; (2) nano-sized droplets scatter less light, enabling transparent or translucent products (e.g., fortified beverages, soups, sauces) to be developed; and (3) they have been shown to have greater bioavailability (McClements and Rao, 2011). The potential use of nanoemulsions for entrapping fish oil has been investigated by Belhaj et al. (2010), Muller et al. (2012), and McClements and Xiao (2012). A Virun nanoemulsion patent, invented by Bromley (2009), revealed a number of formulations for their nanoemulsion preparations, including: fish oil and its concentrates (80% omega-3), algal DHA oil (35% DHA), vitamin E oil, flaxseed oil (50% omega-3), borage oil (22% GLA), and 80% conjugated linoleic acid oil. Typical oil concentrations within their patent ranged from 5 to 10%. Formulations also used surfactant (polysorbate 80) concentrations from 18 to 25%, and a small amount of a biopolymer mixture comprised of xanthan gum, guar gum, and sodium alginate as an emulsion stabilizer. Other potential technologies involve research into microemulsions (Cho et al., 2008; Zheng et al., 2011) and multiple emulsions (Kajita et al., 2000; Choi et al., 2009) as potential delivery systems. Casana et al. (2006) prepared water-in-oil-in-water (W/O/W) emulsions to make omega-3 oil microcapsules. The reverse emulsification technique was used with biopolymers such as sodium alginate, pectin, guar gum, and surfactants to form the shell with calcium ions as a crosslinker to form solidified gel particles. It is claimed that the microcapsules can be used for food applications in emulsion form and can be spray dried for applications in powder form.

37.3 CHARACTERIZATION METHODS

37.3.1 Physical Properties

Encapsulated omega-3 and -6 PUFA-rich powders are typically evaluated based on their loading levels (or payload), encapsulation efficiency, surface oil content, particle size and distribution, flowability and dispersibility, color, moisture content, and water activity (Reineccius, 2004; Drusch et al., 2006, 2007, 2012; Fuchs et al., 2006; Polavarapu et al., 2011). Desired target specifications for a commercial-encapsulated powder are given in Table 37.2, along with recommended methods for analyses; however, caution should be taken with identified levels, as they will differ with wall formulations and processing. Nevertheless, encapsulated powders can also be evaluated using more advanced techniques. For instance, Drusch et al. (2012) applied a number of characterization methods to study the physical characteristics of spray-dried carrier matrices and microcapsules as well as their impact on the stability of a microencapsulated fish oil. The spray-dried carrier matrix was characterized by different physical methods such as: helium pycnometry for

TABLE 37.2 Desired Industry Target Specifications Relating to the Physical Properties of Encapsulated Powders, and Recommended Method for Analyses

Physical Property	Target Specification	Recommended Method of Analysis
Payload	30–70%	AOAC 996.06; one-step extraction method for hard-to-digest powders (Curtis et al., 2008)
Encapsulation efficiency	>98%	Westergaard (2004); Drusch and Berg (2008)
Surface oil content	<2%	GEA Niro Analytical Method A 10 a
Emulsion droplet size	<2 μm	Light scattering method (Malvern or Coulter instruments)
Flowability	N/A < 40°	GEA Niro Analytical Method A 23 a, angle of repose (Drusch et al., 2006)
Dispersibility	N/A	GEA Niro Analytical Method A 6 a
Color	N/A	Hunter colorimeter
Moisture content	<3%	GEA Niro Analytical Method A 1 a to d, or moisture balance
Water activity	<0.3	Water activity meter
Powder apparent density	N/A	ASTM B923-10, helium or nitrogen pycnometer

determination of true powder density; nitrogen displacement for surface area analysis; X-ray photoelectron spectroscopy for determination of elemental surface composition; and positron annihilation lifetime spectroscopy for determination of free volume of the biopolymer matrix. Surface viscoelasticity (i.e., by interfacial rheometry) of protein-stabilized oil–water interfaces was analyzed using dynamic pendant drop tensiometry. Surface accumulation of proteins at the air–water interface led to a modified surface composition of spray-dried carrier matrix particles for microencapsulation. By measuring free volume elements, Drusch et al. (2009b) were able to correlate lipid oxidation to the increase in protein content in the microcapsules leading to an increase in the matrix porosity (indicated by free volume measurements), which is suspected to negatively affect oxygen diffusion.

37.3.2 Chemical Properties and Oxidation Stability

Analyses for determining chemical changes in different raw materials of oils during processing, as well as during storage and in food matrices, are also of utmost importance, as they are the major determinant of shelf-life of the encapsulated powders. Traditionally, oxidation is monitored through the measurement of hydroperoxides, the primary products of lipid oxidation, and aldehydes, the primary products of secondary oxidation products (Frankel, 2005). Peroxide values (PV) are a measure of the level of hydroperoxides. The Global Organization for EPA and DHA (GOED) sets a PV limit of 5 meq/kg for fish oils in their Voluntary Monograph for Omega-3. Hydroperoxides themselves have very little impact on oil flavor, and decompose rapidly into secondary oxidation products causing various undesirable flavors and odors. Anisidine values (AV) are an indicator of the content of the secondary oxidation products. The AV limit set by GOED is 20. In practice, neither PV nor AV are sensitive enough for the determination of oxidation stability in omega-3 and -6 PUFAs alone, or being fortified in food matrices (Frankel, 2005; Sullivan and Budge, 2012). For example, undesirable flavors can be detected in fish oils with a PV of <1 meq/kg (Frankel, 2005). Eidhin and O'Beirne (2010) monitored the oxidative stability of innovative blends of fish oil with camelina oil using chemical properties such as PV, AV, thiobarbituric acid reactive substances (TBARS), and conjugated triene levels (CT). The results demonstrate that there is potential for use of blends of camelina oil with fish oils in food products, as the results show some benefits in terms of reduction of fishy odors.

Matalanis et al. (2011a) prepared fish oil-encapsulated hydrogel microspheres using an O/W/W emulsification method, and compared the lipid oxidation stability of these microspheres with the emulsions stabilized by either Tween 20 or casein. Oxidation rate was determined by using the hydroperoxide method and propanal detection method with gas chromatography, which is a more advanced analysis method than those of wet chemistry. Their results showed that emulsion stabilized by Tween 20 was much less stable than both casein stabilized emulsion and the hydrogel microsphere systems. In another instance, Anwar (2010), and Anwar and Kunz (2011) investigated the stability of microencapsulated fish oil prepared by spray granulation (SG), SG plus fluid bed coating, spray drying, and freeze drying by

TABLE 37.3 Desired Industry Target Specifications Relating to the Chemical Properties of Encapsulated Powders, and Recommended Method for Analysis

Chemical Assays	Oxidation Products	Target Specification	Recommended Method of Analysis
Peroxide value	Primary	5 meq/kg	AOCS Official Method *Cd 8-53*
Thiobarbituric acid reactive substances		N/A	Eidhin and O'Beirne (2010)
Anisidine values	Secondary	20	AOCS Official Method *Cd 18-90*
Conjugated diene levels Conjugated triene levels	Secondary Secondary	<2 mmol/kg N/A	AOCS Official Method *Ti 1a-64* Eidhin and O'Beirne (2010)

measurement of PV and headspace propanal after storage at room temperature and at 3 to 4°C for 6 weeks. The results showed that the PVs were in agreement with the concentration of propanal for most of the uncoated powders stored at low temperature. However, lower stability was detected for the coated powders than for uncoated powders as more hydroperoxides were detected as well as increasing propanal concentration. This is a typical example showing the complex nature of oxidative stability. It is affected by many variables in materials themselves and in processing parameters and the final matrix structure. Desired target specifications for a commercial encapsulated powder are given in Table 37.3, along with recommended methods for analyses.

37.3.3 Shelf-Life and Sensory Studies

Shelf-life of foods fortified with omega-3 and -6 PUFAs is a critical factor determining the fate of the microcapsules or straight oils in that particular application, and affects consumers' acceptance of the food product. The shelf-life reflects indirectly the storage stability of the microcapsules as well. However, most studies focus on the shelf-life of the carrier food. As an example, Iafelice et al. (2008) enriched spaghetti with EPA and DHA with a microencapsulated form and tested their sensory acceptability. It was found that the spaghetti preparation provided $\approx$20% of the recommended daily intake of EPA and DHA with high sensorial acceptability and low loss of their nutritional values after cooking ($<$10%). It was therefore suggested that such spaghetti fortified with EPA and DHA could be used to increase consumption of omega-3 fatty acids and to decrease the dietary omega-6/omega-3 ratio. Krishnan and Prabhasankar (2011) reported the trend in health-based pasta that could serve as a base to carrier nutritional ingredients such as omega-3 fatty acids, among others, and redefine the concept of the next generation convenience food. Fortification of different types of cheeses with omega-3 fatty acids is another subject of studies by various research groups (Bermúdez-Aguirre and Barbosa-Cánovas, 2012).

Sensory assessment of foods fortified with omega-3 fatty acids is typically inherently difficult to correlate with primary and secondary oxidative products generated as a result of oxidation (Frankel, 2005; Jacobsen, 2010; Sullivan and Budge, 2012). The most accurate way to evaluate sensory qualities of oils is to use a taste panel, as humans can be trained to detect low levels of volatile components, down to ppb level for example, that traditional tests of oxidation cannot. A scientific approach is required to select and train the panelists, and calibrate and maintain their accuracy over time (Sullivan and Budge, 2012). Technological advances has meant that analytical instrumentation can act as an alternative to a sensory panel. As the volatile oxidation compounds are most responsible for oil flavors, extraction of and monitoring these compounds in the headspace of samples has become a method of choice in recent years for stability studies. The volatile compounds are extracted by a solid phase microextraction device and measured with gas chromatography-mass spectroscopy to quantify their levels (Guillen et al., 2009; Serfert et al., 2009a, b, 2010).

Jimenez-Alvarez et al. (2008) used a sensory panel with a triangle test method to collect sensory data from emulsion samples with/without single volatile compound such as (Z)-2-hexenal and (E)-4-heptenal. However, they found it difficult to correlate these results with the amounts of volatile components added in the emulsions. One reason for the finding could be attributed to the need for screening and calibrating the panelists. That study (Jimenez-Alvarez et al., 2008) could also serve as an example that food systems are complex systems and many factors could affect the oxidation process as well as the sensory properties people perceive while tasting.

37.4 APPLICATIONS

Fortification of foods with encapsulated omega-3 and -6 fatty acids is on the rise because of advances in encapsulation technologies. Table 37.4 summarizes various commercial-encapsulated omega-3 fatty acid powders produced around the world, along with their key ingredients, loading levels, and shelf-life (Jensen et al., 1995; Nakahara et al., 2005; Trubiano and Makarious, 2005). Today, fortified products include dairy products, baked goods (bread, brownies), snack foods (cereal bars), along with juice beverages (Whelen and Rust, 2006; Garg et al., 2006; Jin et al. 2007). Omega-3 and -6 PUFAs are now being added to infant formulas as an added nutrition in early stage development and growth (Jacobsen, 2011).

Whelen and Rust (2006) surveyed 62 food products that have been fortified with omega-3 fatty acids covering a wide range of food categories. Various forms of omega-3 fatty acids were used for food fortification, including flaxseed oil containing mainly ALA, fish oils rich in EPA and DHA and its concentrates, and algal-derived DHA-rich oils. As the examples showed, the fortification can be implemented with straight oils in certain food applications, but more preferably delivered in the forms of microencapsulated and free-flowing powder products to provide the required protection and/or masking effects to achieve desirable sensory features and textures, as well as the expected storage stability and shelf-life. These food products are summarized in Table 37.5 based on the types of omega-3 fatty acids and food categories. It is interesting to point out a few trends based on these limited but informative data. Among those 62 fortified

TABLE 37.4 Commercial Omega-3 and -6 PUFA-rich Microencapsulated Powders

Manufacturer	Key Ingredients	Payloads (mg omega-3/g powder)	Shelf-life (months)	Linkable Patents
(a) Complex coacervation				
ONC	Gelatin, polyphosphate, or polysaccharides	140–180	18 at 4°C	US 2011/0111020
(b) Spray drying				
Clover/Nu-Mega	Maillard reaction with caseinate, various sugars, starches in alternative formulations	69–143	>6	US 7374788B2 US 2007/0218125
Lipid Nutrition	Carbohydrate, protein	170–195	24 at 5–10°C	WO 2006/006856
Kievit	Caseinate, soy proteins, glucose syrup	190	18 at 5–10°C	EP 1616486A1 WO 2008/111837
Fuji Chemical	Modified starch, gum arabic, glucose syrup	213	N/A	N/A
Wright Group	Modified starch and corn syrup; whey protein and gum arabic	100–150	6 at 4°C	N/A
National Starch/Omega Protein	Modified starch and soy protein	100	6–12 at 5–10°C	US 2005/0233002
Pronova-Polaris	Soy protein, caseinate, glucose syrup	150–180	18 at <15°C	EP 1969952
DSM/legacy Martek	Hydrolyzed gelatin, soy protein, caseinate, glucose syrup, mannitol	100–215	12 at 4°C	N/A
DSM	Starch-coated, caseinate or gelatin, and sucrose	90	12 at <15°C	US6444227
BASF	Gelatin, or caseinate, coated with starch granules	68–83	24 at 8–15°C	US 5460823 WO 94/01001
Kitii Corp.	Calcium salt and surfactant	<100	N/A	WO 2005/090534

TABLE 37.5 Data Analysis for the n-3 and n-6 Fortified Food Products Surveyed by Whelen and Rust (2006)

Types of omega-3 fatty acids	Bread/ Pasta	Cereal	Milks	Eggs	Meats	Juice	Nutrition Bars	Salad Dressings	Infant Formulas	Others
EPA/DHA	3		10	4	2	1	2	2	6	1
ALA	4	4	2	3			3	4		
ALA and EPA/DHA	1		1	2	1			1		
N/A	1		1				1	1	1	
Total number	9	4	14	9	3	1	6	8	7	1

foods, there are 14 milk products, being the number-one fortified food category. This top ranking seems to carry a message that milk product fortification has high consumer acceptability in the marketplace. Of these 14 milk products, 10 of them are fortified with EPA and/or DHA. Another food category that has high DHA fortification is infant formula due to its expected functionality in this food category. In contrast, all four cereal products were fortified with flaxseeds oils with significant high loading levels, which can be difficult to match if using EPA/DHA fortification due to their oxidation features that render them unsuitable for such typical, long shelf-life applications.

Garg et al. (2006) provided a list of foods fortified with omega-3 fatty acids that were available mainly in the Australian food market. These products include Farmers Best™ milk from Dairy Farmers; Heart Plus™ milk drink from Brownes; Wonder™ White DHA bread from Buttercup; HighTop™ bread from Coles; Seachange™ omega-3 spread from AP Foods; and IQ3 Brainstorm™ cereal bars from Biomedical Laboratories, which was an exceptional product that was sold in the United Kingdom. It is worth noting that for each of these omega-3 fortified food products, Garg et al. pointed out that the dose is well below the recommended level for daily intake. Another food fortification example is the ONC coacervation powders that were reported to cover a wide range of food categories, including: bread/bagels, cereal bars, tortillas, frozen dough, and frozen waffles for the bakery category; fresh white milk (HTST), UHP white milk, yogurt, ice cream/frozen yogurt, and UHT milkshakes for the dairy category; fresh 100% citrus juice, UHT orange juice boxes, dry (ss) protein beverage, and frozen citrus juice for the beverage category; frozen alfredo/marinara dinners, baked and ranch beans, pasta sauce, frozen pizza, canned spaghetti, and tomato sauce for the meals category; and chocolate, protein bars, chew candy, gummy candy, fruit preparation, peanut butter, seasoned potato chips, pretzels, granola bar, granola cereal, and popcorn for the confections/snacks category. For many of these food applications, 12 months of shelf-life were reported. In a review article, Taneja and Singh (2012) pointed out of some trends in the marketplace for omega-3 fatty acids products. The supplements sector would see a growth rate of 30% each year. Sales in the fortified food market in the USA alone would jump to $7 billion by 2011 from $2 billion in 2007. In Europe, new product launches were more than doubled in 2007 compared to that in 2005. In contrast to this sharp increase in new product launch, not many of these new products won wide and durable consumer acceptance. Several challenges that could limit the growth of omega-3 fatty acids-enriched foods were discussed, including factors that affect product quality and consumer acceptance, as well as regulatory issues that vary from country to country.

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Chapter 38

Microencapsulation of Vitamins, Minerals, and Nutraceuticals for Food Applications

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38.1 MICROENCAPSULATION AS A TOOL FOR EFFECTIVE DELIVERY OF MICRONUTRIENTS AND NUTRACEUTICALS

Micronutrients are compounds required only in minute amounts that enable the body to produce enzymes, hormones, and other substances essential for proper growth and development. As tiny as the amounts are, however, the consequences of their absence are severe. Their addition into foods has been practiced by the food industry for years as a cost-effective means of alleviating micronutrient malnutrition. Some common examples of micronutrients that have been added to foods include iron, iodine, calcium, zinc, and vitamins A and D, as well as several B vitamins (Black, 2003). Nutraceuticals are physiologically active components naturally present in foods or added to them as functional ingredients, which have a role in the health and well-being of the population. Examples of nutraceuticals that have been used to develop functional foods include polyphenols, omega-3 fatty acids, probiotics, and prebiotics (Hasler, 1998). However, there are compounds that can be classified as either a micronutrient or nutraceutical or both. An example is β-carotene, which acts as a provitamin, a Vitamin A precursor and also as an antioxidant.

As the ultimate goal is effective use, i.e., bioavailability, of these components by the body, the goal in food fortification is to ensure that they are delivered and released at the proper time and location in the body. The bioavailability of many compounds with poor water solubility or low permeability in the small intestine can be improved by appropriate control of food production and consumption. Therefore, innovative delivery technologies are always in demand for generating effective delivery systems that can protect active ingredients, and are able to deliver and release them in a soluble form at an appropriate site in the body. The chapter will focus on the use of microencapsulation techniques for effective delivery of vitamins and minerals through fortified food products, and will discuss the extension of successful technology platforms to the delivery of nutraceuticals in functional food development.

38.1.1 Importance of Microencapsulation in Fortified and Functional Food Development

To take advantage of the benefits from the consumption of essential micronutrients and nutraceuticals, actions are required (1) to ensure stability of the compounds during processing, handling, and storage, as well as in the gastrointestinal tract, and (2) to facilitate controlled release at the site of the GI tract for proper absorption (Champagne and Fustier, 2007). However, most of these compounds have limited solubility and stability (Fang and Bhandari, 2010), or are highly reactive and may interact with many other food components (Li et al., 2010). Additionally, many have unpleasant flavor, taste, or color that detracts from the attractiveness of the food (Kosaraju et al., 2008). These factors impose several limitations on direct addition of micronutrients and nutraceuticals to foods, and therefore the development of effective delivery systems has gained increased urgency in the food industry. Any effective delivery system must be designed so that it does not adversely impact the physicochemical and organoleptic properties of the finished food product in which it is incorporated, while achieving desirable absorption and bioavailability of the active components (Lesmes and McClements, 2009). This has led to numerous attempts to develop food-grade delivery systems to encapsulate, protect, and deliver nutraceuticals and micronutrients through novel food products. Appropriate selection of encapsulation

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00038-8

techniques alleviates many of the problems related to the direct addition of bioactive compounds to foods. Microcapsules have the potential to protect sensitive compounds, eliminate or minimize nutrients, incorporate time-release mechanisms to finished formulations, preserve desirable flavors and aromas or mask unpleasant taste and appearance, and transform potent plant or herb extracts into liquid or solid microcapsules for easy handling (Huang et al., 2010). All these functionalities have been exploited in drug and vaccine delivery in the pharmaceutical sector, and are increasingly used to add value to novel food products in the food industry.

Microencapsulation is not a new idea; it is a technology that has been in use for over 50 years in the pharmaceutical, biological, nutritional, and food science fields. Briefly, it is a process whereby the functional ingredients are protected from their environment by entrapping them within a protective coating material and forming particles with diameters of a few nanometers to a few millimeters. The substance that is encapsulated may be called the active agent or core ingredient, while the substances that provide the protection are called the coating or shell materials. The coating material of encapsulates used in food products should be food grade and able to form a barrier protecting the active agent from adverse effects of moisture, heat, light, oxygen, and other reactive components present in the food matrix (Ghosh, 2006; Zuidam and Shimoni, 2010). The latest advances in drug delivery have their roots in basic microencapsulation, while the technology has opened up to a broad range of applications in other fields. In contrast to drugs, food products have to meet the additional criterion that the addition of the encapsulated bioactive ingredients should not adversely affect or alter the sensory properties of the food vehicle (Champagne and Fustier, 2007). A detailed discussion on these criteria is presented Section 38.2.

Microencapsulation has become increasingly important in the development of fortified and functional foods in recent years. As estimated in "Food encapsulation: a global strategic business report" (Global Industry Analysts, Inc., 2010), the world market share involving the use of encapsulated food ingredients was more than $26 billion in 2009, and is predicted to rise to nearly $40 billion by 2015. For a long time microencapsulation was regarded as too expensive for the food industry to use, until recent advances presented more and more cost-effective preparation methods and scalable, massive production lines that contribute to the affordability of microencapsulation technologies for effective delivery of various bioactive ingredients in novel, value-added food products (Shelke, 2005).

As summarized in many reviews, microencapsulation has been used for protection, modification, and controlled delivery of many food ingredients, including acidulants, flavors, sweeteners, colorants, enzymes and microorganisms, antioxidants, leavening agents, and nutritional ingredients such as vitamins and minerals (Kirby, 1991; Gibbs et al., 1999; Schrooyen et al., 2001; Gouin, 2004). Over the past two decades, numerous microencapsulation techniques have matured, as seen in many practical applications summarized in Part 7 of this book. The most successful applications lie in the encapsulation and delivery of these rather common food ingredients in conventional processed foods, e.g., the "4Bs"—beverages, breakfast cereals, bakery goods, and bars (snacks) (Feder, 2006). More recently, mature processes have been found in extended applications for advanced delivery of rare bioactive ingredients, e.g., various nutraceuticals such as omega-3 fatty acids, probiotics/prebiotics, natural polyphenols, phytosterols, and medicinal/herbal extracts (Zuidam and Shimoni, 2010; Munin and Edwards-Lévy, 2011; Onwulata, 2013).

Specifically, the study and use of microcapsules for the protection and controlled release of nutrients is a growing area of interest to the food science and technology community. As revealed by a recent patent search, there were over 22,000 patents filed globally (WPTO) when "encapsulation of nutrients in food applications" was used as the key words. There were 1483 hits during a search of the US patent database (USPO) when "encapsulation," "nutrient," and "food" were used together in the description/specification of the patents. Detailed search results are compiled in Tables 38.1 and 38.2. Specifically, as indicated in Table 38.2, mainstream suppliers of nutrients and nutraceuticals, such as BASF, DSM, Kraft, Cognis, Balchem, and Firmenich, contribute greatly to this field. Some examples of

TABLE 38.1 US Patent Search Result Based on a Combination of Relevant Keywords

Search Criteria	Number of Hits
"encapsulation" + "nutrient" + "food" in description/specification	1483
"microencapsulation" + "nutrient" + "food" in description/specification	450
"microencapsulation" + "vitamins" + "food" in description/specification	488
"microencapsulation" + "nutraceuticals" + "food" in description/specification	29

TABLE 38.2 US Patent Search Result Based on Major Food Ingredient Suppliers under the Category of "Assignee Name"

Major Nutrient and Nutraceutical Ingredient Suppliers	When "(micro)encapsulation" + "food" used in description/specification + assignee name	
	# of Hits	Relevant Patent Examples
BASF	17	• Chaundy et al. (1998) US Patent 5,767,107—Compositions containing gluten and polysaccharides that contain uronic acid residues useful for **encapsulating fats, oils and solids** • Schmidt et al. (1984) US Patent 4,486,43—**Spray-dried vitamin** powders using hydrophobic silica • Breitenbach and Zettler (2001) US Patent 6,318,650—Method for producing small-particle preparations of **biologically active substances** • Chaundy et al. (1992) US Patent 5,153,177—Process for incorporating a material in a **crosslinked gelatin**, and product therefrom
DSM	7	• Van Den Burg and Groenendaal (2000) US Patent 6,048,557—**PUFA coated solid carrier particles** for foodstuff
Southwest Research Institute	21	• Vasishtha et al. (2010) US Patent 7,794,836—Microencapsulation of oxygen or water sensitive materials • Vail (2008) US Patent 7,381,426—Targeted delivery of bioactive factors to the systemic skeleton
Kraft	45	• Boghani and Gebreselassie (2013a) US Patent 8,389,032—Delivery system for active components as part of an edible composition having selected particle size • Boghani and Gebreselassie (2013b) US Patent 8,389,031—Coated delivery system for active components as part of an edible composition • Ripoll et al. (2006) US Patent 6,989,169—Production of capsules and particles for improvement of food products
Cognis	3	• Rull Prous et al. (2012) US Patent 8,329,672—Oral and/or topical compositions comprising prebiotics and fatty acid • Weitkemper and Fabry (2002) US Patent 6,444,659—Use of mixtures of active substances, containing phytostenols and/or phytostenol esters and potentiators, for the production of hypocholesterolemic agents
Balchem	7	• Pacifico et al. (2008) US Patent RE40,059—Sensitive substance encapsulation • Lee and Richardson (2004) US Patent 6,835,397—Controlled release encapsulated bioactive substances • Pasin (1972) US Patent 3,664,963—Encapsulation process
Firmenich	30	• Elabbadi and Ouali (2013) US Patent 8,383,175—Active ingredient delivery system with an amorphous metal salt as carrier • Benczedi et al. (2011) US Patent 8,017,060—Process for the preparation of extruded delivery systems ("particularly useful for the controlled release of flavoring or perfuming ingredients")
Bio-Dar (LycoRed)	2	• Blatt et al. (2006) US Patent 7,097,868—Stable coated microcapsules
National Starch	16	• Eden et al. (1989) US Patent 4,812,445—Starch-based encapsulation process
Aveka	2	• Hamer et al. (2005) US Patent 6,858,666—Organogel particles • Hamer et al. (2002) US Patent 6,413,548—Particulate encapsulation of liquid beads

innovative delivery technologies for various nutraceuticals including vitamins and minerals from rather small, emerging companies include the following:

- NutraLease™, Ltd. (Mishor Adumin, Israel) has developed a unique, patent-pending technology to produce micelles, which are self-assembled, structured liquid particles with a diameter of 30 nanometers or less. These particles are designed to readily penetrate cell membranes and dramatically increase the bioavailability of the phytonutrients carried and protected by the micelles (Food Product Design, 2006).

- BASF has launched a series of studies to formulate nano-scale health ingredients, including vitamins A, E, D, and K, PUFAs, carotenoids, synthetic lycopene, and co-enzyme Q_{10} (End, 2006; Foley, 2012).
- Aquanova (Darmstadt, Germany) claimed its patented NovaSOL® technology is able to deliver a wide variety of active ingredients in a stable, crystal-clear aqueous solution. The solubilizing system has a colloidal micelle structure, enabling an ultrafine distribution (even below the wavelength of light) of carrying ingredients, including fat-soluble vitamins, omega-3 fatty acid, co-enzyme Q_{10}, isoflavones, flavonoids, carotenoids, phyto-extracts, and essential oils. This system is expected to bring much higher bioavailability of the carrying ingredients compared to other formulations in the market (Aquanova, 2013).
- Proprietary Nutritionals Inc. (Kearny, NJ) launched Benexia™ ALA Powder, a new patent-pending, neutral-tasting, water-soluble omega-3 microencapsulated powder for numerous food applications (Shelke, 2006).
- Longevinex (San Dimas, CA) introduced a micron-sized trans-resveratrol delivery system for enhanced absorption, which is further microencapsulated in an envelope of all natural plant dextrins and starches to preserve resveratrol in its trans-resveratrol form from degradation by light, heat, and oxygen.
- Maxx Performance (Chester, NY) recently introduced a solution that allows manufacturers to add green tea extract (rich in polyphenol antioxidants but with a bitter after-taste) to baked goods and other formulations without compromising flavor.
- LiveTheSource (Ft. Lauderdale, FL) announced its launch of "daily source," the first ever nano-encapsulated liquid vitamin, mineral, and herbal supplement. The company claims the herbal compounds in "daily source" will "provide a substantial increase in nutritional value, first, due to their synergy, and second, due to their nano-encapsulation" (Shelke, 2006).

38.1.2 Microencapsulation Technologies for Developing Fortified and Functional Foods

A number of microencapsulation technologies have been developed for use in the food industry that show promise for the production of fortified and functional foods. Techniques of microparticle formation include physical processes (such as spray drying, spray chilling/cooling, extrusion, rotating pan and fluidized bed coating), and chemical processes (such as coacervation, gelation, co-crystallization, molecular inclusion, and interfacial or *in situ* polymerization), as discussed in detail elsewhere in this book. In many cases, a combination of processes is preferred, e.g., the formation of a single or double emulsion could be followed by spray drying (Gibbs et al., 1999). Appropriate selection of these technologies can lead to the successful delivery of bioactive ingredients to the gastrointestinal tract (Champagne and Fustier, 2007).

The selection of the microencapsulating method depends on the properties of the core and the coating material, the desired release mechanism, processing conditions, and final particle size required (Shahidi and Han, 1993). Many reviews are available in the literature with respect to critical appraisal of various microencapsulation techniques and their applications in different areas (Kirby, 1991; Gibbs et al., 1999; Gouin, 2004). Many of these techniques are the subject matter of Part 3 of this book. As expected, all microencapsulation techniques have advantages and disadvantages. In general, physical techniques are less expensive and easy to scale up, but have the drawbacks of relatively low payloads and imperfect particle properties. In contrast, chemical processes are costly and involve complicated concepts, but typically can provide well-defined particle structures and desired release properties. Chemical processes are often reported in formulating drug delivery systems or for making value-added products. Specifically, in the area of food applications, physical encapsulation techniques are widely used for the purpose of protection and/or effective delivery of various ingredients including flavors, colorants, nutritional ingredients, and other functional additives (Madene et al., 2006).

An effective combination of appropriate coating materials and encapsulation techniques is the key for developing a microencapsulated system as it plays an important role in the physical and chemical properties of the resulting microparticles, such as particle size, porosity, density, flowability, integrity, reactivity/stability, and release properties (Gharsallaoui et al., 2007). For each active ingredient the appropriate choices of process and wall materials depend greatly on the end use of the microencapsulated particles.

38.1.3 Encapsulants Commonly Used for Delivery of Micronutrients and/or Nutraceuticals

Substances that are used in food formulations or are in direct contact with food products should be generally recognized as safe (GRAS), under conditions of their intended use. The amounts added to foods in accordance

with good manufacturing practices (GMPs) should be within the limits approved by the Food and Drug Administration (FDA) (Pavlath and Orts, 2009).

A number of coating or matrix materials can be selected depending upon desired properties of the final microcapsule including stability and unaltered bioavailability, as well as compatibility with selected microencapsulation techniques and high encapsulation efficiency. Among them, proteins, sugars, starches, gums, lipids, and cellulose derivatives are most popular, as covered elsewhere in this book. Ideal coating materials should have a bland, neutral taste and odor, good film-forming properties, low viscosity when present in solutions or suspensions, and desirable gelling and barrier properties (Augustin and Sanguansri, 2008).

As a coating or encapsulating system is designed to protect the core material from environmental factors that may cause its deterioration and to prevent premature interaction between the core material with other food components, it is of paramount importance when selecting the coating material that it be stable under processing and storage conditions.

Another important requirement for the coating or matrix material is that it is able to release the core ingredient under desired conditions. Delivery systems with desired release profiles can be obtained by using biopolymers with or without chemical or physical modifications. These modifications allow the release of core ingredients under controlled rates or at target sites when the encapsulant responds to changes in the environment, such as pH changes or presence of certain enzymes (Augustin and Hemar, 2009).

Enteric coatings, sometimes also called sustained or delayed release coatings, are comprised of pH-sensitive polymers, which remain intact in the acidic environment of the stomach and then become deprotonated and dissolved in basic media or at nearly neutral pH values such as that present in the small intestine (Lin and Kao, 1991). Some examples of enteric coatings include shellac, zein, cellulose acetate phthalate, and other cellulose ester polymers. An example of enzymatic response coatings is proteins crosslinked using transglutaminase. Crosslinking helps improve heat and moisture resistance of the proteins. Microcapsules made of these crosslinked proteins can be broken down by trypsin and chymotrypsin, thus releasing the entrapped content into the small intestine (Yildirim and Hettiarachchy, 1998). Another enzyme-controlled release system relies on the existence of enzyme-producing microorganisms in the colon. The colonic microflora produces a variety of enzymes, including azoreductase, various glycosidases, and, at lower concentrations, esterases and amidases, which can be exploited for colon-specific drug delivery (Liu et al., 2003). By taking advantage of these enzymes, biodegradable coating materials have been developed for enzyme-controlled drug delivery that could be extended to food applications (Leopold, 1999; Nunthanid et al., 2008).

38.2 CRITERIA FOR DEVELOPING MICROENCAPSULATED DELIVERY SYSTEMS FOR MICRONUTRIENTS AND NUTRACEUTICALS

As stated earlier, many factors related to the chemistry of vitamins and minerals require the use of proper encapsulation processes before adding these ingredients to foods. The physicochemical factors influencing the stability of vitamins and their retention in foods include pH, oxygen, light, heat, potential interactions, the presence of transition metals, and packaging (Foley, 2012). These factors define the criteria for developing microencapsulation systems for the effective delivery of specific micronutrients. Obviously, the same principles apply to the delivery of nutraceuticals. While the technology for vitamin/mineral encapsulation has matured over the past decade, the emerging delivery systems for nutraceuticals are under development and/or validation. Among many technical factors, the stability, bioavailability, and sensory characteristics of the microencapsulated ingredients and their performance in the finished food products are considered first, while priority also needs to be given to consideration of cost, technique scalability, and encapsulant material selection as well as regulatory compliance and safety (Foley, 2012). Based on experience in microencapsulation of vitamins and minerals for fortification of staple foods such as salt and rice, the following factors, as discussed below, should be considered (Li and Diosady, 2009).

38.2.1 *In Vitro* Bioavailability of Micronutrients and Nutraceuticals

One of the key objectives of microencapsulation of micronutrients and nutraceuticals is to improve their absorption rates in the body. Methods for bioavailability determination involve human/animal tests (*in vivo*) or simulated experiments performed in a laboratory (*in vitro*). The detailed reviews of these techniques are presented elsewhere in this book, therefore only a brief summary and comparison of the two groups of methods is included here. *In vivo* methods provide direct data of bioavailability and have been used for a large number of nutrients, their delivery systems, and fortified foods. However, cost, ethical restrictions, and the lengthy approval process of protocols when humans and/or animals are used in biological or clinical research greatly limit the use of this type of method. Another drawback of *in vivo* tests relates to

variations in physiological state of participating individuals and possible interactions of the target nutrient with other components in the diet. Thus, as an alternative to *in vivo* tests, *in vitro* methods are extensively used in the food industry since they are rapid, safe, cost efficient, and do not raise ethical or legal issues (Parada and Aguilera, 2007).

In a typical *in vitro* test, the digestion process is simulated under controlled conditions using a variety of enzymes, for example, lingual lipase, amylase, protease, pepsin, pancreatin, and so on; while the final transport and absorption process is commonly assessed using differentiated cell monolayers obtained from human intestinal epithelium (Caco-2 cell cultures) (Gangloff et al., 1996). An understanding of the basic physicochemical and physiological processes that occur as a delivery system passes through the human gastrointestinal (GI) tract is required to develop effective *in vitro* models that accurately simulate digestion (Artursson et al., 1994; McClements and Li, 2010). Mechanical models of the whole digestive system are under development, e.g., by Dr. Paul Singh's laboratory at UC Davis.

Successful *in vitro* models have been developed to evaluate the bioavailability of micronutrients in foods. Specifically, vitamins and many other phytochemicals, which are organic compounds with complex chemical structure and involve complicated metabolic pathways in the body, stand in contrast to essential minerals such as iron, zinc, calcium, and potassium are inorganic elements with simple chemical structure that have relatively straightforward absorption patterns. Therefore, the bioavailability of many minerals can be readily assessed by *in vitro* methods. Gangloff et al. (1996) evaluated the *in vitro* bioavailability of iron in meat using a simulated digestion system; while pepsin was used for the gastric phase, the intestinal phase was simulated by adding pancreatin and bile extract before the uptake of iron by Caco-2 cell monolayers was measured. The authors observed that iron absorption was higher from meat than other media. Zhu et al. (2006) also used this approach to evaluate the iron uptake by Caco-2 cells when comparing different iron compounds (NaFeEDTA, $FeCl_3$, and $FeSO_4$) and examining the influence of other food components such as ascorbic acid (absorption enhancer) and an iron chelating agent (absorption inhibitor). The authors observed similar absorption for all the iron compounds in the absence of ascorbic acid; however, when adding the enhancer the absorption was significantly improved. Similarly, when the inhibitor was added, the absorption of the three iron compounds decreased to a similar extent.

An even simpler test can approximate the iron release rate in the simulated gastric acid (pH 1 HCl solution) based on USP General Chapter 711. This method gives a good initial indication of iron digestibility in the stomach. The good agreement between the results obtained by this method and by *in vivo* tests justifies the use of this technique for quick screening (Swain et al., 2003). With an aid of appropriate *in vitro* bioavailability procedures it is possible to design optimized formulations for an iron microcapsule in order to obtain enhanced absorption of this important mineral in the small intestine (Li et al., 2009a).

38.2.2 Encapsulation Efficiency

Besides bioavailability, another requirement for an effective microencapsulation system for micronutrients or nutraceuticals is acceptable encapsulation efficiency, which is a measure of how well the microcapsules separate the core from the environment. It is generally defined as the ratio (in percentage) between the weight of the core ingredient actually encapsulated and its total weight used in the formulation. There are numerous studies in the literature that involve the use of specific methods to quantify the encapsulation efficiency for various delivery systems.

To confirm whether microencapsulation results in an effective protective layer for micronutrients and nutraceuticals, it is imperative to evaluate the encapsulation efficiency. Romita et al. (2011) evaluated the encapsulation efficiency in spray-dried ferrous fumarate microcapsules coated by one of several biopolymers. The microcapsules were immersed in a pH 7 EDTA solution for 5 minutes, during which time exposed iron leached while the polymeric portion of the capsules remained intact. As the amount of iron leached is directly proportional to the amount of iron exposed, the authors were able to evaluate the microencapsulation efficiency, concluding that microcapsules prepared using hydroxypropyl methyl cellulose (HPMC) have the high coverage and protection of ferrous fumarate.

The same authors also analyzed the encapsulation efficiency of the spray-dried ferrous fumarate delivery system using time-of-flight secondary ion mass spectrometry (TOF-SIMS), which uses a pulsed primary ion beam to desorb and ionize species from a sample surface. The CH_3O ion signal, attributed to the biopolymer, indicated that the microcapsule surface was mainly covered by wall material, while the signal for surface iron was small, suggesting that iron was well enclosed in the system and protected from the environment (Romita et al., 2011).

38.2.3 Microcapsule Morphology and Size

Microencapsulation technologies can allow us to produce micronutrient/nutraceutical delivery systems for food vehicles with different particle size ranges. An important goal is that the particles resemble the physical characteristics of the

selected food vehicles in terms of shape, size, color, and appearance, which ensures the resulting fortified foods have desired physical, chemical, nutritional, and organoleptic properties, ultimately meeting the requirements of consumer acceptability and product shelf-life stability. Pedroza-Islas et al. (1999) reported that the choice of wall materials and their core material loading were critical for obtaining desirable microcapsules, i.e., those that have smooth surface and are spherical in shape with a narrow size distribution.

Among physical/mechanical microencapsulation technologies, extrusion followed by surface coating is flexible and able to readily generate microcapsules with different particle sizes, ranging from several hundred microns to several millimeters, which subsequently ensures that the microparticles can match the size of a wide variety of staple foods. For example, to produce encapsulated ferrous fumarate for inclusion in refined table salts with particle sizes ranging from 300 to 1000 μm, particle agglomeration based on extrusion or fluidized bed technique followed by surface coating was evaluated (Diosady, 2007; Li et al., 2009a). Both size-enlargement techniques were able to produce microparticles approximating the size of salt grains. Surface modifications including coverage of whitening agent followed by a film coating resulted in iron microcapsules with an opaque, white appearance and smooth surface, which ultimately makes them indistinguishable visually when blended into salt. Matching the size and density of the iron microcapsules also reduced the possibility of particle segregation during salt storage and distribution.

For evaluating the morphology and size of microcapsules to be used in food fortification, several microscopy techniques and laser light scattering-based particle size distribution measurements can be used. Scanning electron microscopy (SEM) is most widely used as it allows the analysis of the surface of the particle as well as its size and shape (Prasertmanakit et al., 2009).

38.2.4 Storage Stability

The stability of micronutrients in the microcapsule itself and when added into a food product is of paramount importance when developing effective delivery systems. The stability of the microcapsule determines the protection of the active ingredients from environmental challenges, such as moisture, temperature, or oxygen, and from potential interactions with other food components. Appropriate modeling of environmental conditions is important in predicting stability based on laboratory measurements. For example, the storage stability of double fortified salt (DFS) samples prepared by adding microencapsulated ferrous fumarate into iodized salt was studied over a period of 5 months in Nigeria and Kenya (tropical conditions) (Oshinowo et al., 2004, 2007; Diosady et al., 2006). The study confirmed that the results obtained in the laboratory storage tests under controlled conditions of high temperature and humidity correlated well with field measurements.

Microencapsulated ferrous fumarate was prepared by agglomerating ferrous fumarate with a binder (HPMC or Opadry F®) and finally coated with soy stearine and titanium dioxide as color masking agent. The authors observed the retention of ferrous iron was around 83% as it was protected by the microcapsule from reacting with iodine. Also, the polyethylene film bundle wrapping the packaging material was enough to protect the iodine compound from the environmental conditions, resulting in iodine retention in excess of 85% in most cases. The authors concluded that salt double fortified with iodine and microencapsulated iron can protect both iodine and the ferrous iron during distribution and retail under typical tropical conditions.

38.3 DEVELOPMENT OF FORTIFIED AND FUNCTIONAL FOODS

38.3.1 Importance of Food Fortification in Fighting Micronutrient Malnutrition

Micronutrient deficiencies are common in many developing countries, and typically originated from inadequate food intake, poor dietary quality and diversity, poor bioavailability of vitamins and minerals due to factors such as the presence of inhibitors in the food, improper mode of meal preparation, and potential interactions, as well as the presence of infections in the affected populations (Ramakrishnan, 2002).

As tiny as the required amounts are, the consequences of their absence are severe. Micronutrient deficiencies are now recognized as an important contributor to the increased rates of illness and death from infectious diseases, and of disability such as mental impairment in the vulnerable populations. Severe micronutrient deficiency causes clinical manifestations in humans that are also confirmed in animal studies when using selectively restricted diets. Even mild to moderate deficiencies have important consequences for human health (Black, 2003).

Iodine, vitamin A, and iron are three micronutrients that are of the most concern for global public health; the lack of adequate amounts of these three micronutrients in the diet represents a major threat to human health worldwide, particularly for children and pregnant women in low-income countries. Specifically, iron deficiency is estimated to affect

about 30% of the world population (World Health Organization, 2008) and it is the most important deficiency related to malnutrition in developing countries.

There are three main strategies to address micronutrient malnutrition: dietary diversification, supplementation, and food fortification. Dietary diversification is the most ideal, long-term solution; however, it requires individual compliance, education, and economic improvements. Supplementation has the advantage of promoting rapid changes in micronutrient status in the body; however, its effectiveness may be limited due to gastrointestinal side effects produced by oral administration. On the other hand, micronutrient fortification of foods that are commonly consumed by the population at risk has been demonstrated to be a viable and cost-effective food-based intervention for the control of micronutrient deficiencies (Schrooyen et al., 2001). One successful example is the universal salt iodization program.

Food fortification has been extensively used for many years and has been regarded as one of the most effective public health measures to alleviate and prevent nutritional diseases in developed nations after World War II (Myers, 2005; Teleki et al., 2013). Staple foods, such as salt, sugar, flour, and rice are considered good vehicles for micronutrient fortification since the added micronutrients reach the largest number of people cost-effectively and such fortification has been successfully demonstrated (Diosady et al., 2002; Li and Diosady, 2009; Li et al., 2011b; Malahayati et al., 2011). In addition to staple foods, many popular, processed foods such as milk and dairy products, breakfast cereals, snacks, and beverages are widely fortified with selected vitamins and minerals. Food fortification is very common in developed countries as almost all processed foods are fortified with one or more vitamins and minerals; however, food fortification is less common, and is much needed in many developing countries.

During the development of a successful fortification program, several technical factors need to be considered simultaneously, including the selection of appropriate food vehicles and fortificant forms, as well as determination of fortification levels and appropriate quality assurance and quality control of the fortified food products. Among these, food vehicle selection is the critical factor in determining whether food fortification programs would be more beneficial than other strategies such as supplementation and dietary diversification. Obviously, the selection of an ideal carrier guarantees that the micronutrients reach the largest number of people and the fortification strategy remains as the best long-term approach to cheaply maintaining the desired micronutrient levels in the diet.

It is generally accepted that staple foods, such as salt, sugar, wheat flour, and rice, are good carriers for fortification, since they are regularly consumed by all of the target population at a fairly constant rate, and are relatively inexpensive so that all segments of the target population could afford them. Another important advantage of these foods is that most are manufactured or centrally processed so that the fortification can be implemented in the existing production lines, which already have proper quality assurance/quality control monitoring systems. Global salt iodization is an example of effective programs that improve human nutrition, mainly due to the attributes of the food vehicle—salt. It is universally consumed and is readily and simply fortified, which makes the iodization program affordable. The World Health Organization (WHO) recognizes salt iodization as a spectacularly simple, universally effective, widely attractive, and incredibly cheap technical weapon for overcoming iodine deficiency diseases. UNICEF estimates that 66% of households now have access to iodized salt since its introduction three decades ago, and the cost of iodizing salt is extremely low—only about US$0.05 per person per year (Sullivan et al., 1995).

Pure salt is an ideal vehicle since it contains no other food component that could react with iodine. However, special considerations have to be taken with respect to salt purity and storage conditions, such as relative humidity (RH) and temperature, as they can negatively affect the stability of the added iodine compound (Diosady et al., 1997). While salt is an attractive carrier of micronutrients needed in small quantities, multiple fortification, by adding other components to iodized salt, may result in unwanted interactions and undesirable sensory changes to salt and the food seasoned with it. Therefore, other micronutrients may require physical or chemical protection to prevent these potential interactions. Moreover, efficient delivery systems are also required to ensure the release of the micronutrients at the site of absorption in the gastrointestinal tract. Microencapsulation technologies offer technical solutions to these challenges.

38.3.2 Technical Challenges in Fortification of Staple Foods

A key to the success of fortification of staple foods is the selection of appropriate micronutrient forms. The added micronutrient should not cause unwanted sensory changes, yet have desirable bioavailability, but which may be degraded by the presence of potential inhibitors in the food vehicle (Hurrell, 2002). Many commercial preparations of vitamins and minerals in a free-flowing powder form have achieved the basic requirements on stability and bioavailability, and are appropriate for use in processed foods such as breakfast cereals, beverages, and bakery goods. The only staple food vehicle for direct addition of commercial vitamin preparations is wheat flour. BASF's vitamin A premix for

flour fortification is a typical example (Foley, 2012). However, due to their small particle size, such commercial premixes/pre-blends are unsuited for addition to most granular staple foods.

Staple foods, such as salt, sugar, and rice, are typically presented to the consumer as solids with particle sizes ranging from several microns to several millimeters. To prevent particle segregation, which may result in potential under- or overdosing, micronutrients must be added in forms that either stick to the food carrier, or match its particle size, and, if possible, the particle density of the granular food (Li and Diosady, 2009). Some vitamins and minerals in their natural chemical forms and in commercial preparations could be simply added to selected food carriers as a powder, involving simple methods of solid−solid blending or solid−liquid mixing. These methods are straightforward and low in cost, but usually ineffective in protecting the micronutrients within the fortified staple food. Moreover, the incorporation of these minor ingredients often causes undesirable sensory changes in the fortified foods, such as off-flavors or off-colors. Ignoring sensory effects and physical/chemical properties leads to major concerns regarding product stability and consumer acceptance that may jeopardize the success of a fortification program. Therefore, appropriate technology must be used in designing fortified staple foods.

Successful food fortification processes also require that the added micronutrients are protected, bioavailable, and unnoticeable to the consumer. Thus, the added fortificant consisting of the active ingredient and required coatings or other additives must match the food carrier in color and appearance, and must not alter the food flavor. In order to avoid unwanted reactions between added micronutrients in triple fortified salt, Wegmüller et al. (2006) studied the microencapsulation of potassium iodate, retinyl palmitate, and ferric pyrophosphate in hydrogenated palm oil by spray cooling. In this case, iron and iodine could be encapsulated together as ferric pyrophosphate is a stable yet poorly water-soluble compound that causes negligible iodine loss. During storage for 6 months, iodine losses were approximately 20%, comparable to iodized salt. Stability of retinyl palmitate was excellent, resulting in losses of only about 12%. However, the relatively low bioavailability of this iron compound poses a problem when the triple fortified salt is aimed also to improve the iron status of the target population. To stabilize iodine and iron in DFS, microencapsulation technology was required, as illustrated in Section 38.4.

38.3.3 New Trends of Nutraceutical Delivery through Functional Foods

While the original purpose of food fortification was to correct deficiencies of essential vitamins and minerals in food, there is a growing interest in supplementing foods with other bioactive ingredients such as antioxidants, phytochemicals, omega-3 fatty acids, and probiotic bacteria for added health benefits. Recently, in developed countries trends in fortification have shifted toward functional food development. When used to prevent or cure diseases through food, these compounds are called nutraceuticals. Nutraceuticals are biologically active compounds present in some traditional or unusual food materials, and can be isolated, purified, and encapsulated to form ready-to-use food ingredients. When they are present or added to processed foods with the purpose of health promotion or disease prevention, the products are termed “functional foods.” Since these active ingredients in nutraceuticals and functional foods are also required in small quantities, they can be also termed micronutrients, and all of the technical parameters discussed earlier apply.

Functional foods and/or nutraceuticals are defined in a number of ways in the literature. In current terminology, “nutraceutical” is used for functional components naturally occurring in food materials or bioactive ingredients, which can be extracted, isolated, and further purified from edible or non-edible parts of raw food materials, including the waste streams from post-harvest and post-slaughtering productions; whereas “functional foods” refer to the processed, ready-for-consumption food products containing active nutraceuticals. Nutraceuticals are categorized based on their chemical structures, such as vitamins, minerals, PUFAs, phytochemicals, probiotics/prebiotics, essential amino acids, specialty peptides and proteins, fibers, carotenoids, and polyols (Frost and Sullivan, 2010). On the other hand, functional foods are grouped or actually marketed based on their health-related functions, for instance, weight control (anti-obesity); promotion of cardiovascular health, joint and bone health, and cognitive health; boosting energy and immune responses; lowering levels of cholesterol, blood pressure, and blood sugar (anti-diabetes); or based on targeting consumer groups, for example the elderly, infants, children, pregnant women, and athletes (Tallon, 2007).

It is now clear that a healthy diet, best defined as “eating the right foods in moderate amounts,” can significantly reduce the chances of developing devastating chronic diseases, including cancers, cardiovascular diseases, and diabetes, and subsequently extend life expectancy and improve the quality of life. However, with changes in lifestyle, Americans will find it increasingly difficult to consume all of the required nutrients. In the fast modern lifestyle, more processed food is consumed instead of fresh fruits and vegetables that are rich in many micronutrients and nutraceuticals. At different times of the year and in specific geographical regions, the cost of fresh produce is prohibitive to a significant fraction of the population. Therefore, foods enriched or fortified with nutraceuticals, vitamins, and minerals become viable alternatives for delivering these biologically active compounds essential for good health. As a result, the

functional food industry became one of the fastest growing business sectors worldwide over the past decade, with global functional food sales approaching $110 billion by 2010 with an additional $34 billion in sales of functional beverages (Sloan, 2008).

As revealed by some market surveys, the consumer demand for specific nutraceuticals in functional foods decreases in the order: vitamins and minerals, proteins and amino acids, omega-3 fatty acids, anti-aging/antioxidants, probiotics, botanicals, fiber, and prebiotics (Frost and Sullivan, 2010). This may be related to the current status of scientific research in this field, as more scientific evidence is made available and is more easily accepted by the general public. There is demand for a wider variety of functional foods besides the "4Bs"—breakfast foods, beverages, bakery goods, and nutrition bars, more specifically, through natural, less-processed, whole food formats. In parallel, according to the predictions of the health ingredient supplier industry (Toops, 2012), naturally derived substances, consisting of herbal and botanical extracts and animal- and marine-based derivatives, will see the fastest growth among the major groups of nutraceutical ingredients, followed by micronutrients (vitamins and minerals), proteins, and dietary fibers.

Currently, the majority of studies of nutraceuticals reported in the literature have been focusing on their biological effects, such as weight control, blood sugar reduction, anti-tumor qualities, heart health promotion effects, and GI disorder improving activities. This is more from a pharmaceutical perspective or for the purpose of developing dietary supplements, as summarized by Barton in "Next generation nutraceuticals" (Barton, 2006) and by Tallon in "Key trends in nutraceutical food and drinks" (Tallon, 2007). From the standpoint of functional food development, numerous studies and reviews have emphasized the physical/chemical properties of various nutraceuticals and how they are related to technological functionality during food processing, as summarized by Roberfroid (2000) and Siro et al. (2008). Food industrial associations and governmental agencies are also active in monitoring the changes of consumer demands and reporting the new trends in this area (Sloan, 2008; Agriculture and Agri-Food Canada, 2009). In some way, functional foods can be regarded as a joint venture between conventional food fortification and dietary supplements manufacturing, but certainly falling under different regulations.

There are also numerous studies and reviews in the literature on the food engineering and processing perspective of delivery technologies/systems for various nutraceuticals. However, it seems hard to form a consensus on the types of delivery technologies or formulating systems that would be desirable for each individual nutraceutical. Nonetheless, due to the chemical complexity of many nutraceutical compounds in comparison with commonly used food processing agents, it is generally agreed in this field that the use of microencapsulation to protect, separate, modify, and/or mask these ingredients before incorporating them into formulated food products is almost a necessary practice (Poncelet et al., 2011). When selecting coating or carrier materials or choosing encapsulation techniques, the decision varies case by case, and depends on particular nutraceuticals that are of interest and their end uses in food applications. For example, for delivery and controlled release of nutraceuticals including antioxidants, vitamins, probiotics, fish oil, and proteins, different food-grade coating materials are proposed and tailored to meet the specific requirements of their applications (Garti, 2008; Zuidam and Shimoni, 2010). Meanwhile, nanotechnology-based delivery systems, including micro- and nanoemulsions, structured or self-assembling lipids (liposomes), and complexes or conjugates of biopolymers (proteins and polysaccharides), are also under intensive research for effective delivery and controlled release of selected nutraceuticals (Weiss et al., 2006; Huang et al., 2010). A review by Onwulata (2013) summarizes current trends from an application perspective and suggests that future direction will focus on "personalized" delivery systems, which address an individual's metabolic needs and genetic makeup (nutrigenomics).

The development of novel delivery systems/technologies for various nutraceuticals is under way, but not surprisingly, there are not many patents granted so far. As indicated in Table 38.1, a search of the US patent database with "nutraceutical," "microencapsulation," and "food" used in combination as the keywords resulted in 29 hits. Most of them were filed after 2005 and granted after 2010. Among them, the majority are from emerging ingredient companies or start-up, spin-off research groups. For example:

- "Multi-phase, multi-compartment, capsular delivery apparatus and methods for using the same" (Miller, 2013; US Patent 8,361,497) from Innercap Technologies, Inc. (Tampa, FL) claimed the development of a method for delivering one or more active or medicinal ingredients having diverse physical states (e.g., solid, liquid, gas, or dispersion) into a single dosage, multi-compartment capsule. This patent is a continuation of a series of patents from the same applicant/assignee.
- "Encapsulated labile compound compositions and methods of making the same" (Subramanian et al., 2012; US Patent 8,221,809) is from Martek Biosciences Corporation (Columbia, MD). This patent claims to apply a first encapsulant through spray drying coating and a second encapsulant through prill coating, so as to form an encapsulation system for labile compounds including polyunsaturated fatty acids and vitamins.

- From Response Scientific, Inc. (Princeton, NJ), a patent titled "Medical food or nutritional supplement, method of manufacturing same, and method of managing diabetes" (Webster and Opara, 2011; US Patent 7,943,163) claims a method to manufacture medical foods or nutritional supplements with a specific aim to manage blood glucose levels, prevent the onset of type 2 diabetes, or manage diabetes mellitus. The finished product is made by separate microencapsulation of one or more of the components followed by encapsulation of the individual components.
- Known as a leading contributor to the research in this area for years, the Southwest Research Institute published a patent, titled "Microencapsulation of oxygen or water sensitive materials" (Vasishtha et al., 2010; US Patent 7,794,836), which involves the use of atomization or coacervation methods, wherein a structuring agent was added to a polymer material in forming a barrier coating composition, aiming to decrease oxygen or water permeability through the coating shell.
- Van Lengerich et al. (2010; US Patent 7,803,414) from General Mills claimed, in a patent titled "Encapsulation of readily oxidizable components," the use of a stabilized emulsion to produce shelf stable, solid particles or pellets that can control the release of embedded components, such as omega-3 fatty acids (readily oxidizable agents).

The last two examples are in alignment with market observations (Frost and Sullivan, 2005; Research and Markets, 2007; Global Industry Analysts, 2010), suggesting many key players in this field, including General Mills, Kraft, Nestlé, DSM, and BASF, are also very active in creating novel delivery technologies/systems for various nutraceuticals in broader food applications.

38.4 CASE STUDY: TECHNICAL APPROACHES TO THE FORTIFICATION OF STAPLE FOODS

In a wide variety of staple foods, the use of combinations of microencapsulation techniques is superior to any single method when fortifying with multiple micronutrients. Instead of directly adding micronutrients into the selected food carriers, the active ingredients can be pre-processed into granular premix form that is similar to the food vehicles, such as salt or rice, in terms of physical characteristics. These "engineered" premixes in the form of salt or rice grain-sized particles, containing concentrated nutrients, can be blended into the food in the required ratio to achieve desired dietary intake levels. This approach, which was successfully implemented in multiple-fortified Ultra Rice® and salt double fortified with iron and iodine (DFS), among others, is illustrated in Figure 38.1.

The development of these engineered foods started with investigation of several encapsulation techniques, including physical processes such as extrusion, fluidized bed coating, rotating pan/drum coating, spray drying, and chemical methods such as gelation and microemulsions. For the development of DFS, extrusion was investigated as the agglomeration process due mainly to its low operating cost and ready availability of extrusion equipment in many developing countries. Extrusion agglomeration coupled with surface coating is a feasible approach to the production of stable microparticles with a range of sizes that could match that of many food vehicles.

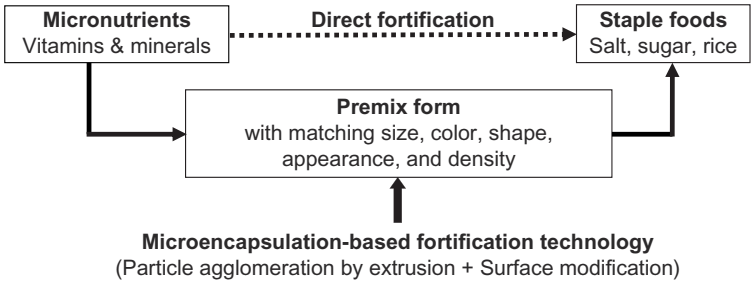

FIGURE 38.1 Micronutrient fortification through "engineered" staple foods (Li, 2009).

38.4.1 Salt

In developing countries, deficiencies of micronutrients such as iodine, iron, and vitamin A often coexist, and their adverse effects are compounded by synergistic physiological effects, and therefore providing two or more of these micronutrients together in a same food vehicle is desirable. In order to take advantage of the global progress in salt

iodization, a premix based on an appropriately designed, microencapsulated iron compound was developed to produce salt double fortified with iodine and iron.

In many developing countries, high temperature and humidity and the presence of impurities in locally produced salt led to a rapid loss of iodine. This was greatly accelerated by interaction when potassium iodate and ferrous sulfate were added together to unrefined salt (Diosady et al., 2002). Potassium iodate in its oxidized sate is reduced by ferrous iron, forming elemental iodine, which sublimes and is lost to the atmosphere. Simultaneously, ferrous iron is oxidized to the less bioavailable ferric form. It is clear that a physical barrier is required in the DFS to keep the two elements separated. Based on this consideration, the Food Engineering Group in the Department of Chemical Engineering and Applied Chemistry at the University of Toronto carried out a wide-ranging research program with a focus on encapsulation as a means to preventing iron and iodine interaction to produce a stable, DFS product (Diosady et al., 2002, 2006; Oshinowo et al., 2004, 2007; Diosady, 2007; Li et al., 2009a, 2010, 2011c; Romita et al., 2011; Yadava et al., 2012). The following sections will discuss the research approaches and progress.

38.4.1.1 Microencapsulation of Iodine by Spray Drying and Fluidized Bed Drying

Fortifying salt with iron along with iodine was first studied by microencapsulating iodine in order to create a protective barrier, since iron compounds are relatively more stable than iodine compounds. The aim of this work was to develop a microcapsule able to prevent iodine loss by sublimation of elemental iodine.

Potassium iodide and potassium iodate were encapsulated by spray or fluidized bed drying/coating processes using five different barriers: dextrin, gelatin, sodium hexametaphosphate (SHMP), fully hydrogenated fat, and purified salt. The utilization of fully hydrogenated fat was complicated as the fat had to be melted first in order to disperse the iodine, and produced odd-shaped solids. On the other hand, gelatin microcapsules stuck to each other, making it difficult to blend it into salt. The best results were obtained with dextrin.

Salt samples were prepared by mixing salt with ferrous fumarate or ferrous sulfate powder, and encapsulated iodine particles. These samples were then stored at high temperature and high humidity, modeled on tropical weather. The best results in terms of stability, appearance, and taste were obtained with samples of salt containing potassium iodide encapsulated in dextrin and ferrous fumarate powder. Microencapsulation preserved iodine for up to 12 months at elevated temperature and humidity (Diosady et al., 2002).

In spite of the acceptable results in terms of chemical stability, the encapsulation of iodine was impractical, since spray drying produces fine microcapsules, which readily segregate from the much larger salt grains, and more importantly, a very substantial investment had already been made in salt iodization through spraying potassium iodate solution onto salt. Therefore, the encapsulation of iron compounds became more attractive.

38.4.1.2 Encapsulation of Ferrous Fumarate to Mimic Salt Grains

Ferrous fumarate is an attractive source of iron, as it is inexpensive, highly bioavailable, and has a bland taste. Unfortunately, it has a strong reddish-brown color. For effective fortification of salt, the iron compound must be added as particles that match the size and preferably the density of salt grains, to prevent segregation during distribution of salt. Accordingly, ferrous fumarate has to be agglomerated, color masked, and encapsulated to produce an effective iron premix for salt fortification. Fluidized bed and extrusion agglomeration were investigated for producing encapsulated ferrous fumarate with particle sizes ranging from 300 to 1000 μm for fortification of refined table salt. With further color masking and film coating, the microcapsules achieved similar physical characteristics to those of salt, making it easy to achieve uniform distribution of iron in salt.

A two-step encapsulation process was developed, starting with particle agglomeration using a fluidized bed followed by lipid coating. Appropriate binders for granulation, encapsulants for coating, and solvents for carrying the encapsulants were investigated. Dextrin was finally selected as a workable binder for agglomerating ferrous fumarate to the target particle size. The particles were then color masked and encapsulated by titanium dioxide suspended in molten soy stearine (a partially hydrogenated lipid). The DFS prepared by such encapsulated iron premix had excellent iodine stability (Li, 2009; Li et al., 2010).

The process was then tested on different scales and in several countries under the sponsorship of the Micronutrient Initiative (MI)—an Ottawa-based NGO with a primary focus on interventions for addressing micronutrient deficiencies worldwide. The field tests have shown that DFS is effective in reducing the incidence of iron deficiency anemia (IDA) and iodine deficiency disorder (IDD) (Li, 2009). The stability tests showed that the salt was acceptable in terms of the organoleptic properties in 11 countries, and it was stable during normal salt distribution and retail in Nigeria and Kenya (Oshinowo et al., 2004, 2007). The encapsulated iron premix production was then scaled up at Glatt Air Techniques,

NJ, from the laboratory process to 5, 60, 120, and finally to large commercial batches of 600 kg using a 1200 L Wurster-type agglomerator/coater (Diosady et al., 2004). Field tests in India, where the school lunches of 3.4 million school children were prepared with DFS made by this process, resulted in the cure of 34% of anemic children—a reduction by some one million (Andersson et al., 2008) DFS is now used in Tamil Nadu with 5.5 million school children.

Fluidized bed agglomeration followed by lipid coating has the advantage of high throughput and low operating cost. However, the microcapsules produced by this process had surface defects, porous texture, low density, and marginally acceptable color, as shown in Figure 38.2. The low density of the iron premix made with the fluidized bed technique leads to a problem when the salt is added to cold water, as the iron particles float and could be unintentionally discarded by consumers as impurities.

Extrusion agglomeration was then explored to produce particles with higher density, reduced porosity, and smoother surface that allow better film coating at lower coat loading. Other advantages of extrusion include its flexibility in forming particles on different size scales, ranging from several hundred microns to several millimeters, which ensures the premix particles can match the size of a wide variety of staple foods (Li, 2009).

During process development, ferrous fumarate powder was first mixed with selected binders such as wheat or rice flour, and water and oil to form an extrudable dough mass. The mixture was then extruded, cut, and dehydrated to obtain cylindrical particles matching the size of typical table salt grains (300−700 μm). These particles were then covered with a thin layer of whitening agent (titanium dioxide), and encapsulated using hydrophilic film coatings, as shown in Figure 38.3 (Li et al., 2011c; Yadava et al., 2012). These studies found that the best binder for preparing an extrudable

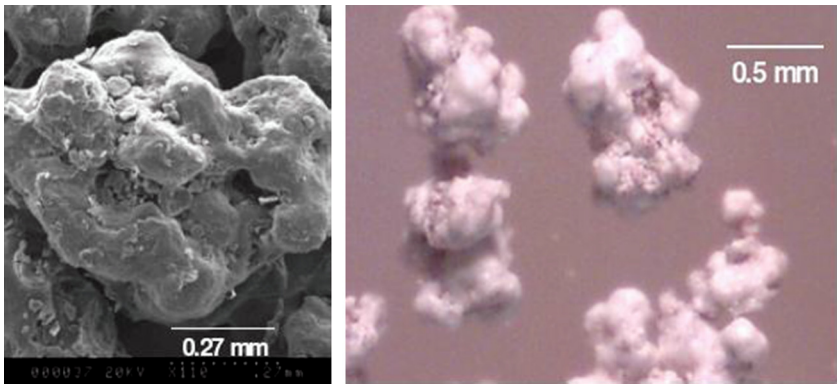

FIGURE 38.2 Surface defects on encapsulated ferrous fumarate made by fluidized bed agglomeration and soy stearine coating (left: SEM image; right: microscopic image under normal light) (Li, 2009).

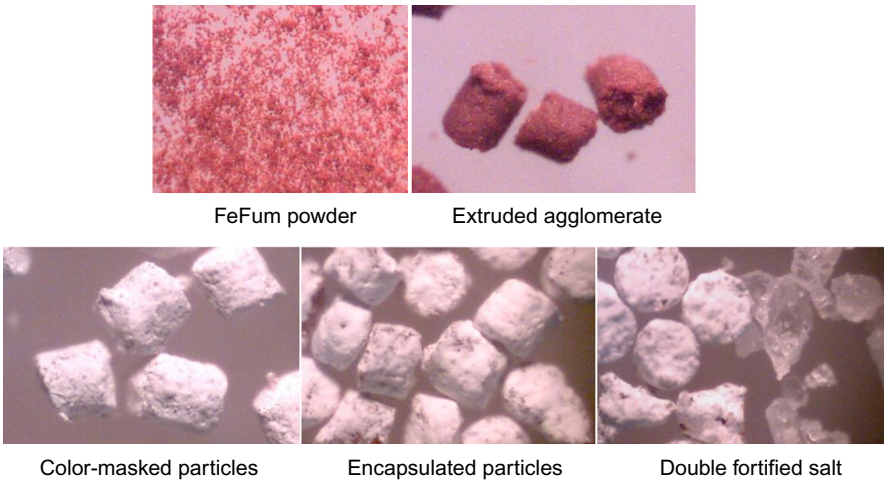

FIGURE 38.3 Evolution of ferrous fumarate premix during the DFS preparation process (digital microscopic images taken under normal room light) (Li, 2009).

dough with high ferrous fumarate content (75%) was durum semolina, and to a slightly lesser extent, wheat flour (Li et al., 2011c). Water and shortening (hydrogenated vegetable oils) in the dough were added at 16–20% and 2.5% levels on a dry basis, respectively, to impart plasticity and lubrication to the dough. The resulting extrudates had a bulk density of 1.40 g cm^{-3} and a particle density of 1.70 ~ 1.85 g cm^{-3}, approximating that of iodized salt (1.86 g cm^{-3}), which simplifies uniform iron distribution in the DFS.

The color-masking and hydrophilic polymer coating were further optimized (Yadava et al., 2012). Several color-masking techniques and polymer coating materials were investigated. HPMC-based, polyvinyl alcohol-polyethylene glycol (PVA-PEG) copolymer-based, enteric and reverse-enteric coatings were tested. Color masking was achieved by blending the extrudates with 25% (w/w) of titanium dioxide until the surface, i.e., the brown color of the particles, was fully covered. This was followed by film coating done in a top-spray lab-scale fluidized bed processor (Uni-Glatt model, Glatt Air Techniques, Ramsey, NJ, USA). Among the coating materials tested, HPMC offered the best protection at a relatively low encapsulation level (10% w/w) compared to other coating materials studied (Yadava et al., 2012).

DFS was then prepared by blending the pre-processed iron premix into iodized salt at a ratio of between 1:150 and 1:200, resulting in an iron concentration of 1000 ppm in DFS. A storage stability test showed that the DFS retained more than 90% of the original iodine and more than 93% of the ferrous iron after 3 months' storage at 35°C and 60% RH (Yadava et al., 2012). Besides the excellent storage stability, the DFS also exhibited desirable particle density, good *in vitro* bioavailability, and acceptable organoleptic properties (Li et al., 2010). Figure 38.3 illustrates the microstructure of ferrous fumarate and its encapsulated versions during the different steps in production of DFS. The process was tested in India on a pilot scale in December 2012.

38.4.1.3 Attachment of Spray-Dried Ferrous Fumarate Microcapsules to Coarse Salt

Coarse, unrefined salt, consumed in some developing countries, is typically of large particles (1–10 mm), which makes the addition of matching-sized iron premix impractical. An alternate approach is to produce tiny iron microcapsules through spray drying. Small particles would be indistinguishable visually (<20 μm) yet readily adhere to the surface of large salt crystals by electrostatic attraction in the absence of moisture, or due to its hygroscopic nature in the presence of moisture, and thus avoid particle segregation.

A stumbling block to this approach is the very low solubility of ferrous fumarate. Thus, a process for encapsulating solid ferrous fumarate particles through spray drying as a suspension was developed. Several food-grade polymers were tested as encapsulating agents through spray drying, including HPMC, sodium carboxymethylcellulose (Na-CMC), gum arabica (GA), and maltodextrin (MD). Sodium fumarate was used as an excipient and titanium dioxide was added as color-masking agent. The effect of operating conditions for spray drying, including inlet temperature, feed solution flow rate, atomizing gas, and aspirator flow rates, were investigated with a goal of achieving a high particle yield and uniform particle size distribution (Romita et al., 2011). The optimized formulation was sprayed in a 10% w/v suspension containing 9% ferrous fumarate, 6% HPMC, 63% sodium fumarate, and 22% TiO_2 (on a dry weight basis) with 150°C as inlet temperature, feed rate of 0.72 L/h, an atomizing gas flow rate of 667 std L/h at 90 psi, and an aspirator flow rate set to maximum flow in a lab-scale Buchi B290 mini-spray dryer. The process resulted in 64–82% microcapsule yield. The encapsulation efficiency was also good as only 7% of the iron was leached out of the spray-dried microcapsules when soaking the iron powder in EDTA solution. The microcapsules also had desirable morphological characteristics—regular and uniform spherical shape—as determined by microscopic examination. As the diffusion rate of the coating polymer was low, sodium fumarate had to be added as an excipient in the spraying suspension thus preventing the collapse and shrinkage of the microcapsules (Romita et al., 2011).

This fine iron premix powder was blended into iodized, coarse salt to produce a DFS with an iron concentration level of 1000 ppm. Due to the small particle size (~20 μm), 92% of the fine particles were able to adhere to coarse, unrefined, commercial salt samples due to their typically high moisture content. The premix particles were visually indistinguishable on the coarse salt crystals. The DFS samples were stable when stored at 40°C and 60% RH for 6 months, retaining more than 80% of the added iodine after 6 months of storage (Romita et al., 2011).

38.4.2 Rice

Encapsulated premix-based fortification was also demonstrated in the development of Ultra Rice®. Ultra Rice is a reconstituted, nutrient-fortified rice premix made by extrusion, to resemble the shape, size, and appearance of common rice kernels. In the 1980s, Dr. James Cox and his son Mr. Robert Cox invented this idea and later obtained a series of

patents for Ultra Rice technology (US Patents 5,034,378 (Cox, 1991); 4,844,936 (Cox and Cox, 1989); 4,362,748 (Cox, 1982)). The Coxes later transferred the patents to PATH (Program for Appropriate Technology in Health—a Seattle-based NGO) in 1997. Reconstituted Ultra Rice grains were made by extrusion of a wet dough mixture containing selected micronutrients, rice flour, and a structural ingredient—sodium alginate. The extruded rice kernel-shaped particles were stabilized by a surface modification step where a calcium solution was sprayed on the rice grain surface and the ensuing crosslinking reaction between alginate and calcium hardened the particles.

The surface-coating process or the diffusion-driven alginate−calcium gelation leads to two problems: (1) the post-extrusion, surface-coating step is hard to control for uniform distribution of calcium ions on the grain surface; and (2) the hardened grains tend to crack and disintegrate during cooking due to starch expansion against the rigid shell. These constraints have greatly hindered the commercialization of the technology. The process was improved by the development of controlled internal gelation (as illustrated in the Figure 38.4). Specifically, sodium alginate and a calcium salt (with limited solubility, e.g., $CaSO_4$) were added to the formulation prior to extrusion. With the aid of appropriate sequestrants, the crosslinking reaction was delayed until completion of the extrusion. The experiments included a preliminary screening for appropriate techniques/materials followed by an optimization study based on statistical experimental design. This approach not only led to a simplified process by removing the post-extrusion coating step, but also resulted in improved grain integrity as all of the alginate could be converted to a completely interconnected structure throughout the grain, which enclosed and protected the added micronutrients within the rice matrix, ultimately improving its commercial acceptability. The most effective internal gelation system is comprised of alginate, calcium sulfate ($CaSO_4$), and sodium tripolyphosphate (STPP) at an optimized ratio of 3:3:0.6 (w/w) (Li, 2009).

When the Ultra Rice premix made by extrusion is mixed with market white rice—typically at a 1:100 ratio—the resulting product, fortified rice, is nearly identical to untreated rice in odor, taste, and texture (as shown in Figure 38.5), making this a simple and powerful fortification system. So far, two successful formulations of Ultra Rice grains have been developed at the University of Toronto, one fortified with vitamin A containing a robust antioxidant system (Li et al., 2009b) and another containing multiple micronutrients including iron, zinc, and several B vitamins (Li et al., 2008, 2011a). This technology has also been field tested in several countries under the sponsorship of PATH, and demonstrated acceptable results in terms of product stability, sensory properties, and clinical effectiveness (PATH, 2007).

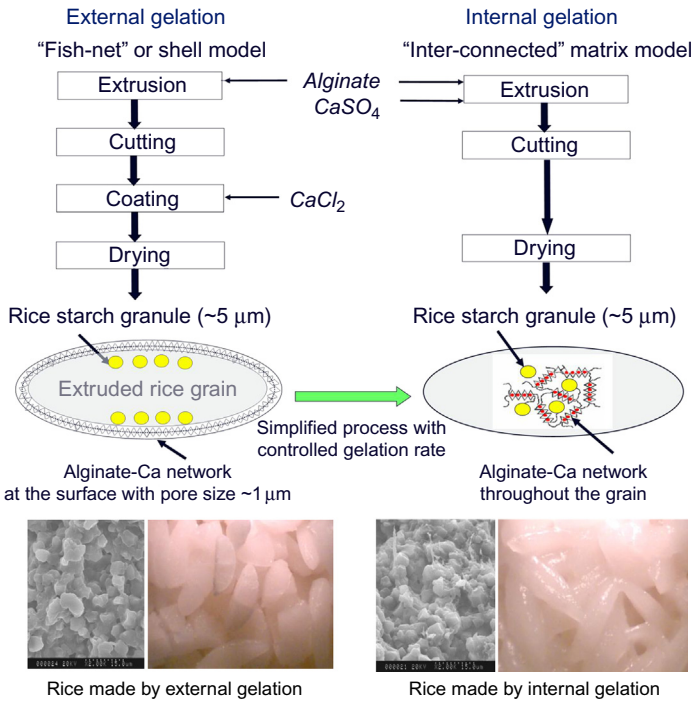

FIGURE 38.4 Schematic process flow for Ultra Rice production using external (left) and internal (right) gelation techniques and microscopic images of rice premix (left: SEM; right: digital microscopic image under normal room light).

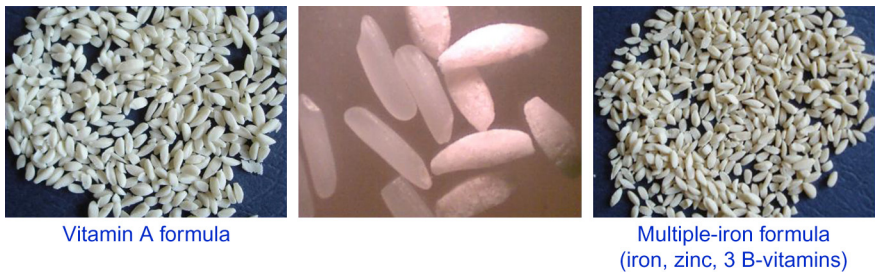

FIGURE 38.5 Ultra Rice premix made by extrusion (digital and microscopic images taken under normal room light).

38.4.2.1 Fortification of Extruded Rice Grains with Vitamin A

Ultra Rice initially started off as a delivery system exclusively for vitamin A. Vitamin A is prone to oxidative degradation by free radicals and isomerization at high temperatures and in the presence of light. Therefore, a stabilizing system containing effective antioxidants had to be included in the extrusion formulation to prevent vitamin A losses. The contribution of the University of Toronto's Food Engineering group was to redesign the antioxidant system to reduce formulation cost and improve vitamin A protection under extreme tropical storage conditions using widely approved food-grade antioxidants (Li et al., 2009b).

Vitamin A readily degrades in the presence of many environmental factors by an apparent first-order mechanism. To develop a stable Ultra Rice, 10 formulations were tested using various combinations of hydrophilic and hydrophobic antioxidants. After a 6-month storage stability test at high temperature and humidity, the best formulation was identified (formulation 9 in Figure 38.6), which retained $\geq 70\%$ of the added vitamin A. It contained BHA and BHT as the fat-soluble antioxidants, ascorbic acid as the water soluble antioxidant, and citric acid and sodium tripolyphosphate (STPP) as metal chelating agent and moisture stabilizer, respectively. The optimized antioxidant system consists of generally accepted food additives with low costs, which greatly improves the commercial viability of Ultra Rice premix as a rice fortificant.

Ideally, rice would be fortified with multiple micronutrients including vitamin A, some B vitamins, and essential elements such as iron and zinc. However, the addition of iron compounds to Ultra Rice, specifically the vitamin A formulation, resulted in a significant increase in the vitamin A degradation rate, mainly due to the catalytic activity of iron on lipid oxidation. In order to minimize the chemical interaction between iron and vitamin A, a coated iron premix was used together with vitamin

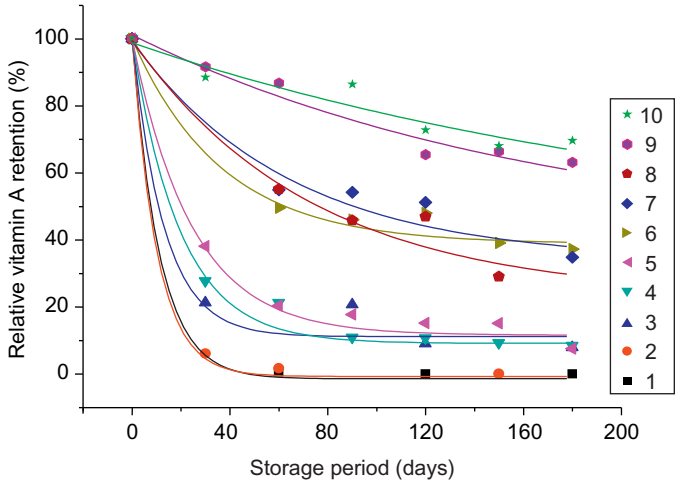

FIGURE 38.6 Relative vitamin A retentions in Ultra Rice premix formulations containing various antioxidant systems over 6 months' storage at 35°C and ~60% RH. Formulation legends: 1—butylated hydroxyanisole (BHA), tocopherol, ascorbic acid, sodium metabisulfite (NaHS); 2—BHA, tocopherol, ascorbic acid; 3—butylated hydroxytoluene (BHT), tocopherol, ascorbic acid; 4—BHA, BHT, tocopherol, ascorbic acid; 5—tert-butylhydroquinone (TBHQ), tocopherol, ascorbic acid; 6—TBHQ, ascorbic acid; 7—TBHQ, ascorbic acid, ascorbyl palmitate, citric acid; 8—BHT, ascorbic acid, ascorbyl palmitate, citric acid, STPP; 9—BHA, BHT, ascorbic acid, citric acid, STPP; 10—TBHQ, ascorbic acid, citric acid, STPP.

A. This led to improved vitamin A retention. Encouraged by the results, the use of coated vitamin A sources (such as a coated retinyl palmitate) was investigated next. Stability was significantly improved to >85% retention of the original vitamin A after 6 months' storage while the rice sensory attributes remained acceptable (Palynchuk, 2010).

38.4.2.2 Fortification of Extruded Rice Grains with Multiple Micronutrients

Based on the optimized antioxidant system, a stable formulation containing iron, zinc, and several B vitamins was developed. The choice of iron compound had great impact on the stability of added vitamins and grain physical characteristics. The effect of several iron sources was studied to see the impact on micronutrient retention, oxidative rancidity/stability, and sensory/physical properties of the premix during storage at 40°C and 60% RH (Li et al., 2008). As expected, the most stable formulation included an insoluble iron source—ferric pyrophosphate (FePP)—which resulted in minimal thiamine loss and acceptable sensory properties. The other iron sources (ferrous fumarate and iron−sodium EDTA) promoted thiamine loss or induced rancidity, respectively. However, as FePP is an insoluble iron compound, its bioavailability in humans is extremely low.

Iron encapsulation has the potential to overcome this major challenge by reducing interactions of iron with other food components that may cause unwanted sensory changes or decreased iron bioavailability. Therefore, the effect of the addition of encapsulated ferrous fumarate with soy stearine as a protective layer was evaluated (Li et al., 2007). After 20 weeks' storage at 40°C and 100% RH, essentially all of the vitamin B1 and ferrous iron were retained; showing that microencapsulation of iron prevents the reaction causing vitamin B1 losses.

When ferric pyrophosphate was used as the iron source, more than 95% of the originally added thiamine and more than 75% of folic acid were retained after 9 months' storage at 40°C and 60% relative humidity. The extruded grains had similar color to polished white rice, as measured by L*, a*, and b* values indicating that a stable fortified rice product with excellent consumer acceptability could be obtained by blending the extruded rice premix into normal market rice (Li et al., 2008).

38.4.3 Application of the Extrusion-Based Microencapsulation Technology Platform to Nutraceutical Delivery through Functional Foods

The extrusion-based microencapsulation technology platform developed for salt and rice fortification enables the production of microencapsulated particles ranging in size from 300 μm to 10 mm, while other unit operations, such as spray drying, freeze drying, and fluidized bed agglomeration, could form smaller particles (10−500 μm). Chemical methods can produce even smaller particles, e.g., nano- or microemulsions, and microfluidic techniques can lead to particles at nano-scales. Appropriate combinations of these physical and chemical methods make it possible to produce microencapsulated particles covering a broad size range for effective delivery of bioactive ingredients or nutraceuticals in food applications.

As shown in the schematic process block diagram (Figure 38.7), chemistry-based microencapsulation techniques, such as gelation, emulsions, or microemulsions, can form nano- or submicron particles carrying selected active ingredients in a liquid phase. After appropriate drying (spray or freeze drying), the active ingredients can be converted to microencapsulated forms as free-flowing powders with particle sizes of 1 to 100 μm. These microparticles, either as

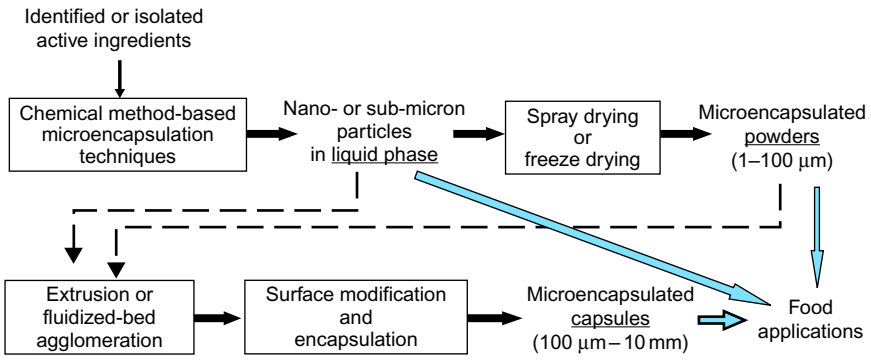

FIGURE 38.7 Schematic process block diagram for developing "engineered" delivery systems of bioactive ingredients for food applications.

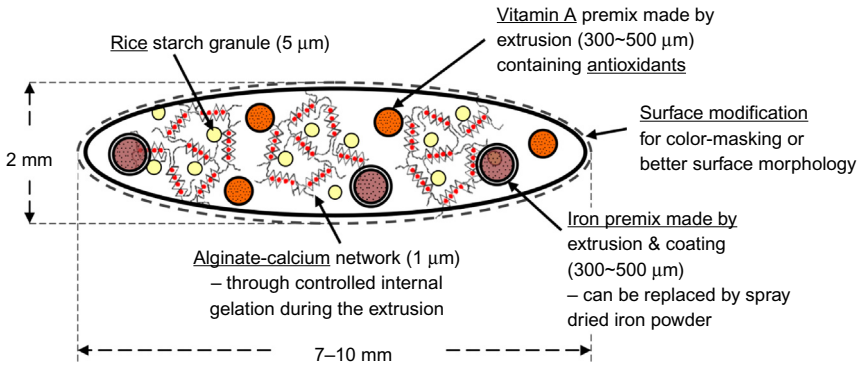

FIGURE 38.8 Schematic structure of Ultra Rice analogues made by extrusion and multiple encapsulation for delivery of multiple nutrients.

encapsulated liquids or as dried powder, can be agglomerated using extrusion or fluidized bed techniques to produce bigger particles ranging from 100 μm to several millimeters in size. The agglomerates thus formed can be further overcoated using a rotating pan/drum coater or a Wurster-type fluidized bed coater to achieve the desired physical/organoleptic properties and chemical stability. The microparticles stabilized in each processing stage can be used in various food applications. For example, vitamin A stabilized in a nanoemulsion or microemulsion may be added directly to dairy products, or can be further processed by extrusion agglomeration to generate salt grain-sized or rice grain-sized particles for fortification of granular foods, as illustrated with a model Ultra Rice grain particle (Figure 38.8).

Current research work by one of the authors, YOL, at California State Polytechnic University, Pomona, involves the investigation of the potential use of plant-derived biopolymers such as dietary fibers and plant protein isolates as building blocks or coating materials for microencapsulating micronutrients (vitamins and minerals) or other nutraceuticals such as phytochemicals, antioxidants, or fermentation by-products from probiotic bacteria. As there are numerous studies in the literature indicating that soluble dietary fibers and plant proteins can help in weight control/management, these compounds would be desirable components of functional food products for overweight or obese consumers. Currently, the food product development literature lacks information on their utilization as delivery matrices for other minor ingredients, focusing only on their utility as bulking agents and excipients.

The novel use of plant-derived biopolymers, e.g., protein isolates and refined dietary fiber extracts, as both biologically functional components (nutraceuticals) and technologically functional components (structural or delivery matrix) for minor bioactive ingredients, will permit the fabrication of integrated delivery systems that combine the multiple functions of protection, targeted delivery, controlled release, and/or enhanced absorption in novel food products.

While stability and bioavailability were of major concern in developing effective delivery systems for vitamins and minerals, the concepts of targeted delivery and controlled release have recently become more attractive to the food industry, especially in functional food development. These concepts originating from oral drug delivery systems, when adapted to food applications, are still mostly focused on nutrient absorption in the body, but the potential use of them in protection of the active components during manufacturing and distribution present further opportunities. Modern food production practice involves many components and multiple processing stages, which require precise control of operation conditions for preserving active ingredients at all stages. Encapsulated ingredients are increasingly used to prevent premature or unwanted interactions. Controlled release of various ingredients, at a targeted stage or at a desired release rate, during food formulating, processing, distribution, even during food preparation, can be critical factors for obtaining desired physicochemical and sensory properties and the ultimate effectiveness of the finished food products. Therefore, a systematic and holistic approach should be followed when developing "smart" delivery systems for nutraceuticals and micronutrients.

38.5 CONCLUSION AND PERSPECTIVES

Clearly, there are many technical challenges to overcome when developing effective delivery systems for micronutrients and nutraceuticals. Potential interactions between the added ingredients and the intrinsic components of the food vehicle, leading to adverse effects on sensory properties of the food vehicle and stability and bioavailability of the added nutrients, are foremost among these. The selection of appropriate food vehicles is of utmost importance as the success

of a fortification program depends on regular consumption of the fortified food by the target populations in uniform amounts. To prevent possible interactions and avoid degradation of the added nutrients while maintaining the desired stability and organoleptic properties of the fortified foods, effective delivery systems typically based on microencapsulation techniques are critical.

Recent research, such as the highlighted program of the Food Engineering Group at the University of Toronto, enhanced the understanding of the importance of interactions between the added components and the food carriers, which will lead to the development of more stable delivery systems for food fortification. An appropriate technology platform must be based on an understanding of microencapsulation, combined with a series of unit operations, such as particle enlargement via extrusion or fluidized bed agglomeration, surface modification, color/flavor masking, and film overcoating by the use of rotating disc or fluidized bed coating processes. The technical approach is adaptable to formulating customized delivery systems for active ingredients in a broad range of applications.

Much current research focuses on extending these technologies to developing "engineered" foods and food ingredients containing both essential micronutrients and desirable nutraceuticals, consequently leading to novel functional food products with added nutritional value for health promotion and disease prevention. Clearly, to achieve this research goal, an integrated approach combining nano-/microencapsulation techniques appropriate to the selected food carriers is essential for meeting the principal fortification criteria: technical and economical feasibility, clinical effectiveness, and consumer acceptance.

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Chapter 39

Taste-Masking and Controlled Delivery of Functional Food Ingredients

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39.1 INTRODUCTION

The World Health Organization (WHO) estimates that 2 billion people worldwide suffer from parasitic infections caused by helminth (parasitic worms in the human intestine) worms. About 520 million people in Brazil, Mexico, China, and India are affected. Half of the incidence is in children aged 7−14 among low socioeconomic populations living in both rural areas and large cities.

It is possible to get rid of these helminths by using a pharmaceutical drug. However, effectiveness of current pharmaceutical treatments is limited by three factors:

1. Reach: this is the ability to consistently reach the affected population.
2. Resistance: Helminth worms develop resistance to common pharmaceutical treatments (Kaminsky et al., 2008).
3. Reinfection: People become reinfected within 4 weeks, and in 3 months accumulate the same level of worms they had before the treatment.

A food solution has the potential to overcome the limitations of pharmaceutical treatments:

1. Reach: This can be maximized by integrating the ingredient into a very commonly consumed food, e.g., rice (similar to iodine in salt) or beverages.
2. Resistance: Technology simultaneously targets three to four receptors to mitigate worm reinfestation, so the probability of successful mutation decreases.
3. Reinfection: The goal is to develop safe dosing that helps to prevent reinfection through daily intake.

Vaccines may provide an alternative, but have low probability of success. The Gates Foundation grant aimed at hookworms may accelerate progress in this area. If successful, it would probably take a long time to develop.

Some essential oils are known to have anti-parasitic property, i.e., they kill the parasites. US Patent Publication No. 2008/0145462 to Enan (Enan, 2008a) describes a composition of an anti-helmenthic oil blend containing 35−45% thymol, 25−35% p-cymene, 1−10% linalool, 1−10% α-pinene, and 20−30% soybean oil. Also, Enan claimed compositions used for insects in another US patent application (Enan, 2008b).

39.2 WHY CONTROLLED DELIVERY?

Incorporating essential oils in food and beverage products at relatively high concentration brings about multiple challenges. For the most part, these challenges necessitate the design and development of a customized delivery system.

1. Form: Essential oils naturally are in a liquid form, and therefore need to be contained in a dry form for ease of use and stability. For the purpose of converting the liquid essential oil into a powder, a protective carrier matrix is needed. When converting liquid oil into a powder, a drying process is required such as spray drying. This is challenging because of the volatility of essential oils. A gentle drying process is needed that would minimize the loss of actives when high temperature is used in the processing procedure.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00039-X

2. Enteric delivery: Why do actives need to be delivered in the intestine (enteric delivery)? It has been established that the efficacy of essential oils for obtaining anti-helmenthic effect is significantly improved when actives are delivered enterically to and near the infection site, i.e., intestine (Enan, 2008a). It has been hypothesized that the actives need to be in physical contact with the target host in the intestinal vicinity. It is also believed that maximum efficacy requires a minimal release in the stomach and slow and sustained release in the intestinal tract. A pH-triggered, targeted, controlled delivery system for certain pharmaceutical active ingredients has been patented by Shefer and Shefer (2010). Several examples of pH-sensitive ingredients are provided in this patent.
3. Taste masking: The blend of essential oils is very aromatic and its flavor/aroma profile is not compatible with that of common food products, especially at the target efficacious dose in the food or beverage products. The sensorial detection threshold of the components of the essential oil is very low (a few ppm). Hence, at these low detection thresholds, even very low water solubility or minute residual oil on the particle surface could pose a product quality challenge. Furthermore, the active ingredients need to be stable in a powdered form over a 12-month shelf-life (distribution in hot/humid tropical regions). Ingredients' inherent flavor/aroma must be undetectable upon opening the package and its inherent flavor/aroma must be undetectable upon dilution of the powder into water, and during the reconstituted food's/beverage's shelf-life (up to 2 hours).

39.3 PRODUCT APPLICATION

Several factors are involved in the selection of an appropriate product for application. Some of those factors are technical, and others are marketing and consumers' socioeconomic status. For the technical factors, the selected application should minimize the time of exposure to water and, therefore, prolong the shelf-life. For that reason, a powdered beverage was selected as a good fit. To be included in a powdered beverage, the particles must be small enough to have minimal organoleptic impact, e.g., gritty mouth feel, minimal incidence of maceration, release of the active compounds leading to undesirable flavor impact, and minimal segregation and/or precipitation to uniformly deliver the intended dose.

The active ingredients in an essential oil can include α-pinene, p-cymene, thymol, and linalool (Enan, 2008a,b, 2011a,b). The volatile nature of some of the active ingredients leads to a very low threshold value for olfactory perception, resulting in an undesirable flavor/taste in the beverage even if a small amount of the actives is released. Use of a corresponding ester, wherever possible, should help in reducing the water solubility and increasing the odor/taste threshold. The esters of linalool and thymol were found to have less negative impact on the flavor of the beverage than the free linalool and thymol. In all microencapsulation processes, there will remain at least marginal surface or free oil. With the desire to mask compounds with low perception thresholds, the surface or free oil must be reduced to levels below perception threshold in the final product matrix and/or in the prepared beverage.

Enteric delivery within a food matrix is achieved by the formation of matrix particles with the internal phase being that of the essential oil blend, with or without a triglyceride as a diluent, and the external phase is that of enteric polymers chosen from the following: shellac, zein, calcium alginate, and denatured whey protein.

39.4 MATRIX TO CORE COMPATIBILITY

It is important to emphasize that when selecting a polymer to form the functional matrix, its compatibility with the actives in the core becomes critical. For example, the most commonly used enteric food-grade polymers are zein and shellac. The traditional practice in using these polymers is to solubilize them in a mixture of water with other solvents such as alcohol. When doing so, the polymer becomes incompatible with the hydrophobic core such as essential oil since it possesses high solubility/miscibility in the solvent. A better solvent for such a matrix is alkalized water. For example, shellac solution can be prepared without an organic solvent when the pH of aqueous shellac solution is raised above the solubility threshold. A twenty-five percent solution of shellac in water can be achieved when the pH is raised above the solubility threshold. The most common practice is to use ammonium hydroxide at a pH of >7.0. A similar practice will apply for solubilization of zein except that the pH of zein solution needs to be raised significantly higher, e.g., to around pH 11 using sodium hydroxide.

In doing so, a phase separation of the core entrapped within the matrix can occur without partitioning into the water phase. Phase separation can be achieved when the pH of the mixture (polymer and essential oil emulsion) is lowered below the solubility threshold of the corresponding polymer. The efficiency of encapsulation/entrapment is dependent on the compatibility between the core actives and the nature of the matrix.

In other situations, modifying the actives present in the core should be considered for improving compatibility with the matrix (without altering intended functionality). For example, modifying the core by increasing the hydrophobicity of selected components of the oil blend had a significant improvement not only in encapsulation efficiency but also in taste masking and final product quality.

The inclusion of esters in place of parent components, where possible, led to an increase in the payload of the parent active components in the final microparticle due to a decrease in the water solubility of the encapsulated oil blend containing esters (Gaonkar et al., 2010). Due to the low water solubility and/or increased hydrophobicity, the ester has higher microencapsulation efficiency than the non-esterified parent components, such as thymol and linalool. The efficiency increases about 50 to about 100% over the efficiency observed when using non-esterified functional components. Furthermore, esters have a higher olfactory perception threshold than the parent components, such that the amount of esters necessary to be perceived is more than the amount of non-esterified thymol and linalool. Although a large volume of water is used during processing, the use of esters of thymol and linalool has reduced leaching from the wet or dry forms of the microparticles. The ability to limit losses during processing allows for the control over the final ratio of components of the functional oil blend in the beverage.

When released and ingested in the intestinal tract, the modified form (e.g., esterified form) of the functional ingredient converts back to the parent form upon hydrolysis and provides the same functional benefits as would be the case the parent functional component had been consumed. Thymol is a crystalline solid at room temperature and the substitution of thymyl ester in place of thymol allowed for easier processing since all actives were in the liquid form in that formulation. Esterification of thymol with long-chain fatty acids was also demonstrated to increase the thymol perception threshold to several folds. In summary, esterification of the two essential oil components in the oil blend made the taste masking less challenging.

39.5 PROCESS OF MICROENCAPSULATION

The process of microencapsulation involves multiple steps: emulsification, particle formation and entrapment of the actives, dewatering (removal of the majority of moisture) and drying, particle sizing, and coating (Akashe et al., 2010a; Gaonkar et al., 2010).

39.5.1 Emulsification

First, a coarse emulsion of an essential oil blend is prepared using the solution of a suitable matrix material(s) such as shellac, zein, caseinate, etc. The coarse emulsion is then homogenized to produce a stable oil-in-water emulsion containing very fine droplets of an essential oil blend. When forming an emulsion, it is important to determine if the matrix polymer being used has emulsification properties. Otherwise, an emulsifier is needed. Most proteins, such as the ones suggested and shellac have emulsification properties. Also, the size of the oil droplets and their stability during wet processing are critical for both the amount of oil blend entrapped within the microparticles (encapsulation efficiency) and the surface oil. The research determined that droplets of submicron size are desirable, and this was achieved with high pressure homogenization and multiple passes.

Light micrographs of the emulsions obtained using different homogenization conditions are illustrated in Figure 39.1. Smaller droplets can be achieved by using higher pressure and two passes. Oil size distributions of the emulsions are illustrated in Figure 39.2 and the values for drop diameter (D10, D50, and D90) are given in Table 39.1. Again, use of higher homogenization pressure and multiple passes provides an emulsion with smaller droplets and higher emulsion stability.

39.5.2 Particle Formation and Entrapment

The emulsion is titrated with an acid, such as citric acid solution, in an amount effective to decrease the pH below the isoelectric or solubility point of the enteric materials causing phase separation and inducing precipitation of the enteric material out of solution with the functional ingredient being microencapsulated therein. This leads to an entrapment of very fine droplets of hydrophobic oil blend in a matrix material, thus creating the slurry of an aqueous solution and precipitate. The slurry includes a particulate precipitate.

It is believed that as the pH of the emulsion drops below the solubility point, enteric materials, such as soy protein, zein, and sodium caseinate, may crosslink to like particles or to one another to form a matrix, the functional, hydrophobic liquid being microencapsulated within the matrix. As a result of the crosslinking, the functional ingredient is homogeneously

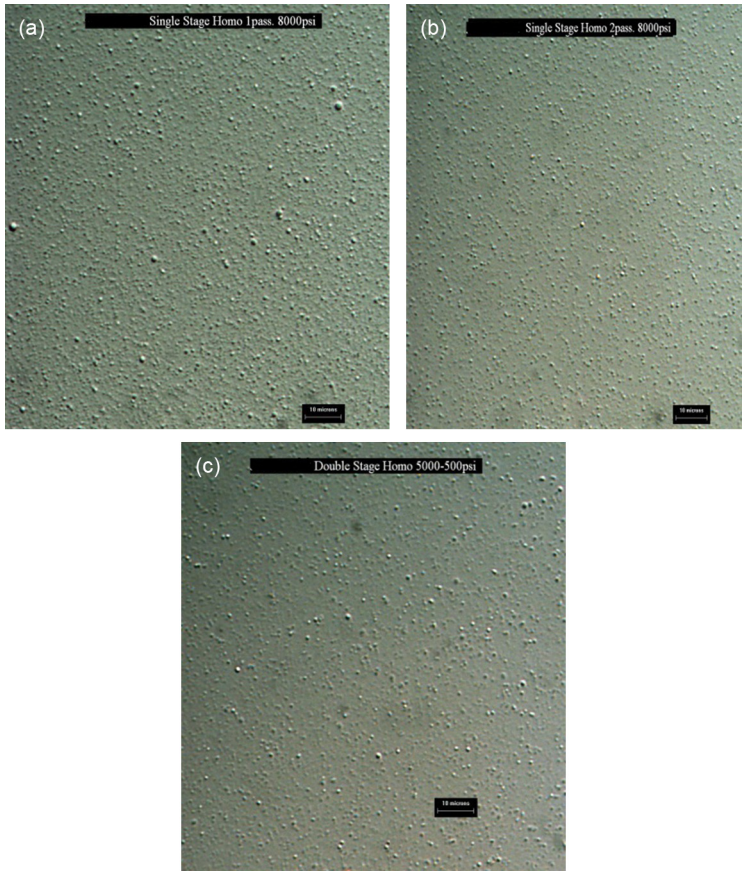

FIGURE 39.1 Micrographs of oil-in-water emulsions prepared using a functional oil blend at three different homogenization conditions: (a) one pass/single-stage homogenizer at 8000 psi, (b) two passes/single-stage homogenizer at 8000 psi, (c) one pass/two-stage homogenizer at 5000/500 psi.

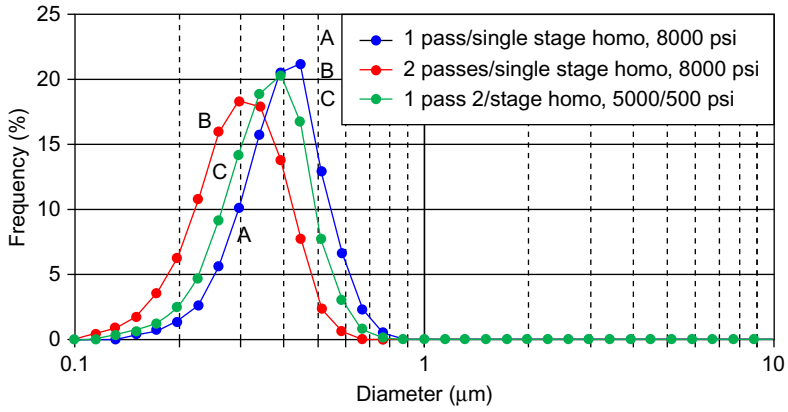

FIGURE 39.2 Oil drop size distributions of oil-in-water emulsions prepared using a functional oil blend: (A) one pass/single-stage homogenizer at 8000 psi, (B) two passes/single-stage homogenizer at 8000 psi, (C) one pass/two-stage homogenizer at 5000/500 psi.

dispersed throughout the matrix. The matrix further provides a seal for the functional ingredient. As a result, the impact of the functional ingredient on the organoleptic qualities of the finished product is generally correlated to any functional ingredient remaining adhered to the outer surface of the enteric material that is not coated (generally called "surface oil").

As indicated above, the acid is added slowly (titrated in) until the target pH is achieved. The pH at which precipitation occurs depends on the polymer used (pH 3−5 for shellac and zein, and 4.5 for caseinate). Particulates were mixed

TABLE 39.1 Drop Size Data for Oil-in-Water Emulsions Prepared Using an Essential Oil Blend at Three Different Homogenization Conditions

Homogenization Conditions	D10 (μm)	D50 (μm) (Median)	D90 (μm)
One pass; single-stage homogenizer (8000 psi)	0.26	0.37	0.51
Two passes; single-stage homogenizer (8000 psi)	0.19	0.28	0.39
One pass; two-stage homogenizer (5000/500 psi)	0.23	0.34	0.46

D10—10% of the particles are less than D10.
D50—50% of the particles are less than D50.
D90—90% of the particles are less than D90.

in at a target pH for enough time to allow for complete curing of the matrix (about 30 minutes). The slurry may be allowed to settle, resulting in a clear division of the liquid or supernatant and the settled particles.

39.5.3 Dewatering and Drying

In this step, precipitate is reclaimed by removing the excess water by filtering, centrifugation, or pressing. The wet cake, thus obtained, is washed and dried to produce a dried cake. Dehydration of the wet cake is achieved by drying, such as fluid bed drying. Fluid bed drying is preferred over other drying methods because it can be carried out at a fairly low temperature. The surface oil on the outer surface of the particulate precipitate is usually less than about 1% by weight of the final product. A surface oil remover may be added to aid in removing residual surface oil from the precipitate as described elsewhere (Akashe et al., 2010b). The surface oil remover can also be added at any point in the preparation after acid titration.

39.5.4 Particle Sizing

Once dry, the powder is sifted to the target size. Our research determined that sizes of 75−250 μm are preferred. This range is suitable for particle coating and the particle size is manageable so as to not cause a sensorial defect in final product application such as a beverage.

Typically, the bigger particles are milled and resifted, and the fines are recycled back into the wet batch process. It is important to select the proper milling equipment, and fine tune milling parameters. Overmilling will produce excess fines (lower yields), and may also generate increased surface oil (actives on the surface of the microparticles). On the other hand, undermilling will result in an inefficient processing throughput. Another problem to watch out for is generating excess heat during grinding if the equipment or parameters are not set properly.

39.5.5 Particle Coating

Particle coating is a process where a film-forming enteric polymer is deposited in a multi-layering process on the surface of each individual particle (Sriamornsak et al., 2011). Typically, this process is carried out in a fluid bed coater and the most common is the Wurster process. In the Wurster process, particles are fluidized and a solution of a coating polymer is sprayed onto the particles from the bottom.

The purpose of particle coating is two-fold: (1) to smooth out the particle surface from the above processing to provide a sealed surface so no leakage of the actives occurs when added to food products such as a beverage, and (2) to strengthen the enteric properties of the particles prepared, and provide the desired enteric release profile.

Based on the extensive research and screening, it was established that two types of coating are needed to satisfy the above objectives. The first coating (inner coating) is prepared with zein (corn protein), and the second coating (outer coating) is prepared with shellac. Enteric properties of shellac (Colz-Berner and Zastrow, 2001; Santos et al., 2002; Signorino, 2003; Farag and Leopold, 2009) and zein (Fu, et al., 1999; Parris et al., 2005) are well documented.

The first and second coatings each have coating thicknesses of about 1 μm to about 10 μm. The coated matrix particles can then be sieved to meet the desired particle size. Additional processing steps after the second coating may include drying to remove moisture from the coated microcapsules to form a material having less than 5% moisture.

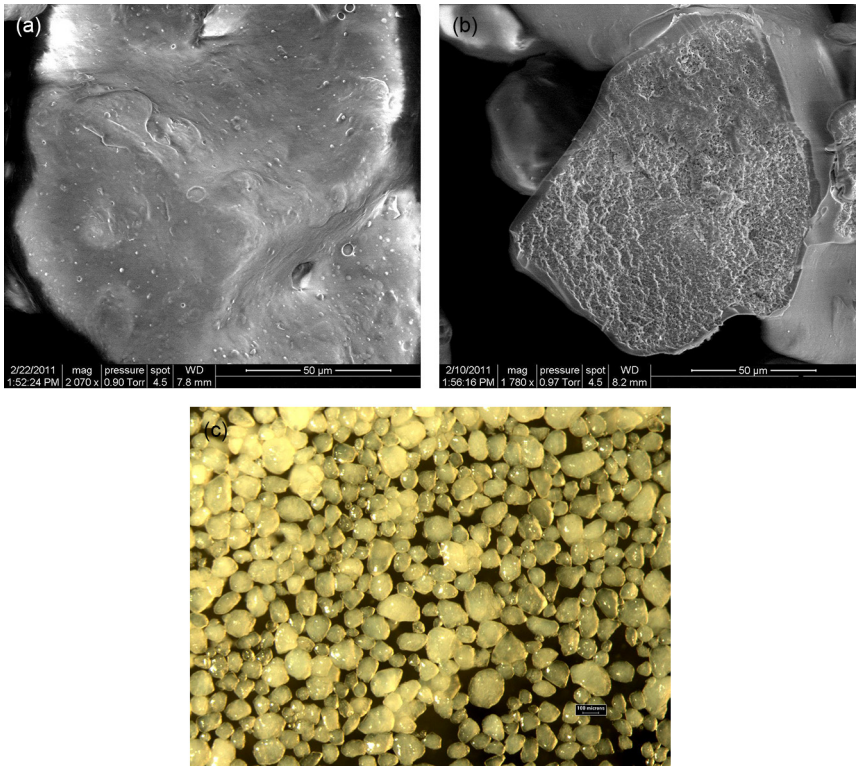

FIGURE 39.3 Light micrographic images of coated (zein and shellac) microparticles prepared using a functional oil blend: (a) micrograph of a coated microparticle, (b) cross-sectional view of a coated microparticle, and (c) coated microparticles.

39.5.5.1 Coating Solution

Solutions of both coating materials can be prepared either in a mixture of water and alcohol or alkalized water. Both solutions may contain suitable plasticizer (glycerin for alcohol/water solvent and sorbitol for alkalized water-based solvent). Solution viscosity is important since it will affect the atomization and throughput. We found that a solution of about 10−12% solids is optimal.

39.5.5.2 Coating Process

The particles are fluidized in a fluid bed coater using a bottom Wurster insert sufficiently to guarantee a circulating upward flow through the insert and downward flow at the outside of the insert. Coating solution is fed with a peristaltic pump, and atomized at a pressure of 1.5−2.0 bar. Air temperature is controlled to maintain a product temperature in the range of 25−35°C.

Light micrographic images of single and multiple microparticles, shown in Figures 39.3a and c, respectively, demonstrate that the microparticles are sealed nicely and that they exhibit a smooth surface. Furthermore, the cross-sectional view of a microparticle presented in Figure 39.3b shows fairly homogeneous coating. Also, the scanning electron microscope image of a single microparticle depicted in Figure 39.4 shows that the coating is around 3−5 μm in thickness.

39.6 CHARACTERIZATION OF MICROPARTICLES

Several analytical tests were developed in order to determine robustness and functionality of the microparticles. Specifications for success criteria were established for each individual test, to provide clear guidance for research direction:

1. Payload, residual surface oil, and proportion of active compounds
2. Amount of actives released (leaked) in a beverage
3. Sensory evaluation

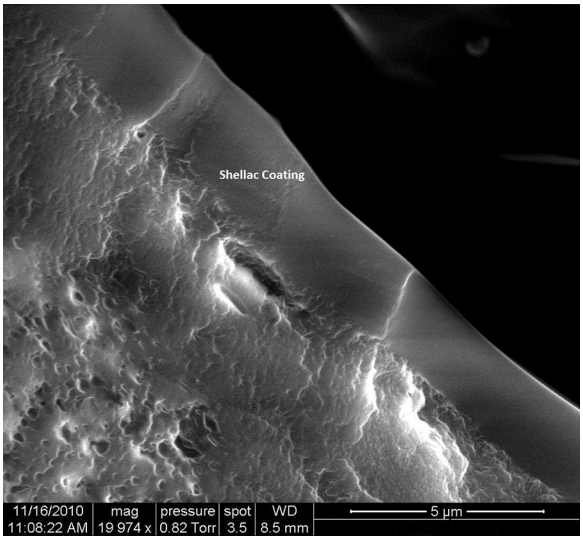

FIGURE 39.4 Scanning electron microscopic cross-sectional image of a single microparticle of a functional oil blend that is coated with zein and shellac.

4. Shelf-life
5. *In vitro* release
6. Efficacy testing (*in vitro* and *in vivo* (preclinical study)).

39.6.1 Payload, Residual Surface Oil, and Proportion of Active Compounds

Methods for analyzing payload and surface oil were developed. The payload analysis involved solvent extraction and gas chromatography analysis. The methodology was fine tuned to provide a very effective extraction and accurate estimation of each compound. Total (composite) payload was calculated based on the sum of the individual component in the essential oil blend. Additionally, the ratio of the components of the oil blend was determined. It is important to maintain the ratio between the components to ensure the integrity of the oil blend during and after microencapsulation.

Residual surface oil was quantified based on a customized methodology developed internally. The testing was based on a quick hexane extraction of the particles followed by gas chromatography analysis.

Payload was consistently analyzed at 22−25% prior to coating. After coating, the payloads were analyzed at 17−20%. Table 39.2 provides information on payload (total and individual components) and surface oil (derived from hexane extractables) of two double-coated (zein and shellac) microparticle samples containing an essential oil blend having four components and zein and caseinate. The proportion of the active compounds was maintained throughout the process and in the final microparticles so as to provide the maximum efficacy.

A testing protocol was designed to set the specifications for an acceptable level of surface oil that would not have an adverse effect on the final sensory quality. Total surface oil of less than 0.5% was required for achieving a product with no off odor/taste. It can be seen from Table 39.2 that the surface oil was negligible in the coated microparticles.

39.6.2 Amount of Actives Released in a Beverage

The amount of the actives released (leaked) in water when cold water was added to the beverage powder containing microparticles of essential oil blend was measured. The method involved filtration to isolate any microparticles, extraction, and gas chromatography analysis of the extract.

39.6.3 Sensory Evaluation

Sensory evaluation in the final product (e.g., beverage) is the most important determinant of knowing if the technology is effective in masking the off taste/odor of the actives. As mentioned before, the research was focused on a powdered

TABLE 39.2 Payload (Total and Individual Components) and Hexane Extractables of Two Double-coated * Microparticles Containing Essential Oil Blend in Zein and Caseinate Matrix

		Component 1	Component 2	Component 3	Ester of Component 3	Component 4	Ester of Component 4	Total Actives
		Weight%	Weight%	Weight%	Weight%	Weight%	Weight%	Weight %
Sample 1	Payload	0.78	1.5	<0.1	2.5	<0.1	14.1	**18.9**
	Hexane extract	<0.001	<0.001	<0.001	<0.001	<0.002	0.070	0.071
Sample 2	Payload	0.75	1.5	<0.1	2.5	<0.1	14.3	**19.1**
	Hexane extract	<0.001	<0.001	<0.001	<0.001	<0.002	0.067	0.068

*Microparticles were coated with an inner coating of zein and an outer coating of shellac.

beverage application. Typically, the microparticle in the powder form is dry blended with the powder beverage in an amount intended to deliver an efficacious dose per serving of the beverage. The aim was to deliver the needed preventive daily intake in one serving per day. A typical dose was estimated based on preclinical testing. The powdered beverage is hydrated in water and consumed within 30 minutes.

Sensory evaluation provided a valuable tool for monitoring and measuring progress of the technology improvements. The goal was achieved when no off taste was detected when the beverage was tasted.

In addition to direct sensory analysis, a quantitative test was developed to correlate directly with sensory evaluation by measuring percent actives released in the beverage at 30 minutes. In this test, after the powdered beverage is hydrated, and held for 30 minutes, the amounts of active components leaked/leached/released into the beverage were quantified. A successful technology should meet the threshold.

39.6.4 Shelf-Life

Technology robustness is determined by how long the microparticles are good for, i.e., shelf-life. For the technology to pass the feasibility test, it needs to pass the shelf-life testing both "as is" and in the final product application, e.g., powdered beverage. Additionally, it should also pass *in vitro* digestion and release tests and *in vitro* and *in vivo* efficacy tests.

39.6.5 *In Vitro* Release

In order to demonstrate that the delivery of the actives is enteric (minimal release of an active in the stomach and complete release in the intestine), an *in vitro* digestion test is carried out. This test is based on a USP dissolution testing method Chapter 711, which has been modified. Chapter 711 is flexible as to which solutions/reagents are used for the digestion medium, depending on the objectives of the test; these can range from simple buffers to more complex systems that use acid/pepsin, pancreatin, etc. We used a multistage system with acid/pepsin for the stomach simulation and pancreatin/bile salts for the small intestine simulation. Chapter 711 specified dissolution apparatus could not be used because of the volatile nature of the active compounds. Hence, the work was done in sealed tubes instead of the specified open dissolution.

Multiple samples were evaluated for static *in vitro* digestion. In this test, samples are incubated for 30 minutes in stomach fluid, and then transitioned for incubation in an intestinal fluid. Samples were pulled out and analyzed at different time intervals.

Figure 39.5 shows percent composite release of essential oil components during the *in vitro* digestion of the microparticles made from the oil blend. A minimal release of the actives was found to occur in the first 30 minutes of incubation in

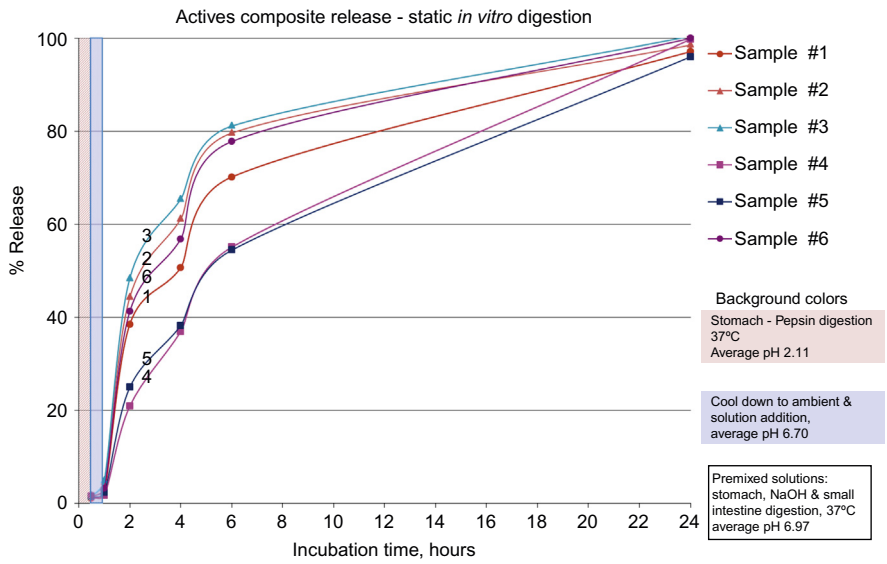

FIGURE 39.5 Percent composite release of functional oil components during the *in vitro* digestion of the microparticles of the functional oil blend.

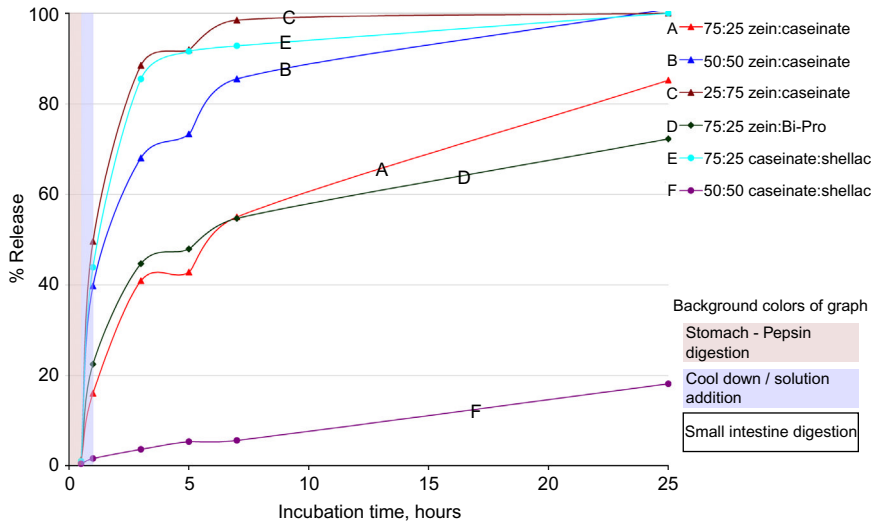

FIGURE 39.6 Percent active released with time in an *in vitro* digestion of microparticles of functional oil containing an ester of the active component in different matrices.

the stomach environment, and then a gradual increase of the actives occurred with an increase in the incubation time in the intestinal environment. Nearly 100% was released after 24 hours of incubation in the intestinal environment.

Figure 39.6 depicts percent release of an active component with time during an *in vitro* digestion of coated microparticles of an essential oil containing components. All the microparticles were coated with an inner coating of zein and an outer coating of shellac. When the matrix is made up of zein and caseinate, the release of thymol decreases as the ratio of zein:caseinate is increased. At the same ratio (75:25), release was similar when caseinate was replaced with whey protein isolate (BiPro). In microparticles made using caseinate and shellac as a matrix, release rate of the active increased with an increase in the ratio of caseinate:shellac in the matrix. It is apparent that the release of the active can be changed by manipulating the composition (matrix) of the microparticles.

39.6.6 Efficacy Testing

Anti-helmenthic efficacy of microencapsulated actives was evaluated against multiple intestinal parasites, including *Hymenolepis nana*, *Trichuris muris*, and *Ascaris*. Most of the testing was done either *in vitro* or *in vivo* with the selected host.

39.6.6.1 *In Vitro* Efficacy Testing

Two tests developed by Dr. Enan at Vanderbilt University (Lei et al., 2010) were utilized mostly for screening of various microparticles and blends of actives. The first test involved *Caenorhabditis elegans*: the microcapsules with the actives were incubated in selected medium on a petri dish in presence of *C. elegans* or other model parasites—the efficacy was measured as percent mortality (number of dead parasites on the plate). The second test involved cyclic AMP (cAMP) activation in a test kit. Intracellular cAMP was activated and caused an increase in calcium ions when the antihelmenthic actives were released and bound to cellular tyramine receptor; both cAMP and calcium can be quantified.

39.6.6.2 *In Vivo* Efficacy Testing (Preclinical Study)

In this test, several feeding studies were conducted with different parasite models in various hosts. The most common is *H. nana* in mouse host. Also, studies involved feeding of *T. muris* in rats and rabbits, and *Ascaris* in a pig as a host. Each study design was controlled, with variations in feeding regimens and dose per day.

Results from both *in vitro* and *in vivo* studies consistently showed efficacy from the prototypes prepared with the invented enteric delivery system. Efficacious dose was established based on a dose−response study and delivered in a single serving of a beverage (details of these studies will be published separately). One needs to add about five times the microencapsulated functional essential oil blend per serving if the payload is 20%.

39.7 SUMMARY

The development of a functional food for the prevention of infection from intestinal parasites was possible with the invention of a suitable controlled delivery system that was capable of entrapping the actives efficiently, masking the taste completely, and delivering the actives in the intestine efficaciously. In addition to selecting the proper polymers, a combination of matrix encapsulation (entrapment) and coating was essential for optimizing the delivery of essential oils in powder beverage application. The *in vitro* and *in vivo* studies consistently showed efficacy.

ACKNOWLEDGMENTS

The assistance received in the areas of analytical, microscopy, processing, and *in vitro* and *in vivo* tests from numerous former colleagues from Kraft Foods, the current team members from Mondelēz International, and Dr. Essam Enan is gratefully acknowledged. In addition, the authors would like to thank the management (Research & Nutrition) of Mondelēz International for permission to publish this work.

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Chapter 40

Microencapsulated Enzymes in Food Applications

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40.1 INTRODUCTION

Enzymes have been used in food applications for centuries (Beckhorn et al., 1965). In early uses, the enzymes were from plant or animal sources such as amylases in grain used in brewing or protease in rennet from animals in the production of cheese. Today, though these enzymes are still used, many new activities have taken advantage of advances in biotechnology and are produced in bulk via fermentation processes. In addition, the majority of enzyme products available in the early years were in the form of liquid products. It was not until the 1970s that immobilized enzymes were first utilized (Olson and Richardson, 1974). Since then more and more applications of immobilization and encapsulation have been utilized for enzymes in food as they provide opportunities to apply activities where previously it was not possible (Zuidam and Nedovic, 2010). Many of the encapsulation approaches have been adopted from other industries such as pharmaceutical, industrial processing, and animal nutrition where the encapsulation protects and delivers the enzyme or active when and where it is required.

Encapsulation can be accomplished through many technologies, covered in detail in previous chapters. These include spray drying, agglomeration or multistage drying, fluid bed granulation or coating, high shear granulation, spray chilling, emulsion polymerization, and liposome formation. Historically, simple processes such as spray drying, agglomeration, or granulation have been the main approaches in food applications as they provide an easy-to-handle material that is stable, reduces exposure, and performs well in most applications. However, more recently advanced forms of encapsulation are being used more and more as delivery of enzyme activities can be tailored or targeted to provide unique properties or advanced processes to formulate food products (Gibbs et al., 1999). This chapter explores the challenges that encapsulation can address for enzymes and their use in food applications.

40.2 FOOD ENZYME MARKET

The food enzyme market continues to grow on an annual basis. Over the period from 2006 to 2010, the growth rate has been greater than 10% per annum (Leatherhead Food Research, 2011). New food applications and identification of new enzyme activities contribute to this factor. Encapsulation is also helping to grow the market as it allows the use of existing enzyme activities in new applications where they were previously not used due to issues with incompatibility, stability, or delivery. As new needs arise, encapsulation is one route to enable to meet these needs.

A survey of enzyme activities that are used in the market and the applications where they are used are listed in Table 40.1.

40.2.1 Enzyme Manufacturers

There are several companies that produce and market enzymes for use in food. Many of these can be found listed as members of various industrial groups such as the Association of Manufactures and Formulators of Enzyme Products

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00040-6

TABLE 40.1 Enzyme Use in Food Applications

Enzyme	Application
Amylase	Baking, brewing, sweeteners
Beta-gluconase	Brewing, juice
Catalase	Dairy
Cellulase	Brewing
Glucoamylase	Sweeteners, baking
Glucose oxidase	Baking, juice
Isomerase	Sweeteners
Lactase	Dairy
Lipase	Dairy, oil production
Pectinase	Juice
Protease	Protein modification, dairy, brewing, baking
Xylanase	Brewing

TABLE 40.2 Producers of Food Enzymes

Company	Location	Website
AB Enzymes	Germany	www.abenzymes.com
Amano	Japan	www.amano-enzyme.co.jp
Biocatalysts	UK	www.biocatalyts.com
Chr Hansen	Denmark	www.chr-hansen.com
DSM	Netherlands	www.dsm.com
DuPont	US, Delaware	www.dupont.com
Novozymes	Denmark	www.Novozymes.com

(AMFEP, www.amfep.org) and the Enzyme Technical Association (ETA, www.enzymetechnicalassoc.org). Several major suppliers are listed in Table 40.2.

40.2.2 Enzyme Production

Enzymes can be obtained and produced from plant and animal sources or through microbial fermentation. Enzymes from plants or animals are typically extracted from the origin and then purified and concentrated to provide a final product for use. The extraction can be done using water or organic solvents by a variety of means (Schwimmer, 1981). This can be an expensive path but serves the need for many food products.

Alternatively and more common today, enzymes are produced by large-scale fermentation processes (Aehle, 2004). This can be advantageous as it allows the producer to enhance the production and ease of purification of the desired enzyme, resulting in a more economical enzyme product. In addition, the producer can genetically modify the enzyme to increase or tailor its activity on a specific substrate. The discovery and production can be generalized in the following steps:

1. Identifying the enzyme that provides the desired activity;
2. Isolating and transforming the gene encoding the enzyme into a chosen production host (bacteria, yeast, or fungus);
3. Growing the host in a fermentation process to maximize expression of the enzyme;

4. Separating the enzyme from the host organism and media components;
5. Purifying and concentrating the enzyme; and
6. Formulating the enzyme into a solid or liquid product.

40.3 ENZYME PROPERTIES AND CHALLENGES

Enzyme proteins are catalysts that carry out specific biochemical reactions in an efficient manner. In the case of food applications, they can be used to modify or enhance processes, textures, flavors, and shelf-life, to name a few (Schwimmer, 1981). The utilization of enzymes in food continues to grow as new activities are discovered and applied (Fernandes, 2010).

Though enzymes are broadly utilized, they are vulnerable to degradation by a variety of means, which can severely reduce or eliminate their ability to carry out any reaction. They are subject to denaturation or modification through temperature, moisture, pH, shear, oxidation, deamidation, glycation, and more. Each of these factors can affect the shelf-life of the enzyme product as well as impact the performance in the application. In some cases, the application takes advantage of these properties to shut down their activity, such as in the use of amylase in bread production, where the enzyme denatures during the baking cycle to halt the breakdown of starch to give the bread desired properties (Livermore et al., 1998). However, the vulnerability of enzymes to these environmental forces requires that the enzymes be formulated so that they maintain their activity during production, storage, shipment, and use. The stabilization of enzyme products can be accomplished through either formulating in a liquid form through the use of sugars, sugar alcohols, buffers, and salts, or by drying the enzymes to arrest their mobility and reduce the kinetics of any modifications. In addition, solid products provide the benefit of being more resistant to microbial contamination. This is especially important in an age where the use of preservatives in food is becoming more restricted globally.

40.3.1 Enzyme Systems

Enzymes are frequently blended with other enzymes or food additives to provide systems that contribute unique properties in the application. In many cases, the materials used in these blends may be incompatible, such as proteases combined with other enzymes leading to proteolysis of the non-protease enzyme. Also, other additives such as some salts or emulsifiers may lead to the denaturation of the enzymes during storage. Both of these factors raise additional challenges when developing these systems. The use of encapsulated enzyme products enables the blends to be created more easily compared to creating blends with liquid enzymes. By isolating or drying the enzyme, reactions with denaturing components are dramatically reduced. The resulting enzyme system can easily be incorporated into food applications for providing added benefits.

40.3.2 Safety and Hygiene

Safe handling is another key aspect for enzymes to consider (Vanhanen et al., 1996). Enzymes like any other protein can create allergies through exposure via inhalation. In order to reduce the potential of exposure, it is important to create encapsulates that are large enough in particle size such that they do not aerosolize well or are not respirable. Alternately, one can include dust reduction additives, such as oils, that will also help to prevent aerosolization during handling and use.

40.4 ENCAPSULATION

The approach to encapsulation of enzymes for use in food applications is dependent on the specific application or challenge. For example, where enzymes are added to dry blends in baking, the enzyme particle must maintain a small particle size in order not to be removed in any subsequent sifting operations. Or the encapsulated enzyme may be needed to deliver the enzyme at a specific point in the application through controlled delivery. This can be achieved through the use of specific coating approaches depending on the trigger of delivery such as temperature or solubility. Regardless of the specific need, encapsulation strategy continues to grow in use and capability (Poncelet et al., 2011). Several of these are outlined in case studies later in this chapter where specific approaches enable improved processes or products.

Many of the encapsulation processes covered in this chapter have been discussed in more detail elsewhere in this book. However, the processing of enzymes raises specific concerns that must be considered when choosing and designing an encapsulation method. These are discussed in more detail in the following sections.

40.4.1 Spray Drying/Agglomeration

Spray drying of enzymes was likely one of the first approaches to creating a dry enzyme product. More details on the spray drying process can be found in Chapters 5 and 6. The powder generated from this process is easily blended into many applications such as baking. Spray drying is considered to be an efficient and low-cost process. However, it brings some significant challenges in the drying of enzymes. Two key considerations are the general thermal labile properties and safety considerations of the resulting powder and exposure of workers.

As discussed previously, enzymes and proteins can be denatured through exposure to high temperatures. The temperature at which this occurs is specific to each protein, but most will denature below 100°C when in the liquid state and become inactive. Once dried, an enzyme is much more stable when exposed to high temperatures. To overcome the denaturing propensity during spray drying, there are two approaches used: process and formulation (Tsotsas and Mujumdar, 2011). During the drying cycle the processing parameters should be adjusted such that the enzyme is exposed to high temperatures for the shortest time possible prior to reaching the dry state. This can be done by reducing the inlet air temperature or decreasing the particle size of the atomized liquid droplets going into the dryer. One way to enable this while allowing the particles sufficient time to fully dry is to use a tall dryer, but this is not always feasible (Mujumdar, 2007).

The other approach of reducing the denaturation of the enzyme is through formulation (Carpenter and Manning, 2002). By combining the liquid enzyme with materials that will protect the enzyme structure from denaturing during exposure to high temperatures, yields of active enzyme will increase. The stabilizing compounds that are generally used include sugars (Allison et al., 1999; DePaz et al., 2000), sugar alcohols, polyols (Gekko and Timasheff, 1981; Bhat and Timasheff, 1992), salts (Arakawa and Timasheff, 1982), and metals (Karunakar et al., 2002). The choice of compounds will depend on each enzyme and will require experimentation to determine which is the best. One approach to understanding which compounds can increase the stability is to utilize differential scanning calorimetry (DSC) (Remmele and Gombotz, 2000). In this analysis, one can explore which compounds increase the melting temperature of the protein. This is a good indication of the ability of a compound to increase the stability of the protein structure. When choosing the compound to use, one must also be aware of how it may impact the drying process and final product. Some polyols, for example, will not "dry" and will require other compounds such as starch to absorb the polyol to ensure that the final product will still have acceptable flow properties. Other properties to consider are if it is hygroscopic, which may lead to shorter shelf-life, or how the material may affect the application, for example, adding an off flavor. The combination of modifying process parameters and enzyme formulation chemicals can ultimately lead to acceptable activity yields and final product properties.

Efficient handling of the spray-dried powders is also a key consideration. Exposure to enzymes can lead to skin irritation or allergic response. The latter is usually the result of inhalation of enzyme aerosols leading to sensitization of the individual and reaction during subsequent exposures (Vanhanen et al., 1996). There are many approaches to modifying the spray-dried powders to reduce the risk of exposure by subjecting the powder to additional granulation process such as adding a dust reduction agent or agglomerating the particles to increase the particle size.

The dust reduction agents that are typically utilized include lipids, oils, or fats. Each of these will modify the properties of the powders, such that the propensity to aerosolize is dramatically reduced. The agent would be added during a subsequent blending process after the powder has been produced. This can be done in a continuous fashion or in two separate batch processes depending on the setup of the production site. One advantage to using the dust reduction agents is that it is a simple approach that requires little material and the agents are usually added as part of the application anyway, such as emulsifiers in baking. A potential negative to this approach is that the flow properties and subsequent dosing into the application can be affected.

Increasing the particle size of the powders by agglomerating is another approach to reducing the likelihood of exposure through inhalation. This can be done in two separate processes or combined in one unit. One approach to agglomerating the particles can be carried out in a fluid bed apparatus as described in US Patent 6,120,811 (Ghani, 2000) where microgranules are created and coated to reduce dust. In the description it is stated that the objective is to generate particles that are low dusting and have suitable particle size and dissolution properties to be useful in baking applications. The approach taken is to use fluidized bed agglomeration to form larger particles from fine-sized starting materials utilizing appropriate binders (sugar, polymer, gum, fat, or even water if the powder itself has binding properties). Once the agglomerates have reached the desired size range (20−400 μm), the process is changed to a coating operation where a food-grade polymer is applied to "envelop the enzyme and to hold the agglomerates, or granules, together."

Another way to generate an agglomerated particle is to utilize equipment that combines spray drying and agglomeration, such as a multistage dryer (MSD) from GEA/Niro. In this unit, air flows in a controlled manner such that

undersized particles are continuously reintroduced to the point of liquid introduction through a recycling loop. This results in particles being formed and agglomerated simultaneously leading to an increased particle size. Regardless of the use of multiple processes or the combined process, the particles generated typically have better flow properties compared to spray-dried powder.

40.4.2 Spray Chilling or Prilling

In the spray chilling process, dry enzyme particles are blended with a molten material and atomized via spinning disk or rotary atomizer into a chamber with cool air flow where they solidify and form spherical particles. If a powder or small particle is used, the final particle that is formed is typically a matrix where multiple dried particles are homogeneously dispersed in the solidified material. If larger particles are used, a core−shell structure is formed. Since the enzyme is already in a dry state going into the process, the impact of the temperature of the molten material on the enzyme is decreased. However, even powders with low moisture content can cause damage to the enzyme. Hence, it is best to use moderate (less than 100°C) temperatures. For both the matrix and core−shell particles, enzyme exposure from the final particle is dramatically reduced (Masters, 1991). In addition, the nature of the continuous phase material that is used can impart controlled delivery through dissolution or temperature triggers.

40.4.3 Spray Coating

Coating of enzyme particles is another common approach in which actives can be introduced into food applications. Spray coating can introduce additional benefits such as controlled release, increased storage stability, or co-delivery of incompatible materials. The enzyme-containing core used in the coating process can be produced by a variety of methods. These include applying an enzyme as a layer to a carrier particle using a fluid bed coater, spray drying and agglomeration, high shear granulation, spray chilling, etc. The resulting enzyme core particle that is created can be further processed to add a coating, thereby modifying the properties of the particle.

The granules produced via spray coating allow the producer to introduce customized properties into food applications. One of these is controlled or targeted delivery. This can be done by utilizing coating materials that will only release as a result of a physical change in the environment. These include pH, temperature or water content, for example. By taking advantage of these changes, the enzyme activity can be introduced into the application at specific steps. Another possibility for delivery is to introduce multiple components in a single particle that may not be compatible with each other under normal storage conditions. These can include reactive systems, such as combining an enzyme with its substrate, where the resulting product of the reaction is the component needed in the application. The enzyme/substrate system can be incorporated on one particle by applying them in separate coating layers that may or may not be separated by a barrier layer. Once the particle is exposed to water, for example, the layers would then dissolve and be allowed to react, generating the desired properties.

Spray coating can introduce forces that may damage the enzyme similar to that in spray drying (DePaz et al., 2002). Temperature and oxygen exposure are two factors to consider. The temperatures are generally lower in spray coating, but the time of exposure can be significantly longer. This is especially true when utilizing a batch mode fluid bed coater. In addition, the amount of air that is utilized is significantly higher compared to spray drying because it is not only the driving force for evaporation, but is also utilized as the power source to fluidize the particles for coating. As a result, oxygen-sensitive enzymes can also suffer damage. Similar to spray drying, the producer can address both of these potential harmful parameters by formulating the enzyme in a matrix that will protect the enzyme from heat and/or oxygen, such as incorporating antioxidants in the latter case.

40.4.4 High Shear/Wet Granulation

Wet granulation processes can also be used to produce enzyme granules for food applications. The processing conditions, especially temperature, are more enzyme compatible in wet agglomeration compared to spray drying or spray coating, for example, but there are other aspects that must be considered. Since the enzyme will be in a wet state during the process, it is key to use formulation ingredients that are compatible with the enzyme activity. In the case of wet granulation, any material that has the potential to modify the enzyme, such as oxidants, certain salts, and chelators, should be avoided. The pH of the system should also be considered.

During the formation of the final particles, either the enzyme can be introduced as a dry powder that can be generated via spray drying, or the liquid enzyme concentrate can be used as the granulation aid. Even if the enzyme is added

as a dry powder, it will rehydrate when the granulation aid, such as water, is added. In addition to any chemical modification that may occur, physical forces such as shear can also damage the enzyme structure and subsequent activity. Optimization of the granulation parameters to minimize the time of granule formation is therefore required. Following the particle formation, the granules must be dried. This can be done in a continuous or batch process. The temperature used to dry the particles is typically much lower than that used in spray drying, so thermal denaturation is less of a factor. However, the time to reach the desired final moisture content is longer, so even moderate temperatures can have an impact.

40.4.5 Extrusion

Similarly to wet granulation, a key aspect during extrusion is the materials used during formulation. However, the enzyme will be in the wet state for a longer time compared to the wet granulation process due to the processing order where the extrusion paste is formed sooner in the process, and therefore the impact of the materials increases. The enzyme will be in the wet state after the dough is formed, through the pressing and extrusion of the dough to particles and any subsequent optional spheronization process. As with the wet granulation, the formed particles must be dried to a desired moisture content. The process and considerations for drying the extruded particles are the same as for wet granulation.

40.4.6 Liposomes

One of the more recent approaches for delivering enzymes into food applications is the use of liposomes. Liposomes have been used in pharmaceutical applications because they can allow the active to penetrate through membranes. In food, liposomes have been used in dairy applications where delivery in fats might be necessary. More details on liposomes can be found in Chapter 13.

Liposomes introduce some unique challenges for enzymes compared to dry state delivery. These can include stabilizing the enzyme in a solution state, protection from shear during production, and denaturation at the water−oil interface. One common approach to generating a liposome encapsulate is to generate a water-in-oil (W/O) emulsion. In order to form useful particle size, shear must be introduced. The shear forces can denature an enzyme as well as introduce significant amounts of heat into the system. Once the liposome is formed, enzymes have the potential to denature at the oil−water interface through the exposure of hydrophobic regions of the enzyme interacting with the lipid interface. Each of the potential destabilizing phenomena can be reduced through formulation and incorporation of emulsion stabilizers into the system.

40.5 FOOD APPLICATIONS

The use of enzymes in food application is diverse (Whitehurst and van Oort, 2010). Enzymes can be used for improving processes, such as filtration in beer production or increasing yields in juice production. They can also be used to improve product quality, such as extending shelf-life of bread and tortillas or flavors in cheese (Panesar et al., 2010). Regardless of the use, the delivery of the enzyme to the application must be done at the correct time and place. Though enzymes can be added as liquids or solids to the application, the following case studies highlight the use of enzyme encapsulation to deliver unique benefits.

40.5.1 Baking

Many different enzyme activities are utilized in baking applications. These include amylases to improve dough characteristics and shelf-life, protease to alter dough and subsequent crumb quality, and lipases to enable emulsifier generation *in situ*. In order to maximize the benefit of the enzyme activities, they must act on the substrate at the appropriate time, be homogeneously distributed throughout the mix, and remain active if stored for any given period. Encapsulated enzyme particles can address each of these areas and some of these approaches are outlined in further detail below.

In the production of bread, it is common to include amylase activity to maintain freshness of the final product. Staling of bread occurs when the amylose from starch crystallizes creating rigid regions that continue to grow with time, leading to the hard unsatisfying properties. This can result in significant amounts of wasted product. When amylase is incorporated during the dough production process, it will clip the amylose such that the crystallized regions are slower to develop, extending the shelf-life and freshness of the product.

In one example, protection of the alpha amylase during baking and subsequent controlled release of the enzyme to react and thus retard staling is demonstrated (Horn, 2003). The patent describes substantially coating a spray-dried amylase powder with an oil, and then incorporating this into the dough production process. The oil protected the enzyme through the baking process allowing it to release and react at an acceptable rate to retard staling. The oil encapsulation worked best when starting with an enzyme powder rather than a microgranule, presumably due to the porosity of the microgranule. This patent is a good example of achieving both thermal protection and controlled delivery of enzyme activity.

Encapsulation via spray coating or spray chilling to protect and target delivery of an amylase is described in the patent by Duesterhoft (Duesterhoft et al., 2012). The process describes using different hydrophobic coatings applied to an amylase-containing core. The resulting enzyme release rates of the particle in aqueous solution can be tailored based on the type and level of coating utilized. When the coated particles were incorporated into dough production, the resulting dough was less sticky and more easily handled compared to dough with uncoated enzyme, as no reaction with the starch took place at this stage. However, the finished baked product had similar characteristics compared to the sample with uncoated enzyme. This is the result of the fact that during the baking process the coating melted, releasing the enzyme and allowing it to react. This is a good example of how the utilization of coatings to ease processing and target delivery can be effectively utilized.

In another use, lipase is encapsulated to eliminate reaction with fats such that no rancidity is formed during storage, thus extending the shelf-life of the product (Plijter and Meesters, 2000). Once the baker adds water to create the dough, the lipase is released allowing it to react with the fats. The encapsulation examples are simple, but effective. A fluid bed coating process is used where first an aqueous lipase enzyme concentrate is coated onto sodium chloride particles. After this, a coating layer of primarily hydroxypropylmethyl cellulose is applied. When the coated particle is mixed into a dry dough composition, no rancidity is observed for over 60 days. In comparison, when a non-coated powdered lipase formulation is incorporated in a similar dough formulation, rancidity is detected after storage of only 2 days. In addition, the lipase activity is not significantly affected during storage.

40.5.2 Sweeteners

The production of syrups and sweeteners from starch requires several enzymatic steps as outlined in Figure 40.1. In the last of these steps, an immobilized glucose isomerase is used to convert glucose to fructose. The heat required to enable good material flow of the glucose syrup and reaction with the enzyme can damage the free enzyme, thus making the process economically unfavorable. By immobilizing the enzyme, it is better protected from the heat. In addition, the reaction can be run in a continuous mode and the enzyme can be reused multiple times leading to an efficient and cost-effective process. When immobilizing glucose isomerase or any enzyme, several factors must be considered. Two key

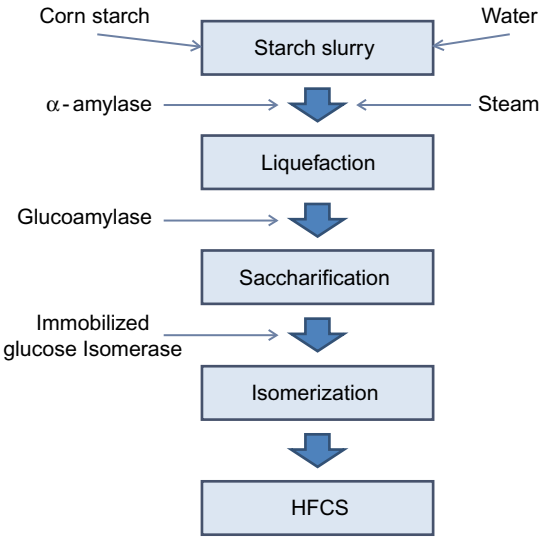

FIGURE 40.1 Schematic of a typical high fructose corn syrup production process highlighting where enzymes are added.

factors that one must insure are that the enzyme's access to the substrate is not limited and that the specific activity of the enzyme is minimally affected.

In an early patent, Sipos describes immobilizing glucose isomerase on a modified cellulose support (Sipos, 1973). The resulting complex is said to have increased thermal stability, higher conversion yield on glucose, and a shift in the pH optimum of the reaction leading to increased activity. The increase in temperature stability can be attributed to stabilizing the native state of the protein by decreasing the mobility of the structure, thus preventing unfolding and aggregation. No reasoning is provided as to why the pH and activity optima are modified in a positive manner, but this may be due to alterations in the enzyme structure resulting from the immobilization.

Another approach is described by Lantero in which a crosslinked mass of cells, enzyme, polyethylene amine, gluteraldehyde, and chitosan is generated and subsequently extruded and dried to create an immobilized enzyme particle (Lantero, 1987). The resulting particles were spherical and easily handled. In addition, the particles were described as "tough" with the ability to stand up to being stacked in a column and continuously cycled through the isomerization process without breaking down. Like the Sipos particles, these encapsulates also showed increased stability by demonstrating an increase in enzyme's half-life at elevated reaction temperatures.

40.5.3 Dairy

Many types of enzyme activities are used in dairy products, including proteases, lipases, and lactases. They serve the purpose of ripening cheese, modifying textures, and contributing to flavor formation and treatments to remove lactose to improve consumer tolerance. However, it is essential to deliver the enzymes at the correct time in the application to maximize the benefit. For example, if proteases are added too early in the curd development in cheese production, it is possible the peptides will continue to be released and ultimately removed with the whey, reducing yield. Through encapsulation and targeted release, this phenomenon can be reduced. Liposomes, emulsion systems, and coated products are commonly utilized to control the delivery of the enzymes in many dairy applications.

In the production of cheese, the flavor generation process is critical as this gives the correct quality desired in the end product. However, it is a costly process given the amount of time. In an effort to speed up the flavor development cycle, the incorporation of free protease was attempted with limited success. By utilizing encapsulated protease, the limitation of free enzyme addition was overcome (Kailasapathy and Lam, 2005). The approach to encapsulation was to create a W/O emulsion where the enzyme was part of the aqueous phase that contained a gum. Once the emulsion was formed, the solution was cooled to solidify the gum droplets to entrap the enzyme. Care was taken to use temperatures that were as low as feasible to reduce the exposure time of the protease to elevated temperatures as this could affect the enzyme stability. The resultant particles were successfully incorporated into the cheese curd. During aging, the protease was released as the capsules broke down and reacted with casein. The reaction rate was directly related to the capsule formulation and the enzyme concentration. Though the capsules did show advantages in the ability to be incorporated into the cheese process, several physical attributes in the cheese were negatively affected. The author suggests that with further optimization of the system, some of these problems may be overcome.

The use of liposomes to encapsulate and deliver enzymes to the cheese process has also been described (Kheadr et al., 2003). The liposomes were formed by using a highly negatively charged proliposome mixture. After combining the mixture with the enzyme, the liposomes were induced by the addition of deionized water. The enzyme-containing liposomes were separated from the free enzyme by centrifugation. The entrapment efficiency was relatively low, 20−40%, but sufficient to study the effect of the liposomes on the aging process of the cheese. The authors found that they were successful in reducing the aging process, thus resulting in good flavor generation without impacting textural properties through the use of a combination of liposomes containing either protease or lipase. The study demonstrates that both lipase and protease can be successfully entrapped in liposomes and the resulting liposomes can be incorporated into the cheese production process. In addition, this shows that two enzymes that are usually not compatible with one another can be physically separated from each other, incorporated as a blend to the application, and subsequently benefit from a synergistic effect that could not have occurred if the enzymes were combined prior to entrapment.

The use of lactase to treat dairy products or as supplements is a common approach to allow lactose intolerant individuals to enjoy dairy products. Protecting the enzyme during processing and subsequent use is essential to maximizing its benefit. Soloman demonstrated how it can be protected in low pH environments by applying functional coatings using spray coating (Soloman, 2013). In this application several different coating systems were developed including shellac, gum acacia, and hydroxypropylmethyl cellulose and combinations thereof. The coating systems were able to protect the lactase better than uncoated lactase under acidic conditions. In addition, it was able to perform adequately in reducing lactose to its components.

40.5.4 Food Supplements

Though not directly added as part of food processing, enzyme supplements have been developed to help aid in the digestion of foodstuffs to maximize the nutritional benefit to individuals whose digestive system may be compromised. Several of these supplement products are provided as enzyme blends or food systems, such as infant formulas. The blended aspect raises the need to protect the enzymes from environmental conditions, such as pH and moisture, but also from any incompatible ingredients during storage. Encapsulation and coating systems can provide solutions to these challenges.

Two different enzyme blend systems containing protease, amylase, and lipase are described where one offers improved stability of the enzymes in aqueous environments (Freedman and DaSilva, 2010) and another that provides enteric coated benefits (Ross and Kang, 2008). In the first case, the inventor describes utilizing enzyme crystals, either in their free form or crosslinked and then subsequently encapsulated using polylactic-co-glycolic acid (PLGA) or protein. The claimed enzyme particles are said to minimize release when mixed with water, allowing for good release once consumed. In the second case, enzymes are coated with enteric polymers such as hydroxypropylphthalate or methyl cellulose using a fluidized bed spray coating. The resulting particles should allow the enzyme to survive passage through the stomach and then release in the intestinal tract and act on the food substances. Though no data are shown, enteric coating is a common approach to enable passage through the acidic portion of the digestive tract effectively.

In the last example, an infant formula is created that can contain both nutritional components as well as a plurality of enzyme activities including protease, amylase, lactase, fructase, sucrase, or lipase. In this case, enteric coatings are utilized once more to allow the enzymes to react in a digestive environment where they will be most effective (Pabst, 1999). In addition to using enteric materials (PLGA or proteins) to coat the enzyme the patent references including stabilizers for the enzymes with a focus on materials that are common to infant formulas such as casein, sucrose, and lactose. By utilizing encapsulated enzyme particles, the inventor was able to create a stable enzyme/nutritional powdered infant formula that is stable during storage, then targets the release of the enzymes where they will be most effective.

40.6 CONCLUSION

The use of enzymes in food processing is long standing and continues to grow as more applications are discovered. Traditionally, enzymes have been provided as liquid or powder formulations as they are easily incorporated into the point of use. However, these delivery methods do not address all the needs such as worker safety, stability, and controlled release, to name a few. New uses in microencapsulation have been shown to effectively deliver enzymes in unique ways while providing reduced risk of exposure and superior stability. Microencapsulation of enzymes will continue to expand in food applications as it can provide unique properties that are otherwise not achievable.

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Chapter 41

Commercial Applications of Microencapsulation and Controlled Delivery in Food and Beverage Products

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41.1 INTRODUCTION

As global food consumption habits continuously change, microencapsulation of food ingredients has become an extraordinary tool in providing solutions to consumers' demand for more convenient, nutritious, safe to eat, natural, energy-providing, experiential, and interactive foods and packaging. As a result, microencapsulation has become a versatile and indispensable technology with application across the whole food and beverage spectrum, ranging from health and wellness, flavor and taste creation, to interactive food and packages, and food safety (Figure 41.1). These technologies have enabled a diverse range of ready-to-eat or drink products in the marketplace (Rathor and Nupoor, 2012; Jadupati et al., 2012; Sri et al., 2012; Gbassi and Vandamme, 2012). From the convenience standpoint, people are attracted to food that will save them time, physical energy, and/or mental energy (Botonaki et al., 2007; Anonymous, 2012a,b). A whole range of ready-to-eat (RTE) food and beverage products are offered in the market in part because of the availability of microencapsulation of food ingredient technologies, allowing for specific processing, packaging, distribution, longer shelf-life, and bioavailability of specific nutrients after consumption. In addition to providing information on commercial products, this chapter will also discuss microencapsulation technologies that have a potential for marketing new products.

In the RTE meat and poultry industry, microencapsulation technologies allow for the incorporation of food safety ingredients, as well as heat-, oxygen-, and moisture-sensitive constituents. As a result, RTE meat and poultry products in the market are of higher quality and have a longer shelf-life (Abril and Wills, 2008; Bontenbal, 2008; Claude and McAninch, 2008; Stiles et al., 2012). These technologies are mainly based on the controlled delivery of organic acids in meat, such as lactate and sodium diacetate, which result in an increased resistance against the growth of bacteria, in particular as *Listeria monocytogenes*, *Clostridia*, *Salmonella*, and spoilage bacteria such as *Lactobacilli*. In RTE raw fish such as salmon, controlled released through microencapsulation of nucleases or proteases (e.g., lactic acid bacteria) has been effective in deactivating antagonistic bacteriophages used for food safety (Lin et al., 2011). Similar controlled release technologies have been used to enhance salmon preservation and quality through the feeding of microencapsulated astaxanthin, the main pigment providing the desirable reddish-orange color to salmon and other crustaceans, as well as providing antioxidant activity (Higuera-Ciapara et al., 2006; Kazuaki-Hirasawa et al., 2010).

In the bakery and confectionery industry, there has been an increasingly high level of reliance on microencapsulation of sensitive ingredients to provide unique desirable attributes such as texture, taste, flavor, appearance, nutrition, and longer shelf-life. Microencapsulation of ingredients has played an important role in the ability of the bakery and confectionery industry to tailor-make products to quickly adapt to consumer lifestyles and eating trends. An example is the availability of ready-to-bake dough in the market, which has become very popular due to the convenience of being able to make freshly baked products at home (Hahn, 2010). The commercial availability of these products is in part due to the ability to microencapsulate key ingredients such as cinnamon and other flavorings, for example, herbs and spices. Encapsulation of these particulates using, for instance, fat barriers helps in providing storage stability and in avoiding

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00041-8

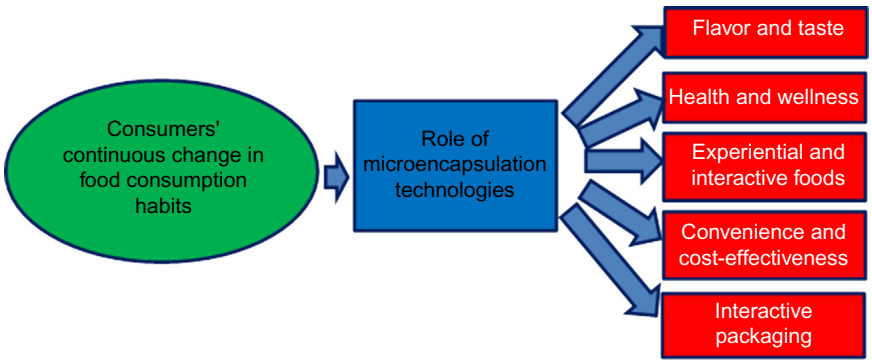

FIGURE 41.1 Innovation in microencapsulation and controlled delivery has a significant role in producing commercially relevant food applications to keep pace with consumers' continuous evolution in consumption habits.

enzymatic and chemical deterioration, which would otherwise result in gray dough (Hahn, 2010). A particular example is Flavorshure®, developed by the Balchem Corporation, a specialty formulated cinnamon ingredient for the baking industry (www.foodnavigator.com/Science-Nutrition/Encapsulated-cinnamon-hits-the-baking-industry). Cinnamic acid is known as an antimicrobial volatile and a major component of the cinnamon aroma. The encapsulation of cinnamon flavoring prevents interaction of cinnamic acid with yeast allowing the dough to rise.

In the gum and candy business, commercialization of products with unique functionalities is possible in part as a result of significant advancements in the microencapsulation of sensitive ingredients. These include oral anti-odor or antimicrobial compositions and taste-masking applications (Nevo, 2010), a protective microencapsulation system for highly volatile additives to minimize losses during process and storage (Gregson and Sillick, 2012), and the delivery of cooling agents (Amundson et al., 2007). Commercially available examples include the TheraBreth® brand from the Wrigley Corporation, which incorporates microencapsulated cinnamic aldehyde as an antimicrobial agent. Similarly, Trident® from Mondelez International and Extra® from the Wrigley Corporation are prototype chewing gum brands containing menthol compositions, which provide a cooling sensation during consumption. Alternatively, a cooling effect is achieved by producing chewing gum containing a physiological cooling agent such as microencapsulated acyclic carboxamide, and by controlling its release during chewing (Wolf et al., 2007).

Commercially available instant products, such as coffee, cocoa, chocolate, lemonades, tea, juices, energy drinks, purées, and milk powder, are important beneficiaries of the advancement in microencapsulation technologies (Barnekow et al., 2008; Celeste, 2009; Frascareli et al., 2012, Rocha et al., 2012; Nazzaro et al., 2012). Benefits include: flavor enhancing, increase of solubility or instantability, antimicrobial control, aroma or odor masking, sustained energy release, appearance modifiers, and many other characteristics that appeal to consumers. In instant coffee, for instance, microencapsulation permits the protection of unstable, character impact molecules such as thiols, unsaturated aldehydes, and ketones, which are critical in providing a coffee flavor profile similar to roasted coffee. Additionally, microencapsulated coffee oils can be utilized in the flavoring of other foodstuffs such as candy, cakes, and puddings. The development of coffee microcapsules or granules with internal voids containing pressurized gas provides instant coffee with the added benefit of forming foam on its surface after reconstitution (Zeller et al., 2010; Wyss et al., 2010; Boehm et al., 2010).

Technological advancement in alginate-based microencapsulation has provided a boost to the dairy and frozen dessert business, leading to diversification in this subsector. Increasingly, consumers are more attracted to specialized frozen confectioneries with particular inclusion systems that render value added textural benefits, as well as health promoting attributes such as use of probiotics, certain vitamins, and antioxidants. These inclusion systems are only possible through the utilization of gel-forming processing systems such as calcium alginate hydrogel production (Aldred and Chamberlain, 2012).

The functional food industry has gone thorough vigorous expansion over the last decade, as a result of not only an increased interest of consumers for healthier foods, but also because of significant progress in ingredient microencapsulation technologies. Forecasts for functional foods growth range between 8.5 and 20% per year, or about four times that of the food industry in general, and it is expected to be about $100 billion in United States alone for 2013 (Smith and Charter, 2010). Recent development in controlled delivery technologies such as functional hydrogels, nanoemulsions, and nanoparticles has enabled the preservation of beneficial food structures and nutrients for proper biological functions when consumed and assimilated (Pernetti, 2009; Gaonkar, et al., 2010; Sanguansri and Augustin, 2010; Onwulata, 2012). A popular approach in protecting sensitive bioactive food ingredients is through protein-based microencapsulation (Amaya-Llano and Ozimek, 2010). Whey proteins, for instance, produce unique microencapsulating structures due to their ability to form emulsion and gel compositions (Sanguansri and Augustin, 2010; Brodkorb and Doherty, 2012).

41.2 FLAVOR AND TASTE

A great deal of innovation in microencapsulation of food and beverage ingredients related to flavor and taste has been undertaken during the last decade. Resulting new technologies have rendered a whole host of industrial applications leading to commercial products, with value addition in areas such as flavor enhancing, odor masking, long lasting, sustained release, burst or quick release, and flavor stabilization. An example of a flavor enhancing system includes shelf-stable aroma-producing compositions used in foods that are heated. Such formulations are achieved by utilizing a diverse range of microencapsulation mechanisms (Celeste, 2009; Gaonkar and Ludwig, 2008; Given, 2009; Grover et al., 2010; Nonaka and Takahashi, 2011; Robinson et al., 2012). A commonly used system for hot beverages are the fat matrices with melting point sufficiently high to entrap and protect the aroma but low enough to release it only after heating the beverages, such as coffee, tea, or chocolate milk. Other flavor enhancing microencapsulation compositions include coacervation of oppositely charged polyelectrolytes such as alginates and calcium alginate, which will release flavor only after being exposed to certain pH conditions.

Similarly, microencapsulation technologies for masking flavor or taste have been developed. The principle behind flavor or taste masking has been based on the design of protective compositions that impede interaction of targeted molecules with particular receptors on the tongue (Nedovic et al., 2011; Deepak et al., 2012). These technologies have been particularly useful in cases where the active is meant to be delivered in the digestive tract, for example, use of enteric-coated bioactive components in the intestine (Akashe et al., 2010).

Another flavor microencapsulation of commercial value is delayed/sustained/long-lasting systems, particularly in gum and candy, as well as confectionery products. There is a variety of chewing gums in the market with varying effect in taste and release time such as sustained or burst release, as well as nutritional and medical substances such as teeth whitening agents (Maggi, 1994; Castro and Johnson, 2006, 2007; Atarés et al., 2010; Suzuki et al., 2011; Sandra et al., 2012; Gebreselassie et al., 2012). Examples of chewing gum brands commercially available with longer-lasting flavor release include Orbit® and Trident. Long-lasting flavors or fast, burst-type flavor release is achieved by multilayering microparticles with flavor-loaded film forming agents. Generally, compositions with carriers having higher solubility in organic solvent, good film-forming properties, and low water solubility (e.g., ethyl cellulose) will particularly control the release of flavors.

41.3 HEALTH AND WELLNESS

Consumers' demand for natural and less processed foods pertains to focusing on a more functional and personalized nutrition. In fact, a significant portion of consumers are interested only in foods' functional ingredients. This type of market niche has presented a tremendous opportunity for innovation in the field of controlled delivery of functional food ingredients. Specialized systems for microencapsulation of sensitive functional ingredients have enabled the incorporation of health-promoting bioactives in an array of food products. Popular food and beverage choices with health-promoting attributes include: ready-to-drink (RTD) teas, sport drinks, dairy-based drinks, energy shots and stick packs, enhanced water, fortified snacks and confectionery, low-sodium foods, food supplements, and oral health-promoting chewing gums and candies. Microencapsulation techniques allowing the protection of probiotics, readily oxidable components such as vitamins, unsaturated lipids, and other functional actives have been paramount in this effort (Robert, 2010; Perlman, 2011; Van Lengerich et al., 2011; Nakhasi and Corbin, 2012; Lathan et al., 2012; Fang et al., 2012; Wang and Bohn, 2012). Sensitive ingredients used in functional foods, energy-boosting, and food supplements in general include caffeine, taurine, D-ribose, B vitamins, flavoring, flavor masking, antioxidants, sweeteners, essential electrolytes (sodium, potassium, chloride, calcium, phosphate, and magnesium), pre/probiotics, fruit extracts, botanicals, colors, and stabilizers.

An important source of amino acid, nowadays popular across the food and beverage industry, is whey proteins. Microencapsulation has in part enabled the design of effective protection and delivery systems containing whey proteins and their derivatives for use in beverages, foods, and supplements. For instance, by microencapsulating hydrolyzed whey protein having bitter sulfur-containing amino acids such as cysteine and methionine, it is possible to prepare foods and beverages with high added nutritional value by masking the bitterness of these amino acids and other ingredients with undesirable flavor and taste (Gaonkar et al., 2010; Nedovic et al., 2011; Rizvi and Manol, 2011; Deepak et al., 2012; Pereyra and Mutilangi, 2012). Correspondingly, microencapsulation of omega-3 fatty acids enables the masking of its undesirable fishy off odor, thereby allowing for its use in a wider range of foods and beverages (Dekker and Husken, 2010; Van Lengerich and Walther, 2010; Gbassi and Vandamme, 2012). See Chapter 36 for further discussion on the microencapsulation of probiotics, and Chapter 37 for details on masking and protection of omega-3 and -6 fatty acids.

41.4 EXPERIENTIAL AND INTERACTIVE EFFECTS

Through the art and science of microencapsulation, nowadays consumers can find food and beverage products in the marketplace with particular experiential and interactive attributes. These characteristics include color and flavor change, cooling and warming effects, foam-producing, mouth-popping hydrogel beads, long-lasting flavors, intelligent packaging, multicolor hydrocolloids, and effervescence. Flavor-changing chewing gum and candies are available in the market (see Chapter 34 for more details).

Microencapsulation of a gas under high pressure provides sound effects. For example, in Pop Rocks®, carbon dioxide gas at high pressure is encapsulated in a glassy food polymer matrix. After placing Pop Rocks on the tongue, a popping sound is produced. Pop Rocks coated with a moisture barrier is included in B&J's KaBerry Kaboom ice cream to produce a crackling sensation in the mouth when the ice cream is consumed.

Sequential change in color and flavor of a beverage (Ludwig et al., 2006) and particle motion in a beverage (Gaonkar and Ludwig, 2006) have also been reported. In Kool-Aid® Magic Twists, the beverage powder changes color after the addition of water. For example, Changin' Cherry® is a green-colored powder that changes into blue drink with a cherry flavor. In Mystery Colorz Cheetos®, the tongue is dyed green or blue when cheese puffs come into contact with the saliva in the mouth. In another product called Magicolor® Quaker Oatmeal cereal, color magically appears as the milk is added to the cereal.

In beverages, for instance, gas-infusing or turbulence-inducing microparticles have been developed to provide froth or foamed texture to instant cappuccino and other coffee mixes, instant refreshing beverage mixes, instant milkshake mixes, and the like, when combined with water, milk, or other suitable liquid (Zeller et al., 2010; Keller, 2010; Boehm et al., 2010). Similarly, some beverage containers provide a flavor delivery system capable of bringing a burst of flavor when drinkers place their mouth to portions of the beverage container (Norris et al., 2010). Furthermore, microencapsulation of fruit juices into hydrogels is also possible, by creating liquid juice beads through the crosslinking of the alginate polymer in the presence of calcium. These juice hydrogels with mouth-popping effects are very popular in frozen yogurts. The same principle is used to produce juice caviar by mixing sodium alginate with well-known commercial food products such as Jell-O® and ice cream. One example of an RTD beverage containing suspended macrobeads is Orbitz® drink marketed by the Clearly Canadian Beverage Corporation. Density of the beverage is adjusted so as to suspend the beads in the beverage. The macrobeads are usually prepared by crosslinking a food polymer. It is possible to include color, flavor, or a functional component in the gel beads.

Long-lasting flavor and cooling effects are highly desirable attributes in chewing gums, which have been addressed utilizing microencapsulation of cooling and refreshing agents such as various carboxamides (Wolf et al., 2007; Bardsley et al., 2010; Kazimierski and Kraut, 2011). Chapter 34 is exclusively dedicated to flavor release in chewing gum.

41.5 INTERACTIVE PACKAGING

In the packaging field, microencapsulation technologies have also propelled the smart or interactive packaging market. Specially designed packaging, with smart nanocomposites embedded in it, allows for packages to interact with foods and the environment, playing a dynamic role in food safety, food preservation, and consumption (Brody et al., 2008). More details pertaining to the role of microencapsulation and packaging can be found in Chapter 41.

Straws, edible films, and caps are used as a delivery vehicle in some commercial products. The flavor is included in the edible film, and flavor is released as soon as the film starts dissolving after contact with the saliva on the tongue. The edible, soluble films can be made using pullulan, starch, cellulose derivatives, proteins, and their mixtures. Sipahh® straws are claimed to flavor a carton of milk. In this product, microencapsulated flavors and sweeteners, called "unibeads," are packed between two filters in the straw. Unibeads dissolve as the liquid is sucked through the straw, thus releasing the actives (e.g., flavors and sweeteners). In BioGaia's LifeTop® telescopic straw, a functional agent such as probiotics is embedded within an adhering agent such as oil or fat on the inner wall of the straw. The functional agent is consumed when water or beverage is sipped through the straw. This is an ideal way to protect the moisture-sensitive ingredient before it is consumed. LifeTop Caps by BioGaia protect and deliver moisture-sensitive functional components in a similar way. Here, thin aluminum foil protects the active within the cap and upon screwing the cap onto a water bottle the aluminum seal is broken and the active is released into the water, thus producing an enhanced beverage. Again, this is a good way to deliver a moisture-sensitive functional ingredient such as flavor, color, probiotics, and so on because the functional ingredient is protected until ready to be consumed.

41.6 TRENDS AND OUTLOOK

Consumers are increasingly interested in foods with health-promoting and value added microencapsulating systems that are able to deliver nutraceuticals or pharma foods (Onwulata, 2012; Amin et al., 2012; Dey, 2012). Targeted delivery of foods, such as enterically protected functional bioactives, oral health-promoting actives, and foods that swell to provide satiety sensation, seem to be of high interest. There is current development in designing micro- and nanotechnologies that enable the delivery of unique ingredients through foods, tailored to individual genetic prototypes, according to specific metabolic requirements. These areas are referred to as nutrigenomics and metabolomics (Wang and Bohn, 2012).

Natural, balanced diets continue to gain interest by consumers (Wang and Bohn, 2012). Natural balanced food and beverages will greatly benefit from microencapsulation innovations able to protect natural and less processed ingredients through specialized physical encapsulation techniques. Similarly, energy-boosting and sustained energy food products are poised to continue escalating in the marketplace. In addition to traditional microencapsulation technologies such as spray drying, spray chilling, hot melting, extrusion, and coacervation, other newer technologies are gaining interest, which might play an important role in future innovations of commercial relevance in the food and beverage industry. These include bioactive tagging such as PEGylation, nanoemulsions, and electrospinning for the generation of nanofibers and nanotubes, which is the result of both electrospraying and the conventional solution of dry spinning.

Looking forward, food and beverage manufacturers will increasingly face the challenge of having to produce novel products to address consumer demands and the ever more aggressive role of supermarkets producing their own brands. Microencapsulation technologies (Table 41.1) will continue to be a dependable tool in creating differentiation through the development of value adding ingredients and processes, particularly in areas such as food safety, nutrigenomics, and metabolomics.

TABLE 41.1 Examples of Available Microencapsulation Technologies Rendering Flavor, Taste, Texture, and Interactive Benefits of Commercial Application in the Food and Beverage Industry

Food and Beverage Ingredients	Microencapsulation Mechanism	Function	Commercial Applications
Character impact compounds such as thiols in coffee and esters in fruits	Heat-tolerant coating, isoelectric precipitation	Flavor enhancing/ potentiation	Coffee, tea, juice, soup
Omega-3 fatty acids, probiotics, prebiotics	Heat and moisture-tolerant coating, isoelectric precipitation	Flavor/odor masking and protection from moisture and heat	Beverage, nutritional bar, cereal
High vapor pressure esters, fruity volatiles, mint flavors	Coacervation of oppositely charged polyelectrolytes	Long-lasting flavor	Chewing gums
Fruit flavors, mouth-refreshing aromas	Hot-melt particle formation	Sustained release	Chewing gums, energy drinks
Esters, fruity volatiles, mint flavors with higher vapor pressure	Hydrophilic coating carriers, flavor potentiators, emulsion systems, vapor pressure suppressors	Burst/quick release, flavor stabilization	Chewing gum, energy drinks
Cheese ripening enzymes, gut health promoting cultures	Enzyme tagging technologies, PEGylation, enzyme immobilization	Cheese ripening and flavor producing	Cheese and other dairy products
Carbonate, pressurized air	Gas-containing inclusion systems, cyclodextrin, carbohydrates, proteins	Foam and turbulence forming in beverages	Foamed coffee, juices
Juices, yogurts, ice cream	Sodium alginate microencapsulation, coacervation systems	Hydrogels and mouth-popping juices	Juices and dairy desserts
Spoilage by-product reacting agents	Nanocomposite microencapsulation	Color change, food safety, interactive and intelligent packaging	Interactive and intelligent packaging
Probiotics, prebiotics, gut health compounds	Protein-based microencapsulation	Enteric release of probiotics	Beverages, baked goods

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Chapter 42

Inventing and Using Controlled-Release Technologies

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42.1 INTRODUCTION

Today, food manufacturers, industry experts, and others continue looking for newer and better ways to use controlled-release (CR) (a.k.a. sustained-release, continuous-release, etc.) technologies to enhance the value of their products. Unfortunately, there appears to be a lack of available technology that works, in other words, there is no apparent technology or group of technologies that will satisfy the exact, prespecified release profile. What this means is that the manufacturer must apply an existing technology in a novel way, or invent (Fritz, 1989) a new technology (Michalko, 1998).

This chapter addresses two approaches:

1. How to reexamine existing technologies for a novel application.
2. How to use an apparently unrelated technology to create a new CR technology from it.

Thus, the discussion in this chapter is based on the encapsulator's (i.e., the encapsulating company's) point of view. In some cases, the reader (the encapsulator) may be looking for some form of controlled release, but that form has not been specified. In others, the reader may be interested in workable methodologies, but he/she has no specific applications. Or, the reader may simply be scientifically curious.

In any case, the term *microencapsules* is being used in this discussion for brevity, and in a very broad sense includes any form of controlled release, macro or micro.

42.2 A NEEDS-BASED PROCESS

The process of finding a suitable existing or new CR technology (Kydonieus, 1980) begins with the need(s) of the encapsulator's end-user. The encapsulator is not looking for problems to solve; rather the end-user has approached him/her with a need to satisfy. This means that both the end-user and the encapsulator have to determine that each has sufficient resources to solve the problem—resources that are expressed in terms of money, calendar time, and man-hours (Zuidam and Nedovic, 2010).

In some cases, the end-user may have high expectations and little capital. Some end-users may say, "I cannot pay you now, but I'll buy a whole lot from you in the future if it works." To avoid this potential lose−lose situation, the encapsulator should view the process as an "engineering trial," never using the word "sample." Samples are often assumed to be free of cost to the end-user. If true, the cost must be paid by the encapsulator. This is a quick way to financial disaster under the guise of a technical success and commercial failure. An "engineering trial" implies that the cost will be borne by the end-user.

A primary concern is the end-user's ability to pay for both development and subsequent small production quantities. The process involved in determining the cost for production quantities, large and small, is addressed in Chapter 30.

A.G. Gaonkar, N. Vasisht, A.R. Khare, R. Sobel (Eds): Microencapsulation in the Food Industry. DOI: http://dx.doi.org/10.1016/B978-0-12-404568-2.00042-X

42.3 DEVELOPMENTAL PRINCIPLES

Early in the process, it is highly recommended that a systems approach be used to find the best CR technology. The systems approach is one of four developmental principles, as follows:

1. **Use a systems concept**. Essentially, it is the best way to do business and perhaps the only way to ensure success. Every participant in the process (the encapsulator, the needs-based customer, etc.) knows that for every business outcome, there is a specific cause that can be managed and improved. The only way to solve a specific CR problem is to look at the whole system involved in the manufacturing process.
2. **Avoid intuition**. Be quantitative. Use a release profile.
3. **Ask the experts**. Seek help from experienced CR professionals, many of whom have had similar problems (e.g., manufacturers, food industry experts, CR service providers, etc.).
4. **Assign value to applications knowhow (i.e., formulation experience)**. Knowledge of specific CR formulations and industry-specific applications for those formulations are premium assets that should be quantified—even when feasibility studies are being performed.

42.4 RELEASE PROFILE

The creation of the release profile begins with a timeline. The timeline starts with the development of the job-specific "controlled-release dosage form," that is, at the beginning of the manufacturing process. This timeline will include the following:

1. **Manufacturer of the microcapsules**. Questions to ask: Can the microcapsules be made in sufficient quantities? Are the economics acceptable to the user?
2. **Suitable formulation**. Questions: Will the microcapsules be stable? Will they retain their desired properties during manufacturing? If stability is an issue, this could mean changing the current or anticipated process to avoid excessive shear in mixing, and so on. It may also mean changing the formulation.
3. **Packaging**. Here, the microcapsules (product) may be subjected to excessive shear in pumping, flowing, and valve operation.
4. **Distribution**. Here, the microcapsules (product) are subjected to environmental conditions that may not be controllable. Long-term storage may occur.
5. **Consumption**. Key questions: How long will this product remain on the customer's shelf? What are the environment conditions during this time? Temperature? Humidity? Sunlight?

42.5 OTHER ISSUES

Once the timeline is drawn and annotated, the end-user should address the following issues—issues that can have an impact on the overall need for microcapsules:

1. Why is controlled release needed in the first place (Brownlie, 2007)? Is there a real performance problem, or is there just a desire to satisfy company marketing employees who want/need to use words on the packaging that substantiate the company's product claims?
2. Can controlled release be avoided? Could simple reformulation of the product enhance product stability? Can reformulation of the product, combined with a simpler encapsulation, such as simple matrix encapsulation, work as well or better?
3. Is process selection a means to an end? In spite of the technical fascination, fixation on the process could be very costly.
4. Avoid situations involving water—either retaining it or keeping it away. Water is a small molecule, and it goes virtually everywhere. Also, the molecular weight of air is greater than that of water.
5. Perform a cost analysis. Look at an overall cost analysis. Some core material (deliverable) is lost in processing. The cost of controlled delivery may be offset by the use of less material in the formulation. Examine intangible factors with marketing, such as customer acceptance and perception of product variability.

42.6 RELEASING THE CORE

At the far end of the timeline is the delivery of the active ingredient (core) to the consumer. How is the core to be released? Essentially, all release mechanisms can be grouped into three categories:

1. **Diffusion**. The wall or matrix remains intact, but acts as a diffusional membrane. In actual practice, this is never perfect when compared to the theory for first order release. Most release curves are somewhere between first and second order release because there is an assembly of particles of varying size, wall formation, and wall thickness. Transdermal patches do, however, work this way.
2. **Fracture**. The wall or matrix is broken open and a burst, or dumping effect, occurs. Most applications of controlled release use this mechanism. In other words, the wall or matrix is simply a microcontainer until full release of the contents occurs. Scratch-n-sniff fragrances work this way.
3. **Ablation**. This occurs when the wall or matrix melts, dissolves, or abrades away. Spray-dried matrix particles containing flavor in powdered beverage products work this way.

In the end, the user must recognize that the objective, simply stated, is this: The deliverable must be released at the time they want, the place they want, and the rate they want. The specifications of time, place, and rate are shown in the release profile.

42.7 DEVELOPING A NEW TECHNOLOGY

Frequently, a single CR technology does not solve the problem (Versic, 2013). It does not give the user the desired results, in spite of adjusting conditions on the timeline. It now becomes necessary to review the existing method to determine what can be used and/or what changes need to be made with these technologies.

Table 42.1 helps simplify this process. The table shows an idealized version of how 11 of the better known, and commercially successful, time-release technologies may have been developed.

TABLE 42.1 Former Process Development

Base Technology	Year	Addition	Controlled Release
Spray drying of emulsions	1872	Emulsified oils, 1925 Special disk, 1949, 1987	Spray-dried flavors, matrix, and reservoir structures
Extrusion of synthetic fibers into a bath	1920	Flavor oils, 1957	Sunkist process
Fluid bed drying	1922	Top, side, bottom spray nozzle and partition, 1965	Wurster coating, agglomerization
Emulsion polymerization	1920	Add oil to polymer, 1989	Advanced polymer systems, Polytrap™
Biomass briquetting (solids compaction)	Ancient	Add ribbons, liquid core, 1934	RP Scherer
Spun sugar (cotton candy/candy floss)	1897	Emulsify oil into hot melt, 1989	Fuisz
Droplet stabilization with surfactants	1880	Form a massive wall, 1951	Coacervation
Parylene coating of macro-objects	1964	Tumbling of powder, 1983	Parylene
Polycondensation of nylon	1935	Droplets in a water medium, 1968	Interfacial polymerization
U/F resins	1925	Coacervation formation, *in situ*, 1970	U/F, PMU (polymethylene urea)
Pan coating	19th century	Specialty coating, e.g., enteric wall "tunnel coating"	Enhanced release, continuous process

The table presents the base technology, the year it was developed (if known), the processes that were added that altered the original technology, and the resulting CR product(s) or process(es).

42.8 PUBLIC KNOWLEDGE

To determine if this technology is viable for a particular, needs-specific application, additional research on the origins of these technologies is appropriate. Looking back prior to 1976 (i.e., the beginning of the Electronics Age), the major source of this earlier information is issued patents—a long-standing source of public knowledge. Note that patents are records of inventions, not necessarily complete documents on how the technology works. The preferred embodiment often does not work because it requires an unspecified "skill in the art." The following is a complete listing of public knowledge resources for existing CR processes:

1. **Patents going back into the 1950s**. The previous comments apply; however, it should be noted that the patent literature in the 1980s and later contains excessive detail and is of questionable value. The problem is not too little information, but too much information that is undecipherable.
2. **Published books beginning in 1963, continuing into the 1980s** (Anderson, 1963; Ranney, 1969; Gutcho, 1972, 1976, 1979).
3. **Journals and publications from professional societies.** The Controlled Release Society (CRS) started in 1972; the International Microencapsulation Society (IMS) dates to 1984; and the BioEncapsulation Research Group (BRG) was founded in 1989.
4. **Market surveys.** These are professionally assembled and carefully researched, and include information on various technologies, as well as names of companies and institutions that specialize in these technologies.
5. **Short courses.** These represent an excellent opportunity to meet acknowledged leaders in the field (Versic, 2011; IFT Annual Meeting Short Course, 2012; Bioencapsulation Research Group, 2013a,b; International Society of Microencapsulation, 2013; Bioactives World Forum, 2013). Often, the presenters are available for off-the-record discussions, one of which could justify the entire outlay of time and costs for the course. Sometimes, the presenter is available for a timely, but brief and focused, telephone conversation after the course is completed.
6. **Advertising and public relations.** Encapsulation companies, especially in pursing business, will provide Internet information on their processes. Because such information is easily changed, this should be downloaded and saved.
7. **Existing applications.** There are numerous CR consumer products on the market today. Based on their nominal cost, it is desirable to maintain a collection of these. The difficulty is in locating those products with the "CR" labels on them.
8. **Provider libraries.** Even with due diligence, thoughtful science, and the aforementioned sources for public knowledge, it is not always possible to develop a successful CR microcapsule. These formulas may only be available from the companies that manufacture them (Versic, 2012). Typically, these CR providers scan current, developed CR technologies, and save those in a binder (electronic or paper). The purpose is to save information that may have no current value (other formulas developed in the process), but may provide the exact dosage form needed for a future application. Successful CR providers should maintain a library containing the listed aforementioned sources of public knowledge, plus a compendium of these types of articles.

42.9 CONCLUSION

Even as food manufacturers and others continue looking for new applications for CR technologies, it is apparent that there is a lack of available technology that allows them to satisfy their exact requirements. As a result, they must either apply an existing technology in a novel way or invent a new technology.

First, finding the right CR process for industry-specific or job-specific applications requires the use of solid developmental principles. Use a systems concept to ensure success. Be quantitative; use a release profile; and lay out a timeline to accomplish the task. Ask experts in the industry who have had similar problems. Quantify applications knowhow, and use it. In other words, learn from others who have learned about developing the process and using it effectively and profitably.

Use public knowledge resources that are available, such as patents, books, professional journals, market surveys, short courses, advertising and public relations communications, and product information.

In developing the specific application, also seek out the expertise and resources of successful CR providers who have developed similar applications. They have strong oral traditions; they maintain laboratory notebooks; they maintain in-

house libraries (containing both comprehensive and obscure information); and they maintain thorough in-house documentation of their applications knowhow. Finally, the invented technology should allow for freedom-to-operate (FTO) and not infringe on any previous patents.

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