Encapsulation Technologies for Active Food Ingredients and Food Processing

Nicolaas Jan Zuidam • Viktor A. Nedović
Editors

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Chapter 1
Introduction

Nicolaas Jan Zuidam and Viktor A. Nedović

Consumers prefer food products that are tasty, healthy and convenient. Encapsulation, a process to entrap active agents into particles, is an important way to meet these demands by delivering food ingredients at the right time and place. For example, this technology may allow taste and aroma differentiation, mask bad tasting or bad smelling components, stabilize food ingredients and/or increase their bioavailability. Encapsulation may also be used to immobilize cells or enzymes in the production of food materials or products, as in fermentation or metabolite production.

This book provides a detailed overview of the technologies used in the preparation and characterization of encapsulates for food active ingredients to be used in food products, processing, or production. This book aims to inform people, with both a limited and an advanced knowledge of the field, who work in the academia or R&D of companies on the delivery of food actives via encapsulation and on food processing using immobilized cells or enzymes.

The first part of the book reviews the general encapsulation technologies – food-grade materials and characterization methods for encapsulates.

Chapter 2 by Zuidam and Shimoni introduces the readers to the most common encapsulation technologies and the general criteria to select a proper encapsulation technology for a certain application.

Chapter 3 by Wandrey, Bartkowiak, and Harding discusses food-grade materials to be used for encapsulation.

Chapter 4 by Zhang, Law, and Lian describes the principle behind the methods used to characterize properties of encapsulates, including their applications. Furthermore, the release mechanism of actives from encapsulates are also described.

The second part of the book discusses encapsulates of active ingredients, i.e., aroma, fish oil, minerals, carotenoids, enzymes, peptides, and probiotics, for specific food applications. The group of actives is chosen so that they represent different classes of actives. This part of the book is intended to serve as a guide to a food

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scientist or developer looking for a specific solution to fulfill his or her needs. As encapsulation technologies may change rapidly, emphasis is laid on strategy, assuming that strategy does not change as fast. Most chapters include application possibilities of the encapsulation technologies in specific food products.

Chapter 5, written by Zuidam and Heinrich, highlights aroma or flavor encapsulates. Most of the food encapsulates used are aroma encapsulates. This chapter also discusses how encapsulates could be used to retain aroma during production, storage, and cooking of food products and to release aroma during eating.

Chapter 6, written by Beindorff and Zuidam, discusses fish oil microencapsulates. In this chapter, the authors provide an overview of possible encapsulation technologies used for fish oil and the criteria to select them for different food applications.

Chapter 7 on iron encapsulation is written by Zimmermann and Windhab, and covers the use of encapsulates containing iron and other micronutrients for food fortification of, e.g., salt and staple cereals.

Chapter 8, authored by Ribeiro, Schuchmann, Engel, Walz, and Briviba highlights the use of encapsulates in delivering carotenoids. Carotenoids are instable, natural pigments that are insoluble in water and hardly soluble in oil. Formulation of carotenoids in emulsions or encapsulates influences these characteristics, and improves their bioavailability.

Chapter 9 reviews the encapsulation of enzymes and peptides, including the different types of drying and agglomeration processes. The author, Meesters, also provides examples of their use in industry.

Chapter 10 discusses the encapsulation of probiotics by Manojlović, Nedović, Kailasapathy, and Zuidam. These living and large actives need to survive the food process, storage, and food intake before they can be useful. Examples of the use of encapsulated probiotics in food products are provided.

The last part of the book describes immobilization technologies of cells or enzymes for use in food processing and production.

Chapter 11, authored by Verbelen, Nedović, Manojlović, Delvaux, Laskošek-Čukalović, Bugarski, and Willaert, shows how immobilization of yeast cells can be used in the fermentation of beer.

Chapter 12 reviews how encapsulation of microbial cells can be used for alcoholic and malolactic fermentation of wine and cider. It is written by Kourkoutas, Manojlović, and Nedović.

Chapter 13 by Champagne, Lee, and Saucier describes how immobilization of cells and enzymes can be utilized in dairy and meat fermentation processes.

Chapter 14 presents the view of Breguet, Vojinovic, and Marison on the use of encapsulates for food bioconversions and metabolite production.

The editors are grateful to all the authors for their willingness, time, and effort in contributing to this book! Without their contributions, the book would not have been of such an outstanding quality. We would also like to thank Prof. Denis Poncelet from ENITIAA (France), who as President & Coordinator of Bioencapsulation Research Group and the EU-sponsored action COST 865, supported the idea of writing this book. Many authors who contributed are active in these networks. Finally, many thanks to the editorial staff at Springer for their valuable help throughout this project.

We hope you will enjoy reading this book and that it may help you in choosing the right encapsulation solution to fulfill your need!

Chapter 2
Overview of Microencapsulates for Use in Food Products or Processes and Methods to Make Them

Nicolaas Jan Zuidam and Eyal Shimoni

2.1 Definitions and Benefits of Microencapsulates in Food Products

Encapsulation may be defined as a process to entrap one substance within another substance, thereby producing particles with diameters of a few nm to a few mm. The substance that is encapsulated may be called the core material, the active agent, fill, internal phase, or payload phase. The substance that is encapsulating may be called the coating, membrane, shell, carrier material, wall material, external phase, or matrix. The carrier material of encapsulates used in food products or processes should be food grade and able to form a barrier for the active agent and its surroundings. Please see Chap. 3 for more information on this.

Two main types of encapsulates might be distinguished, i.e., the reservoir type and the matrix type (see Fig. 2.1). The reservoir type has a shell around the active agent. This type is also called capsule, single-core, mono-core or core-shell type. Application of pressure can lead to breakage of the reservoir type of encapsulates and thus to the release of its contents. Poly- or multiple-core type of encapsulates with several reservoir chambers in one particle also exist. The active agent in the matrix type is much more dispersed over the carrier material; it can be in the form of relatively small droplets or more homogenously distributed over the encapsulate. Active agents in the matrix type of encapsulates are in general also present at the surface (unless they have an additional coating, see Fig. 2.1), in contrast to those in the reservoir type. For simplification, Fig. 2.1 shows only spherical shaped encapsulates, but they can also be cylindrical, oval or irregular shaped.

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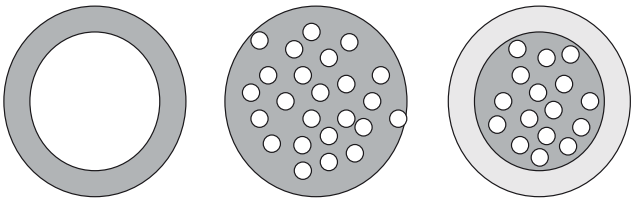

Fig. 2.1 Reservoir type (*left*), matrix type (*middle*), and coated matrix type (*right*) encapsulates. The latter is a combination of the first two. Only spherical shaped encapsulates are shown but other forms are also possible. Here the active is indicated in white, and the carrier material in gray. The active in the matrix type of encapsulates might be in the form of tiny droplets or is dispersed at the molecular level throughout the particle

Encapsulates might also be defined by their particle size, e.g., nanoparticles, microcapsules, microreservoir, etc.

The possible benefits of microencapsulated ingredients in the food industry could be:

- Superior handling of the active agent (e.g., conversion of liquid active agent into a powder, which might be dust free, free flowing, and might have a more neutral smell)
- Immobility of active agent in food processing systems
- Improved stability in final product and during processing (i.e., less evaporation of volatile active agent and/or no degradation or reaction with other components in the food product such as oxygen or water)
- Improved safety (e.g., reduced flammability of volatiles like aroma, no concentrated volatile oil handling)
- Creation of visible and textural effects (visual cues)
- Adjustable properties of active components (particle size, structure, oil- or water-soluble, color)
- Off-taste masking
- Controlled release (differentiation, release by the right stimulus)

Such benefits should overcome the following possible negatives:

- Additional costs
- Increased complexity of production process and/or supply chain
- Undesirable consumer notice (visual or touch) of the encapsulates in food products
- Stability challenges of encapsulates during processing and storage of the food product.

Because of these possible negatives, encapsulates should generally not be seen as a first option when designing food formulations. Only when other, simple options fail one may consider encapsulation. Nevertheless, because encapsulates facilitate formulations of food products that are healthier, tastier and more convenient, the demand for encapsulation has been growing since the last few decades (Frost and Sullivan 2005).

2.2 Encapsulation Processes

This section aims to provide a short overview of commonly used processes to encapsulate food active agent. It is certainly not a complete list. More details about these processes can be found in the references, and their use for specific applications can be found in the other chapters of this book.

Many encapsulation processes are based on making first droplets of the active (in gas, liquid or powder form) and these droplets are subsequently surrounded by carrier material in a gas or liquid phase via different physico-chemical processes (see Table 2.1 and below). The preparation of melt extrudates, liposomes, inclusion complexation technologies, and the use of natural encapsulates like yeast cells (see Chap. 5) might be the exceptions.

Table 2.1 Overview of common microencapsulation processes

Technology	Process steps	Morphology	Load (%)	Particle size (μm)
Spray-drying	1. Disperse or dissolve active in aqueous coating solution 2. Atomize 3. Dehydrate	Matrix	5–50	10–400
Fluid bed coating	1. Fluidize active powder 2. Spray coating 3. Dehydrate or cool	Reservoir	5–50	5–5,000
Spray-chilling/ cooling	1. Disperse or dissolve active in heated lipid solution 2. Atomize 3. Cool	Matrix	10–20	20–200
Melt injection	1. Melt the coating 2. Disperse or dissolve active in the coating 3. Extrude through filter 4. Cooling and dehydrating	Matrix	5–20	200–2,000
Melt extrusion	1. Melt the coating 2. Disperse or dissolve active in the coating 3. Extrude with twin-screw extruder 4. Cool	Matrix	5–40	300–5,000
Emulsification	1. Dissolve active and emulfiers in water or oil phase 2. Mix oil and water phases under shear	Matrix	1–100	0.2–5,000
Preparation of emulsions with multilayers	1. Prepare o/w emulsions with lipophilic active in oil phase and ionic emulsifiers 2. Mix with aqueous solution containing oppositely charged polyelectrolytes 3. Remove excess of free polyelectrolytes (option) 4. Repeat steps 2 and 3	Reservoir	1–90	0.2–5,000

Table 2.1 (continued)

Technology	Process steps	Morphology	Load (%)	Particle size (μm)
Coacervation	1. Prepare o/w emulsions with lipophilic active in oil phase 2. Mix under turbulent conditions 3. Induce three immiscible phases 4. Cool 5. Crosslink (optionally)	Reservoir	40–90	10–800
Preparation of microspheres via extrusion or dropping	1. Dissolve or disperse active in alginate solution 2. Drop into gelling bath	Matrix	20–50	200–5,000
Preparation of microspheres via emulsification	1. Emulsify water with biopolymer in oil phase 2. Add gelling agent under shear	Matrix	20–50	10–1,000
Co-extrusion	1. Dissolve or disperse active in oil 2. Prepare aqueous or fat coating 3. Use an concentric nozzle, and press simultaneously the oil phase through the inner nozzle and the water phase through the outer one 4. Drop into gelling or cooling bath	Reservoir	70–90	150–8,000
Inclusion complexation	1. Mix carrier, active and water together 2. Incubate and dry if necessary	Molecular inclusion	5–15	0.001–0.01
Liposome entrapment	1. Disperse lipid molecules in water, with active agent in lipid or water phase 2. Reduce size by high shear or extrusion 3. Remove free active (option)	Various	5–50	10–1,000
Encapsulation by rapid expansion of supercritical fluid (RESS)	1. Create a dispersion of active and dissolved or swollen shell material in supercritical fluid 2. Release the fluid to precipitate the shell onto the active	Matrix	20–50	10–400
Freeze- or vacuum drying	1. Dissolve or disperse active agent and carrier material in water 2. Freeze the sample 3. Drying under low pressure 4. Grinding (option)	Matrix	Various	20–5,000
Preparation of nanoparticles	Various methods, see text	Various	Various	0.1–1

2.2.1 Spray-Drying and Agglomeration

Spray-drying is one of the oldest processes to encapsulate active agent. It is so common in foods that it is not always perceived as an encapsulate, e.g., aroma in a spray-dried form. Spray-drying of active agent is commonly achieved by dissolving, emulsifying, or dispersing the active in an aqueous solution of carrier material, followed by atomization and spraying of the mixture into a hot chamber (see Fig. 2.2 and Barbosa-Cánovas et al. 2005; Gharsallaoui et al. 2007). During this process a film is formed at the droplet surface, thereby retarding the larger active molecules while the smaller water molecules are evaporated. Optionally, one may also spray-dry active agent in organic solutions like acetone or ethanol; however, this is used much less for environmental and safety reasons (which also increase the costs).

Spray-dryers in the food industry are usually atomizing the infeed with a high-pressure nozzle or centrifugal wheel (also called rotary atomizer) and operate with a cocurrent flow of air and particles to give minimal overheating of the particle. This latter is important if the contents are heat sensitive or somewhat volatile (as is the case with aromas). However, cocurrently-dried particles are likely to be more porous than ones prepared in the counter-current mode.

The size of the atomizing droplets depends on the surface tension and viscosity of the liquid, pressure drop across the nozzle, and the velocity of the spray. The size of the atomizing droplets also determines the drying time and particle size.

The temperature of the droplet surface corresponds at any point in the dryer to the "wet bulb" temperature of the gas phase surrounding the droplet as long as the particle surface is wet. The wet bulb temperature under standard spray-drying conditions is of the order of 50°C. By controlling the air-inlet temperature

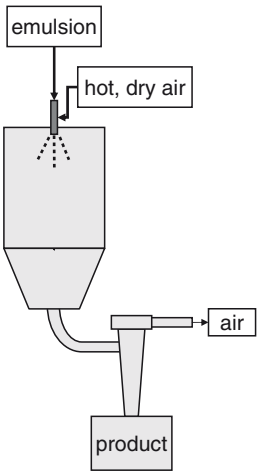

Fig. 2.2 Set-up of a spray-dryer with a cocurrent flow. The dried product is collected in a cyclone at the end

(typically 150–220°C), the flow rate, the feed rate, the feed temperature, and evaporative cooling, it should be ensured that the droplet temperature never exceeds 100°C. This temperature might be indicated by the air outlet temperature, which is typically 50–80°C. The larger the spray-dryer, the longer the residence time of the particle in the dryer (typically 5–100 s) and hence the larger the maximum size of the droplets that can be dried. Atomizing nozzles are usually mounted to spray downward, but it is also possible to spray upward like a fountain, which permits somewhat larger droplets to be dried because of the larger residence time of the droplet.

During the drying process a film is formed at the droplet surface and the concentration of ingredients in the drying droplet increases. Finally, a porous, dry particle is formed.

The carrier material used should meet many criteria, such as protection of active material, high solubility in water, molecular weight, glass transition, crystallinity, diffusibility, good film forming properties, good emulsifying properties, and low costs (Gharsallaoui et al. 2007). Examples from literature include natural gums (gum arabic, alginates, carrageenans, etc.), proteins (dairy proteins, soy proteins, gelatin, etc.), carbohydrates (maltodextrins and cellulose derivatives) and/or lipids (waxes, emulsifiers).

Conventional spray-dried encapsulates release their active agent immediately upon addition to water (which may also depend on the porosity of the particles). However, recent introductions of more hydrophobic and/or cross-linked carrier materials may provide a more gradual release upon dilution in water. Examples of these are denatured proteins, cross-linked proteins or cross-linked biopolymers.

Please see the reviews of Reineccius (2001, 2004), Gouin (2004), Barbosa-Cánovas et al. (2005), Desai and Park (2005), Gharsallaoui et al. (2007) and Jafari et al. (2008) for further, general information about encapsulation of food active agent by spray-drying.

For many applications, larger particles than those produced by spray-drying (in general about 10–150 μm) might be desirable. This might be achieved by agglomeration or granulation (Barbosa-Cánovas et al. 2005; Ortega-Rivas 2005). In general, this can be achieved in any equipment creating random movements. An option is fluidized bed spray granulation (also called spray-bed-drying), in which a spray-drying step is followed in one or two steps by a secondary agglomeration step in a fluid bed (Fuchs et al. 2006; see also the next section). Another option is to spray-dry onto another carrier powder (Fuchs et al. 2006). In both cases, the spray-dried particles are not fully dried after the first stage, and therefore remain sticky to facilitate agglomeration during the second phase. Alternatively, a binder solution (e.g., water) can be sprayed onto powder particles during high shear or tumbling (Litster 2003; Barbosa-Cánovas et al. 2005), or in a fluid bed (Uhlemann et al. 2002; Barbosa-Cánovas et al. 2005; Ortega-Rivas 2005).

An alternative process for the preparation of large particles is pressure agglomeration or compaction, in which spray-dried material is compressed under high pressure in extruders or presses, maybe together with additional maltodextrin, into lumps and then crushed into small pieces of about 0.7–3.0 mm (Barbosa-Cánovas

et al. 2005; Ortega-Rivas 2005; Uhlemann et al. 2002). This process is useful for applications in which encapsulates should not segregate within food products.

2.2.2 *Fluid Bed Coating*

Fluid bed coating is a technique in which a coating is applied onto powder particles in a batch process (see Fig. 2.3) or a continuous set-up. The powder particles are suspended by an air stream at a specific temperature and sprayed with an atomized, coating material. With time, each particle will be gradually covered every time it is in the spraying zone. The coating material must have an acceptable viscosity to enable pumping and atomizing, must be thermally stable and should be able to form a film over a particle surface. In general, 5–50% of coating is applied, depending on the particle size of the core material and application of the encapsulate.

The coating material might be an aqueous solution of cellulose derivatives, dextrins, proteins, gums and/or starch derivatives, and the evaporation of its water content is then controlled by many factors such as the spray rate, the water content of the coating solution, the air flow, the humidity of the air inlet in the chamber, and the temperature of the coating solution, atomized air, and the material in the chamber (Dewettinck and Huyghebaert 1999; Guignon et al. 2002; Teunou and Poncelet 2002, 2005a). Often a so-called Würster set-up is used, in which the coating is sprayed in an inner column from the bottom (see Fig. 2.3, left picture). The air flow

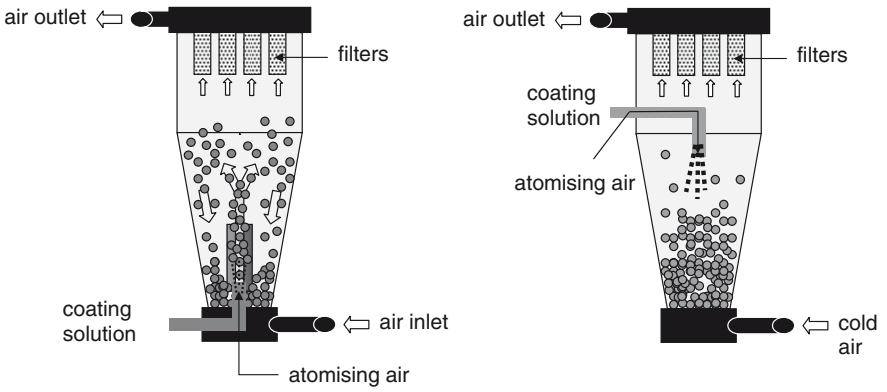

Fig. 2.3 Fluidized bed coating is achieved by an upwards air flow through a bed of particles and spraying a liquid with shell material. Here a so-called Würster coating set-up is shown at the *left*, in which process the coating material is sprayed onto powder particles within an inner column which brings the particles into circulation. On the *right*, a set-up is shown in which a coating solution is sprayed from the top onto powder particles. Other set-ups are also possible, which may include bottom-spraying without the inner column, side spraying with rotating disk and continuous configurations

rate is typically 80% in the center flow in the inner column and 20% in the periphery, which brings the powder particles into circulation. This increases the drying rate and reduces agglomeration. The bottom spray reduces the distance between the powder and the drops of coating solution, thereby reducing the risk of premature drying of the coating.

Alternatively, a molten lipid can be used as a coating material which can be either applied from the bottom or the top (see for the latter configuration the right picture in Fig. 2.3). Examples of lipids used are hydrogenated vegetable oils, fatty acids, emulsifiers and/or waxes. Care must be taken to prevent solidification of the lipid before it reaches the powder. This might be done by heating not only the storage vessel from which the molten lipid is pumped, but also the line, the nozzle, and atomizing air. Once in the chamber, the rate of congealing (solidification) is controlled by the application rate and the cooled, inlet air (often 10–20°C below its melting point). Product temperature too close to the melting temperature of the fat may result in sticky particles and thus agglomeration. At lower product temperature the congealing might occur before complete spreading so the coating might contain defects and pores.

The particles to be coated by fluid bed should ideally be spherical and dense, and should have a narrow particle size distribution and good flowability. Spherical particles have the lowest possible surface area and require less coating material for the same shell thickness than nonspherical ones. Sharp edges could damage the coating during handling. Fine and low-dense particles might face the risk of accumulating on the filter bags in the top of the machine.

Alternative air suspension coating technologies, e.g., pan coating, have been described by Teunou and Poncelet (2005b). In general, one applies a coating to make the powder more resistant to humidity. If desired, more than one coating can be applied on the powders (with increasing costs).

2.2.3 Spray-Cooling or Spray-Chilling

Spray-chilling or spray-cooling is another technology to produce lipid-coated active agent (Kjaergaard 2001; Uhlemann et al. 2002; Gouin 2004). The active agent might be soluble in the lipids, or be present as dry particles or aqueous emulsions. Firstly, droplets of molten lipid(s) are atomized into a chilled chamber (e.g., via nozzle, spinning disk or (centrifugal) co-extrusion), which results in solidification of the lipids and finally their recovery as fine particles. The initial set-up of spray cooling is quite similar to spray-drying (see Sect. 2.2.1), but no water is evaporated here. In the spray-chilling technique, the particles are kept at a low temperature in a set-up similar to the fluidized bed spray granulation (see Sect. 2.2.2), on which molten lipid droplets may adhere to already hard lipid particles before solidification. In general, the melting point of the lipid used is in the range of 34–42°C for spray-chilling, and higher for spray-cooling.

Rotating disk is another atomization method for the preparation of solid lipid particles (Sparks and Mason 1987). A suspension of particles in molten lipid is spread on the disk, followed by separation of coated particles by atomization at the

edge of the rotating disk. The disk may be flat or bowl-shaped and can be heated. The drops solidify when falling from the disk. Depending on the droplet size and melt characteristics a certain falling height is required. The size of the particles depends on the core particles, melt viscosity, melt temperature, disk configuration and the rotational speed.

2.2.4 Melt Injection and Melt Extrusion

Carbohydrate materials can be mixed with an active when molten, at a temperature above 100°C, then pressed through one or more orifices (extrusion) and finally quenched to form a glass in which active agent have relatively little mobility. In general, the glass transition of encapsulates made by extrusion is between 30 and 70°C.

Basically, two processes to encapsulate active agent in a carbohydrate melt can be distinguished. One is melt injection, in which the melt (composed of sucrose, maltodextrin, glucose syrup, polyols, and/or other mono- and disaccharides) is pressed through one or more orifices (filter) and then quenched by a cold, dehydrating solvent. This is a vertical, screwless extrusion process. Generally isopropanol, and also liquid nitrogen, is used as the dehydrating solvent. The coating material hardens on contact with the dehydrating solvent, thereby encapsulating the active (Porzio 2004). The size of the extruded strands is reduced to the appropriate dimensions inside the cold solvent during vigorous stirring, thereby breaking up the extrudates into small pieces. Any residues of active agent on the outside will be washed away by the dehydrating solvent. Encapsulates made by melt injection are water-soluble and have particle sizes from 200 to 2,000 µm.

Encapsulation in a carbohydrate melt can also be achieved by using an extruder with one or more screws in a continuous process (see Fig. 2.4). This process is called

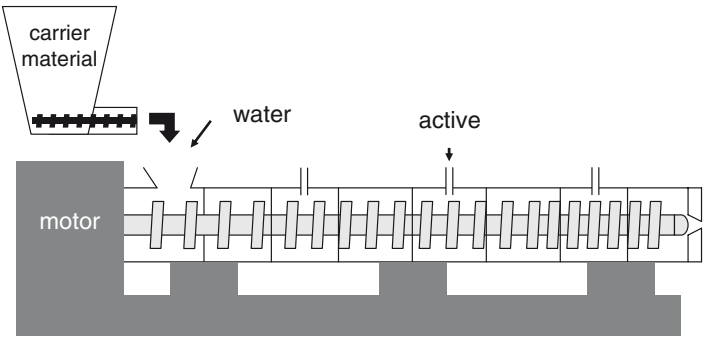

Fig. 2.4 Scheme of a melt extruder. Most often, extruders with two screws are preferred. Each section can be temperature controlled. The carrier material is commonly added via a twin screw feeder in the first section, and water and active can be added simultaneously or later

melt extrusion, and it can be regarded as a process very similar to melt injection; the main differences are that, in general, melt extrusion utilizes screws in a horizontal position and that the extrudates are not surface washed. Extruders are thermomechanical mixers that consist of one or more screws in a barrel. Most often, double screw extruders equipped with sinusoidal screws (self-wiping) are preferred for encapsulation. It is common to characterize the extrusion screws by the length/diameter (L/D) ratio, typically between 20 :1 and 40 :1. Transport of material within the extruder takes place by rotational, and sometimes oscillatory, movement of the screws. In the beginning (the feed zone), the screw design is such that a low pressure is generated to homogenize the feeding. In the subsequent zone(s), a gradual increase in pressure is achieved via the screw design to melt, further homogenize, and compress the exrudate. In the final part of the barrel, a constant screw design helps to maintain a continuous high pressure to ensure a uniform delivery rate of molten material out of the extruder. Most of the time, the barrel is also divided into sections to allow for section-controlled variation in temperature. At the end of the barrel, a "pre die" and "die head" determine the shape of the final product (e.g., sheets, ropes or threads). It can be equipped with a chopper/cutter to obtain granular extrudates. Alternatively, these can be obtained via postpreparation equipment like grinders or mills, see Ortega-Rivas 2005. Extrudates can be composed of starch, maltodextrins, modified starches, sugars, cellulose ethers (like hydroxypropyl cellulose or hydroxypropyl methyl cellulose), proteins, emulsifiers, lipids, and/or gums. Often, melt extrudates for use in food products are composed of "thermoplastic" starch (Yilmaz 2003; Yilmaz et al. 2005). Native starch is composed of semicrystalline granules with a size of 1–100 μm (see also Sect. 3.2.1.1). It consists of amylose and amylopectin, both containing only α-D-glucose units. Thermoplastic starch is obtained by destructuring the semicrystalline starch via simultaneous application of heat (around 110–120°C) and mechanical forces. The presence of plasticizers, such as water or glycerol/polyols, enables further processing. Plasticizers also influence other properties, such as glass transition temperature of the material, its solubility and morphology (Yilmaz et al. 1999 and references therein). Unmodified thermoplastic starch dissolves quickly in water. Modified starches that are tailor-made (using physical, chemical or enzymatic routes) might be used to make encapsulates more water resistant (Zasypkin and Porzio 2004). In addition, additives (such as plasticizers and other constituents in the formulation) and in situ modifications (e.g., heat treatment, surface modification, and induction of (local) crystallinity) might also be used to get relatively water-insoluble extrudates. Addition of the active ingredient might be in the mixing/dispersing zone of the extruder (at about halfway in the scheme of Fig. 2.4). This minimizes the residence time of the active ingredients and avoids the relatively high temperatures required to plasticize starch in case starch is still in its granular form. The active can be added as a gas, liquid, emulsion, or powder. Morphology of the obtained formulations will depend on the properties of the active agent as well as the matrix. In case the matrix and the active agent are compatible a single-phase morphology can be obtained, where the matrix behaves as a solvent. In case of incompatibility a two-phase morphology is likely to be obtained. For example, lipophilic compounds may mix well with starch modified with hydrophobic groups, in contrast to hydrophilic ones.

Alternatively, pre-encapsulation and surface modification are possible. The encapsulation efficiency (and the release kinetics) will depend on adequate mixing and dispersion of the encapsulant within the matrix. The use of emulsifiers may allow better control of these characteristics (Yilmaz et al. 2001). Unfortunately, the active load of extruded encapsulates is relatively low (typically less than 10%), which may have an impact on their cost-in-use.

2.2.5 Emulsification

Emulsions are kinetically rather than thermodynamically stable two-phase systems and ultimately, both the oil and water phase will separate. Proper formulation design of both phases and the interface, including choice of ingredients like emulsifiers, might prevent that (McClements 2005; Appelqvist et al. 2007). Emulsions are commonly made under high shear with, e.g., homogenizer, colloid mill, high shear mixer, or stirred vessel preferably equipped with baffles (see Fig. 2.5 for the latter).

Plain emulsions can be used as a delivery vehicle for either water soluble and/or lipophilic active agent in food products (Appelqvist et al. 2007). There are two considerations that must be taken into account when formulating an emulsion for controlled delivery. First, the emulsion system must be (storage) stable right up to the point

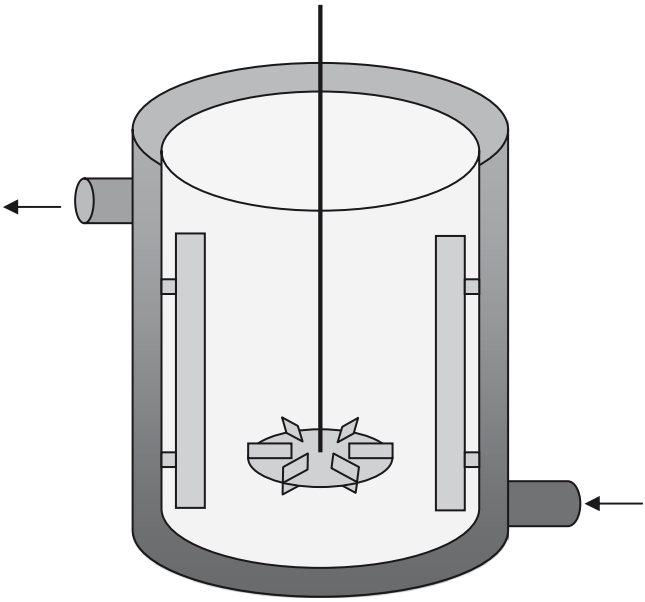

Fig. 2.5 Set-up of a stirred, double-wall vessel with 3–4 baffels and Rushton-impeller, which might be used for the preparation of emulsions or complex coacervates. This set-up can be used at both a lab scale and a factory scale

of application. Second, on application the emulsion should behave in a manner consistent for achieving the desired delivery. In many (but by no means all) cases this equates to the "making and breaking" of emulsions for stability and subsequent delivery.

Water soluble food active agent might be encapsulated in water-in-oil (w/o) emulsions or double emulsions of the type w/o/w (Appelqvist et al. 2007). Furthermore, oil-in-water (o/w) emulsions may affect taste (e.g., salt) by changing the aqueous phase volume and thus the concentration of taste molecules in water, and by suppressing contacts of salt with taste receptors. Lipophilic active agent (e.g., aroma, cartonenoids such as lycopene and beta-carotene, plant sterols, vitamin E, dietary fats) might be protected and delivered to consumers via o/w emulsions (Appelqvist et al. 2007; see also Chaps. 6 and 8).

Several technologies have been developed to produce highly uniform emulsion droplets (see Link et al. 2004; McClements 2005), such as reduction of polydispersity of already formed emulsions (including repeated fractionation and shearing immiscible fluids between uniformly separated plates; Mabille et al. 2003), or single-drop technologies like microfluidics. Monodispersed emulsions may have a more defined behavior and release pattern of entrapped active agent than polydispersed ones. This can be very important in pharmaceutics and when the emulsions are used as a template to make new materials for, e.g., electronics. Currently, it is not clear whether this would constitute a real advantage in food systems.

Oil-in-water emulsions might be dried by, e.g., spray-drying (see Sect. 2.2.1) or freeze-drying (see Sect. 2.2.13) to provide a powder. Such dry emulsions might be encapsulates or an instant formulation of beverages or other food products. Emulsion droplets might also be prepared during the processing of encapsulates (such as extrudates or co-extrusion, see Table 2.1), or act as templates for further processing (such as complex coacervates, microspheres or emulsions with multilayers; see Table 2.1 and below).

Another use of emulsions is the emulsification of molten fat or wax in water at a temperature above the melting temperature of the fat, followed by cooling during mixing (Mellema et al. 2006). This might be an alternative process to the spray-chilling/spray-cooling process described in Sect. 2.2.3. However, if the active is (partly) water-soluble, then it might not be (fully) encapsulated within the fat.

2.2.6 Preparation of Emulsions with Protein and/or Biopolymer Multilayers

A layer around "primary" emulsions with ionic emulsifier(s) can be formed by adsorbing oppositely charged polyelectrolytes to form "secondary" emulsions with a two-layer interface. This procedure can be repeated to form emulsion droplets with three or more layers at their interface (Guzey and McClements 2006). Removal of excess free polyelectrolytes by, e.g., centrifugation or filtration between the steps might be necessary.

This procedure is also called layer-by-layer (LBL) electrostatic deposition technique. It is a relatively new technique and its full potential is under investigation. It is a simple preparation technique at lab scale, but quite laborious at larger scales. Examples include emulsions with multilayers composed of β-lactoglobulin– ί-carrageenan, β-lactoglobulin–pectin, or sodium dodecyl sulfate (SDS)–chitosan–pectin.

2.2.7 Coacervation

Coacervates are made via a liquid–liquid phase separation mechanism of an aqueous solution into a polymer-rich phase (known as coacervate) and a polymer-poor phase. According to the number of polymer type(s) present, the process can be identified as (simple) coacervation when only one type of polymer is involved or complex coacervation when two or more types of polymers of opposite ionic charges are present. The coacervates used to encapsulate active agent are most often of the complex type. Their shell is frequently composed of gum arabic and gelatin. The technology was developed by National Cash Register Co. in the 1950s and was the basis of carbonless copy paper, the first commercial product with microencapsulates.

Complex coacervates are commonly made from an o/w emulsion with gelatin and gum arabic at a 1:1 w/w ratio and at a 2–4% w/w of each polymer dissolved in the water phase via adjusting the pH from neutral to about 4 under turbulent conditions in a stirred vessel (see Fig. 2.5) at >35°C, a temperature above the gelation temperature of gelatin (Gouin 2004; Lemetter et al. 2009). This creates three immiscible phases (oil, polymer-rich, and polymer-poor phase), and the polymer-rich phase droplets will deposit on the emulsion surfaces because of interfacial sorption. Alternatively, complex coacervation can be induced by dilution instead of pH adjustment; oil is emulsified in a 8–11% (w/w) gelatin solution, followed by addition of gum arabic and dilution water (Thies 2007). Upon cooling well below 35°C (Lemetter et al. 2009), the deposited gelatin and thus the shell will solidify. Factors like polymer concentrations, pH, turbulence of the system, emulsion size, ionic strength, and temperature affect the preparation process. After cooling, there is an option to crosslink the shell with, e.g., glutaraldehyde (Tabor et al. 1992; not allowed in Europe for food applications) or transglutaminase (Thies 2007). Finally, the coacervates are isolated and washed (if needed) via filtration or sedimentation (if their density is higher than the density of water, which depends on the relative amount of shell compared to the oil core) and might be dried by spray-drying or fluid bed drying. Optionally, gum arabic can be replaced by other negatively charged molecules like carboxymethylcellulose, pectin, carrageenan, alginate and alginate derivatives, or polyphosphate (Bakker et al. 1999; Gouin 2004; Thies 2007), or gelatin can be replaced by whey proteins (Weinbreck et al. 2003). Gelatin most often has a beef or pork origin, but as a Kosher or Halal alternative fish gelatin might be used. Each polymer combination operates at unique conditions in terms

of pH, temperature, ionic strength, polymer levels, molecular weight, charge density, cooling rate, etc. Complex coacervates often have a very typical, oval shape.

Simple coacervation has been used less to encapsulate active agent. Examples are the encapsulation of o/w emulsions in gelatin where solubility is reduced by temperature or sodium sulfate, in 0.2 wt. % chitosan by increasing the pH with 0.1–1.5 wt. % sodium hydroxide (Hsieh et al. 2006), and in an aqueous solution of hydroxypropyl methylcellulose or methyl cellulose where simple coacervation was induced by addition of maltodextrin (Porzio and Madsen 1997; the maltodextrin also functioned as spray-drying carrier material for double coating).

2.2.8 Preparation of Microspheres by Extrusion or Emulsification

Microspheres are microbeads composed of a biopolymer gel network entrapping an active. The microspheres are commonly prepared in the presence of the active, but postloading of blank microspheres containing oil droplets with, e.g., aroma is also an option. Calcium-alginate gel is the best known gelling system used for the preparation of gel beads to encapsulate a wide variety of active agent, such as oil droplets containing aroma, cells, probiotics, yeast, or enzymes to name a few. These active agent are relatively large in size, as smaller ones will diffuse easily through the porous biopolymer network. Gelation of alginate in the presence of divalent cations can be easily controlled and does not require heating like other gelling biopolymers like agarose, agar, or carrageenan. Microspheres are commonly made via two different routes (Krasaekoopt et al. 2003; Gouin 2004):

(a) *The extrusion or dropping method*: This method consists of dropping droplets of an aqueous solution of 0.6–4 wt. % sodium alginate and active into a gelling bath of 0.05–1.5 M calcium-chloride solution. The dripping tool can be simply a pipette, syringe, vibrating nozzle, spraying nozzle, jet cutter, atomizing disk, coaxial air-flow, or electric field (see Fig. 2.6 and also Zhang et al. 2007 for dropping and spraying set-ups). In general, particles with a diameter between 0.2 and 5 mm can be made depending on the dripping tool and the viscoelasticity of the alginate solution. Alternatively, the extrusion or dropping method can be used with a concentric nozzle (co-extrusion), to prepare core-shell type of encapsulates with a lipophilic core and a shell of a gel network (see Sect. 2.2.9). In a recent study by Prüsse et al. (2008), different common bead production technologies were analyzed to check their ability to process fluids of different viscosities. Each of the technologies is suitable for the production of spherical microspheres (800 μm in diameter) from low-viscous sodium alginate solutions (up to 2% w/w), whereas high-viscous alginate solutions (≥3% w/w sodium alginate) cannot be processed with the vibration technology anymore. With the electrostatic, jet cutter, and coaxial air-flow technologies microsphere production was possible and a narrow size distribution was always achieved. However, the shape of the microspheres produced by coaxial air-flow was nonspherical and

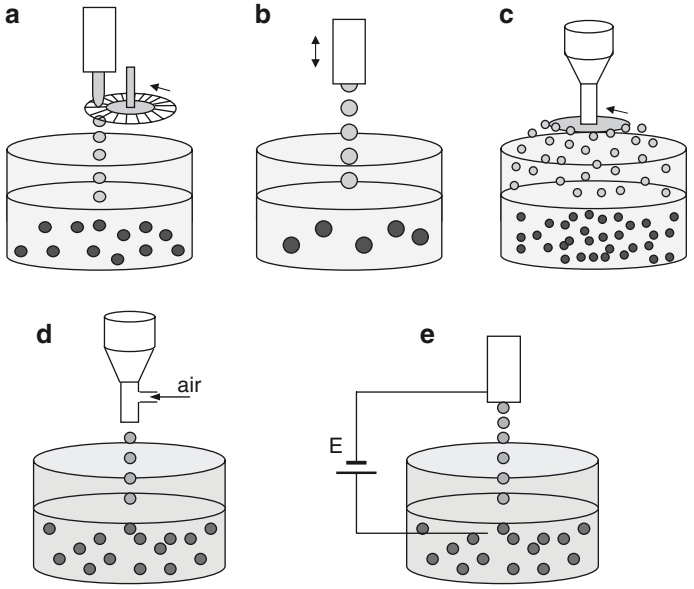

Fig. 2.6 Set-ups of three different ways of making microspheres. Aqueous solution of, e.g., sodium alginate and active are atomized by jet-cutter (**a**), pipette or vibrating nozzle (**b**), atomizing disk (**c**), coaxial air-flow (**d**), or electrostatic potential (**e**). The droplets fall into a batch of 0.05–1.5 M calcium chloride, resulting in instantaneous formation of calcium alginate microspheres

deformed egg-like or drop-like microspheres were obtained from 3 and 4% (w/w) sodium alginate solutions, respectively. In addition, extrusion technologies were compared with respect to productivity. The microsphere production rates of the coaxial air-flow and the electrostatic technology are very low. Thus, these technologies are limited to small/lab-scale applications when only a few grams of material have to be processed. The vibration technology exhibits up to 50 time higher production rates than the aforementioned technologies. Vibration systems, thus, are suitable for lab-scale as well as larger scale applications, assuming that multinozzle devices are used for larger scales. The JetCutter technology is suited both for lab-scale and large- up to industrial-scale microsphere production. Instead of calcium-alginate, one may prepare microspheres with other compositions, e.g., by dropping 4% κ-carrageenan into 0.3 M potassium chloride, or heated gelatin, agarose, or agar solution into a cold bath.

(b) *The emulsion method*: This technique utilizes emulsions to make microspheres. Several variants exist. One may add calcium chloride to an emulsion of water droplets of an alginate solution and active in vegetable oil. This results in the "break-up" of the emulsion and microbeads are formed by the gelation of the alginate droplets. Alternatively, both alginate and calcium (in an insoluble form such as calcium carbonate) can already be present in the water phase of the emulsion. Upon addition of an oil-soluble acid (such as acetic acid) the pH

decreases, liberating free calcium ions in the system and initiating the gel formation of alginate droplet with calcium. Delta-glucono-lactone can also be employed for slower gelation kinetics, if needed. Another variant of the emulsion method is the preparation of a water-in-oil emulsion first, with calcium ions in the water phase, and second, addition of an aqueous alginate solution during stirring which produces a phase inversion, and calcium alginate begins to deposit on the newly formed drops (Casana Giner et al. 2006). Another colloid might then be added that will deposit on the surface of the microspheres (e.g., xanthan gum) and then a primary surfactant is added to reduce the size of the water in the oil drops. Agglomeration or deagglomeration may occur (depending on the process conditions) and finally the microspheres are hardened at an elevated temperature (75°C for 120 min). Gelling materials other than alginate can also be used in the emulsion technique, such as κ-carrageenan (gelation upon cooling with potassium ions), chitosan (crosslinking by addition of anions), gelatin (crosslinking by mixing with anionic polysaccharides, such as gellan gum, at neutral pH, followed by adjusting the pH to make gelatin positively charged), and pectin (chemically or physically crosslinked).

The emulsion method has the advantage that it can produce smaller microspheres (10 μm–1 mm) than the extrusion method (0.2–5 mm). It is also easier to scale-up. However, the emulsion method might be more expensive if vegetable oil has to be removed (and recycled), and the microspheres have to be washed sufficiently to eliminate the residual vegetable oil on the surface.

The presence of chelating agents (e.g., phosphate, lactate, citrate or bicarbonate) may interfere with the encapsulation process or alter the integrity of the calcium-alginate gels beads added into wet products. Posthardening for long periods of time in a solution with the crosslinker (e.g., storage of calcium-alginate microspheres for 1 day in 0.2 M calcium chloride solution), coating (e.g., with chitosan or poly-L-lysine, which are both not food grade), cross-linking with cationic polymers (e.g., chitosan), incorporation of additives (e.g., microcrystalline cellulose, hydrophobic starches) in the gel network, and/or modification of oil reservoir (if applicable) might be applied to modify the properties of the microspheres.

2.2.9 Co-extrusion

Co-extrusion is an extrusion technology which utilizes a concentric, multifluid nozzle, which may be stationary, rotating, or vibrating, It can be utilized to prepare spherical microbeads with a hydrophobic core of active agent and a hydrophilic or hydrophobic shell produced by interfacial gelling (e.g., with calcium-alginate or potassium-carrageenan) or cooling (e.g., gelatin or fat). Different set-ups are possible:

– Some equipment (e.g., from Inotech, Brace, Nisco) utilizes a vibrating multi-fluid nozzle to produce 80–1,500 micrometer particles. The technology is based on the principle that a laminar liquid jet is broken into equal-sized droplets by a superimposed vibration.

– Some other equipment is based on centrifugal co-extrusion, which leads to the formation of round beads at the edge of the nozzle due to Raleigh instabilities.
– The nozzle might also be submerged into a moving carrier and cooling fluid (see Fig. 2.7; Uhlemann et al. 2002). The submerged set-up prevents disruption of the shell upon contacting the cooling liquid. The capsules can be about 1–8 mm with typically a 70–95% load (aroma, fish oil, vitamins, freeze-dried probiotics dispersed in oil, etc.).
– Another option is to make use of a dual-feed spraying nozzle in combination with ultrasonic atomization (e.g., from Sono-Tek), which allows one to spray-dry immediately in the air after atomization takes place.

2.2.10 Inclusion Complexation

Molecular inclusion is the association of the active in a cavity-based material. The best known example is cyclodextrin (Hedges 1998; Szente and Szejtli 2004; Regiert 2008). Cyclodextrins are cyclic oligosaccharides of 6–8 D-glucose molecules, which are enzymatically joined through alpha 1–4 linkages in such a way that they to form a ring (see Fig. 3.7 in the next chapter). Some properties of cyclodextrins are listed in Table 2.2. Cyclodextrins containing six, seven, or eight glucose molecules are referred to as α-, β- and γ-cyclodextrin, respectively. Their diameters are about 14, 15 and 17 Å, respectively. Cyclodextrins have a lipophilic inner pocket of about 5–8 Å, in which an active molecule with the right size can be reversibly entrapped in an aqueous environment. However, this characteristic limits

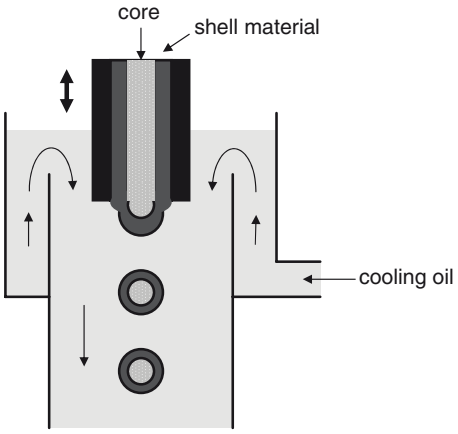

Fig. 2.7 Set-up of submerged co-extrusion with a vibrating nozzle placed into the carrier and cooling oil. The cooling oil is circulating and core-shell encapsulates are isolated from the cooling oil by, e.g., filtration

Table 2.2 Properties of cyclodextrins (Most data taken from Regiert (2008))

	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Number of glucose units	6	7	8
Molecular weight (g/mol)	973	1,135	1,297
Crystal water content (wt. %)	10.2	13.2–14.5	8.1–17.7
Molecule diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
Solubility in water at 25°C (g/mol)	14.5	1.85	23.2
Hydrolysis of α-amylase	Negligible	Slow	Fast
Crystal water content	10.2	13.2–14.5	8.13–17.7

its loading capacity. Loading of cyclodextrins can be achieved by coprecipitation of the complex in aqueous solutions (essentially a laboratory method), by using a slurry of partially dissolved cyclodextrin (upto 45% w/w), by using a paste with 20–30% water, or by dry mixing (Hedges 1998). Temperature, time, the amount of water, and the particular active and cyclodextrin control the loading rate and efficiency. β-cyclodextrin is the most common one. The cyclodextrins might be branched enzymatically to increase their water solubility. Unfortunately, the use of cyclodextrin might be limited by regulatory rules. In Japan, cyclodextrins are regarded as a natural product. In the USA, α-, β- and γ-cyclodextrin have GRAS status. However, in the EU β-cyclodextrin is allowed in a limited number of products (chewing gum, potato, cereal, flour or starch based snacks, and in water-based flavored drinks; <1 g/kg) and α-cyclodextrin only has a regulatory status as a novel food since February 2007. Novel food status for the use of γ-cyclodextrin in the EU has been filed but not given as yet.

Other examples of molecular inclusion might be the entrapment of lipids by amylose (see Sect. 2.3) and the use of ligand-binding proteins (De Wolf and Brett 2000), such as the milk protein β-lactoglobulin. This protein belongs to the superfamily of lipocalins, together with retinol-binding protein and odor-binding proteins (Guichard 2006; Tromelin et al. 2006). β-lactoglobulin has a hydrophobic pocket, which binds fatty acids and aroma molecules in a pH and temperature dependent manner.

2.2.11 Liposome Entrapment

Liposomes consist of at least one closed vesicle composed of bilayer membranes which are made of lipid molecules, such as phospholipids (lecithin) and cholesterol (see also Sect. 3.2.3.4 and Fig. 3.19). They form when (phospho)lipids are dispersed in aqueous media and exposed to high shear rates by using, e.g., microfluidization or colloid mill. The underlying mechanism for the formation of liposomes is basically the hydrophilic–hydrophobic interactions between phospholipids and water molecules. Active agent can be entrapped within their aqueous compartment at a low yield, or

within or attached to the membrane at a high yield. The particle size ranges from 30 nm to a few microns. Small vesicles tend to aggregate or fuse and may end up growing into micron-size particles during storage, which might be prevented by electrostatic repulsion (e.g., by addition of charged lipids in the membrane) or steric stabilization. Liposomes are currently mainly studied and used as advanced, pharmaceutical drug carriers (Torchillin and Weissig 2003), and their use in foods (Were et al. 2003; Gouin 2004; Taylor et al. 2005; Kosaraju et al. 2006; Mozafari et al. 2006; Takahashi et al. 2007) is quite limited due to its chemical and physical instability upon storage in especially emulsified food products, low encapsulation yield, leakage upon storage of liposomes containing water-soluble active agent, and the costs of raw materials (Zuidam et al. 2003). Liposomes are now used as drug delivery systems. For food applications, however, liposomes have mainly been studied to enhance ripening of hard cheeses and other applications in the food industry are very limited.

2.2.12 Encapsulation by Using Supercritical Fluid Technology

Supercritical fluids exist above a critical temperature and pressure at which the substance's liquid and gas phases are indistinguishable (Thies et al. 2003; Martin Del Valle and Galan, 2005). Their properties are intermediate to those of liquids and gases – liquid-like densities, gas-like viscosities, gas-like compressibility, and higher diffusivity and mass transfer than liquids. Many compounds can be brought into a supercritical state, such as water, propane, nitrogen, and carbon dioxide. The last one is probably the most interesting solvent for use in an encapsulation process, since it is environmentally friendly, it minimizes the use of organic solvent and water, and can be applied at reasonable pressures and temperatures (<30°C). It is actually applied to improve existing encapsulation processes:

- When supercritical fluid is released through a small nozzle, the abrupt pressure drop causes the supercritical fluid to evaporate or to transform into a much poorer solvent. Dissolved or swollen shell material will precipitate onto active agent dispersed in the supercritical fluid. This process is called Rapid Expension of Supercritical Solutions (RESS). Carbon dioxide is an apolar solvent, and therefore only shell materials like fat or wax will solubilize well in it. Hydrophilic proteins (e.g., gelatin) or polymers (e.g., cellulose, hydroxypropyl methylcellulose) can swell in it or might be solubilized by using cosolvents.
- Spray-drying of solvents containing supercritical carbon dioxide (also called supercritical assisted atomization, SAA) operates at relatively low temperatures, which might be beneficial for temperature-sensitive materials such as proteins or volatile flavors. Cosolvents might also be necessary here to dissolve active agent like proteins.
- Active agent and carrier material dissolved in organic solvent may be sprayed into supercritical fluid, thereby extracting the solvent from the incoming spray droplets and coprecipictating the active agent and carrier material. This process is called aerosol solvent extraction (ASES).