Chapter 17

Nanocarriers for Plant-Derived Natural Compounds

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Chapter Outline

1. INTRODUCTION

Plant-derived natural compounds have been used for the treatment of many diseases since ancient times. Nowadays, natural compounds having both antioxidant and antimicrobial activities are widely used in medical, cosmetic, food, and pharmaceutical industries. Their antioxidant activities contribute to protecting against oxidative damage of biologically important cellular components such as proteins, membrane lipids, and also DNA. In addition, phytochemicals act as antimicrobial agents because phytochemicals, secondary metabolites of medicinal plants, are also a kind of defense mechanism of the plant. Polyphenol structures are among the phytochemicals that are found in large amounts in most of the plants. However, the extraction of bioactive phenolic compounds from plant materials is the first and significant step for identification of bioactive phytochemicals in medicinal plants. Another important issue is the preservation of their bioactivity during their processing conditions and storage. The main problem of using plant-derived natural compounds is their degradation in the gastrointestinal system before reaching the circulation system, which limits the area of usage of these compounds. Therefore it is necessary to apply encapsulation systems to maximize the potential therapeutic benefits of natural compounds. Encapsulation provides good protection for sensitive compounds present in plant extracts against oxidation and dehydration reactions, which reduce the bioactivity of natural compounds. Many biopolymers have been widely studied as an alternative encapsulating nanocarrier material since synthetic polymers have many undesired properties. This chapter will discuss nanoencapsulation methods and advances in carrier systems for plant-derived natural compounds.

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2. PROPERTIES OF POLYPHENOLS

2.1 Antioxidant Properties of Polyphenols

Phenolic compounds are bioactive substances widely distributed in all vascular plants ranging from simple to complex structures. Their structures possess one or several benzenic cycles as a derivative of shikimic acid and polyacetate metabolism (Munin and Edwards-Lévy, 2011). Plant polyphenols possess a broad spectrum of biological properties, including antioxidant, antiinflammatory, antibacterial, and antiviral functions (Fang and Bhandari, 2010).

Polyphenolic compounds possess antioxidant properties because of their interaction with proteins or with ions and radical scavenging activity. They chelate potential prooxidant metal ions (Fe^{3+}, Al^{3+}, Cu^{2+}) or trap reactive oxygen species (ROS) (Leopoldini et al., 2011). Basically, the action of phenolic compounds as antioxidants respectively acts as free radical acceptors. Thus they inhibit or delay the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals (R) and suppressing the formation of ROS (Dai and Mumper, 2010).

$$R^{\bullet} + POH \rightarrow RH + PO^{\bullet} \tag{17.1}$$

The phenoxy radical intermediates (PO•) are less stable because they form a resonance structure. Thus the phenoxy radical intermediates also continue to interfere with chain-propagation reactions by reacting with other free radicals.

$$PO^{\bullet} + R^{\bullet} \rightarrow POR \tag{17.2}$$

Phenolic compounds have dominant and ideal structure chemistry for free radical scavenging activities. Because of their phenolic hydroxyl groups that are prone to donating a hydrogen atom or an electron to a free radical, phenolic compounds exhibit strong antioxidant capacity. Another property of a polyphenolic nature that contributes to its antioxidant capacity is a high tendency to chelate metal ions. Free radical formation is prevented by redox-active transition metals (Leopoldini et al., 2011). Chelation of iron ions and suppression of superoxide-driven Fenton reaction may be driven by polyphenols. Eqs. (17.3) and (17.4) indicate that harmful ROS stems from Fenton reaction. In this reaction, two different oxygen radical species are formed as an effect of oxidation and reduction of iron(II). The key role of the Fenton reaction takes part in the oxidation of membrane lipids and amino acids, whereas its presence may also lead to pathologies related to ROS such as carcinogenesis, neurodegenerative diseases, and atherosclerosis.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \tag{17.3}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H^{+} \tag{17.4}$$

Antioxidants contribute to protect against oxidative damage of biologically important cellular components such as proteins, membrane lipids, and also DNA from ROS attacks. Free radicals and ROS are released continuously during the essential aerobic metabolism as unwanted metabolic by-products. The role of antioxidants may directly react with an inactive free oxygen radical. Antioxidants show functions as terminators of free radical chains, or chelators of redox-active transition metal ions that are capable of catalyzing lipid peroxidation (Al-Mustafa and Al-Thunibat, 2008; Wellwood and Cole, 2004). There are many pathways by which antioxidant can intercept free radical oxygen species in biological systems, such as acting as reducing agents, inducing the preparation of antioxidative enzymes, or suppressing the production of oxidative enzymes, i.e., cyclooxygenase, telomerase, lipoxygenase, by complexing the protein with noncovalent bond formation and hydrophobic interactions (Naasani et al., 2003; Su et al., 2007). The activity of these enzymes is responsible for inhibiting free radical oxygen species under normal circumstances, but the enzymes can deform or the gene of the enzymes cannot make transcription during stress conditions. Antioxidant activity can prevent stress response.

2.2 Antimicrobial Properties of Polyphenols

Phytochemicals with antioxidant activity may show prooxidant behavior under the setting of pathogenic microorganisms. It is thought that the toxicity of bioactive polyphenols to microorganisms is associated with the sites and number of hydroxyl groups they have (Das et al., 2010). In addition, some researchers have observed that more highly oxidized phenols are more inhibitory against pathogenic microorganisms (Paiva et al., 2010). According to research, there are many mechanisms of antimicrobial action of phytochemicals, yet they are also not fully understood. It is thought that flavonoids inhibit cytoplasmic membrane function, while they are able to change cell morphology with damage formation of filamentous cells (Cushnie and Lamb, 2005). Moreover, they may inhibit DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydrate activities, thus the synthesis of DNA and RNA is inhibited (Cushnie and Lamb, 2005; Paiva et al., 2010). Some

compounds (e.g., Tannins) have been reported to have antimicrobial activity by binding to polysaccharides or enzymes on the surface of cells. On the other hand, Terpenes directly cause membrane disruption, and coumarins can reduce the cell respiration of microorganisms (Cowan, 1999). It is important to note that not only a single compound responsible for observed microbiological activity but also the combination of compounds may show bioactivity when they interact in a synergistic manner.

2.3 Application of Polyphenols

Natural antioxidants are in high demand because of their potential in health improvement and disease prevention, and their developed safety and consumer acceptability (Bellik et al., 2012). The properties of antioxidants in medicinal plants depend on which plant phytochemical contains secondary metabolites. In addition, concentration and composition of a phytochemical are related to antioxidant activity. Plants, the main sources of antioxidants, comprise a great diversity of compounds. These compounds, which are phytochemicals, vary in structure and the number of phenolic hydroxyl groups and their position, which leads to variation in their antioxidative capacity (Buchanan et al., 2000). Phytochemicals are classified as carotenoids, alkaloids, nitrogen-containing compounds, organic sulfur, and phenolic compounds, based on their biosynthetic origins. The most studied phytochemicals are the carotenoids and polyphenolics.

Polyphenols present a wide range of pharmacological attributions. Phenolic compounds are known for their antioxidant activity, which is useful for diabetes mellitus or preservation against cancer. Interestingly, several studies have revealed that some polyphenols, such as tannins or flavonoids, cause oxidative strand breakage in DNA in the presence or absence of metal ions (Nobili et al., 2009; Ziech et al., 2012). The reason for this is that cancer cells are known to include high amounts of copper ions. When exposed to redox reactions with polyphenols, cancer cells generate ROS and phenoxyl radicals lead to the breakdown of the structure of DNA, lipid, or protein (Sawadogo et al., 2012), therefore polyphenols act as prooxidants. The antioxidant capacity of phenolic compounds has also been shown to play a role in reducing risks of cardiovascular disease, neurodegenerative diseases, or osteoporosis, which are conditions related to oxidative stress (Fang and Bhandari, 2010).

2.4 Encapsulation of Polyphenols

Polyphenols have gained accelerated trends in various fields ranging from therapeutics to food additives. However, they have limited utilization depending on loss of antioxidant activity. Antioxidant activities of polyphenols are effective only when active compounds preserve their bioactivity and stability. Their major disadvantage is that they are sensitive to environmental factors, such as chemical, physical, and biological conditions, which play an important role in the maintenance of their activities. Loss of activity leads to bioavailability problems in the case of therapeutic usage. The term bioavailability is the dose fraction available at the site of action (Acosta, 2009). This term generally refers to a dose fraction that enters the bloodstream. Bioavailability, which is related to the integrity of the compounds, shows variation according to administration route. Orally administered natural products exhibit lower absorption and bioavailability. As a therapeutic, when used at the same concentration, they exhibit higher efficacy for in vitro conditions than for in vivo conditions. The effectiveness of these natural products depends on preserving the bioavailability of the active ingredients (Bell, 2001). Instability resulting from inappropriate storage conditions of compounds before usage (i.e., storage without sheltering from light, oxygen, etc.) may also affect bioavailability as well as insufficient gastric residence time, low permeability and solubility in the gastrointestinal system, degradation of first pass metabolism before reaching saturation level in bloodstream, environmental conditions in the presence of low pH, and limitation of the bioactivity of polyphenols caused by enzymes within the system. Topical use of natural polyphenols is also affected by external environmental factors because of their sensitivity. Oxidation of polyphenols may lead to unwanted effects such as color and odor changes observed externally, which may also indicate activity loss (Munin and Edwards-Lévy, 2011).

Freeform polyphenolic compounds are prone to activity loss because of the aforementioned factors as well as exhibiting limited solubility in aqueous solutions. They also have a characteristic unpleasant taste that needs to be masked before use. Therefore utilization of polyphenolic compounds requires a protectant formulation to maintain structural integrity, extending its half-life and bioactivity of the compound until consumption (Chen et al., 2006). This formulation should also augment solubilization, increase bioavailability of the compound to exhibit its benefits, as well as provide control over release properties. The degradation profile of the formulation affects the release kinetics such as burst and sustained or controlled release, which may be modified according to the case implemented. Targeted delivery of compounds may also be accomplished by tailoring the surface properties of the formulation and

producing stimuli-sensitive release systems such as pH-sensitive carriers or thermal-sensitive pharmaceuticals (Choi et al., 2010; Jiang et al., 2013). Encapsulated polyphenols can be utilized to overcome these deficiencies and maximize the potential therapeutic benefits of antioxidants (Nedovic et al., 2011). Criteria for selection of encapsulation material should cover the following: functionality that encapsulates should provide to the final product, potential restrictions for the coating material, concentration of encapsulates, type of release, stability requirements, and cost constrains.

3. NANOENCAPSULATION TECHNIQUES

Encapsulation methods can be employed for conservation and controlled release of bioactive compounds. Nanoencapsulation is one of the most preferable techniques to preserve bioactivity. The comprehensive advantages of nanoencapsulation are transfer of compounds in a targeted manner and effective absorption from cells.

The difficulties of working with plant-derived natural compounds are their low stability and bioavailability, their chemical degradation during storage, and their sensitivity to ultraviolet light and oxygen. To improve these properties, a number of techniques have been developed. The one focused on in this chapter is nanocarrier systems and their application areas. Nanocarrier systems are useful ways to improve delivery of bioactive molecules such as antioxidants, vitamins, lycopene, fatty acids, and minerals.

Encapsulation is a method where one or more ingredients are immobilized in some form of matrix or wall. This matrix could be in the form of a solid or liquid phase, which could be homogeneous or heterogeneous. The ingredient is immobilized in the matrix, which is usually called the core or active part of the material, and the outside of the capsule is called the shell, wall and encapsulant, or carrier material. By reducing the particle size, delivery properties, solubility, and bioavailability of the nutraceutical can be improved depending on the increased surface area per unit volume. The nutraceutical compounds can be either lipophilic or hydrophilic affecting their solubilities in water. Hydrophilic compounds such as ascorbic acid and polyphenols are soluble in water. However, they are insoluble in lipids and organic solvents. Lipophilic compounds such as lycopene, beta-carotene, lutein, and phytosterols are insoluble in water but soluble in lipids and organic solvents. In the determination of the release rate and release mechanism of the bioactive compounds, solubility is the major property. Hydrophilic compounds indicate faster release rates and the determination of their release kinetics is done by the suitable composition of erosion and diffusion mechanisms. Lipophilic compounds have poor solubility and low dissolution rates, which is caused by incomplete release by an erosion mechanism. Choice of the carrier material is based on physicochemical properties such as molecular weight, solubility, diffusivity, and viscosity. Also operational cost should be considered. The encapsulation technique is employed to protect the bioactive compounds, to increase the time of storage, and to preserve the end product. Also to control the release profiles and reach the objective area, encapsulation techniques are used. Nanoencapsulation is defined as both packing the bioactive compounds and entrapping natural compounds in carrier dimensions in nanoscale (Fig. 17.1).

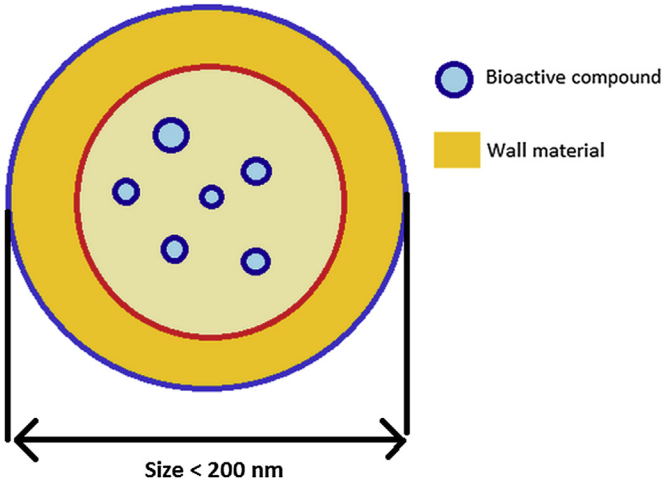

FIGURE 17.1 Schematic structure of nanocapsules including bioactive compounds.

4. METHODS FOR THE PREPARATION OF CARRIERS

4.1 Emulsification Techniques

Emulsion technology is usually used for encapsulation of natural bioactive compounds in aqueous solution to produce a nanoemulsion. Dispersing one liquid in another immiscible liquid by using a preferred emulsifier to obtain thermodynamically stable colloidal systems of nanometric size is called nanoemulsions with droplet sizes from 50 to 1000 nm.

The kinetic stability of nanoemulsions is very high because of their small droplet size. High kinetic stability is of real interest for encapsulation purposes and places a critical part in the retention of surface oil content of the product. Nanoemulsions cannot occur spontaneously because by their nature they are nonequilibrium systems. Energy input requirements of these systems can originate from mechanical devices or the chemical potential of the components. Homogenizers, ultrasonicators, and microfluidizers that provide high speed, high pressure, and high shear stirring can be utilized for nanoemulsion production. With the help of these methods, the required energy to prepare nanoemulsions is provided. Microfluidization uses high pressure (up to 20,000 psi), which forces the liquid toward the interaction chamber. The interaction chamber has microchannels in a special configuration. The collision chamber is fed with microchannels by emulsion; as a result, fine nanoscale emulsion droplets occur. It was reported that microfluidization was the best emulsification method based on the maximum encapsulation efficiency and smaller emulsion droplet size. Ultrasonication is also an emulsification technique. This technique reduces the emulsion droplet size with minimum recoalescence by increasing energy input. High-pressure homogenization is another technique in which a mixture is pushed with high pressure (100−2000 bar) and high shear stress to help the fragmentation of particles into the nanometer range. There are two different homogenization types governed by operating temperature: hot homogenization in elevated temperature and cold homogenization below room temperature. By increasing the homogenization pressure, cycle, and temperature, droplet sizes can be decreased by up to 50%. All the emulsification methods as mentioned earlier can reduce the droplet size. Therefore the emulsification technique is one of the most efficient nanoencapsulation techniques. However, to produce the particles in powder form, additional drying techniques are needed.

Comparing nanoemulsions with emulsions of larger particle size, the lack of flocculation, sedimentation, and creaming combined with large surface area and free energy provide advantages over emulsions of larger particle size. Their large interfacial area, delivery of natural bioactives, and their transport positively influence their targeting to specific sites. Reducing the droplet size to the nanoscale leads to impressive properties such as optical transparency and unusual elastic properties.

When it comes to oil-soluble nutraceuticals or bioactives, the nanoemulsion technique offers great potential to encapsulate a high concentration. Nanoemulsions can be used in liquid phase or by using drying operations such as spray drying and freeze drying to form a powder. Depending on the method of solvent removal, the emulsification technique has two variations: emulsion diffusion and emulsion solvent evaporation.

4.1.1 Emulsion Diffusion Method

The emulsion diffusion method (EDM) is an efficient technique to encapsulate both lipophilic and hydrophilic bioactives. With this technique, high encapsulation efficiency, uniform size distribution, high reproducibility, and easy scaling-up procedure are achieved. EDM was first published by Quintanar-Guerrero's group. The process of emulsion diffusion is an organic phase, which contains biopolymer and oil in an organic solvent. The aqueous phase is prepared separately from organic solvent. Next, emulsion is produced using a mechanical shearing method. Because of its polarity, an encapsulant may occur in either the aqueous or organic phase. By adding water, organic solvent leads to very fast elimination of less than 20 ms, including in the oil phase. As a result, biopolymers and oil are separated. Also particle size reduction, biopolymer precipitation, and nanocarrier formation occur. The advantages of this method make it easy for the nanoencapsulation of different natural bioactives. However, the disadvantage of the process is that the final product might contain some residual organic solvents, which may have potential toxic effects (Kakran and Antipina, 2014).

Dissolving polyphenols in polyglycerol oleic acid ester, adding ethanol, and stirring them with vegetable oil in a homogenizer results in ethanol-in-oil or ethanol-oil-water-type emulsions (Fang and Bhandari, 2010), which can be used as polyphenol delivery systems. Their polyphenolic nature leads to low solubility in water and oil. These emulsions provide high concentration of polyphenols to reduce lipid oxidation or increase lipid stability.

Han et al. (2009) developed an oil/water emulsion for a camptothecin derivative, 10-methoxy-9-nitrocamptothecin (MONCPT). Camptothecin derivatives are known as anticancer drugs, taking action as a topoisomerase I inhibitor. Among them, 9-nitrocamptothecin possesses a potent activity with low toxicity, but difficulty in its preparation limits its research and clinical application. To overcome preparation, release, and stability issues, a potential parenteral delivery

system was developed for MONCPT. Nanoemulsion can release MONCPT in a sustained manner, with longer half-life compared to freeform. MONCPT nanoemulsions also exhibited a significant increase in cytotoxic activity, by 23.6- and 28.6-folds, respectively, when applied to an S180 sarcoma cell line and A549 lung carcinoma cells. In vivo antitumor activity of MONCPT nanoemulsions also exerted a dramatic increase of 93.6% when compared with MONCPT injection that resulted in 24.2% suppression.

4.1.2 Emulsification Solvent Evaporation Technique

The emulsion solvent evaporation method is extensively used in the development of particulate carriers. It consists of the formation of a simple or double emulsion and the following evaporation of the organic solvent, which leads to the precipitation of the polymer and the obtaining of the particles (Fig. 17.2). Polymer solution emulsified into the aqueous phase and polymer evaporation are steps of the emulsification solvent evaporation technique. Nanospheres, which are obtained by polymer precipitation after evaporation, are the end products of this method.

To be more precise, the polymer is first dissolved in a volatile and nonmiscible organic solvent such as dichloromethane, chloroform, or ethylacetate. Then, the organic phase is dispersed by high-speed homogenization or by sonication in an aqueous phase that contains a surfactant. After an oil/water emulsion is obtained, evaporation of the organic solvent allows its diffusion to the outer phase leading to the formation of particles. Emulsion solvent evaporation is generally used for the encapsulation of hydrophobic drugs (O'Donnell and McGinity, 1997). If the active pharmaceutical ingredient is hydrophilic, the double-emulsion technique will be more appropriate. Another step in addition to the emulsion solvent evaporation technique consists of the dispersion of the primary emulsion (generally, a water/oil emulsion) in a second aqueous phase, which is essential before organic solvent evaporation (Giri et al., 2013). In both cases, evaporation of the organic solvent is procured by stirring the emulsion at room temperature or under high-temperature and low-pressure conditions. The obtained particles can then be harvested by ultracentrifugation or filtration then washed and lyophilized. The size of the capsules can be arranged by changing the stir rate, type, and amount of dispersing agent, viscosity of both organic and aqueous phases, and temperature. Mostly, used polymers are polylactic acid (PLA), poly(lactic-*co*-glycolic acid) (PLGA), ethylcellulose, and polycaprolactone (PCL). PLGA was employed to encapsulate curcumin, epigallocatechin gallate (ECGC), and ellagic acid. Curcumin, a hydrophobic polyphenol derived from the herbal spice *Curcuma longa* L. has many bioactive properties, including antioxidant, antiinflammatory, and anticarcinogenic activities as well as reducing beta-amyloid plaque formation in Alzheimer's disease. Because of its poor absorption by oral administration, Tsai et al. (2011) optimized PLGA nanoformulation of curcumin. Intravenous and oral studies showed that

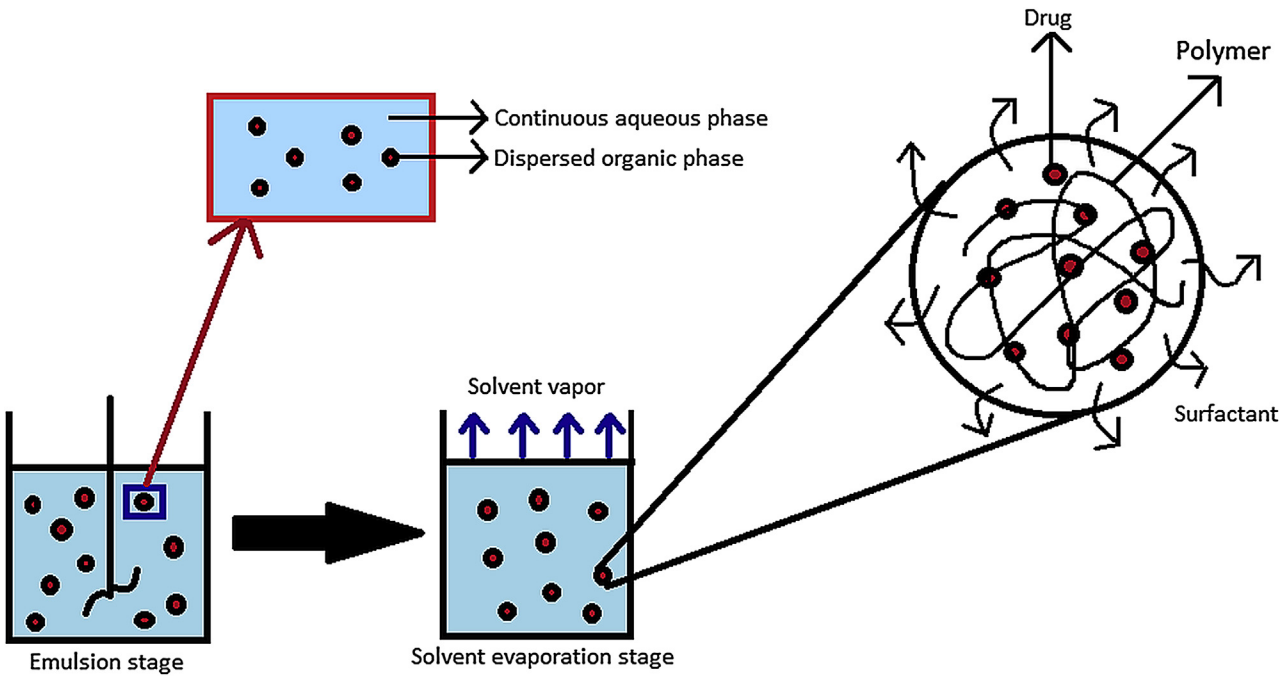

FIGURE 17.2 Formation of nanoparticles by emulsion solvent evaporation method.

nanoformulated curcumin caused an increase of 55% and 21-fold, respectively. Also the oral bioavailability of nanoformulated curcumin at 22-fold higher than conventional indicated that PLGA nanoformulation may enhance the bioavailability of water-insoluble polyphenol in functional food and nutraceuticals.

The antioxidant molecule quercetin was encapsulated on poly-D,L-lactide (PLA) nanoparticles by Kumari et al. (2010) to improve its poor aqueous solubility and stability. Quercetin is abundantly found in varying concentrations in berries, onions, and apples. Because of the number and position of free hydroxyl groups in its structure, the antioxidant capacity of quercetin is higher than rutin and trolox. PLA was chosen for encapulation because of its high hydrophobicity, biodegradability, biocompatibility, low toxicity, strong mechanical strength, and slow drug release. In this study, quercetin was loaded into PLA nanoparticles with an encapsulation efficiency of 96.7% and loading of 19.4% depicted by high-performance liquid chromatography (HPLC) analysis. Release kinetics showed that 40%−45% of quercetin was released within 0−0.5 h showing rapid burst release, which could be attributed to the fraction adsorbed close to the surface. After the first burst release effect, nanoparticles exerted sustained release during 96 h. After that point it was found that 87.6% of quercetin was released. High encapsulation efficiency and biphasic release profile may present quercetin-loaded PLA nanoparticles as a candidate for bioavailable nanomedicine.

The double-emulsion solvent evaporation method was also employed to synthesize nanoparticles for pomegranate extract by Shirode et al. (2015). A polymer layer was formed using a PLGA−PEG complex. The obtained nanoparticles exhibited monodispersion with a negative surface charge. Intracellular uptake of Alexa Fluor-conjugated nanoparticles was observed in MCF-7 cells during 24 h, which reached the highest level at the end of 24 h of incubation, indicated by fluorescence intensity. The IC_{50} value of pomegranate extract in nanoencapsulated form is 2.5-fold of its free form. All these findings indicate that encapsulation of pomegranate polyphenols in PLGA−PEG nanoparticles enhances their bioefficacy.

4.2 Advantages and Disadvantages of Nanoemulsions as Drug Delivery Systems

4.2.1 Advantages

The attraction of nanoemulsions for application in personal care and cosmetics as well as in health care is because of the following advantages. Small droplet size is utilized to reduce gravity force, which overcomes sedimentation issues during storage, and prevents flocculation, which enables dispersion within the solution. Efficient delivery of bioactive compounds through the skin can be accomplished by the large surface area of the emulsion system. Uniform deposition as well as enhanced spreading, penetration, and wetting are provided by the small size of droplets and low surface and interfacial tension, respectively. Because of the properties given earlier, nanoemulsions can be employed for fragrant delivery in alcohol-free perfume applications.

Recent literature indicates that encapsulation techniques lead to improvements in the stability and availability of natural compounds in vivo and in vitro, when compared to their free form. These techniques also provide optimization strategies for administration routes. Future studies will focus on targeted codelivery of natural compounds by using more than one ingredient to exhibit a synergistic effect and enhance the efficacy toward therapeutic use.

4.2.2 Disadvantages

In spite of the advantages, nanoemulsions also have disadvantages. In many cases, preparation of nanoemulsions requires special application techniques, such as the use of high-pressure homogenizers as well as ultrasonics. Such equipment (e.g., the microfluidizer) became available only recently. There is a perception in the personal care and cosmetic industry that nanoemulsions are expensive to produce. Expensive equipment is required as well as the use of high concentrations of emulsifiers. Lack of demonstration of the benefits that can be obtained from using nanoemulsions when compared with classical macroemulsion systems is also one of the disadvantages, especially for proceeding with clinical trials. Lack of understanding of the interfacial chemistry involved in the production of nanoemulsions is another drawback in the industry (Lovelyn and Attama, 2011).

4.3 Spray Drying

The spray-drying technique is achieved by the transformation of materials from a liquid to a solid state. This technique was widely used for drying liquid formulations such as solutions, emulsions, or suspensions. It has gained enormous interest since its application had been extended to the development of microparticles (Palmieri et al., 2001). As Fig. 17.3 shows, practically, a solution or a suspension of the drug in a solvent also contains the polymer, and is sprayed from a nozzle in a

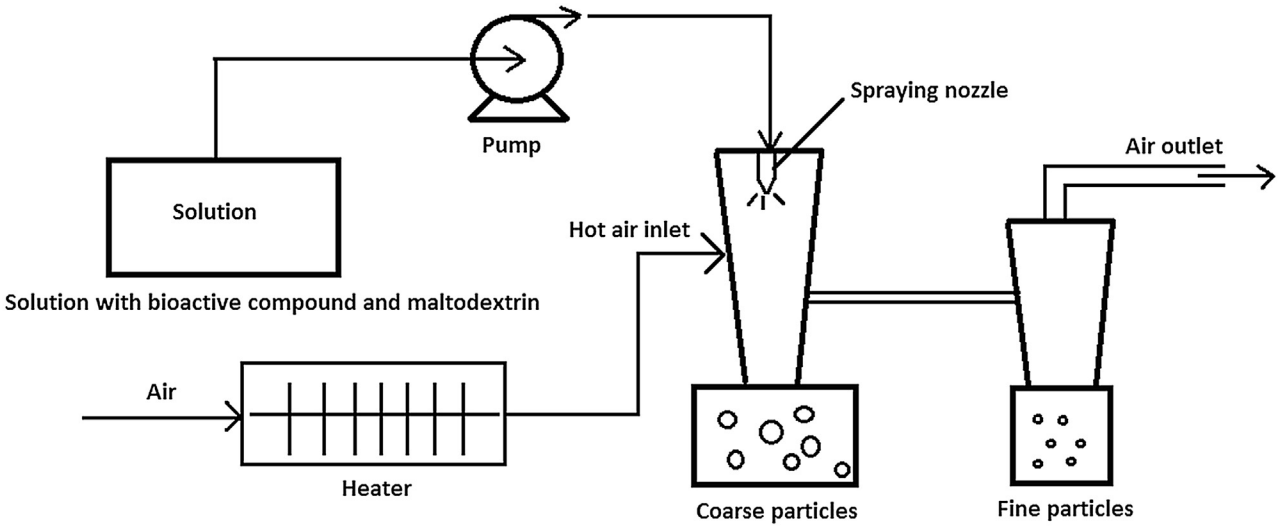

FIGURE 17.3 Spray-drying process.

hot drying medium. Atomization transforms the liquid stream into fine droplets. Following a high surface-to-volume ratio favors efficient and rapid drying of the droplets and their transformation into particles. When the polymer is not used, the acquired particles are called nanocrystals (Keck and Müller, 2006) or microcrystals depending on their size. With this method, a number of advantages such as ease of scale-up and mild processing conditions are achieved, but these come with disadvantages such as the high cost of spray dryers. The key parameters that have to be controlled in the process are inlet and outlet temperatures, spray flow, and the volume of the air inlet (Yashwant and Deepack, 2009).

The most common polymers, which are known as wall materials, in this process are gum arabic, maltodextrin, and modified starch (Munin and Edwards-Lévy, 2011). Maltodextrins are widely used for encapsulation of flavors. Calvo et al. (2010) performed a study on olive oil, comparing the influence of wall material components, selected as sodium caseinate, gelatin, gum arabic, starch, lactose, and maltodextrin. Encapsulation efficiency was at most 53% with the gelatin, gum arabic, maltodextrin, or sodium caseinate−maltodextrin conjugates. Robert et al. (2010) also stated that maltodextrin was found to preserve anthocyanidins as a result of their study on pomegranates, because of its thermal protective properties. Zhang et al. (2007) also formed a mixture of maltodextrin and gum arabic and performed encapsulation of procyanidin extracted from grape seeds, which reached an encapsulation efficiency of 88.84% and proved that no change was exerted on procyanidin structure by HPLC analysis. Rocha-Guzmán et al. (2010) accomplished encapsulation of oak extract (*Quercus resinosa*), which is very rich in polyphenols, within a matrix of sodium caseinate and lactose. The end product exhibited high antioxidant activity at very low phenolic concentrations, indicated by results of deoxy-D-ribose assay that measured OH radical inhibition.

4.4 Coacervation

Coacervation is one of the most easily applied techniques for the production of nanocarriers. Formation of the coacervate phase by polyelectrolyte mixture and its deposition of active material underlies the coacervation technique. Based on the number of polymer types used, the process can be called simple coacervation and complex coacervation. The power of the interaction between the biopolymers and the nature of the complex is affected by many factors such as pH, concentration, ionic strength, biopolymer type, and the ratio of biopolymers.

The common driving force for the coacervation method is electrostatic attraction between oppositely charged molecules. In addition to the electrostatic interactions between biopolymers of opposite charges, hydrogen bonding and hydrophobic interactions inversely affect complex coacervation. The functional efficiency of produced nanocapsules depends on the chemical nature and surface characteristics of the biopolymeric shell. The efficiency of nanoencapsulation is increased with the increasing of surface charges (Fang and Bhandari, 2010).

Nanocapsules ranging between 100 and 600 nm can be obtained by the coacervation technique. The drying technique determines the size of nanoparticles. Gelatin, acacia gum, and chitosan are the most commonly utilized wall materials. Chitosan and alginate can be used together because of their opposite charges. The easy solubility of chitosan at low pH is

prevented by the alginate network since alginate is insoluble at low pH conditions. Deladino et al. (2008) utilized this property of chitosan–alginate mixtures to encapsulate yerba mate (*Ilex paraguariensis*) extract. This polyphenolic extract was composed of caffeoyl derivatives and flavonoids as chlorogenic acid, caffeic acid, dicaffeoylquinic acid, rutin, quercetin, and kaempferol. More than 85% encapsulation efficiency was accomplished with chitosan–alginate complexes. Efficiency was lower in chitosan beads at 50%, since active compound was lost during immersion in chitosan. Glucan, as a thermoreversible gelling agent, was employed to encapsulate blackcurrant extract by either cooling the glucan gel or dropping into oil (Xiong et al., 2006). Anthocyanidin recovery was measured between 73% and 79%.

4.5 Nanoprecipitation Technique

The nanoprecipitation method is based on spontaneous emulsification of the organic phase. The organic phase can be composed of dissolved polymer, drug, and organic solvent. Polymer precipitation and diffusion of organic solvent constitutes the basis of nanoprecipitation. Nanocapsules and nanospheres around 100 nm in diameter can be obtained by solvent displacement.

Govender et al. (1999) performed a study to assess parameters for incorporation of a water-soluble drug, procaine hydrochloride, into poly(DL-lactide-*co*-glycolide). Findings indicated that the pH of the aqueous phase, replacement of the hydrochloride group of the drug with the dihydrate group, and incorporation of polymer or fatty acid derivatives into formulation were the parameters that altered the encapsulation efficiency. Increase of pH of the aqueous phase from 5.8 to 9.3 increased encapsulation efficiency from 65.1% to 93.4%, which might be attributed to a lower degree of ionization and lower solubility, as well as replacing procaine hydrochloride with procaine dihydrate. Fatty acid formulated options with caprylic acid and lauric acid groups also enhanced drug encapsulation efficiency significantly in contrast to the slight effect of poly(methyl methacrylate-*co*-methacrylic acid) on drug entrapment. Drug entrapment of the nonencapsulated system was 11%, while it showed an increase to 22%, 34.8%, and 18.4% in the presence of caprylic acid, lauric acid, and poly(methyl methacrylate-*co*-methacrylic acid) groups, respectively. This study indicated that enhancer factors are required for efficient entrapment of water-soluble drugs by the nanoprecipitation method. Alternatively this is an efficient method to nanoencapsulate lipophilic drugs because of the miscibility of the solvent with the aqueous phase (Ezhilarasi et al., 2012). Stability against degradation and related sustained release of nanoparticles exhibit enhanced cellular uptake and bioavailability.

Resveratrol, quercetin, and epigallocatechin-3-gallate (EGCG) were employed for nanoprecipitation in individual studies. Shao et al. (2009) incorporated resveratrol, which has been reported to elicit cell cycle arrest, differentiation, and apoptosis, into polyethylene glycol (PEG)-PCL-based nanoparticles. Encapsulation efficiency was achieved at 90%, as well as performing sustained release profile and high cellular uptake that led to 80% cell death in glioma cells. Quercetin, which is a well-known flavonoid found in apples, onions, Ginkgo biloba, and red wine, was encapsulated with polyvinyl alcohol (PVA) and Eudragit E (EE) (aminoalkyl methacrylate copolymers) by Wu et al. (2008). Highest yield (99.3%) and lowest mean particle size (81.9 ± 0.26 nm) were achieved when the quercetin:PVA:EE ratio was 1:10:10, which may indicate that a high proportion of PVA could provide sufficient stabilization to the nanoparticle system, and reduce its particle size and size distribution. The release rate of quercetin–polymer conjugate was also found to increase by 74-fold when compared with the free form. Siddiqui et al. (2009) performed encapsulation of green tea polyphenol EGCG with PLA-PEG nanoparticles and observed that encapsulated EGCG exerted proapoptotic and angiogenesis inhibitory effects 10-fold when compared to the free form, indicated by the difference in IC_{50} doses. They also performed a xenograft study to observe the in vivo effect. Tumor volume of the mice treated with nanoencapsulated and nonencapsulated ECGC was found to be 707 and 854 mm^3, respectively. Nanoencapsulated ECGC provided a significant decrease in tumor size.

4.6 Rapid Expansion of Supercritical Solutions Technique

The rapid expansion of supercritical solutions (RESS) method, along with all the techniques relying on supercritical fluids, presents the common advantage of not needing the use of toxic organic solvents and surfactants. This technique can be used for encapsulation purposes and also for size reduction. A fluid is supercritical when it is maintained at a temperature and pressure that are higher than those of its critical point. At this state the fluid has very interesting properties for separation and reaction because its density is close to a liquid density and it presents mass transfer properties. Because of these special characteristics, principal parameters tied to the fluid density may be controlled, particularly the solvent properties. In fact, variation of the pressure enables the dissolution of molecules when the density is close to liquid density and allows the precipitation of the same substances if the density is close to a gas density. Carbon dioxide is the most used fluid in supercritical fluid technology because of its nontoxic, nonflammable, and environmentally acceptable properties.

Moreover, it can be transformed into the supercritical state easily (Yashwant and Deepack, 2009). In the RESS technique, the drug alone is first dissolved in supercritical CO_2 (SC CO_2) in a high-pressure chamber. The solution is passed through a nozzle, leading to an instant decrease of the pressure and consequently a precipitation of the natural compounds alone or embedded in the polymer matrix if a polymer is added. RESS is very efficient for producing submicron particles containing active molecules for drug delivery. The studies show that dissolution rates and solubility of a lot of active pharmaceutical ingredients were enhanced. However, the application of the RESS process is severely limited because polymers in general have very limited solubility in SC CO_2 at temperatures below 80°C. Also the operating pressure in RESS is usually above 200 bar, which leads to less attractive economical results.

4.7 Polymer Coating/Encapsulation Method

The coating or encapsulation of nanoparticles has been found to be of particular interest for the controlled release of drugs, genes, and other bioactive agents. Controlled release systems provide the benefits of protection from rapid degradation, delivery to the target site, control of the release rate, and extended duration of bioactive agents.

Insulin encapsulation by a polymer represents an example of this method. Insulin is generally administered by injection. Therefore all tissues are exposed to insulin excessively, which may trigger overstimulation of diabetic complications, whereas liver receives only a small portion. Following the study that represented poly(isobutylcyanoacrylate) nanocapsules as a delivery agent for active insulin (Damge et al., 1988), Aboubakar et al. (2000) investigated the degradation resistance of insulin nanocapsules in the gastrointestinal tract. Nanocapsules prepared by Texas Red-labeled insulin were tracked under fluorescence microscopy and it was observed that the nanocapsulated system reached 90% encapsulation efficiency. Release kinetics in phosphate-buffered saline exhibited 10 min rapid release of 10% of the insulin, followed by sustained release of 3% insulin for the next 5 h, which showed similarity in gastric fluid. On the contrary, 75% of the insulin was rapidly released within 30 min in intestinal medium, indicating that intestinal enzymes were capable of degrading the polymer wall.

By combining the advantages of both the RESS method and polymer coating, new studies have been investigated. Using the SC CO_2 as an antisolvent (SAS) for nanoparticle coating/encapsulation processes causes a heterogeneous polymer nucleation with the nanoparticles acting as nuclei and a subsequent growth of polymer on the surface of the nanoparticles induced by mass transfer and phase transition (Wang et al., 2001). Using SC CO_2 in an SAS process, CO_2 is an ideal processing medium because of its relatively mild critical conditions ($T_c = 304.1$ K, $P_c = 7.38$ MPa). Furthermore, carbon dioxide is nontoxic, nonflammable, relatively inexpensive, and recyclable. The SAS process is based on the principle of SC CO_2—induced phase separation in which the solute precipitates because of a high supersaturation produced by the mutual diffusion of organic solvent into SC CO_2 and vice versa when an organic liquid solution comes into contact with SC CO_2. An important feature of the SAS process is that the organic solvent can be almost completely removed by simply flushing with pure CO_2. Thus dry particles are produced after a CO_2 extraction step (flushing) following feeding of the organic solution (Randolph et al., 1993).

Sosa et al. (2011) employed a semicontinuous near-SAS process to coprecipitate green tea polyphenols with polylactide—PCL copolymer to overcome the short half-life issue of a polyphenolic nature. Optimization was performed for the process parameters such as pressure, temperature, and concentration ratio of the blend of polymer and polyphenol. These parameters were investigated for comparison of yield, polyphenol content, particle morphology, and size of the coprecipitated product. Mean particle size was 5 μm and exhibited a homogeneous size distribution with a low agglomeration degree. HPLC results showed that 90% of ECGC and 100% of epicatechin were retained in the coprecipitates while caffeine was present at 13% in the final product. Release kinetics indicated that 30% of the antioxidants are burst released within the first 4 h, followed by progressive release in 90 h. Burst release effect can be attributed to diffusion through the matrix while the release of the rest depends on degradation of the matrix.

Essential oil encapsulation by zein nanospheres was performed by Parris et al. in 2005. Spice essential oils are valuable products because of their antimicrobial properties against bacteria, yeast, and fungi, attributed to the ability to disrupt membrane integrity. Lambert et al. (2001) stated that oregano essential oil, thymol, and carvacrol disrupted the membrane by altering the pH gradient and electrical potential and led to permeabilization of nuclear stain ethidium bromide. Zein was employed to encapsulate essential oils to investigate the potential of the polymer as a site-specific delivery agent to maximize its antimicrobial effect (Parris et al., 2005). Stability of the zein particles was determined in the presence of pepsin at pH 3.5 to mimic the stomach environment. It was observed that they were completely dissolved after 52 h, which is a very long period when compared with gastric emptying time between 1 and 4 h. These results demonstrate that zein particles protect essential oils by preventing their release. Nonenzymatic release rate was also affected by the content of the solution. Ethanol presence in phosphate-buffered saline, pH 7.2, promoted release of essential oil caused by zein solubility

in ethanol. Zein particles exhibited burst release profile by releasing 60% of the oil within 4 h, followed by sustained release during the next 20 h. Release was completed at the end of the elapsed time period. Release properties at pH 7.2 also offered release kinetics in the duodenum. Obtained zein particles might potentially have been used for oral administration because of their stability in stomach and preferable digestion in the intestines, which can be used for probiotic formulations.

Although zein utilization is dominant in food packaging and coating, it also has an application area in the pharmaceutical industry because of its biodegradability and hydrophobicity as well as barrier function (Miyoshi et al., 2005). Demchak and Dybas (1997) encapsulated abamectin, a macrocyclic lactone that is used as a pesticide, with zein to overcome the rapid degradation of the drug in the presence of air and sunlight. The reason for rapid degradation was the oxidation of the diene chromophore of the abamectin. Their results showed that zein functioned as a physical oxygen barrier and quencher of oxygen that provided photostability by retarding the interaction between oxygen and the chromophore structure. Liu et al. (2005) also performed a study to encapsulate ivermectin, an effective parasiticide used in farm animals. They employed zein microspheres for drug targeting and overcame the disadvantage of hydrophilicity by using a hydrophobic protein.

4.8 Electrospinning

Electrospinning is a popular and cost-effective method to produce novel fibers with diameters from less than 3 nm to over 1 mm. An electrostatic force is applied to the polymeric solution to produce nanofiber. The diameters of the fibers are from 50 to 1000 nm during the electrospinning process. The polymer solution is kept in the syringe and pumped in the opposite direction to the surface tension. Then, a conical shape occurs at the tip of the syringe known as a Taylor cone. By increasing the electric potential, the surface tension is overcome and a jet forms, which splits from the Taylor cone. The solvent evaporate and the jet form the nanofibers. The nanofibers can be collected from a stationary or rotary collector (Fig. 17.4).

The electrospinning process is generally affected by solution, process, and ambient conditions. The fiber morphology and fiber diameter are changed with all of these parameters, which affect their end use (Amiraliyan et al., 2009). To produce the nanofibers in desired dimensions, optimization of the parameters is important. The increase in molecular weight of the polymer results in an increase in viscosity. The high-viscosity solution results in an increase in fiber diameter up to a critical point in viscosity, where the solution cannot be pumped out of the syringe to form polymer jet as well as hardening the polymer solution and clogging at the tip of the syringe. The volume of the solution in the tip of the syringe is controlled alternatively with the optimization of increment of the feed rate, which in turn affects the fiber diameters. Fiber

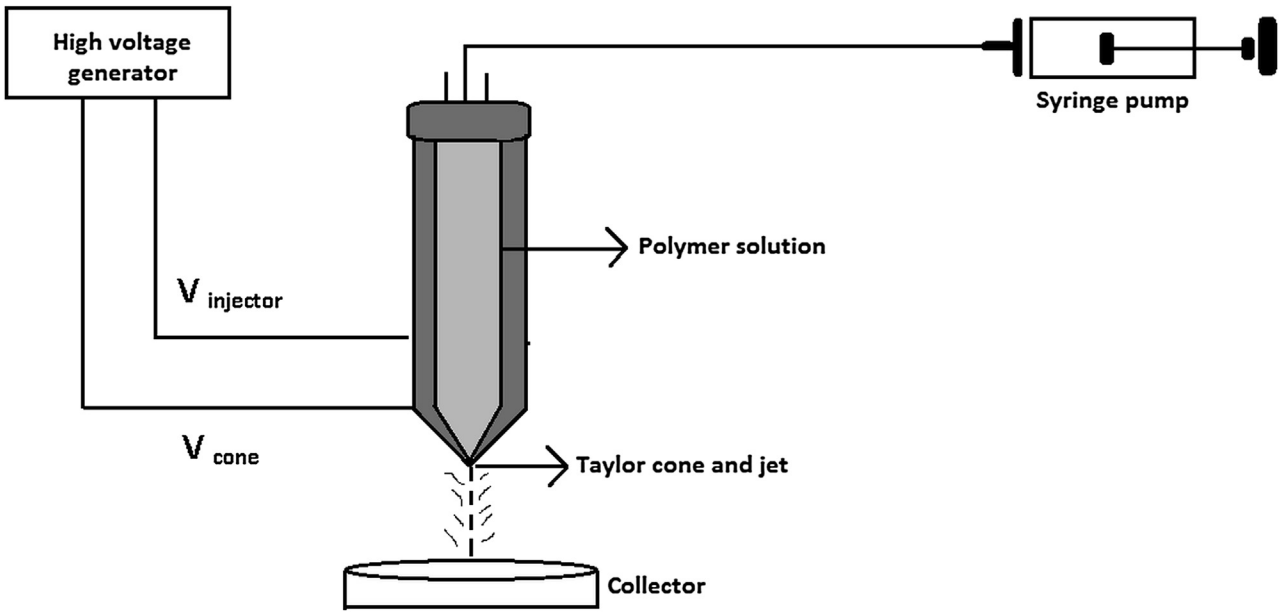

FIGURE 17.4 Electrospinning set-up.

diameter is related to the jet size, elongation of the jet, and evaporation rate of the solvent. Elasticity of the fluid determines nanofiber morphology. Elongation of the jet and evaporation of the fluid together change the shape and charge per unit area carried by the jet. Polymer skin formed on the liquid jet also affects the morphology. Koombhongse et al. (2001) explained that after the skin is formed, the solvent inside the jet escapes and the atmospheric pressure tends to collapse the tube-like jet. The circular cross-section becomes elliptical and then flat, forming a ribbon-like structure. Shape and continuity of the fibers are also related to the amount of molecular chain entanglements in the polymer solution. Sufficient molecular entanglement can prevent polymer jet breakup, allow the electrostatic stresses to further elongate the jet and draw it into fibers (Wang et al., 2006). Also the increase in temperature causes a decrease in viscosity of the solution but makes vaporization of the solvent easier, which augments formation of uniform fibers. In addition to this, low viscosity causes a decrease in surface tension. Because of this, fiber length and surface area of the fiber decrease. The dielectric property of the solution is also a parameter in fiber formation. As the dielectric property of the solution is higher, bead formation is minimized and increase in fiber diameter is observed.

Depending on the basis of electrospinning, stability of the Taylor cone is affected by the voltage applied. The increase in applied voltage increases the amount of charge, which causes an unstable Taylor cone. In addition, the jet elongates further, resulting in a decrease in fiber diameter. At a lower voltage, fine fibers are obtained because of the increase in duration of fiber formation. Voltage and viscosity of the polymer should be balanced to reach a stable jet and desired fiber diameter.

Various polymers are used in electrospinning processes to produce scaffolds for tissue engineering and drug delivery applications. Utilized polymers range from natural polymers such as collagen, gelatin, chitosan, silk fibroin, and hyaluronic acid to synthetic polymers such as PLA, PCL, polyethylene oxide, and their copolymers. The use of polymer nanofibers for biomedical and biotechnological applications has some intrinsic advantages. They can mimic the native extracellular matrix and are therefore considered as promising tissue engineering scaffolds (Zhang et al., 2005). Nanofibrous 3D scaffolds have high porosities and surface area-to-volume ratios that make them suitable for cellular adhesion and proliferation as well as diffusion and adhesion of proteins, growth factors, and enzymes that influence function (Nisbet et al., 2009). This property also eliminates the accumulation of by-products produced caused by degradation of polymeric materials (Zamani et al., 2013).

Functionality of polymer scaffolds can be achieved by incorporation of therapeutic bioactive compounds, which can be antibiotics, proteins, small molecules, and DNA (Chakraborty et al., 2009). Therapeutic compounds and fiber structure can be integrated in three ways regarding the order of process, and can be summarized as follows (Zhang et al., 2005):

1. Mixing the polymer solutions with bioactive compounds prior to electrospinning.
2. Covalently conjugating or coating nanofibrous matrices with bioactive compounds after electrospinning.
3. Encapsulating bioactive compounds in the core of fibers with a core−shell structure through coaxial electrospinning (Zhang et al., 2009).

Burst release can be observed with the first and second methods, which are preferred for emerging treatment, whereas the third method may be utilized for controlled release regarding long-term treatment (Zhang et al., 2005).

Wound dressings functionalized with bioactive compounds can serve as an example of therapeutic delivery applications. Functionalization of silk mats was performed to enhance wound healing by using epidermal growth factor to enhance the wound-healing process by stimulation of proliferation and migration of keratinocytes. Katti et al. (2004) incorporated cefazolin into electrospun PLGA nanofibers as an antibiotic delivery system for wound treatment, by mixing both the polymer and the drug in the same solvent. In another study performed by Verreck et al. (2003), two kinds of poor water-soluble drugs (itraconazole and ketanserin), having potential use in wound healing and topical drug delivery, were loaded in water-soluble (hydroxylpropyl-methylcellulose) and water-insoluble (polyurethane) nanofibrous polymer carriers.

The size and shape of electrospun fibers to perform targeted applications such as cancer treatment can be tailored. Green tea polyphenols constitute a candidate therapeutic because of its fewer side effects compared to other chemotherapeutics, but it is prone to oxidation, which limits the utilization. Shao et al. (2011) developed PCL/multiwalled carbon nanotube composite nanofibers fabricated via an electrospinning technology to enhance the structural stability of green tea polyphenols. In vitro degradation and polyphenol release were found to be correlated with the amount of polyphenol loading. Green tea polyphenol-loaded nanofibers also exhibited cytotoxic activity to Hep G2 hepatoma cells, while it exerted low cytotoxicity for normal osteoblast cells. Li et al. (2014) also employed green tea polyphenols combined with dexamethasone to provide an effective treatment against keloids, which are fibroproliferative lesions that occur at areas of cutaneous injury. Dexamethasone has been demonstrated to suppress fibroblast proliferation, and green tea polyphenols have been shown to have an antimicrobial effect because of their high antioxidant capacity. Resulting electrospun PLGA fiber meshes fabricated for codelivering of dexamethasone and green tea polyphenols resisted bacterial infection in

accordance with green tea polyphenol loading amount. An in vitro compatibility study performed by NIH 3T3 fibroblast cells indicated that PLGA was compatible with the fibroblasts, and addition of the dexamethasone/green tea polyphenol complex did not alter this property. To investigate the treatment effect in vivo, a keloid model of nude mice was created. After treatment for 8 weeks, it was observed that pure PLGA fiber mesh did not cause a decrease in keloid volume, while dexamethasone-loaded fibers caused shrinkage in keloids ($5.12 \pm 0.48\ mm^3$) when compared with the control ($4.01 \pm 1.08\ mm^3$). Addition of green tea polyphenols to dexamethasone also increased the shrinkage effect ($5.75 \pm 0.39\ mm^3$) when compared with dexamethasone-loaded fibers.

Ma et al. (2011) employed chitosan and polyethylene oxide blend polymer solutions to incorporate with paclitaxel after the electrospinning process. Chitosan was chosen for its biological properties such as biocompatibility, lack of toxicity, and antimicrobial effects. It was blended with polyethylene oxide to fine tune its biodegradability in aqueous media. Paclitaxel, being a mitotic inhibitor with a mechanism on microtubule degradation and used in cancer chemotherapy, was incorporated into the polymer blend because of its poor solubility in water. After immersion of chitosan−polyethylene oxide-blended fibers into paclitaxel, hyaluronic acid was used to encapsulate chitosan nanofibers. Interaction of negatively charged hyaluronic acid and positively charged chitosan polymers exhibited crosslinking without any side effects of aldehyde derivatives. Interaction of oppositely charged polymers was indicated by −N-H shift in the Fourier transform infrared spectrum. Fluorescence imaging was employed to investigate paclitaxel loading on fibers, which was distributed uniformly inside the fibers and in the encapsulated sections. Because of the high surface area of porous electrospun fibers, diffusion throughout the structure was enhanced, as indicated in the burst release profile of paclitaxel from the electrospun fibers. Burst release of paclitaxel was followed by an equilibrium stage after 48 h because of prevention of drug release resulting from interaction between positively charged chitosan and negatively charged hyaluronic acid. Porous structure and surface roughness of the electrospun fibers enhanced adhesion and proliferation of cells according to a study performed on DU145 prostate cells. Paclitaxel-loaded fibers were also observed for drug efficacy in terms of cell viability. Cells exhibited a tendency toward adherence on the fiber surface and the released amount of paclitaxel was sufficient to inhibit cell growth, indicated by a decrease in viable cell number. This study indicated that interaction between the polymers in the blend affected the morphology of the material obtained and release properties of the incorporated drug could be tailored according to application area.

4.9 Electrospray Technique

The need for controlled delivery of therapeutic molecules has increased an interest in the investigation of polymeric particles as biodegradable reservoirs. Many techniques exist for producing these delivery particulate systems, with emulsion/evaporation-based methods being the most extensively used. In emulsion techniques, aqueous/organic interface and shear stresses are two disadvantages. During the process there are issues such as degradation of surrounding molecule (such as denaturation of proteins) and instability of molecules. Also entrapped molecules differ in terms of therapeutic function and physicochemical properties, demonstrating a different degree of stability and sensitivity to stress. In other techniques, extended time of exposure to organic solvents and residual traces from solvents or other processing agents in the final delivery particulate system create concern because of the nature of the end product. Low drug loading efficiency, scale-up limitations, fabrication issues of producing small-sized particles, and difficulties in incorporation of hydrophilic content can also be considered as disadvantages of common methods such as solvent evaporation, spray drying, coacervation, and emulsification (Zamani et al., 2013). Such factors can affect the nature and stability of the encapsulated molecules, limiting their performance both in vitro and in vivo. To overcome these drawbacks, the electrospraying technique has been used. This method is also termed electrohydrodynamic atomization. Although electrospraying is a well-established technique in the field of mass spectrometry, it has only been applied to the loading of molecules in the last decade and its understanding and optimization are still relatively new with respect to biological loading. Electrospraying is a similar method compared with electrospinning; a high voltage is applied to a liquid infused through a capillary nozzle. The electric charge generated on the droplet competes with the surface tension of the droplet, causing the droplet to break up in nano- to microdroplets, which go thorough solvent evaporation. Then, the resulting particles are dried and can be collected. Therapeutic molecules can be incorporated into the polymer solution prior to electrospraying resulting in loaded particles (Bock et al., 2012).

Electrospraying has many advantages in the encapsulation of bioactive molecules. First, the usage of high temperature is not required. Because particles are instantaneously dried during the process an additional drying process is not needed. Also in drug delivery systems, monodispersity of the particles is a desired property. Monodispersity of the particles ensures the control of release profiles. Electrospraying provides the control of size distribution of the particles by producing monodisperse particles and using suitable parameters.

The electrospraying process can reduce the denaturation of the bioactive molecules. Also this process has many alternatives in the choice of polymers, bioactive molecules, and system apparatus. For the highly sensitive bioactive molecules, to reduce the exposure to organic solvent, coaxial electrospraying can be applied. With coaxial electrospraying, core−shell capsules are formed and a polymer matrix surrounds the core of capsule in an aqueous solution.

Encapsulation of the bioactive molecules has become a favored method in controlled delivery to the target cell and tissue sites. To maintain the stability of the therapeutic molecules, natural polymers can be used as encapsulation material. Protein hydrogels, especially gelatin, are utilized to form hard and soft capsules for drug encapsulation. Biodegradability and ability to form a network in aqueous media because of its chain entanglements make gelatin a candidate to be utilized for controlled drug release applications (Okutan et al., 2014). Gomez-Mascaraque et al. (2015) proposed gelatin for the encapsulation of EGCG, a water-soluble antioxidant. Different gelatin concentrations were tested prior to EGCG incorporation and it was found that except for the 20% (w/v) concentration, pseudospherical particles were obtained. At the highest concentration of 20%, spheres were transformed into fibers, which could be attributed to viscosity gradient. Increase in viscosity resulted in residual fiber formation. After optimization of spherical formation, EGCG was added to a polymer solution prior to electrospraying, at a theoretical concentration of 10% (w/w). Morphology of the resulting spheres did not change but the incorporation of EGCG was observed with Fourier transform infrared spectroscopy. Protein interaction with polyphenol molecules by hydrogen bonding and hydrophobic interactions resulted in the shift of the amide A and amide III band to lower wavenumbers. Band shifts could be assumed as a proof of intermolecular interactions, which may be attributed to stabilization of the gelatin particles. Stabilization was also confirmed by a release study performed in phosphate-buffered saline at pH 7.4 as a simulated biological fluid, which resulted in burst release caused by the swelling of gelatin and was followed by a slower sustained release. Antioxidant activity of the encapsulated molecule was maintained within 10 days, indicating the protection of EGCG from degradation in alkaline solutions, while antioxidant activity of the free form of EGCG was fully lost after 4 days. Slow degradation rate and preserved antioxidant activity can be attributed to stabilization effect of polymer on polyphenolic structure because of intermolecular interactions.

Lee et al. (2010) also utilized hydrophilic EGCG for the dual capillary electrospray method, in comparison with lipophilic budesonide, a steroid used to reduce inflammation. PLGA was chosen as the coating material because of its low toxicity and well-defined controlled release kinetics. Two coaxially aligned capillaries consisted of a drug solution in the inner capillary and a polymer solution in the outer capillary. Drug encapsulation efficiencies were found to be 56.3% and 17.1% for budenoside and EGCG, respectively. EGCG was highly soluble in water, which resulted in loss of material instead of being encapsulated by PLGA.

4.10 Layer-by-Layer Technique

In recent years, the multilayer technique for increasing the stability of microcapsules has gained popularity. This technique involves the formation of multiple layers of biopolymers at the interface using a layer-by-layer (LbL) electrostatic deposition technique. Applying double-layer techniques for producing multiple emulsions can efficiently coat oil particles during emulsification and result in improved stability to environmental stresses of encapsulated ingredients. Combination of spray drying of double-layer water/oil/water multiple emulsions showed the best morphology, highest microencapsulation efficiency, and highest total carotenoids retention, and a high biopolymer blend (gum arabic, mesquite gum, and maltodextrin)-to-primary emulsion ratio also produced high microencapsulation efficiency. An LbL self-assembly strategy has been used to increase the mechanical stability of microcapsules through noncovalent interactions; the only drawback of this method is that the assembly process has to be repeated a number of times to meet the mechanical requirements. The films are formed by depositing alternating layers of oppositely charged materials with wash steps in between. This method can be achieved by using various techniques such as immersion, spin, spray, and electromagnetism. LbL offers several advantages when compared with other thin film deposition methods. LbL is simple and inexpensive (Noshad et al., 2015). There are a wide variety of materials that can be deposited by LbL, including polyions, metals, ceramics, nanoparticles, and biological molecules, which give a wide variety of application areas. Another important quality of LbL is the high degree of control over thickness, which arises because of the variable growth profile of the films, and directly correlates with the materials used, the number of bilayers, and the assembly technique. Shutava et al. (2009) obtained (−)-EGCG-incorporated layer structures by this method. ECGC was inserted in the core, which was surrounded by layers of various polymer blends. LbL-coated nanoparticles exhibited sustained release and retention of antioxidant activity indicated by ABTS assay results. In addition to antioxidant activity, nanoparticles also blocked the production of hepatocyte growth factor from cancer cell lines MBA-MD-231 as effectively as free EGCG.

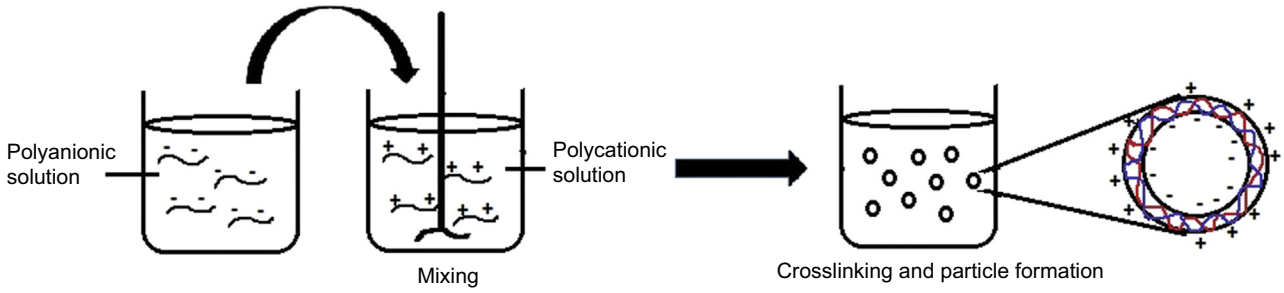

FIGURE 17.5 Cross-linking and particle formation by ionic gelation method.

4.11 Ionic Gelation Method

Ionic gelation is considered as a mild process because use of toxic organic solvents and surfactants is avoided. The technique is based on electrostatic interaction between an oppositely charged polymer and a polyelectrolyte. Practically, a solution of the charged polymer is added dropwise under stirring to an oppositely charged polyelectrolyte, which causes the crosslinking of the two entities and thus the obtaining of the particulate form (Fig. 17.5).

pH is the most effective parameter that changes the protonation degree of molecules rapidly and which no longer allows the triggering of the ionic gelation process (Jafarinejad et al., 2012). Many other parameters may influence the properties of the obtained particles such as stirring rate and the ambient temperature at which the process of crosslinking takes place.

Especially, pH is most effective in the ionic gelation method; the disadvantage of this technique is restriction of desired conditions for delivery.

Catechin, with its low stability in alkaline conditions, was chosen as a candidate antioxidant to be used in the ionic gelation procedure by Dube et al. (2010). Its therapeutic uses were comprised of antiinflammatory, vasodilatory, neuroprotective, and chemopreventive effects. In this study, catechin and (−)-epigallocatechin were immobilized within chitosan tripolyphosphate nanoparticles. Stability profiles of encapsulated catechin and (−)-epigallocatechin in 50 mM potassium hydrogen phosphate buffer, pH 7.4, indicated that after 24 h, encapsulated forms of catechins were degraded to 50% of their initial concentration, while it took 8 h to reach the same value for the free form of catechin. Liang et al. (2011) also prepared carboxymethyl chitosan and chitosan hydrochloride nanoparticles as carriers of tea polyphenols to overcome its low stability in vivo. Tea polyphenols have been shown to inhibit the development of cancer in a wide range of animal models such as oral, esophageal, forestomach, stomach, intestinal, colon, skin, liver, bladder, prostate, and breast cancer. Encapsulation efficiency in terms of drug content was higher when the carboxymethyl chitosan:chitosan hydrochloride:tea polyphenol ratio was 3:3:1 than when it was 2:2:1. This result could be attributed to interactions of polyphenols with the chitosan matrix. Adsorption of polyphenol on chitosan was favorable to coat polyphenols and prevent the extract from escaping into aqueous solution. Increasing amounts of chitosan could strengthen the chitosan skeleton surrounding the polyphenolic compound.

5. CONCLUSIONS

The main problem of using plant-derived natural compounds is their degradation in the gastrointestinal system before reaching the circulation system, which limits the area of usage of these compounds. Therefore it is necessary to apply encapsulation systems to maximize the potential therapeutic benefits of natural compounds.

Nanoencapsulated systems have the advantage of high drug encapsulation efficiency because of optimized drug solubility in the core, low polymer content compared to other nanoparticulated systems such as nanospheres, drug polymeric shell protection against degradation factors, and the reduction of tissue irritation caused by the polymeric shell.

Advancements in utilization of plant-derived natural compounds in combination with nanotechnology will allow us to achieve the treatment of various diseases effectively. In conclusion, we can say that nanotechnology combined with multifunctional nanocarriers with specific abilities to carry one or multiple natural products will find many practical applications for medical treatments in the future.

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