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REVIEW ARTICLE

Controlled Drug Delivery by Biodegradable Poly(Ester) Devices: Different Preparative Approaches

Rajeev Jain,[1,*] Navnit H. Shah,[2] A. Waseem Malick,[2] and Christopher T. Rhodes[1]

[1]*Department of Applied Pharmaceutical Sciences, The University of Rhode Island, Kingston, Rhode Island 02881*
[2]*Hoffmann-La Roche Inc., Pharmaceutical R & D, 340 Kingsland Street, Nutley, New Jersey 07110*

ABSTRACT

There has been extensive research on drug delivery by biodegradable polymeric devices since bioresorbable surgical sutures entered the market two decades ago. Among the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) such as poly(lactide) (PLA), poly(glycolide) (PGA), and especially the copolymer of lactide and glycolide referred to as poly(lactide-co-glycolide) (PLGA) have generated tremendous interest because of their excellent biocompatibility, biodegradability, and mechanical strength. They are easy to formulate into various devices for carrying a variety of drug classes such as vaccines, peptides, proteins, and micromolecules. Most importantly, they have been approved by the United States Food and Drug Administration (FDA) for drug delivery. This review presents different preparation techniques of various drug-loaded PLGA devices, with special emphasis on preparing microparticles. Certain issues about other related biodegradable polyesters are discussed.

INTRODUCTION

A controlled drug action may be achieved by either chemically modifying the drug moiety (e.g., prodrug) or by formulating it in a specific way to control its release. Oral controlled-release dosage forms, depending upon the drug employed, can provide efficacy for about 24 hr (1). Oral dosage forms may not be feasible in cases in which the drug undergoes extensive degradation in the gastrointestinal tract (GIT), exhibits significant first-pass effect, or is poorly absorbed. Of serious concern are the problems associated with the oral administration of pep-

*To whom correspondence should be addressed. NanoSystems L.L.C., 300 Horizon Dr., King of Prussia, PA 19406. Fax: (610) 313-5171. e-mail jainr@nanosys.com

tide/protein drugs which are subject to attack by the acidic and enzymatic environment in the stomach and the enzymes from the brush border membrane of the intestine. The high molecular weight and size also impedes the effective transportation of peptide/protein drugs across the GIT membranes. The main drawback of oral dosage forms is the short transit time of approximately 12 hr through the GIT (2). In addition, if the drug is absorbed only through a specific area of the GIT, the duration of action could be less than 12 hr (2).

If a drug cannot be administered orally because of any of the above reasons, a parenteral route of delivery is an alternative. One advantage that a parenteral controlled-release dosage form has over oral controlled-release dosage forms is patient compliance (2). Although an oral dosage form might have a good bioavailability, a long-acting parenteral dosage form that is safe and efficacious for days, weeks, or months could be beneficial because it ensures that the patient is receiving medication. Also, a parenteral controlled-release dosage form is preferred over a conventional parenteral dosage form for chronic treatment for which routine multiple injections could be inconvenient and painful. Parenteral controlled-release dosage forms are also effective in site-specific drug delivery, thereby improving the drug's efficacy and reducing its toxicity. The main disadvantage of these dosage forms is that once administered, they cannot be easily removed (2). This could be a problem for the patient if a drug was no longer needed, or if it caused an undesirable reaction.

To avoid inconvenient surgical insertion of large implants, injectable biodegradable and biocompatible polymeric particles (microspheres, microcapsules, nanocapsules, nanospheres) could be employed for parenteral controlled-release dosage forms (1). Microparticles of size less than 250 μm, ideally less than 125 μm, are suitable for this purpose (2). Biodegradable polymers are natural or synthetic in origin and are decomposed in vivo, either enzymatically or nonenzymatically, to produce biocompatible, toxicologically safe byproducts which are further eliminated by normal metabolic pathways (3). Drugs formulated in polymeric devices are released either by diffusion through the polymer barrier, by erosion of the polymer material, or by a combination of both diffusion and erosion mechanisms (4). The polymers selected for the parenteral administration must meet several requirements such as biocompatibility, drug compatibility, suitable biodegradation kinetics and mechanical properties, and ease of processing, (4,5).

Although a wide variety of natural and synthetic biodegradable polymers have been investigated for drug targeting or prolonged drug release, only a few of them are actually biocompatible. Natural biodegradable polymers such as bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatin, and hemoglobin have been studied for drug delivery (1). The use of these natural polymers is limited because of their relatively high costs and questionable purity (1).

In the past two decades synthetic biodegradable polymers have been increasingly used to deliver drugs because they are free from most of the problems associated with natural polymers (1–8). Poly(amides), poly(amino acids), poly(alkyl-α-cyano acrylates), poly(esters), poly(orthoesters), poly(urethanes), and poly(acrylamides) have been used to prepare polymeric devices to deliver drugs (1–7). Among them, the thermoplastic aliphatic poly(esters) such as poly(lactide) (PLA), poly(glycolide) (PGA), and especially the copolymer of lactide and glycolide referred to as poly(lactide-*co*-glycolide) (PLGA) have generated immense interest because of their excellent biocompatibility and biodegradability (1–17). They are also easy to formulate into drug carrying devices and have been approved by the United States Food and Drug Administration (FDA) for drug delivery use (13–17).

This review provides a comprehensive overview of different preparation techniques of various drug-loaded PLGA devices, with special emphasis on preparing microparticles. Certain issues about other related biodegradable polyesters such as PLA and PGA are discussed as well.

HISTORICAL DEVELOPMENT OF DRUG DELIVERY USING PLGA

The discovery and the synthetic work on low molecular weight oligomeric forms of lactide and/or glyceride polymers was first carried out several decades ago (3,5). The methods used to synthesize high molecular weights of these polymers were first reported by Lowe (3).

During the late 1960s and early 1970s a number of groups published pioneering work on the the utility of these polymers to make sutures/fibers (2,3,5,12). These fibers had several advantages such as good mechanical properties, low immunogenicity and toxicity, excellent biocompatibility, and predictable biodegradation kinetics (2,3,5,12). The wide acceptance of the lactide/glycolide polymers as suture materials, made them an attractive candidate for biomedical applications such as ligament reconstruction, tracheal replacement, ventral herniorrha-

phy, surgical dressings, vascular grafts, and nerve, dental, and fracture repairs (3,5,9).

The biodegradation, biocompatibility, and tissue reaction of PLA and PLGA have been extensively investigated and well documented by many researchers (5,14). The first work on parenteral controlled release of drugs using PLA was reported by Boswell, Yolles, Sinclair, Wise, and Beck (3,5). Since then a large amount of literature on drug delivery using PLA, and especially PLGA, has been published. Various polymeric devices such as microspheres, microcapsules, nanoparticles, pellets, implants, and films have been fabricated using these polymers for the delivery of a variety of drug classes.

SYNTHESIS OF PLGA COPOLYMER

Low molecular weight PLGA can be prepared by direct condensation (polyesterification) of lactic and/or glycolic acids (5,12). Temperatures as high as 130–190°C are required for the condensation process and the water generated is removed by boiling, using vacuum, purging with nitrogen, or azeotropic distillation with an organic solvent (3,12). An acid catalyst such as antimony oxide increases the reaction rate if used at reaction temperatures below 120°C, but above this temperature water removal is the rate-limiting step (3). This method yields PLGA having molecular weight of ~10,000 (12). The low molecular weight PLGA has limited biomedical application, because of its poor mechanical strength and faster degradation (3).

Intermediate and high molecular weight PLGA (~10,000–40,000) can be prepared by the ring-opening polymerization of the cyclic dimers (cyclic diester of lactic and/or glycolic acids) as the starting materials (3,5,12,14). The advantage of this method is that no water removal/dehydration method is needed in the polymerization system (3). The cyclized monomer(s) and the linear form of the polymers produced can also be readily purified (3). Compounds of lead, tin, cadmium, zinc, antimony, and titanium have been used as catalyst to initiate the polymerization process (12,14). Acid-catalyzed bulk polymerization (melt method) for 2–6 hr at approximately 175°C is generally employed for preparation of PLGA from lactide and glycolide monomers (3). The molecular weight of the resultant PLGA is determined by the concentration of the catalyst added (12). Monomer purity of 99.9% or greater and monomer acidity of 0.05% or less are required with the starting lactide and glycolide materials (5). Low humidity levels in the processing area are also important (5).

PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF PLGA

It is important to understand the physical, chemical, and biological properties of the polymer before formulating a controlled drug delivery device. The various properties of the polymer and the encapsulated drug directly influence other factors such as the selection of the microencapsulation process, drug release from the polymer device, etc. (1).

PLA can exist as the optically active stereoregular polymer (L-PLA) and a optically inactive racemic polymer (D,L-PLA) (1,5,9). L-PLA is found to be semicrystalline because of the high regularity of its polymer chain, and D,L-PLA is an amorphous polymer because of irregularities in its polymer chain structure (3,9). Hence, the use of D,L-PLA is preferred over that of L-PLA because D,L-PLA enables more homogeneous dispersion of the drug in the polymer matrix (9,13). PGA is highly crystalline because it lacks the methyl side groups of PLA (3,9). Lactic acid is more hydrophobic than glycolic acid and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water, and subsequently degrade more slowly (1,3,13).

The molecular weight and polydispersity index of the polymer are factors that affect the mechanical strength of the polymer and its ability to be formulated as a drug delivery device (3,5,12). These properties may also control the polymer biodegradation rate and hydrolysis (3,12). The commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to their molecular weights (3).

The degree of crystallinity of the PLGA polymer directly influences its mechanical strength, swelling behavior, capacity to undergo hydrolysis, and subsequently its biodegradation rate (3). The resultant crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain (1). PLGA polymers containing a 50:50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing a higher proportion of either of the two monomers (5,12). PLGAs prepared from L-PLA and PGA are crystalline copolymers, and those from D,L-PLA and PGA are amorphous (3,5). Gilding and Reed determined that PLGAs containing less than 70% glycolide are amorphous (18). The degree of crystallinity and the melting point of the polymers are directly related to the molecular weight of the polymer (3,5).

The glass transition temperature (T_g) of the PLGA copolymers is above the physiological temperature of

37°C and hence they are glassy (3,5). Thus, they have a fairly rigid chain structure which gives them significant mechanical strength to be formulated as drug delivery devices (3,5). Jamshidi et al. reported that T_g of PLGAs decreases with a decrease of lactide content in the copolymer composition and with a decrease in the molecular weight of the PLGA (19).

It is important for the PLGA polymers to have considerable mechanical strength because the drug delivery devices formulated using them are subjected to significant physical stress (3,5). Different factors such as the molecular weight, copolymer composition (lactide/glycolide ratio), crystallinity, and geometric regularity of individual chains significantly affect the mechanical strength of the polymer (1,3,5).

In vitro and in vivo, the PLGA copolymer undergoes degradation in an aqueous environment (hydrolytic degradation or biodegradation) through cleavage of its backbone ester linkages (1–3,5,12,13). The polymer chains undergo bulk degradation occurs at a uniform rate throughout the PLGA matrix (3,13). Thies and Bissery reported that the PLGA biodegradation occurs through random hydrolytic chain scissions of the swollen polymer (20). The carboxylic end groups present in the PLGA chains increase in number during the biodegradation process as the individual polymer chains are cleaved; the end groups are known to catalyze the biodegradation process (3,5). The biodegradation rate of the PLGA copolymers is dependent on the molar ratio of the lactic and glycolic acids in the polymer chain, molecular weight of the polymer, the degree of crystallinity, and the T_g of the polymer (3,5,13). A three-phase mechanism for the PLGA biodegradation has been proposed (21):

1. First, a random chain scission process occurs. The molecular weight of the polymer decreases significantly, but no appreciable weight loss and no soluble monomer products are formed.
2. In the middle phase a decrease in molecular weight accompanied by rapid loss of mass and soluble oligomeric and monomer products are formed.
3. Soluble monomer products are then formed from soluble oligomeric fragments. This phase is that of complete polymer solubilization.

The extent, if any, of the role of enzymes in the PLGA biodegradation is unclear (3,5). Most of the literature indicates that the PLGA biodegradation does not involve any enzymatic activity and is purely through hydrolysis (3). However, some investigators have suggested an enzymatic role in PLGA breakdown based upon the difference in the in vitro and in vivo degradation rates (5).

The PLGA polymer biodegrades into lactic and glycolic acids (1–3,5,12,13). Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water (1–3,5,9). In a study conducted using ^{14}C-labeled PLA implant, it was concluded that acetic acid is eliminated through respiration as carbon dioxide (22). Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and is eventually eliminated as carbon dioxide and water (3).

METHODS OF PREPARING VARIOUS PLGA DEVICES

Microparticles

A number of microencapsulation techniques have been developed and reported to date. The choice of the technique depends on the nature of the polymer, the drug, the intended use, and the duration of the therapy (1,2,4,5,10). The microencapsulation method employed has the following requirements (1,2,23):

1. The stability and biological activity of the drug should not be adversely affected during the encapsulation process or in the final microsphere product.
2. The yield of the microspheres having the required size range (up to 250 μm, ideally <125 μm) and the drug encapsulation efficiency should be high.
3. The microsphere quality and the drug release profile should be reproducible within specified limits.
4. The microspheres should be produced as a free-flowing powder and should not exhibit aggregation or adherence.

Solvent Evaporation and Solvent Extraction Process

Single Emulsion Process

This is essentially an oil-in-water (o/w) emulsion process. The polymer is first dissolved in a water-immiscible, volatile organic solvent (dichloromethane [DCM] is most commonly used). The drug is then added to the polymer solution to produce a solution or dispersion of the drug particles (particle size of the drug added to be <20 μm) (4). This polymer–solvent–drug solution/dispersion is then emulsified (with appropriate stirring and temperature conditions) in a larger volume of water in

the presence of an emulsifier [such as poly(vinyl alcohol) (PVA)] to yield an o/w emulsion. The emulsion is then subjected to solvent removal by either an evaporation or extraction process to harden the oil droplets (10). In the former case the emulsion is maintained at reduced pressure or at atmospheric pressure and the stir rate is reduced to enable the volatile solvent to evaporate (4,10). In the latter case the emulsion is transferred to a large quantity of water (with or without surfactant) or other quench medium, into which the solvent associated with the oil droplets is diffused out (4,10). The solid microspheres so obtained are then washed and collected by filtration, sieving, or centrifugation (4). The microspheres are then dried under appropriate conditions or are lyophilized to give the final free-flowing injectable microsphere product.

Note that the solvent evaporation process in a way is similar to the extraction method, in the sense that the solvent must first diffuse out into the external aqueous dispersion medium before it can be removed from the system by evaporation (4,10). The rate of solvent removal by the extraction method depends on the temperature of quench water or other medium, ratio of emulsion volume to quench water/medium volume, and the solubility characteristics of the polymer, solvent, and dispersion medium. The rate of solvent removal by evaporation method strongly influences the characteristics of the final microspheres and depends on the temperature, pressure, and the solubility parameters of the polymer, solvent, and dispersion medium (10). Very rapid solvent evaporation may cause local explosion inside the droplets and lead to formation of porous structures on the microsphere surface (10). The solvent removal by extraction method is faster (generally <30 min) than the evaporation process and hence the microspheres made by the former method are more porous in comparison to those made from the latter method under similar conditions (10).

The biggest drawback of the o/w emulsification method is poor encapsulation efficiencies of moderately water-soluble and water-soluble drugs (1,4,10). The drug can diffuse out or partition from the dispersed oil phase into the aqueous continuous phase and microcrystalline fragments of the hydrophilic drugs are deposited on the microsphere surface and dispersed in the PLGA matrix (24,25). This results in poor trapping of the hydrophilic drug such as salicylic acid and initial rapid release of the drug (burst effect) (1). The o/w emulsification process is therefore widely used to encapsulate lipid-soluble drugs such as steroids (1).

To increase the drug loading of water-soluble drugs, an oil-in-oil (o/o) emulsification method was developed (1,10,26). A water-miscible organic solvent such as acetonitrile is employed to solubilize the drug in which PLGA or PLA are also soluble. This solution is then dispersed into an oil such as light mineral oil in the presence of an oil-soluble surfactant such as Span to yield the o/o emulsion. Microspheres are finally obtained by evaporation or extraction of the organic solvent from the dispersed oil droplets, and the oil is washed off by solvents such as *n*-hexane. This process is also sometimes referred to as the water-in-oil (w/o) emulsification method (1).

A number of formulation and process factors affect microsphere formation. The main variables that influence the microencapsulation process and the final microsphere product are (*a*) the nature and solubility of the drug being encapsulated; (*b*) the polymer concentration, composition, and molecular weight; (*c*) the drug/polymer ratio; (*d*) the organic solvent used; (*e*) the concentration and nature of the emulsifier used; (*f*) the temperature and stirring/agitation speed of the emulsification process; and (*g*) the viscosities and volume ratio of the dispersed and continuous phases (1,4,5,10).

Solvents. Selection of dispersed and continuous phase is important for successful microsphere formation and to achieve high drug encapsulation efficiencies. For the solvent evaporation/extraction method the dispersed phase selected should be immiscible or only slightly miscible with the continuous phase and must have a boiling point lower than that of the continuous phase (4). Bodmeier and McGinity showed that water-miscible solvents such as acetone and dimethyl sulfoxide do not form microspheres upon emulsification (27). Typically, DCM and water are used as dispersed and continuous phases, respectively.

DCM is widely used because it is a good solvent for the polymers and because its high volatility enables it to be easily removed by evaporation.

A major problem with the use of DCM is its potential toxicity (28). Chlorinated solvents in general are considered hazardous to the environment and undesirable for use in manufacturing processes (28). Chern et al. reported the use of ethyl acetate to prepare PLGA microspheres by the solvent extraction process (29). Sah et al. produced microspheres by a two-step extraction process using methyl ethyl ketone (MEK) (10) times more soluble than DCM in water) as the solvent for PLGA (28,30). Rapid diffusion of MEK into the extraction medium and migration of water into the oil droplets pro-

duced hollow microspheres having volume mean diameter of 96 μm and 60–77% drug entrapment (28,30). The authors concluded that water-immiscibility of the dispersed phase is not an absolute requirement for the solvent evaporation/extraction process (28,30).

For the o/o emulsification method, acetonitrile (26,31–38) is generally used as the dispersed phase. Other solvents such as acetonitrile/water mixture (24), DCM (39), and *N*,*N*-dimethyl formamide (DMF) (40–42) have also been used. The continuous phase consists of oils such as light mineral oil (26,33–36,38,39), cotton seed oil (24), liquid paraffin (40–42), silicon oil (31), and machine oil (37). When prepared by solvent extraction method, heptane has been most commonly used as extraction medium (32-38). Thanoo et al. prepared microspheres from PLA using DCM, glycerin, and isopropanol/water mixture as the dispersed phase, continuous phase, and extraction medium, respectively (43). The same group also reported preparation of PGA microspheres using hexafluoroacetone, carbon tetrachloride, and dioxan as the dispersed phase, continuous phase, and extraction medium, respectively (43).

Van Hamont et al. concluded that the particle size of the microspheres is a balance of the following two opposite actions: (*a*) higher weights of the external oil phase tend to produce larger diameter microspheres due to slowing of the solvent evaporation process, and (*b*) decrease in polymeric droplet coalescence because an increase in viscosity of the oil phase tends to decrease the diameter of the microspheres (37).

Sometimes a solvent mixture rather than a solvent alone is employed as the dispersed phase (43). Such a solvent mixture consists of a water-immiscible solvent such as DCM (44,45) or chloroform (46,47) and a water-miscible solvent such as acetone (44,46,47), methanol (45), ethanol (4), or propylene glycol (4). The water-miscible solvent provides rapid solvent removal and faster polymer precipitation and hardening (44–47). Coombes et al. used a DCM/acetone mixture in the solvent evaporation process and concluded that the solvent removal process is rapid and causes entrapment of the stabilizer molecules by physical chain entanglement and thus their stabilizing capacity is enhanced (44). Use of DCM alone, however, produces a slow solvent evaporation process, allowing entrapped stabilizer molecules to diffuse out into the external aqueous phase with consequent loss of their stabilizing capacity (44). Thanoo et al. prepared PLGA microspheres using a mixture of two water-immiscible solvents, DCM and chloroform, by the solvent evaporation process (43). Polard et al. reported that because of poor solubility of morphine in DCM, and good solubility in methanol, methanol was used as hydrophilic cosolvent (45). As the fraction of methanol was increased in the DCM/methanol mixture, more morphine dissolved in the organic phase and this enhanced the drug entrapment in the microspheres as a result of faster precipitation of the polymer (45). However, when the percentage of methanol in the solvent mixture exceeded 60%, the polymer could not be dissolved (45). Spenlehauer et al. employed DCM/cyclohexane (10:1) mixture for producing PLA microspheres (48). Cyclohexane is less volatile than DCM and hence evaporation of DCM from the emulsion droplets leads to entrapment of cyclohexane in the microspheres, resulting in formation of porous surface structures during the final removal of cyclohexane (48).

Sansdrap and Moës found that the increase in the external aqueous phase volume did not affect the final microsphere size but an increase in the dispersed DCM volume decreased the size with narrow size distribution because of the decrease in the viscosity of the internal phase with increasing volume (49).

Emulsifiers. During the solvent evaporation/extraction process, there is a gradual decrease in the volume and subsequent increase in the viscosity of the dispersed oil droplets (4). This affects the droplet size equilibrium and the droplets tend to coalesce and produce agglomerates during the early stages of solvent removal (1,4,10). This problem could be rectified by adding a small quantity of a droplet stabilizer (emulsifier) in the continuous phase (1,4,10). The emulsifier provides a thin protective layer around the oil droplets, and hence reduces their coalescence and coagulation (10). As the solvent is removed, the emulsifier continues to maintain the spherical shape of the oil droplets and prevents their aggregation, until the microspheres are hardened and isolated as discrete particles (4).

The physicochemical properties and the concentration of the emulsifier strongly influences the microsphere size, shape, and drug encapsulation efficiency. The emulsifiers most commonly used in the solvent evaporation/extraction process are the hydrophilic polymeric colloids and/or anionic or nonionic surfactants (4,10). PVA is by far the most commonly used emulsifier (25,29,30,33–35,45–47,50–63) in the o/w emulsion method. Others include poly(vinylpyrrolidone) (PVP) (4), alginate (4), gelatin (4), methylcellulose (MC) (25,51,54,64,65), hydroxyalkyl cellulose (4,10), hydroxypropylmethylcellulose (HPMC) (49), polyoxy-

ethylene derivatives of sorbitan fatty esters (Tweens) (4,10), cetyltrimethyl ammonium bromide (4,10), and fatty acid salts such as sodium oleate (43,66–68). For o/o method, oil-soluble emulsifiers such as polyoxyethylene fatty ethers (Brijs), Spans, and lecithins have been used (1,4,10).

The appropriate type and concentration of the emulsifier for a particular process is apparently commonly determined by trial-and-error basis, although optimization techniques clearly have potential in this area. For most of the emulsifiers, the microsphere size decreases with an increase in emulsifier concentration (4,49). Beyond a certain concentration, the emulsifier is ineffective because an optimal packing concentration for the emulsion is achieved, i.e., condensed monolayer (40). Wakiyama et al. investigated the emulsifying action of sodium alginate in comparison with gelatin and concluded that sodium alginate produced a relatively more viscous aqueous phase and hence yielded relatively smaller microspheres compared to those produced by the same amount of gelatin (69). Fong et al. found that when sodium hydroxide was added to the aqueous continuous phase, the ionization of the emulsifier sodium oleate was increased, which resulted in higher drug encapsulation efficiencies and smaller, spherical, but highly porous microspheres (70). Jalil and Nixon studied the effects of oil-soluble emulsifiers (Spans and Brijs) on the size of microspheres prepared by o/o emulsion and concluded that more hydrophilic emulsifiers produced smaller microspheres (71). Coombes et al. prepared PLGA microspheres using various grades of poly(oxyethylene)–poly(oxypropylene) (PEO–PPO) copolymers as the surfactants (44). The solvent removal led to entrapment of these surfactant molecules by physical chain entanglement and because of their location at the microsphere surface. The authors stated that the PEO–PPO chain length, structure, and conformation influenced the surface coverage of the microspheres, the strength of surfactant attachment, and its overall performance (44).

A combination of emulsifiers has sometimes been used to achieve the necessary emulsifying action (25,51,72). Cavalier et al. reported that a combination of PVA and MC yielded PLA microspheres having maximum sphericity and drug entrapment compared to formulations that used these individual colloids alone (25). This was due to improvement in the rheological properties of the combined emulsifiers as compared to their properties when used alone. A similar finding was reported by Spenlehauer et al. When the theoretical drug loading ranged from 0 to 30%, 0.25% aqueous PVA solution produced microspheres in the size range 25–50 μm (51). However, for drug loading in the range of 50–60%, 0.25% MC or a PVA/MC (50:50) mixture was necessary to produce the microspheres (51).

Polymer. The polymer type, its molecular weight, and tile concentration used strongly influence the characteristics of the final microspheres. Cavalier et al. reported that a decrease in PLA concentration (increase in drug/PLA ratio) resulted in higher drug content in the microspheres (25). The same group also reported slightly higher drug content for PLGA (65:35) microspheres against those for PLA microspheres (25). Coombes et al. reported a decrease in polydispersity and particle size of the microparticles as the PLGA concentration was decreased (44). In another study, drug content of PLA (molecular weight 2000) microparticles was higher than that of PLGA (molecular weights 9000 and 12,000) and PLA (molecular weight 9000) microparticles due to the rapid rate of polymer precipitation at the droplet surface (45). The particle size increased from 1.0 μm for PLGA (RG 505), to 1.1 μm for PLGA (RG 858), to 1.5 mm for PLA (R 208) microspheres (73). The drug entrapment was, however, the same for RG 505 and R 208 (2.8% w/w), and for RG 858 it was slightly higher (3.2% w/w) (73). In another study, microspheres prepared from 16% w/w PLGA had many structural defects, and those prepared from 5.3% w/w had little structural defects but were aggregated and formed lumps (40). Despite an increase in the PLGA molecular weight from 6600 to 19,000, microspheres with uniform particle size and no structural defects were produced (40). In a peptide adsorption study, Calis et al. found that with increase in microsphere concentration (and hence PLGA concentration) the time for maximum peptide adsorption decreased (66). Delgado et al. reported that values of certain polymer parameters such as polydispersity and degradation index (a measure of polymer erosion) are directly related to the weight-average molecular weight (M_w) of the PLA polymer used for microencapsulation (61).

Drugs. The biggest disadvantage of the o/w emulsification method is poor encapsulation of water-soluble drugs (1,4,10). The o/w emulsification process is therefore recommended to encapsulate lipid-soluble drugs (1). Several investigators have tried various modifications of the o/w method to minimize partitioning and thereby increase the entrapment of water-soluble drugs.

Bodmeier and McGinity achieved higher entrapment of ionizable drugs such as diazepam and quinidine by using pH external aqueous phase (pH 12), in which the loss resulting from ionization of these drugs was reduced (74). Similarly, Wakiyama reported higher drug encapsulation efficiencies for butamben and dibucaine when the aqueous phase consisted of 1% alkali (high pH solution) (69). However, tetracaine under similar conditions became ionized and exhibited poor drug entrapment (69). Polard et al. used an external phase having a pH of 9 to prevent the solubility of morphine in water and thereby reduced its partitioning in the external aqueous phase (45). Contrary to these results, Vaughan et al. reported that increasing the pH of the external aqueous phase to 10 did not increase the loading efficiency of lidocaine (33).

The loss of drug can also be minimized by presaturating the aqueous or organic phase with the same drug. The drug content of quinidine in PLA microspheres increased with an increase in quinidine content in the dispersed organic phase (74), and tetracaine entrapment increased with prior saturation of the aqueous phase (69).

Sah et al. reported that the encapsulation efficiency of PLGA microspheres decreased with an increased theoretical loading of the drug (progesterone) due to rapid partitioning of the drug in the external aqueous phase from the dispersed organic phase (which contained MEK) (30). Polard et al. showed that with an increase in drug loading, the drug content of the microspheres increased, but their encapsulation efficiencies and the microsphere recovery yield decreased (45). Drug entrapment was also higher when the drug was present in suspension form as compared to when present in solution form (45). A similar result was reported by Cavalier et al., where an increase in the drug/polymer ratio resulted in an increase in the drug content of the microspheres (25). Thanoo et al. showed an increase in the drug incorporation efficiency and the microsphere yield within an increase in the theoretical drug loading (43).

Rosilio et al. reported that for progesterone loading of 0–30%, the microsphere (prepared by o/w method) size was in the range 25–50 μm, and for 35% loading it increased to 50–75 μm (5). A different observation was made by Tsai et al. who prepared microspheres by the o/o method (26). Despite an increase in the drug loading from 3.65 to 13.80%, the microspheres exhibited an average size of 95 μm, with a relatively narrow size distribution (26). In another study, an increase in nifedipine (a water-insoluble drug) loading resulted in subsequent increase in its content in the PLGA microspheres but did not influence the mean particle size (49).

Calis et al. carried out peptide adsorption studies and concluded that in dilute peptide solutions, peptide–PLGA interaction favored monolayer adsorption which fitted the Langmuir adsorption, and at higher peptide concentration, peptide–peptide interaction was favored, resulting in multilayer adsorption which fitted the Freundlich model (66). In another study, Duggirala et al. showed that with increased protein loading, the adsorption of protein on PLGA microspheres increased up to a definite value and then remained constant because of saturation of the microsphere surface (monolayer coverage) by the protein (75).

Bodmeier and McGinity showed by a scanning electron microscopy (SEM) study that for PLGA microspheres, the surface changed from a smooth texture at low drug content to a porous honeycomb-like structure at higher drug loading (74). In another study, PLGA microcapsules containing 8% progesterone showed a smooth external morphology, and those containing 21% drug exhibited textured and irregularly shaped surface features (30). When the theoretical progesterone loading was increased from 10 to 50%, the microsphere surface changed from a smooth, uniform appearance to an irregular surface containing well-defined progesterone crystals and numerous pores (51).

In a study carried out by Benoit et al., increase in the encapsulated drug amount resulted in a gradual decrease in the T_g of PLGA polymer from 48.3 to 12.9°C (52). The authors concluded that the drug was molecularly dispersed in an amorphous form in PLGA (formation of a stable solution) and thus strongly plasticized the polymer (52). A similar interaction phenomenon between the drug and PLGA has been reported by Crossan and Whateley (53) and Richey and Harris (65). Rosilio et al. concluded that below 35% loading, progesterone is molecularly dispersed in the PLGA glass (51). At 35% and above, crystal domains of the steroid appeared and two crystalline forms, α and β, could be detected (51). Bodmeier and McGinity (74) and Cavalier et al. (25) also reported similar results of a molecular dispersion of the drug in the polymer glass.

Process. Sah et al. prepared microspheres by a two-step extraction–hardening process using MEK as a solvent for the PLGA polymer (external aqueous phase was presaturated with MEK) (28,30). In the first step, the emulsion was transferred into 250 ml of aqueous PVA solution in which MEK was extracted out (30). In the next step the microcapsules were transferred into 500 ml

of aqueous PVA solution for complete hardening of the microcapsules. The authors concluded that the initial extraction rate of MEK was critical for successful microencapsulation (30). Also, the particle size of the microspheres decreased when an increasing amount of MEK was predissolved in the external aqueous phase before the emulsification process (28). Giordano et al. used DCM-saturated 1% PVA aqueous phase to make PLGA microspheres (56). Rosilio et al. prepared microspheres from PLGA in which the solvent (DCM) was removed by an interrupted process (51). DCM evaporation was interrupted after a definite period and the aqueous phase (continuous phase) was completely removed by several decantation washings. The DCM evaporation was then continued until the microspheres were obtained. This method was developed to minimize formation of emulsifier-assisted drug crystals at the microsphere surface and to achieve higher drug loading (51). Cowsar et al. produced microspheres from PLGA by two techniques: solvent extraction–solvent evaporation and solvent evaporation–solvent extraction (47). In the former case, most of the acetone was first allowed to diffuse out from the dispersed organic phase (chloroform–acetone mixture) into the external aqueous phase, followed by gradual evaporation of the residual solvents to give the final microspheres. In the latter case, the o/w emulsion was first subjected to solvent (DCM) evaporation for a certain period until semisolid droplets were obtained and the residual DCM was removed by the extraction process in a large volume of water. Microspheres from the evaporation–extraction process were less porous and exhibited better encapsulation than those prepared from extraction–evaporation process (47).

Vaughan et al. (33,34) and Pak et al. (35) compared the effects of the solvent extraction versus the evaporation process on the final microsphere product. Microencapsulation of lidocaine base by the evaporation process gave product with a yield of 65–80%, volume mean diameter of 120–130 μm, drug content of 4–10%, smooth and nonporous surface, and only 30–70% loading efficiency (because of the solubility of lidocaine in the external aqueous phase) (33). The extraction process, however, yielded microspheres having lidocaine content in the range of 5–20%, particle size of 7–10 μm, smooth but very porous particles, and 100% loading efficiencies (33). The authors used the salt form of the drug (lidocaine hydrochloride), and not lidocaine base for the extraction process (33). An extraction process using lidocaine base resulted in encapsulation efficiency of less than 10% (33). The same group also reported a better product from the extraction process for the drug ketoprofen in terms of drug content, loading efficiency, particle size, and surface feature compared to the evaporation process (34). Contrary to these results, Pak et al. reported slightly lower drug contents for PLGA microspheres prepared from the extraction process as compared to the evaporation process (35).

Some investigators have compared the microspheres produced from the o/o method against those produced from the o/w process (24,39). Wada et al. reported that the o/o method produced L-PLA microspheres having smooth spherical surface and higher drug entrapment because of a reduction in partitioning of drug in the external oil phase (24). The o/w process on the other hand gave a poor product with drug particles sticking out from the surface and poor drug entrapment (24). Contrary to these results, Menegatti et al. stated that the o/w process produced microspheres having average size of 38.4 μm with no aggregation, as compared to the o/o method which yielded a poor product having severe aggregation (39).

The rate of temperature rise and the operating temperature for solvent evaporation strongly influence the microsphere product. Kyo et al. reported that the solvent evaporation at the rate of 0.5 and 2.0°C/min, in an o/o process yielded PLGA microspheres having many structural defects compared to evaporation at 0.2°C/min which produced fewer defects (40). Wakiyama et al. found that the organic solvent removal by heating at 40°C produced a viscous aqueous phase and resulted in relatively larger microcapsules than those produced by removing solvent by vacuum at room temperature without any heat (69). Tice and Gilley determined that very rapid DCM evaporation would cause DCM to boil off from the emulsion droplets, yielding microspheres with cracks and pin holes (76). Jalil and Nixon stated that when a temperature of 85°C was used (above the boiling point of the solvent acetonitrile), highly porous microspheres, having internal honeycomb-like structure, were produced (77). Van Hamont et al. found a predictable linear increase in the average PLGA microsphere size as the temperature of the continuous oil phase (various grades of machine oil) was increased from 20 to 30°C during the evaporation (of acetonitrile) phase of the o/o emulsification process (37). This linearity was lost as the temperature was increased from 30 to 40°C because of changes in the solubility of acetonitrile in oil (37). By heating an o/w emulsion for 2 hr at 50°C, Vaughan et al. could increase the drug loading efficiency from 20–30 to 75–85% (34). Evaluation of hydrocortisone stability in

PLA microspheres at different temperature/time storage conditions revealed no drug degradation (25).

Generally, increasing the stirring rate decreases the microsphere size and narrows the size distribution. Crossan and Whateley prepared PLGA microspheres in tile size range of 40–60 μm by using an overhead paddle stirrer and stirring for 4 hr at room temperature (53). Modification of this system by addition of a baffle reduced microsphere size to 20–40 μm (53). A similar result was reported by Bodmeier and McGinity (78). The side baffles reduced the effective diameter of the vessel and hence led to the formation of smaller emulsion droplets. Also, the baffles reduced the turbulence in the suspension mixture, thereby increasing the stability of the droplet suspension and the product yield. Further size reduction (5–10 μm) was achieved first by high-speed stirring (1500 rpm) for 10 min using a Silverson homogenizer, followed by magnetic stirring for 18 hr to enable complete evaporation of DCM (53). Rosilio et al. found that for a drug loading of 0–30%, a stirring speed of 480 rpm was required and for a drug loading of 50–65%, a stirring speed of 645 rpm was necessary to produce the microspheres (51). Coombes et al. stated that increasing the stirring rate of the emulsion resulted in a decrease in polydispersity of the PLGA microspheres but not in a decrease in their particle size (44).

Double-(Multiple) Emulsion Process

The double-emulsion process is essentially a water-in-oil-in-water (w/o/w) method and is best suited to encapsulated water-soluble drugs such as peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs such as steroids (1,4,5). A buffered or plain aqueous solution of the drug (sometimes containing a viscosity building and/or stabilizing protein such as gelatin) is added to an organic phase consisting of PLGA and/or PLA solution in DCM and is vigorously stirred to form the first microfine w/o emulsion. This emulsion is added and gently stirred into a large volume of water containing an emulsifier such as PVA to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by either an evaporation or extraction process. In the former case, the emulsion is maintained at reduced pressure or at atmospheric pressure and stirred to enable DCM to evaporate. In the latter case, the emulsion is transferred to a large quantity of water (with or without surfactant) with stirring, into which DCM is diffused out. The solid microspheres so obtained are then washed and collected by filtration, sieving, or centrifugation. These are then dried under appropriate conditions or are lyophilized to give the final free-flowing microsphere product.

Some groups reported using ethyl acetate as the polymer solvent and hydrophilic stabilizers such as Pluronic F68, PEG 4600, BSA, HSA, or sodium glutamate for protein/peptide drugs (79). Singh et al. used a blend of PVA and PVP in the outer aqueous phase to make PLA/PLGA microspheres (80). Cohen et al. used an outer aqueous PVA phase saturated with DCM to prepare PLGA microspheres (81). Alpar et al. reported preparation of PLA microspheres in which the inner aqueous phase contained MC in addition to PVA or PVP (82,83). They found that particles containing PVP were more hydrophobic, exhibited higher drug loading and encapsulation efficiency, and showed decreased burst effect compared to those containing PVA (82,83). The addition of a stabilizing polymer (BSA) reduced the net encapsulation efficiency of the protein drug (82).

A number of hydrophilic drugs such as the peptide leuprolide acetate, a luteinizing hormone-releasing hormone (LH-RH) agonist (84–89), vaccines (21,79–81,83, 90–124), proteins/peptides (82,125–138), and conventional molecules (139–151) have been successfully encapsulated by this method. Various formulation and process variables significantly affect the final microsphere product and the drug release from it.

The primary w/o emulsion. Ogawa et al. concluded that the encapsulation efficiency of the drug in PLA and PLGA microparticles increased with the increase in viscosity of the inner aqueous phase (containing gelatin) and also by increasing the viscosity of the whole w/o emulsion (by decreasing the amount of DCM) (84). The authors concluded that the high viscosity prevented the migration of the inner aqueous phase to the outer water phase due to local demulsification produced by the vigorous stirring (84). Similar results were reported by Jeffery et al., who also found an increase in the microparticle size with the increase in viscosity of the inner aqueous phase (92). However, increasing the viscosity of the inner aqueous phase by adding PVA had no effect on the drug entrapment or the particle size of the final microparticles (92). Jeffery et al. and others have reported that an increase in particle size and drug entrapment was observed following an increase in the internal aqueous phase volume (92,118). Crotts and Park stated that the volume of the inner aqueous phase drastically affected the morphology of the final microspheres and the subsequent drug release from them; those prepared from 5.6% aqueous phase fraction were dense and non-

porous, and those prepared from 22.7% aqueous phase fraction were porous (118). Alonso et al. reported that incorporation of a lipophilic surfactant, L-α-phosphatidylcholine (by dissolving it in chloroform and adding this solution to the DCM phase) produced more hydrophobic microspheres, causing reduction of the microsphere size and increase in particle porosity because of better stabilization of the inner w/o emulsion (100). Other researchers also reported use of L-α-phosphatidylcholine (112). In another study, a decrease in the DCM phase volume yielded particles with dense core (81).

The entrapment efficiency of the drug increased with a decrease in drug loading and an increase in particle size (84). However, other groups have found no relationship between encapsulation efficiency and drug loading (92,104). Jeffery et al. reported that an increase in the antigen/PLGA ratio resulted in an increase in drug entrapment by PLGA and a small increase in the mean particle size of the final microparticles (92). Also, an SEM analysis revealed that at low antigen/PLGA ratios, smooth particles were produced, but at higher ratios the particles were pitted and some particles had collapsed (92). The authors attributed this to high surface concentration of antigen which became soluble in the surrounding external phase, leaving a pitted surface; in some cases this caused the microparticles to collapse (92). In another study small particles were produced when the volume ratio of DCM to PLA was low (84).

Jeffery et al. reported the effect of hydrophobicity (molecular weight) of the polymer on the entrapment of the antigen; more hydrophobic (high molecular weight of 53K) PLGA, showed relatively lower entrapment levels of the drug than less hydrophobic 22K PLGA (92). However, Alonso et al. found no relationship in the encapsulation efficiency with respect to polymer composition (PLA versus PLGA) and molecular weight (3K versus 100K) (79). Okada et al. reported that an increase in the content of water-soluble oligomers (free acid content) in PLA resulted in an increase in the burst release of the encapsulated drug (87). An increase in the T_g of the PLA and PLGA microspheres was also observed with an increase in drug loading (87). Alonso et al. indicated an increase in the microparticle size with an increase in the molecular weight and the concentration of the polymer (79,100). Benoit et al. found that microparticles prepared from PLGA were relatively larger and exhibited higher drug entrapment efficiency as compared to those prepared from poly(caprolactone) (PCL) (124). Hilbert et al. used an aqueous liposomal suspension as the inner aqueous phase and prepared microencapsulated liposomes (109). These showed a higher burst effect as compared to the normal microspheres due to the amphiphilic nature of the phospholipids which generated a porous matrix surface (109). Sah et al. reported that microcapsules containing PLA 5000 (molecular weight 5000) or PLGA 5000 (molecular weight 5000) added to PLGA 75:25 microcapsules exhibited increased degradation rates as compared to those containing PLGA 75:25 alone (117, 119,120). The authors found that PLA 5000 plasticized PLGA 75:25 and facilitated its faster degradation (120). Other groups also reported the effect of PLGAs having different molecular weight and lactide/glycolide ratio on the final microparticle size, drug entrapment, and the degradation rate of the polymer (94,106).

Microparticles loaded with greater amounts of drug produced a greater burst release of the drug due to an increase in the number of channels formed by the hydrophilic drug (84,87,120). Alonso et al. reported that the microparticles prepared by the double-emulsion method (drug dissolved in the inner aqueous phase) produced more regular microspheres with better control over drug release than those prepared by the powder dispersion method (drug powder dispersed in DCM phase) (100). Reich noted that the encapsulated protein drugs decrease the interfacial tension between the inner aqueous phase and the DCM phase of the o/w emulsion (123). The properties of the protein drug have a substantial effect on its entrapment and release, thus leading to a different optimum for different protein/polymer combinations (123).

Cohen et al. reported that for microspheres in which the inner emulsion was prepared using low shear (e.g., vortex mixing), the particles were large in size and the drug encapsulation was low compared to microspheres in which the inner emulsion was prepared using high shear (e.g., probe sonication), which yielded smaller particles with higher encapsulation efficiency (81). However, Sah et al. reported no effect of the shear rate (to prepare the o/w emulsion) on the encapsulation efficiency and the final particle size of PLA/PLGA microcapsules; particles prepared from low shear rate were, however, more porous than those prepared from high shear rate (116).

The double w/o/w emulsion. Ogawa et al. reported that smaller microparticles were produced when the mixing speed during emulsification of the w/o emulsion into the double w/o/w emulsion was increased (84). A similar result was reported by Uchida and Goto, who also

found a decrease in the drug loading efficiency with an increase in the stirring rate (105). An increase in the external phase volume also led to a decrease in the particle size of the microparticles (84). Jeffery et al. reported an increase in the drug entrapment and the particle size with an increase in the external aqueous phase volume (92).

Jeffery et al. found a reduction in particle size as the concentration of PVA increased in the external aqueous phase; the entrapment of the drug was, however, not affected (92). The authors attributed this to unstable emulsion droplets at low PVA concentration, resulting in the formation of larger microparticles compared to those prepared from high PVA concentration (92). Singh et al. investigated the residual PVA content in PLGA microparticles and concluded that various process parameters such as volume and concentration of the aqueous PVA solution and the number of washes in the microencapsulation process could control the residual levels of PVA within the acceptable limits (99).

Alonso et al. compared microsphere preparation by two methods in which the final organic solvent (DCM) was removed by evaporation and by extraction into 2% aqueous isopropanol solution; no major difference was found in the physical characteristics and the controlled drug release properties of the resultant microspheres. The extraction technique, however, yielded the microspheres in only 30 min (79,100). Other groups also reported use of 2% aqueous isopropanol solution to remove the solvent (113).

Drugs. Researchers at Takeda Chemical Industries reported successful encapsulation of leuprolide acetate, a LH-RH agonist (for treating endometriosis), into PLGA microparticles by the double-emulsion method (84–89). A pseudo-zero-order release profile (for 1 month) after administering PLGA-loaded leuprolide acetate in rats through subcutaneous (s.c.) and intramuscular (i.m.) routes (85,86,88) and a 3-month release profile following an s.c. injection (87) was reported by these researchers.

There is much interest in delivering vaccines through PLA/PLGA microparticles and copious literature has been published on this aspect (13,16,28,30,32–36,38,40, 41,54,55,64,65,74,100,102,106–109,114,142,152). A number of vaccines/immunogenic agents such as ovalbumin (30,32–36,42,64,65,152,153), *Dermatophagoides pteronysinuss* for hyposensitization therapy (38,40), cholera toxin (41), tetanus toxoid (79,100,106,114), ricin toxoid (102), HIV vaccine (102), birth control vaccine (107,109), BSA (39,54,55,81,95,124,139,140–143,149,151,153), influenza toxin (28), rotovirus (13), adenovirus (16), lysozyme (39), and *Schistosoma mansomi* against Schistosomiasis (93) have been successfully delivered by encapsulation into PLA/PLGA microparticles. These have been delivered by s.c., i.m., and oral route to provide pulse as well as sustained immune response for days, weeks, and months.

In addition to vaccines, other peptide/protein-based drugs and certain synthetic drugs have also been successfully loaded into PLA/PLGA microparticles by the double-emulsion method and administered for prolonged release effect (82,125–151).

Phase Separation (Coacervation)

The coacervation method consists of decreasing the solubility of the encapsulating polymer by addition of a third component to the polymer solution in an organic solution (1,4,5). At a particular point, the process yields two liquid phases (phase separation): the polymer containing coacervate phase and the supernatant phase depleted in polymer. The drug that is dispersed/dissolved in the polymer solution is coated by the coacervate. Thus the coacervation process includes the following three steps: (*a*) phase separation of the coating polymer solution, (*b*) adsorption of the coacervate around the drug particles, and (*c*) solidification of the microspheres (154).

First, the polymer is dissolved in an organic solution. The water-soluble drugs such as peptides and proteins are dissolved in water and dispersed in the polymer solution (w/o emulsion). Hydrophobic drugs such as steroids are either solubilized or dispersed in the polymer solution. An organic nonsolvent is then added to the polymer–drug–solvent system and stirred, which gradually extracts the polymer solvent. As a result the polymer is subjected to phase separation and it forms very soft coacervate droplets (size controlled by stirring) which entrap the drug. This system is then transferred to a large quantity of another organic nonsolvent to harden the microdroplets and form the final microspheres, which are collected by washing, sieving, filtration, or centrifugation, and are finally dried (4,154).

The phase separation method, unlike the o/w emulsification method, is suitable to encapsulate both water-soluble and water-insoluble drugs, since it is a nonaqueous method. However, the coacervation process is mainly used to encapsulate water-soluble drugs such as peptides, proteins, and vaccines. The addition rate of first nonsolvent should be such that the polymer solvent is extracted slowly, so that the polymer has sufficient time

to deposit and coat evenly on the drug particle surface during the coacervation process (4). The concentration of the polymer used is important as well, because concentrations that are too high would result in rapid phase separation and nonuniform coating of the polymer on the drug particles. Because of the absence of any emulsion stabilizer in the coacervation process, agglomeration is a frequent problem in this method (4). The coacervate droplets are extremely sticky and adhere to each other before the complete phase separation or the hardening stages of this method. Adjusting the stirring rate or temperature, or the addition of an additive is known to rectify this problem (4).

Unlike the solvent evaporation/extraction process, the requirement of solvents for the polymer are less stringent because the solvent need not be immiscible with water and the boiling point can be higher than that of water (4). DCM, acetonitrile, ethyl acetate, and toluene have been used in this process (152–163). The nonsolvents affect both the phase separation and the hardening stages of the coacervation process. The nonsolvents should not dissolve the polymer or the drug and should be miscible with the polymer solvent (152–160). The second nonsolvent should be relatively volatile and should easily remove the first viscous nonsolvent by washing. Some of the oils used as the first nonsolvent are silicone oil, vegetable oils, light liquid paraffin, low molecular weight liquid polybutadiene, and low molecular weight liquid methacrylic polymers (4,152–163). Examples of the second nonsolvent include aliphatic hydrocarbons such as hexane, heptane, and petroleum ether (4,152–163).

In the coacervation process the phase equilibrium is never reached and hence the system is constantly out of equilibrium (4). Therefore, the formulation and process variables significantly affect the kinetics of the entire process and ultimately the characteristics of the final microspheres. In a classic article, Nihant et al. investigated the effect of several process factors on the coacervation process (154). With an increase in the aqueous phase/organic phase volume ratio from 0.02 to 0.12% w/w, the "stability window" (an area in the phase diagram where the dispersed aqueous phase is efficiently coated by the coacervate) was unmodified and got only lightly narrower (154). An SEM photograph revealed that the morphology of the particles changed from a spherical shape for 0.02 ratio to a deformed shape at higher ratio 0.12. Above a water content of 0.12, the microspheres became brittle and spontaneously released the encapsulated drug solution during filtration (154). With a decrease in the stirring rate from 800 to 400 rpm for the aqueous drug dispersion in PLGA/DCM solution, the particle size increased from 40.0 to 51.5 μm and for 300 rpm no microparticles were formed (154). Similarly, with a decrease in the stirring rate from 200 to 130 rpm for the phase separation by adding silicone oil, the particle size increased from 40.0 to 58.0 μm, and for 100 rpm no microparticles were formed. For the addition rate (of silicone oil) of 18 ml/min, microparticles of the size 40.0 μm were formed and their size decreased to 39.1 μm when the addition rate was decreased to 5.7 ml/min (154). However, with further decrease in the addition rate to 0.65 ml/min, the particle size increased to 53.1 μm and aggregates were formed, and in certain cases no microparticles were formed. The authors concluded that microencapsulation by coacervation is a complex process that depends on the interplay of several kinetic parameters (154). In another paper, the same group reported the effects of weight, volume, composition, and viscosity of the coacervate and supernatant phases on the size distribution, surface morphology, and internal porosity of the final microparticles (162).

Other groups also reported microencapsulation by coacervation (152,153,155–161,163). Vidmar et al. induced phase separation of a drug–PLA–DCM suspension by addition of *n*-heptane to give particles in the range of 50–500 μm, and in another study they used chloroform instead of DCM to dissolve the polymer (described in reference 1). Nakano et al. used an ethyl acetate solution of PLA/carboxymethylethylcellulose blend and suspended the drug particles in it prior to inducing phase separation by adding ethyl ether to finally give smooth microspheres having a mean size of 16.4 μm (described in reference 1). Fong et al. carried out microencapsulation at low temperature; the drug was suspended in PLA/toluene solution at –65°C and phase separation was induced by dropwise addition of isopropanol with constant stirring to yield microspheres in the range of 25–50 μm (164). Mandal et al. added the suspension of water-soluble diltiazem or metoprolol in PLGA/DCM solution to a silicone oil/DCM solution (1:6 ratio) with stirring, and the coacervates obtained were hardened by petroleum ether to yield microspheres with high encapsulation efficiencies (155). Ruiz et al. concluded that the polymer properties such as hydrophobicity or chain length, viscosity of the silicone oil used, the concentration of the polymer, and the polymer solvent/silicone oil ratio greatly affected the overall coacervation process and thereby the characteristics of the final microsphere product (165). Leelarasamee et al. reported prepa-

ration of PLA microcapsules by solvent partitioning to achieve phase separation (160). A solution of hydrocortisone and PLA in DCM was slowly injected into a mineral oil stream with a constant injection rate and needle size. As DCM partitioned into the mineral oil phase, the polymer precipitated and encapsulated the drug. The microcapsules were finally washed with hexane and they had a size of 250 μm with 90% yield (160).

Spray-Drying

Injectable biodegradable PLA and PLGA microparticles were successfully prepared by double emulsion and phase separation as discussed in the previous sections. The coacervation method tends to produce particles that are agglomerated, there is difficulty in mass production, the method requires large quantities of organic solvent, and it is difficult to remove residual solvents from the final microsphere product (166). The double-emulsion method on the other hand requires many steps, rigid control of the temperature and viscosity of the inner w/o emulsion, and it is difficult to encapsulate higher concentrations of hydrophilic drugs (1,166). Contrary to these methods, the spray-drying method is very rapid, convenient, easy to scale up, involves mild conditions, and is less dependent on the solubility parameter of the drug and the polymer (1,166,167).

Wise et al. reported the preparation of PLGA microcapsules in which a solution of PLGA, hexafluoro-2-propanol, benzene, and the drug was sprayed to produce particles of less than 125 μm (168). Bodmeier and McGinity prepared microspheres by a spray-drying method in which water-soluble drug (theophylline) was suspended or a water-insoluble drug (progesterone) was dissolved in a PLA/DCM solution and then spray-dried to produce particles of less than 5 μm (169). Because of incompatibility of the hydrophilic drug and PLA, needle-shaped crystals grew on the microsphere surface, and the progesterone/PLA solution gave smooth particles. The nature of the solvent used, temperature of the solvent evaporation, and presence of PLA microspheres during the spray-drying process affected the polymorphic form of progesterone. A major problem encountered with this technique was the formation of fibers due to insufficient force available to break up the polymer solution. An efficient dispersion of the filament into polymer droplets was dependent on the type of polymer and the viscosity of the spray solution. Other groups have also reported successful preparation of PLGA and PLA particles using the spray-drying technique (167,170–174).

Wagenaar and Müller spray-dried a solution of the polymer, DCM, and the drug piroxicam to yield microspheres which were hollow (no solid core) (167). DL-PLA microparticles were more spherical and smooth than those made from DL-PLGA. The microspheres were in the size range of 1–15 μm, with an high drug encapsulation efficiency of 99.0% (167). Men et al. showed that PLGA microparticles prepared using the spray-drying technique produced particles in the size range of 1–15 μm and drug loading of 4.4–6.6 μg/mg microspheres, and PLA microspheres prepared using the coacervation technique yielded particles having diameter in the range of 20–90 μm with relatively low drug loading of 3.5 μg/mg microspheres (173). In order to protect a hepatitis vaccine from the harmful effects of the solvent, a mixture of the antigen powder and a hydrophilic polymer, hydroxypropylcellulose (HPC), was first spray-dried to produce core microparticles (174). These were then suspended in PLGA/ethyl acetate solution and spray-dried to yield double-walled microparticles in the size range of 4–22 μm. The first coating layer of HPC protected the antigen from solvent during the second encapsulation process with PLGA (174).

The spray-drying method might cause a significant loss of the product due to adhesion of the microparticles to the inside wall of the spray-drier apparatus, and can also produce agglomeration of the microparticles (166). In order to rectify these problems, a novel double-nozzle spray-drying technique was developed which involved use of mannitol as an antiadherent (166). A solution or a dispersion (w/o emulsion) of the drug in PLGA solution was sprayed from one nozzle and from another nozzle an aqueous mannitol solution was sprayed simultaneously, and the process completed to produce the final microspheres. The surface of the spray-dried microspheres was coated with mannitol and the extent of agglomeration was decreased (166). This method also produced microspheres with higher yield and encapsulation ratio compared to those prepared from the double-emulsion method (166).

Miscellaneous Methods

Drug-free microsphere preparation by an interfacial deposition technique was described by Makino et al. (175). First, 50 ml of *n*-hexane was emulsified in 1.5% w/w aqueous Pluronic F68 solution to yield an o/w emulsion. Then 20 ml of 1.5% w/v solution of D,L-PLA or L-PLA in DCM was added dropwise to the emulsion and stirred, resulting in precipitation of the polymer and formation of 1.5 μm size microcapsules on complete

solvent evaporation (175). Wichert and Rohdewald prepared microparticles by a new melting method (176). The polymer D,L-PLA (molecular weight 2000 or 16,000) was first melted in the presence of the drug vinpocetine at 180°C to obtain a homogeneous mixture. This was then emulsified in a hot aqueous Tween 80 solution. Microspheres were obtained from this emulsion by either centrifugation or spray-drying. For the first method, the emulsion was poured into ice water, the suspension was subsequently centrifuged, and the particles obtained were then lyophilized. For the second method, PVA was added to the hot emulsion and the emulsion was then spray-dried to obtain the final microparticles. The particle size depended on the molecular weight of the polymer and the isolation method, and was found to be in the range of 2-22 μm. The authors also found that during the microparticle preparation, the molecular weight of PLA was reduced by 12% (176).

A novel low-temperature method for preparing PLA and PLGA microspheres was reported by Khan et al. (177) and the group at Alkermes, Inc. (178,179). First, the protein powder and optional excipients were suspended in the PLA/PLGA solution in acetone, ethyl acetate, or DCM. This suspension was then sprayed into a vessel containing liquid nitrogen overlaying a frozen extraction solvent such as ethanol. The liquid nitrogen was subjected to evaporation, causing the polymer solvent from the frozen droplets to be extracted by then liquid ethanol. Microspheres were then filtered and the residual solvents evaporated by filtration. The microspheres were 50–60 μm in size with drug encapsulation efficiency more than 95% (177–179). Sam et al. described a new spray desolvation method using nontoxic solvents (180). The micronized drug was first suspended in a PLGA solution in acetone. This suspension was then atomized ultrasonically in an ethanol bath to induce coagulation of droplets. After 30 min, ethanol was replaced by water to cause hardening of PLGA, and the microparticles so formed were then collected and dried under vacuum. The volume average diameter of the microparticles was 77 μm with a drug encapsulation efficiency of 30–60% for water-soluble hexapeptides and 90% for water-insoluble estradiol (180).

Iwata and McGinity reported preparation of multiphase microspheres of D,L-PLA and D,L-PLGA containing water-soluble drugs (181,182). An aqueous drug solution containing gelatin and Tween 80 was emulsified into soybean oil containing aluminum monostearate and Tween 80 (o/w emulsion). This was dispersed in PLA/PLGA solution of acetonitrile to form the double w/o/"w" emulsion. This was then dispersed in light mineral oil containing Span 80 to form the w/o/"w"/o multiple erosion, which was then agitated to evaporate and remove acetonitrile. The hardened microspheres were filtered and washed with *n*-hexane and water, and finally dried under reduced pressure. Encapsulation efficiencies of 80–100% were obtained by this method and were higher as compared to microspheres made by the conventional o/o solvent evaporation method, in which the drug partitioned in the polymer/acetonitrile phase (181,182).

To delay the release of protein from PLGA microspheres and to maintain its biological activity, Madiba and Wu et al. combined the PLGA microspheres with BSA-loaded liposomes (183). The authors first prepared protein-loaded liposomes consisting of 1:2 molar ratio of cholesterol and dioleolyphosphatidylcholine. Then a 6% w/w PLGA (75:25) solution in ethyl acetate was made. The above liposome suspension was added to the polymer solution and the mixture was homogenized to produce a w/o emulsion. This emulsion was then transferred to 85 ml of 3% w/v aqueous PVA solution containing 5 ml ethyl acetate and the mixture was stirred to form the double w/o/w emulsion. The solvent was removed by double extraction in ice-cold water and the microspheres so obtained were finally filtered and dried, and were found to be in the range of 30–100 μm. The authors did not provide any information on the drug encapsulation efficiency or the stability of the encapsulated BSA (183).

To prevent water-mediated inactivation of encapsulated vaccines, oil-based PLGA microcapsules were designed, consisting of an oily core of antigen (tetanus toxoid), in which the protein is dispersed surrounded by an outer PLGA shell (184). The antigen powder was first dispersed in mineral oil (1:100 powder:oil ratio) and the microcapsules were then obtained by the conventional o/w solvent extraction/evaporation method. Microcapsules prepared using 50:50 PLGA had a mean size of 112 μm and an encapsulation efficiency of 63%, and those made from 75:25 PLGA were 129 μm in mean size with an encapsulation efficiency of 86% (184). Hariharan and Price reported encapsulation of the protein ovalbumin (OVA) by a novel colloidal suspension method (185). First, OVA was precipitated out of water using acetone to produce a colloidal suspension of OVA. The water was then removed by dialysis and PLGA (50:50) was dissolved in the acetone suspension, which was then emulsified in heavy mineral oil in the presence of magnesium stearate. Evaporation of solvent produced microspheres containing colloidally dispersed OVA. The

microspheres were 90–180 μm in size and had encapsulation efficiency of 89–94% (185). Abraham and Burgess described PLGA microsphere preparation using a novel rapid solvent evaporation method (186). The drug, chlordiazepoxide, and PLGA were first dissolved in DCM and then dispersed in aqueous 0.01% PVA solution to give an o/w emulsion. Water at 70°C was added to this mixture, thereby raising the temperature of the system above the boiling point of DCM, causing its rapid evaporation and subsequent formation of PLGA microspheres (186).

Burns et al. described a new continuous encapsulation process for preparation of progesterone and estradiol containing D,L-PLA microspheres (187). The process was the same as the traditional o/w solvent evaporation method, except that it had been converted from a batch mode to a more cost-efficient continuous process, which according to the authors, reduced several problems associated with scaleup and process validation. There have also been reports of preparation of PLA and PLGA microparticles by using supercritical fluid (compressed carbon dioxide) extraction/expansion techniques (188).

Nanoparticles

Injectable microparticles from PLA and PLGA were successfully prepared to deliver drugs such as peptides, proteins, and vaccines over a period of days, weeks, or even months at a constant rate depending upon the degradation behavior of the polymer employed (189). However, because of their large size, it was impossible to direct the drug to target tissues via systemic circulation or across the mucosal membrane (11). Following oral administration, particles less than 500 nm can cross the M cells in the Payer's patch and the mesentery on the surface of GIT mucosa, delivering the drug to the systemic circulation (11).

Nanospheres and nanocapsules can be prepared by the same methods as those described for microparticles, except that manufacturing parameters are adjusted to obtain nanometer size droplets (10). This is obtained by using a relatively small ratio of the dispersed phase to the dispersion medium and a substantially higher stirring speed (10). Song et al. prepared PLGA nanoparticles by o/w emulsification/solvent evaporation technique to produce 150-nm nanoparticles, drug loading of 15.5% w/w, encapsulation efficiency of 62%, and yield of 85% (190). Many formulation/process variables affecting microsphere production also influence nanoparticle production, in more or less a similar way. Dawson and Halbert prepared nanoparticles by the o/w emulsification technique and used response surface methodology to determine the effect of some variables on the size distribution of PLGA nanoparticles (191). The authors found that the homogenization pressure and PLGA concentration have a linear and thus predictable effect on both size and polydispersity of the particles. Tween 80 concentration and the DCM (organic phase) concentration had a greater effect on the diameter and polydispersity (191).

Müller et al. described a novel method of preparing PLA and PLGA nanoparticles (192). Magnetite (used for magnetic resonance imaging) was first dispersed in ethanol by sonication. This dispersion was then incorporated in the polyester polymers and the temperature was slowly increased while the mixture was stirred to remove ethanol (192). Heating and stirring was continued to melt PLA/PLGA polymer to facilitate homogeneous dispersion of magnetite. On solidification, the magnetite–polymer mixture was ground. (192). PLA/PLGA nanoparticles were then obtained by high-pressure homogenization of the magnetite-containing polymer particles dispersed in aqueous poloxamer 188 solution (192). Allémann et al. prepared PLA nanoparticles by a reversible salting-out process using a cross-flow filtration technique which involved the use of magnesium salts (193). A 90% drug entrapment was achieved by this method.

Niwa et al. reported nanospheres production for indomethacin and 5-fluorouracil (water-insoluble and water-soluble drugs, respectively) and for nafarelin acetate (NA), an LH-RH analog by a novel spontaneous emulsification–solvent diffusion method (194,195). The drug and PLGA were first dissolved in acetone/DCM mixture and then emulsified in an aqueous PVA solution using a high-speed homogenizer (o/w emulsification). The rapid diffusion of acetone in the aqueous phase resulted in faster deposition of polymeric film on the droplet and yielded nanospheres less than 500 nm (194,195). The same group also reported nanospheres production for NA by the emulsion–phase separation method in an oil system (196). An aqueous solution of NA was emulsified in acetone/DCM mixture containing dissolved PLGA using a homogenizer at 15,000 rpm. To this was added a mixture of triester oil (caprylate and caprate triglyceride) and hexaglycerine condensed ricinoleate (HGCR), which resulted in phase separation of PLGA. PLGA coacervates precipitated around the aqueous emulsion droplets and were hardened by evaporation of the solvent to yield nanospheres in the range of 500–800 nm (196). Murakami et al. reported preparation of PLGA latex by a method based on double coacervation of PVA and PLGA (197). PLGA was first dissolved in a mixture of acetone and DCM, ethanol, or methanol. This PLGA solution was then dispersed in aqueous PVA solution by

stirring (197). The latex dispersion was then freeze-dried to obtain powder latex with a size of approximately 300 nm. The authors concluded that the coacervated (adsorbed) PVA molecules prevented the aggregation of PLGA nanoparticles due to steric hindrance (197).

Following intravenous administration, the nanoparticles are taken up by cells of the mononuclear phagocyte system, mainly the Kupffer cells of the liver and the spleen macrophages, and are essentially lost (198,199). This is considered as the major hurdle to target delivery of drugs to other organ/tissue sites within the body (198,199). Several groups have tried to address this problem. Gref et al. prepared polyethylene glycol (PEG)-coated nanospheres by employing an amphiphilic diblock copolymer of PLGA (75:25, lactide:glycolide) and PEG (molecular weight 5000–20,000) (198). The nanospheres were prepared by a single-step o/w solvent evaporation technique, in which the PEG fraction migrated to the surface of the nanospheres forming a protective cover. After 5 min of the injection of coated or uncoated nanospheres, 15% of coated nanospheres were found in the liver and 60% were found in the blood with a significantly improved circulating time (198). In comparison, 40% of uncoated nanospheres were found in liver and only 15% were found in the blood. Also, 4 hr post-injection, 30% of coated nanospheres were still circulating in the blood and the plain nanospheres had completely disappeared from the circulation. Blood circulation time for the nanoparticles increased as the molecular weight of the PEG increased from 5000 to 20,000 because of increased thickness of the protective PEG layer which prevented their opsonization (198). Stolnik et al. (199) and Dunn et al. (200) reported preparation of PLGA nanospheres by o/w emulsification/solvent evaporation and further coated them with the diblock copolymer PLA/PEG (ratio of 2:5 and 3:4). The coated particles had increased hydrophilicity and decreased surface charge (as determined by measuring their surface zeta potentials) and were sterically stabilized particles. These particles exhibited reduced protein adsorption and liver uptake and increased blood circulation time as compared to uncoated PLGA nanospheres (199,200). Leroux et al. also reported similar improved site-specific drug delivery of nanoparticles after coating them with PEG (193). Other groups have also reported successful preparation of PLGA nanoparticles (201–204).

Other Devices

Many groups reported preparation of drug-loaded implants from PLA or PLGA (205–209). Kunou et al. designed nail-like ganciclovir-(GCV) incorporated D,L-PLGA implant (length 5 mm, diameter 1 mm) for intraocular drug delivery to treat cytomegalovirus retinitis (210). Wang et al. reported preparation (by compression) of 5-fluorouracil D,L-PLGA subconjunctival coated and uncoated implants/matrices (5 mg, diameter 2.5 mm, thickness 1.2 mm) using drug/PLGA ratios of 9:1, 8:2, and 7:3 (211).

In order to study the release profile of zidovudine (AZT), Mandal et al. prepared tablets by compressing physical mixture of AZT and PLGA (50:50) and AZT-loaded PLGA microcapsules (212). Other groups also reported preparation of PLA/PLGA compressed tablets, some involving the use of heat (213–215).

Schmitt et al. reported preparation of amaranth-incorporated pellets (diameter 3.7 mm, thickness 3.1 mm) from D,L-PLGA which was purchased from three different sources: DuPont, Birmingham Polymers, and Henley Chemicals, Inc. (216). The authors prepared the pellets by melt-pressing spray-dried PLGA with a 4-mm standard concave punch and die set (216). Tracy et al. prepared drug-free PLGA pellets by a similar method but used a Carver Laboratory Press instead (217).

Schade et al. described preparation of aqueous colloidal D,L-PLGA dispersion by a spontaneous emulsification–solvent diffusion technique followed by drying of these dispersions to form biodegradable latex films (218). In order to study the effect of hydrophilic excipient on the drug release from the hydrophobic PLGA (50:50) film, Song et al. prepared double-layer films (150 μm thickness), in which the drug releasing layer consisted of drug/hydrophilic additive/PLGA (10:10:80 ratio) and a protecting layer consisting of PLGA only (219). A combined solvent-casting and melt-compression method was utilized by prepare these films (219). In an effort to study drug-induced D,L-PLA hydrolytic degradation, Li et al. prepared caffeine-incorporated PLA circular plates (1.5 mm thick) and films (0.3 mm thick) (220). Other researchers have also described drug delivery through PLGA films (221,222).

Hsu et al. reported preparation of low-density PLGA (85:15) foams, having high interstitial void volume. First, PLGA was dissolved in glacial acetic acid at different concentrations (223,224). The solutions were then frozen and lyophilized and the solvent recovered in a dry ice/acetone-cooled trap to give foams that had leaflet or platelet structures. Lyophilization helped to achieve control of the specific gravity and interstitial void volume of the foam. The drug isoniazid (INH) was then impregnated into the foams, by immersing a weighed quantity of foam into an aqueous INH solution of known concentration and finally lyophilizing the foam mixture to re-

move water. The foams were then subjected to matrices preparation by different methods using high-pressure extrusion to prolong in vitro release of INH and to promote the understanding of the release mechanism (223,224). Lee et al. described preparation of biodegradable drug-incorporated L-PLA porous membranes (10 mg, 1 × 1 cm) for periodontal therapy by an in-air drying phase inversion technique which involved use of solvents such as DCM and ethyl acetate (225). Whang and Healy described preparation of BSA-incorporated D,L-PLGA scaffolds having different pore sizes and very high porosities by an emulsion freeze-drying method (226). Pore size was controlled by varying polymer inherent viscosity and/or volume fraction of the dispersed aqueous phase and/or polymer concentration (w/v %) (226). Ovalbumin-loaded D,L-PLA granules were prepared by emulsification (o/w emulsion) of the vaccine followed by lyophilization of the emulsion and then compression-molding the powder into rods (227). These were then ground by pestle and mortar into granules which were then sized using the appropriate sieves (20–100 μm) (227,228).

In situ Formed Implants

The manufacturing processes for PLGA microparticles discussed in the previous sections suffer from drawbacks such as (*a*) the microspheres need to be reconstituted (suspended) in an aqueous media before they could be injected in the body, (*b*) the hazards and environmental concerns associated with the use of organic solvents such as methylene chloride for the solubilization of PLGA polymer, and (*c*) residual organic solvents remaining in the final microsphere product. Although PLGA implants have been fabricated to deliver a variety of drug classes, they have not received much commercial success, primarily because of difficulty in administration; they require minor surgical incision or a special type of pellet injector (trocar), thereby causing inconvenience to the patients.

In order to improve patient acceptance, a novel implant system has been developed which is intramuscularly or subcutaneously administered as a liquid and subsequently solidifies in situ (229,230). First, PLA or PLGA is dissolved by heating in water-miscible, biocompatible solvent (this may also act as a plasticizer for the polymer). The polymer solution is then cooled under ambient conditions and the drug is dispersed into it by homogenization, or alternatively, the drug is dissolved in a solvent (such as propylene glycol) which is miscible with the polymer solvent and water. This polymer–solvent–drug system has a viscous consistency but is sufficiently syringeable to be injected i.m. or s.c. by conventional syringe and needle. When injected, it comes in contact with water from aqueous buffer (in vitro condition) or physiological fluid (in vivo condition) and as a result the polymer precipitates and forms a gel matrix (solidifies), entrapping the drug (in situ/in vitro or in situ/in vivo implant formation). The polymer solvent dissipates and diffuses out of the system and water diffuses into the polymer matrix. Because of the water-insoluble nature of the polymer, it precipitates/coagulates to form a solid implant in situ, from which the drug is released in a controlled fashion.

A number of groups including one at Atrix Laboratories extensively reported drug delivery using this method (229–246). These researchers employed a combination of a host of biocompatible solvents and biodegradable polyesters in addition to PLA and PLGA to deliver a variety of therapeutic drug classes (229–246).

Although this implant system precludes the need for any surgery for its administration, it has a number of disadvantages: (*a*) the safety of solvents such as *N*-methyl-2-pyrrolidone (NMP) used to formulate these systems is questionable and not well documented, (*b*) the injection of these liquid implant systems and their subsequent solidication produce nonuniform matrix implants having variable consistency and geometry, and (*c*) because of the formation of matrix implants having inconsistent texture, shape, and size, the drug release from them is variable and unpredictable. An alternative and attractive approach, therefore, would be to use nonaqueous solvents to produce in situ microspheres.

STERILIZATION OF PLGA-BASED DEVICES

Sterilization of the final formulation containing the lactide and/or glycolide polymers is an important issue often overlooked in the early stages of drug delivery system development. Terminal sterilization and aseptic processing are two main methods reported for sterilization of PLGA-based products.

Steam sterilization usually involves subjecting the product to steam at 121°C for at least 20 min or 115°C for 30 min (5,12). This method cannot be used with PLGA systems because at higher temperature/pressure condition the polymer softens, melts, and leads to deformation of the matrix form, and undergoes hydrolysis (4). Heat sterilization involves exposing the product to higher temperatures for longer periods of time which are de-

structive to both the polymer and the entrapped drug (12). Sterilization using a gas such as ethylene oxide can be achieved when heat/steam sterilization is harmful to the formulation (4,5,12). However, ethylene oxide is known to soften and plasticize these polymers. Also, the residual gas vapors left in these devices were found to be mutagenic, carcinogenic, and allergic (4,5).

Radiation sterilization (^{60}Co γ rays) has been used in several cases to sterilize formulations containing lactide and/or glycolide polymers (4,5,12). Effect of radiation on PLGA has been a subject of various investigations (26,53,57,59). Subjection of PLGA to γ irradiation produced dose-dependent polymer chain breakdown, molecular weight loss (decrease in inherent viscosity), increased in vitro and in vivo bioerosion rates, and increased drug release kinetics (59). Crossan and Whateley (53) and Spenlehauer (247) et al. reported that irradiation by γ-rays did not in any way influence the drug release rates. In another study, exposure of PLA to 100 Gy γ-rays using ^{60}Co did not affect the microsphere structure, release rate, or the drug stability (26).

Aseptic processing is an effective but somewhat expensive technique for formulations containing PLGA polymers (4,5,12). Because of the excellent solubility of these polymers in a number of organic solvents, they can be filter-sterilized. The drug delivery system can then be formulated in a clean room environment using Good Manufacturing Practice (GMP) protocols.

CONCLUSION

Drug delivery from biodegradable PLGA polymers has generated immense interest because of the polymers' excellent biocompatibility and biodegradability. PLGA polymers are also easy to formulate into drug carrying devices and have been approved by the FDA for drug delivery use. The various biodegradable PLGA devices fabricated from different techniques are versatile in terms of the various classes of drugs encapsulated, the different time period of their release, and the diverse routes of their delivery. PLGA microparticles, in particular, are important drug delivery systems for which various drug release profiles can be achieved by adjusting the PLGA composition, molecular weight, drug loading, microparticle size, porosity, and other factors. The newer techniques for fabricating PLGA devices such as the in situ formed implants are evidence of continued efforts by researchers throughout the world to optimize the drug delivery through PLGA polymers. Some drugs formulated into PLGA microparticles and other devices have already been introduced into the market and many more are undergoing clinical trials.

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