CONTROLLED DRUG DELIVERY FROM A NOVEL INJECTABLE IN SITU FORMED BIODEGRADABLE PLGA MICROSPHERE SYSTEM

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CONTROLLED DRUG DELIVERY FROM A NOVEL INJECTABLE \textit{IN SITU} FORMED BIODEGRADABLE PLGA MICROSPHERE SYSTEM

BY
RAJEEV JAIN

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN APPLIED PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND 1998
DOCTOR OF PHILOSOPHY DISSERTATION

OF

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1998
ABSTRACT

A novel method for in situ preparation of injectable biodegradable microspheres from the copolymer poly(lactide-co-glycolide) (PLGA) without incorporating unacceptable organic solvents is described. The delivery system is a dispersion of PLGA microglobules ("premicrospheres" or "embryonic microspheres") in an acceptable vehicle mixture (continuous phase) and whose integrity is maintained by use of appropriate stabilizers. A solution of PLGA, triacetin, drug, PEG 400, and Tween 80 (Oil Phase 1) are added dropwise with continuous homogenization to miglyol 812-Span 80 solution (Oil Phase 2), thereby inducing phase separation (coacervation) of PLGA and forming PLGA microglobules (containing the drug) dispersed in the continuous phase. This novel drug delivery system (NDDS) is a dispersion and has a viscous consistency but is sufficiently syringeable. When injected, it comes in contact with water from aqueous buffer or physiological fluid and as a result, the microglobules harden to form solid matrix type microparticles entrapping the drug (in situ formed microspheres). The drug is then released from these microspheres in a controlled fashion.

This novel microencapsulation process overcomes some of the disadvantages associated with the existing methods by: (i) excluding the use of unacceptable organic solvents and using acceptable vehicle mixture instead to prepare biodegradable PLGA microspheres, (ii) forming drug containing PLGA microglobules ("premicrospheres" or "embryonic microspheres") which could be considered as precursors to the final microsphere product; these on coming in contact with water harden to form discreet PLGA microspheres which subsequently exhibit non-variable, predictable, and controlled drug release profile, and (iii) precluding the need for reconstitution of the PLGA microspheres before their administration.

The composition, rationale, and optimization of the NDDS is described here. The characteristics of this NDDS were affected by various formulation
variables such as: (i) the PLGA concentration and type, (ii) the substitution of the continuous phase by a fresh Oil Phase 2, (iii) the concentration of PEG 400 and the encapsulated drug, (iv) the addition of an hydrophilic excipient (mannitol), and (v) the types of encapsulated drugs and the vehicles added to the system. The characteristics of the NDDS were reproducible and were not affected by a 15 days/4°C storage condition. Also, the formulation, process, and the storage (15 days/4°C) conditions did not adversely affect the physical stability of the encapsulated proteins.

Besides producing injectable in situ formed microspheres, this novel microencapsulation process can be modified to yield injectable in situ formed implant or isolated microspheres. Thus this novel microencapsulation process is versatile and it can produce various drug loaded injectable biodegradable PLGA devices having different characteristics.
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To my wife Harsha for her love, encouragement, support, and everlasting patience
PREFACE

This dissertation has been written in the Manuscript Format as per the guidelines issued by The Graduate School at the University of Rhode Island. This option was most suitable to present my results in several sections.

Section I constitutes the Objectives and Introduction of this dissertation. The manuscripts in Section II are the core of this dissertation. Section III consists of the Conclusion and Final Remarks of this dissertation.
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LIST OF PUBLICATIONS

Manuscript I has been submitted for publication in the Drug Development and Industrial Pharmacy/Biotechnology Drug Development.

Manuscripts II, III, and IV will be submitted for publication in the Journal of Controlled Release, Pharmaceutical Research, and International Journal of Pharmaceutics respectively, pending patent submission.
SECTION I
OBJECTIVES

The main intention of this research project were to achieve controlled drug delivery of micromolecules and macromolecules, such as proteins, from a novel injectable biodegradable poly(lactide-co-glycolide) (PLGA) microsphere system. This system would overcome some of the disadvantages associated with the traditional methods for controlled drug delivery. On injection, the system would come in contact with water from aqueous buffer or physiological fluid and as a result, form solid matrix type microparticles entrapping the drug (in situ formed microspheres); the drug would be released from these microspheres in a controlled fashion.

The specific objectives of this research project were as follows:

1. To develop a novel method for controlled delivery of drugs from an in situ forming biodegradable PLGA microsphere system.
2. To evaluate the effects of various formulation variables on the characteristics of this system.
3. To determine the effects of formulation, process and storage conditions on the reproducibility and stability of this system as well as the stability of the encapsulated proteins.
4. To modify this novel microencapsulation process, to produce in situ formed implant or isolated microspheres and also compare the characteristics of the three biodegradable devices: in situ formed implant v/s in situ formed microspheres v/s isolated microspheres.
INTRODUCTION

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

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 like bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatin, and hemoglobin have been studied for drug delivery. The use of these natural polymers is limited due to their higher costs and questionable purity.

In the past two decades synthetic biodegradable polymers have been increasingly used to deliver drugs, since they are free from most of the problems associated with natural polymers. Poly(amide)s, 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. Amongst them, the thermoplastic aliphatic poly(esters) like 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 due to their excellent biocompatibility and biodegradability. Also PLGA has been
approved by the U.S. FDA for a number of clinical applications including surgical sutures and as controlled-release microspheres. PLGA is shown to be biocompatible and degrades to toxicologically acceptable lactic and glycolic acids that are eventually eliminated from the body. Release of drugs from PLGA microspheres occurs by two mechanisms: (i) diffusion of the drug through a tortuous, water-filled path in the polymer matrix and (ii) matrix bioerosion (bulk hydrolytic degradation) after undergoing sufficient hydration. The actual release is a combination of both the processes.

There is a particular interest in controlled delivery of macromolecules like peptides and proteins through PLGA microspheres. Although a wide variety of pharmacologically useful peptide and protein based drugs have been recently developed by genetic engineering, their therapeutic use is restricted due to certain disadvantages: (i) on oral consumption they are subject to attack by the acidic and enzymatic environment in the stomach and the enzymes from the brush border membrane of the intestine, (ii) their high molecular weight and size impede their effective transport across the gastrointestinal membranes, and (iii) they have a short biological half-life and on injection they are quickly metabolized and eliminated. To achieve sustained blood levels of these drugs, minimize their denaturation or degradation, and to extend their biological half-life, their delivery by encapsulation in PLGA microspheres has become an interesting approach.

The literature on PLGA microspheres is full of different techniques describing their manufacture, where the microspheres are produced in a free-flowing, powder form. Some of the methods reported are: (i) single/double emulsification followed by solvent removal by evaporation or extraction, (ii) phase separation (coacervation), and (iii) spray-drying. Most of these manufacturing processes suffer from drawbacks such as: (i) the microspheres need to be reconstituted (suspended) in an aqueous media, before they could be injected in the body, (ii) the hazards and environmental concern associated with the use of organic solvents like methylene chloride for the solubilization of PLGA polymer, and (iii) residual organic solvents remaining in the final microsphere product.
Shah et al and researchers from Atrix Laboratories, Inc. (Fort Collins, CO) have described a novel implant system which is parenterally administered as a liquid and subsequently solidifies into a gel matrix (implant) in situ, from which the drug is released in a controlled manner. Although this implant system precludes the need for any surgery for its administration, it has a number of disadvantages: (i) the safety of solvents like N-methyl-2-pyrrolidone, used to formulate these systems is questionable and not well documented, (ii) the injection of these liquid implant systems and their subsequent solidification produce non-uniform matrix implants having variable consistency and geometry, and (iii) due to formation of matrix implants having inconsistent texture, shape and size, the drug release from them is variable and unpredictable.

The present process of microsphere formation is based on the principle of coacervation. This method overcomes the problems faced by the above systems by forming a dispersion of PLGA microglobules (“premicrospheres” or “embryonic microspheres”) in an acceptable vehicle mixture (continuous phase) and whose integrity is maintained by use of appropriate stabilizers. A solution of PLGA, triacetin, drug, PEG 400, and Tween 80 (Oil Phase 1) are added dropwise with continuous homogenization to miglyol 812-Span 80 solution (Oil Phase 2), thereby inducing phase separation (coacervation) of PLGA and forming PLGA microglobules (containing the drug) dispersed in the continuous phase. This novel drug delivery system (NDDS) is a dispersion and has a viscous consistency, but is sufficiently syringeable. When injected, it comes in contact with water from aqueous buffer or physiological fluid and as a result, the microglobules harden to form solid matrix type microparticles entrapping the drug (in situ formed microspheres). The drug is then released from these microspheres in a controlled fashion. This novel microencapsulation method can be modified to produce other biodegradable PLGA devices exhibiting controlled drug delivery.
SECTION II
MANUSCRIPT I

CONTROLLED DRUG DELIVERY BY BIODEGRADABLE POLY(ESTER) DEVICES: DIFFERENT PREPARATIVE APPROACHES
ABSTRACT

There has been extensive research on drug delivery by biodegradable polymeric devices since bioresorbable surgical sutures entered the market two decades ago. Amongst the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) like poly(lactide) (PLA), poly(glycolide) (PGA), and especially the copolymer of lactide and glycolide referred to as poly(lactide-co-glycolide) (PLGA) have generated interest due to their excellent biocompatibility, biodegradability, and mechanical strength. Also, 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 Food and Drug Administration (FDA) for drug delivery.

This review presents different techniques of preparation 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 where the drug undergoes extensive degradation in the gastrointestinal tract, exhibits significant first-pass effect, or is poorly absorbed. Of serious concern are the problems associated with the oral administration of peptide/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. Also their high molecular weight and size impede their effective transportation across the gastrointestinal tract membranes. The main drawback of oral dosage forms are the short transit time of approximately twelve hours through the gastrointestinal tract (2). Further, if the drug is absorbed only through a specific area of the gastrointestinal tract, the duration of action could be less than twelve hours (2).

If a drug cannot be administered orally due to 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 or 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 conventional parenteral dosage form for chronic treatment where routine multiple injections could be inconvenient and painful. Parenteral controlled release dosage forms are also effective in site-specific drug delivery, thereby improving its 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 worse 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 non-enzymatically to produce biocompatible, toxicologically safe by-products which are further eliminated by normal metabolic pathways (3). Drugs formulated in polymeric devices are released either by diffusion through the polymer barrier, or 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 like 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 like 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 due to their higher costs and questionable purity (1).

In the past two decades synthetic biodegradable polymers have been increasingly used to deliver drugs, since 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). Amongst them, the thermoplastic aliphatic poly(esters) like 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 due to their excellent biocompatibility and
biodegradability (1-17). Also they are easy to formulate into drug carrying devices and have been approved by the FDA for drug delivery use (13-17).

This review provides a comprehensive outlook on different techniques of preparation of various drug loaded PLGA devices, with special emphasis on preparing microparticles. Certain issues about other related biodegradable polyesters like PLA and PGA have been 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 glycolide polymers was first carried out several decades back (3, 5). The methods 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 had published pioneering work on 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 like ligament reconstruction, tracheal replacement, ventral herniorrhapthy, surgical dressings, vascular grafts, 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 an ocean of literature on drug delivery using PLA, and especially PLGA has been published. Various polymeric devices like 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 like 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, 12). This method yields PLGA having molecular weight of \( \sim 10,000 \) (12). The low molecular weight PLGA has limited biomedical application, due to 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). Also the cyclized monomer(s) and the linear form of the polymers produced can 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 two to six hours at around 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). Also important are the low levels of humidity in the processing area (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 like 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 in nature due to high regularity of its polymer chain while 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 L-PLA as it 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 the 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 which affect the mechanical strength of the polymer and its ability to be formulated as a drug delivery device (3, 5, 12). Also these properties may 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 50:50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing higher proportion of either of the two monomers (5, 12). PLGAs prepared from L-PLA and PGA are crystalline copolymers while those from D, L-PLA and PGA are amorphous in nature (3, 5). Gilding and Reed have pointed out that PLGAs containing less than 70% glycolide are amorphous in nature (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 (Tg) of the PLGA copolymers are above the physiological temperature of 37°C and hence they are glassy in nature (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. have reported that Tg of PLGAs decrease with decrease of lactide content in the copolymer composition and with decrease in their molecular weight (19).

It is important for the PLGA polymers to have considerable mechanical strength since the drug delivery devices formulated using them are subjected to significant physical stress (3, 5). Different factors like 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 and the degradation occurs at uniform rate throughout the PLGA matrix (3, 13). Thies and Bissery have 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; these are known to catalyze the biodegradation process (3, 5). The biodegradation rate of the PLGA copolymers are 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 Tg of the polymer (3, 5, 13). A three phase mechanism for the PLGA biodegradation has been proposed (21):

1. Random chain scission process. The molecular weight of the polymer decreases significantly, but no appreciable weight loss and no soluble monomer products 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 formed from soluble oligomeric fragments. This phase is that of complete polymer solubilization.

The extent, if any, on 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 lactic 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

1. 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 must include the following requirements (1, 2, 23):

(i) The stability and biological activity of the drug should not be adversely affected during the encapsulation process or in the final microsphere product.

(ii) The yield of the microspheres having the required size range (upto 250 µm, ideally < 125 µm) and the drug encapsulation efficiency should be high.

(iii) The microsphere quality and the drug release profile should be reproducible within specified limits.
(iv) The microspheres should be produced as a free flowing powder and should not exhibit aggregation or adherence.

A. **Solvent Evaporation and Solvent Extraction Process**

**(1) 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) 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 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 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 stirring rate 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 diffuses (4, 10). The solid microspheres so obtained are then washed and collected by filtration, sieving, or centrifugation (4). These are then dried under appropriate conditions or are lyophilized to give the final free flowing injectable microsphere product.

It should be noted 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 could 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, the solvent, and the dispersion medium. The rate of solvent removal by evaporation method strongly influences the characteristics of the final microspheres and it depends on the temperature, pressure, and the
solubility parameters of the polymer, the solvent, and the 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). Solvent removal by extraction method is more 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 would diffuse out or partition from the dispersed oil phase into the aqueous continuous phase and microcrystalline fragments of the hydrophilic drugs get deposited on the microsphere surface and dispersed in the PLGA matrix (24, 25). This would result 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 like 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 like 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 presence of an oil soluble surfactant like 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 like n-hexane. This process is also sometimes referred as 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).

(i) 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 have shown that water miscible solvents like 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 due to its high volatility it can 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 environment and undesirable for use in manufacturing processes (28). Chern et al. have 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 produced hollow microspheres having volume mean diameter of 96 µm and 60 to 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 o/o emulsification method, acetonitrile (26, 31-38) is generally used as the dispersed phase. Other solvents like acetonitrile/water mixture (24), DCM (39), N,N-dimethyl formamide (DMF) (40-42) have also been used. The continuous phase consists of oils like 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).

In an article van Hamont et al. have 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 due to increase in viscosity of the oil phase tend 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 like 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 DCM-acetone mixture in the solvent evaporation process and concluded that solvent removal process is rapid and causes entrapment of the stabilizer molecules by physical chain entanglement and thus enhancing their stabilizing capacity (44). Use of DCM alone, however, results in 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. have prepared PLGA microspheres using a mixture of two water-immiscible solvents. DCM and chloroform by the solvent evaporation process (43). Polard et al. have reported that due to 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 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 have found that the increase in the external aqueous phase volume did not affect the final microsphere size while increase in the dispersed DCM volume decreased the size with narrow size distribution due to the decrease in the viscosity of the internal phase with increasing volume (49).

(ii) 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 vinyl pyrrolidone (PVP) (4), alginate (4), gelatin (4), methyl cellulose (MC) (25, 51, 54, 64, 65), hydroxyalkyl cellulose (4, 10), Hydroxypropylmethyl cellulose (HPMC) (49), polyoxyethylene derivatives of sorbitan fatty esters (Tweens) (4, 10), cetyltrimethyl ammonium bromide (4, 10), and fatty acid salts like 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 increase in emulsifier concentration (4, 49). Beyond a certain concentration, the emulsifier is ineffective, due to achievement of an optimal packing concentration for the emulsion, i.e. condensed monolayer (40). Wakiyama et al. have investigated the emulsifying action of sodium alginate in comparison with gelatin and have concluded that sodium alginate produced a relatively more viscous aqueous phase and hence yielded relatively smaller microspheres as 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). Coombs et al. prepared PLGA microspheres using various grades of poly(oxyethylene)-poly(oxypropylene) (PEO-PPO) co-polymers as the surfactants (44). The solvent removal led to entrapment of these surfactant molecules by physical chain entanglement and 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).

Some times a combination of emulsifiers have been used to achieve the necessary emulsifying action (25, 51, 72). Cavalier et al. have reported that a combination of PVA and MC yielded PLA microspheres having maximum sphericity and drug entrapment as 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 0-30%, 0.25% aqueous PVA solution gave microspheres in the size range 25-50µm (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).

(iii) Polymer

The polymer type, its molecular weight, and the concentration used strongly influence the characteristics of the final microspheres. Cavalier et al. have 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. have reported a decrease in polydispersity and particle size of the microparticles as the PLGA concentration in it was decreased (44). In a study, drug content of PLA (molecular weight 2000) microparticles was higher than PLGA (molecular weights 9000 and 12000) 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 same for RG 505 and R 208 (2.8% w/w) while 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 while those prepared from 5.3% w/w had little structural defects but were
aggregated and formed lumps (40). Inspite an increase in the PLGA molecular weight from 6600 to 19000, microspheres with uniform particle size and no structural defects were produced (40). Udupa and Chandrashekar have reported a decrease in drug content and increase in microsphere size with increase in PLGA/drug ratio. 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. have reported that values of certain polymer parameters like 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).

**iv) 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 (74 and more). Bodmeier and McGinity achieved higher entrapment of ionizable drugs like diazepam and quinidine by using high pH external aqueous phase (pH 12), where the loss due to 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 got 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 reducing its partitioning in the external aqueous phase (45). Contrary to these results, Vaughan et al. have 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 increase in quinidine content in the dispersed organic
phase (74), while tetracaine entrapment increased with prior saturation of the aqueous phase (69).

Sah et al. have reported that the encapsulation efficiency of PLGA microspheres decreased with increasing 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. have shown that with increase in drug loading, the drug content of the microspheres increased but their encapsulation efficiencies and the microsphere recovery yield decreased (45). Also drug entrapment was higher when the drug was present in suspension form as compared to when present in the solution form (45). A similar result was reported by Cavalier et al., where an increase in the drug/polymer ratio resulted in increase in the drug content of the microspheres (25). Thanoo et al. have shown an increase in the drug incorporation efficiency and the microsphere yield with increase in the theoretical drug loading (43).

Rosilio et al. have 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 (51). A different observation was made by Tsai et al. who prepared microspheres by o/o method (26). Inspite 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, while at higher peptide concentration, peptide-peptide interaction are favored, resulting in multilayer adsorption which fitted the Freundlich model (66). In another study, Duggirala et al. have showed that with increased protein loading, the adsorption of protein on PLGA
microspheres increased up to a definite value and then remained constant due to saturation of the microsphere surface (monolayer coverage) by the protein (75).

Bodmeier and McGinity have shown 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 while 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 Tg 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 phenomena between the drug and PLGA has been reported by Crossan and Whateley (53) and Richey and Harris (76). Rosilio et al. have 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) have also reported similar results of a molecular dispersion of the drug in the polymer glass.

(v) 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 where 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 were critical for successful
microencapsulation (30). Also, the particle size of the microspheres decreased when 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 where 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). Coinsar 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 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) have compared the effects of the solvent extraction v/s evaporation process on the final microsphere product. Microencapsulation of lidocaine base by the evaporation process gave product with an yield of 65-80%, volume mean diameter of 120-130 μm, drug content of 4-10%, smooth and non-porous surface, and only 30-70% loading efficiency (due to 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 had used the salt form of the drug.
(lidocaine hydrochloride), and not lidocaine base for the extraction process (33).
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 as against the evaporation process (34). Contrary
to these results, Pak et al. have reported slightly lower drug contents for PLGA
microspheres prepared from 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.
have reported that o/o method gave L-PLA microspheres having smooth spherical
surface and higher drug entrapment due to 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. have 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 influences the microsphere product. Kyo et al. have 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 as against
evaporation at 0.2°C/min which produced fewer defects (40). Wakiyama et al.
have 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 have pointed out 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 have stated that when temperature of
85°C were used (above the boiling point of the solvent acetonitrile), highly
porous microspheres, having internal honey-comb 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 due to 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 (49). Crossan and Whateley prepared PLGA microspheres in the size range of 40-60 µm by using an overhead paddle stirrer and stirring for four hours at room temperature (53). Modification of this system by addition of a baffle reduced their 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 lead to formation of smaller emulsion droplets. Also, the baffles reduced the turbulence in the suspension mixture, thereby increasing the stability of droplet suspension and the product yield. Further size reduction (5-10 µm) was achieved by first 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 out that for a drug loading of 0-30%, a stirring speed of 480 rpm was required and for a drug loading of 50-65%, stirring speed of 645 rpm was necessary to produce the microspheres (51). Coombes et al. have stated that, increasing the stirring rate of emulsion resulted in decrease in polydispersity of the PLGA microspheres but not in their particle size (44).

(2) Double (multiple) emulsion process

The double emulsion process is essentially an water-in-oil-in-water (w/o/w) method and is best suited to encapsulated water-soluble drugs like
peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs like steroids (1, 4, 5). A buffered or plain aqueous solution of the drug (sometimes containing a viscosity building and/or stabilizing protein like gelatin) is added to an organic phase consisting of PLGA and/or PLA solution in DCM with vigorous stirring to form the first microfine w/o emulsion. This emulsion is added gently with stirring into a large volume water containing an emulsifier like PVA to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by either 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 have reported using ethyl acetate as the polymer solvent and hydrophilic stabilizers like 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. have used an outer aqueous PVA phase saturated with DCM to prepare PLGA microspheres (81). Alpar et al. have reported preparation of PLA microspheres in which the inner aqueous phase contained MC besides 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 as 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 like 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
have been successfully encapsulated by this method. Various formulation and process variables significantly affect the final microsphere product and the drug release from them.

(i) The primary w/o emulsion

Ogawa et al. have 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 have been 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). In a study, Crotts and Park have 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 while those prepared from 22.7% aqueous phase fraction were porous in nature (118). Alonso et al. have 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 due to better stabilization of the inner w/o emulsion (100). Other researchers have also reported use of L-α-phosphatidylcholine (112). In another study it was found that a decrease in the DCM phase volume yielded particles with dense core (81).

The entrapment efficiency of the drug increased with decrease in drug loading and increase in particle size (84). However other groups have found no
relationship between encapsulation efficiency and drug loading (92, 104). Jeffery et al. have 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 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 and 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. have 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 v/s PLGA) and molecular weight (3K v/s 100K) (79). Okada et al. have 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). Also, increase in the Tg of the PLA and PLGA microspheres was observed with an increase in drug loading (87). Alonso et al. have pointed out an increase in the microparticle size with an increase in the molecular weight and the concentration of the polymer (79, 100). In a study, Benoit et al. found that microparticles prepared from PLGA were relatively larger and exhibited higher drug entrapment efficiency as compared to those prepared from 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 amphiphilic nature of the phospholipids which generated porous matrix surface (109). Sah et al. have reported that microcapsules containing PLA5000 (molecular weight 5000) or PLGA5000 (molecular weight 5000) into PLGA 75:25 microcapsules exhibited
increased degradation rates as compared to those containing PLGA 75:25 alone (117, 119, 120). The authors found that PLA5000 plasticized PLGA 75:25 and facilitated its faster degradation (120). Other groups have 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 amount of drug, gave a greater burst release of the drug due to increase in the number of channels formed by the hydrophilic drug (84, 87, 120). In a study, Alonso et al. have 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 powder dispersion method (drug powder dispersed in DCM phase) (100). Reich has 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 has a substantial effect on its entrapment and release thus leading to a different optimum for different protein/polymer combinations (123).

Cohen et al. have 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 as 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).

(ii) The double w/o/w emulsion

In a study Ogawa et al. have 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 increase in the stirring rate (105). Also an increase in the external phase volume led to a decrease in the particle size of the microparticles (84). Jeffery et al. have reported an increase in the drug entrapment and the particle size with 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 formation of larger microparticles as 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 like 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. have 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 have also reported use of 2% aqueous isopropanol solution to remove the solvent (113).

(iii) Drugs

Researchers at Takeda Chemical Industries have reported successful encapsulation of leuprolide acetate, a luteinizing hormone-releasing hormone (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 s.c. and i.m. routes (85, 86, 88) and a three-month release profile following a s.c. injection (87) has been reported by these researchers.
There is a lot of interest in delivering vaccines through PLA/PLGA microparticles and immense 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, 155). A number of vaccines/immunogenic agents like ovalbumin (30, 32-36, 42, 64, 65, 155, 160), *Dermatophagoides Pteronyssinus* 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, 160), influenza toxin (28), rotovirus (13), adenovirus (16), lysozyme (39), and *Schistosoma mansoni* 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.

Besides 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).

**B. 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 which is dispersed/dissolved in the polymer solution is coated by the coacervate. Thus the coacervation process includes the following three steps: (i) phase separation of the coating polymer solution, (ii) adsorption of the coacervate around the drug particles, and (iii) solidification of the microspheres (152).

First, the polymer is dissolved in an organic solution. The water-soluble drugs like peptides and proteins are dissolved in water and dispersed in the polymer solution (w/o emulsion). Hydrophobic drugs like steroids are either solubilized or dispersed in the polymer solution. An organic nonsolvent is then
added to the polymer-drug-solvent system with stirring 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, 152).

The phase separation method, unlike the o/w emulsification method is suitable to encapsulate both water-soluble as well as water-insoluble drugs, since it's a non-aqueous method. However the coacervation process is mainly used to encapsulate water soluble drugs like 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, since too higher concentrations would result in rapid phase separation and nonuniform coating of the polymer on the drug particles. Due to 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, 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 since 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 like 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. have investigated the effect of several process factors on the coacervation process (152). With 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 only got slightly narrower (152). An SEM picture revealed that the morphology of the particles changed from a spherical shape for 0.02 ratio to a deformed one at higher ratio of 0.12. Above water contents of 0.12, the microspheres became brittle and spontaneously released the encapsulated drug solution during filtration (152). With 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 (152). Similarly with 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 (152). 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 (152). In another paper, the same group
has 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 have also reported microencapsulation by coacervation (153-161, 163). Vidmar \textit{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 $\mu$m and in another study they used chloroform instead of DCM to dissolve the polymer (1). Nakano \textit{et al.} used an ethyl acetate solution of PLA/carboxymethyl ethyl cellulose blend and suspended the drug particles in it prior to inducing phase separation by adding ethyl ether to finally give smooth microspheres having mean size of 16.4 $\mu$m (1). Fong \textit{et al.} carried out microencapsulation at low temperature, where 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 $\mu$m (164). Mandal \textit{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 (153). In an article, Ruiz \textit{et al.} have 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 \textit{et al.} have reported preparation of PLA microcapsules by solvent partitioning to achieve phase separation (159). 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 $\mu$m with 90% yield (159).

\textbf{C. Spray Drying}
Injectable biodegradable PLA and PLGA microparticles have been successfully prepared by double emulsion and phase separation as discussed in the previous sections. The coacervation method tends to produce particles which 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 is difficult to encapsulate higher concentration 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 Chen prepared microspheres by spray drying where a 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). Due to incompatibility of the hydrophilic drug and PLA, needle shaped crystals grew on the microsphere surface, while 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 breakup 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
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Figure 1-Schematic representation of the novel modified microencapsulation process to produce various injectable biodegradable PLGA devices.
Figure 2a-Optical micrograph of the \textit{in situ} formed PLGA implant
Figure 2b-Optical micrograph of the *in situ* formed microspheres
Figure 2c-Optical micrograph of the isolated microspheres
Table I-Percentage cytochrome c encapsulation efficiency and particle size studies of the various injectable biodegradable PLGA devices

| Injectable biodegradable PLGA device | Percentage cytochrome c encapsulation efficiency | Particle size (µm) |
|-------------------------------------|-----------------------------------------------|-------------------|
| *In situ* formed implant            | 52.8                                          | —                 |
| *In situ* formed microspheres       | 74.5                                          | 98.6              |
| Isolated microspheres               | 90.3                                          | 84.3              |
Figure 3- *In vitro* cytochrome c release from various injectable biodegradable PLGA devices.
SECTION III
CONCLUSION AND FINAL REMARKS

The salient features of the novel microencapsulation process and the drug delivery system described in this research project are as follows:

(1) The system excluded the use of unacceptable organic solvents like methylene chloride and used acceptable vehicle mixture instead to prepare biodegradable PLGA microspheres.

(2) The system formed drug containing PLGA microglobules ("premicrospheres" or "embryonic microspheres") which could be considered as precursors to the final microsphere product; these on coming in contact with water hardened to form discreet PLGA microspheres (in situ formed microspheres) which subsequently exhibited non-variable, predictable, and controlled drug release profile.

(3) Unlike the traditional methods, this system precluded the need for reconstitution of the PLGA microspheres as they are formed in situ.

(4) Various formulation variables affected the characteristics of this system.

(5) The formulation and process conditions did not adversely affect the physical stability of the encapsulated protein drugs.

(6) Besides in situ forming microspheres, the novel microencapsulation method can be modified to produce in situ formed implant or isolated microspheres; these drug loaded devices exhibited different characteristics.

(7) This research project makes a significant overall contribution to the knowledge of the underlying theoretical principles of drug delivery.
through biodegradable devices and in particular, problems associated with protein drug delivery.

(8) The novel nature of the system provides a high probability that a patent application would be filed.
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