Chapter 1

Biodegradable Microparticles of Peptide Drugs Using Polylactide Polymers

H. P. Bhagwatwar

1.1 ABSTRACT

Presently, peptides and proteins are available in plenty due to advances in synthetic chemistry, fermentation technology and recombinant technology. Most of these molecules have to be administered daily because of their short half-lives in-vivo, and parenterally because of their inherent chemical and physical instability, for extended periods of time causing discomfort to the patients and resulting in non-compliance. The challenge facing the pharmaceutical scientist lies in the development of controlled release formulations capable of reducing the frequency of dosing while providing reproducible, controlled plasma levels of the drug over extended periods of time. A few such products based on microencapsulation with biodegradable polymers are now available with quite a few more under development. Several researchers have reviewed the different methods of microencapsulation. But, a logical approach to the development of a process for the encapsulation of peptides for controlled release is missing. This chapter is an attempt to describe the logical development of the water-in-oil-in-water technique of microencapsulation for peptide drugs within biodegradable polymers.

1.2 INTRODUCTION

Peptides/polypeptides have gained considerable interest in the treatment of different disease conditions. Although various routes of administration (nasal, parenteral, vaginal, oral, transdermal, etc.) have been investigated for their delivery most products are administered parenterally because of their inherent physical and chemical instability and short biological half-lives (Lee, 1991). Usually a solution or a suspension formulation of the peptide (e.g. insulin, leuprolide, busereline, calcitonin, etc.) is administered intravenously, subcutaneously or intramuscularly. Repeated administration is essential to achieve prolonged effects over extended periods of time. Thus, controlled release parenteral dosage forms, which reduce the frequency of administration and minimize the fluctuations in plasma levels caused by repeated injections are essential.

Certain devices allow the continuous administration of a solution /suspension of the peptide at programmed rates over extended periods of time (external or implantable infusion pumps). These systems require implantation through surgery, causing discomfort to the patient and result in loss of patient compliance. Similarly, after the completion of drug release the systems may require surgical recovery. Alternatively, peptides could be entrapped in implants (gosereline acetate, Zoladex, ICI Pharmaceuticals) which has to be injected under the abdominal skin under local anesthesia. A drug delivery system which can be administered through a normal 22-23gauge needle and which will not need surgical recovery after complete drug release is required. Biodegradable polymers are alternatives to these above-mentioned systems for the delivery of peptides.

A liquid injectable system capable of forming a biodegradable implant was developed (Dunn et al, 1994). The system comprises a biodegradable polymer dissolved in a biocompatible organic solvent such as N-methyl pyrrolidone, with a drug dissolved/dispersed in the polymer solution. The mixture upon being injected subcutaneously/intramuscularly through a conventional 22-23 gauge needle comes into contact with body fluids and causes the precipitation of the polymer forming an implant to entrap the drug substance for prolonged release through biodegradation. The system has the disadvantage that the implant formed varies in shape and size depending upon the site of injection.

Another viable alternative to this system is biodegradable drug delivery systems based on microencapsulation. Microencapsulation is a process by which a drug substance is entrapped within discrete free-flowing polymeric particle microencapsule products (Sanders et al, 1986; Mason-Garcia et al., 1988; Ogawa et al., 1988 a, b, c; Ruiz et al., 1989; Csernus et al., 1990; Cohen et al., 1991; Heya et al., 1991; Hermann & Bodmeier, 1995; Bittner et al., 1998).

Several methods of microencapsulation are known including solvent evaporation, phase separation, spray drying, supercritical fluid extraction, etc. Of these methods, the solvent evaporation method has attracted the most attention because of its ease of use and scale-up, lower residual solvent potential etc.

Table 1.1 : Commercially available controlled release peptide formulations

Drug substance	Brand name	Company
Leuprolide acetate	Lupron Depot	TAP Pharmaceuticals
Goserelin acetate	Zoladex	I.C.I.
Triptorelin	Decapeptyl	Debiopharma
Busereline acetate	Bigonist	Laboratoires Cassenne

A recent article which describes the United States Food and Drug Administration (USFDA) viewpoint on microencapsulated products for parenteral depot administration (Niu & Chiu, 1998) emphasizes the following : 1. polymer/copolymer, 2. organic solvents, 3. copolymer-peptide complexes, 4. sterilization, 5. in-vitro-in-vivo correlation's, 6. particle size, 7. diluent - suspending vehicle

The purpose of this chapter is to highlight the water-in-oil-in-water (w/o/w) emulsion solvent evaporation method for encapsulation of peptides within polylactide polymers and to serve as a guideline for the rational development of such a product. An attempt is made to bring out the interplay of the formulation and processing parameters affecting this process keeping the USFDA viewpoints in perspective.

1.3 DEVELOPMENT OF A MICROENCAPSULATION PROCEDURE

The oil-in-water (o/w) solvent evaporation method, also known as "in-water drying", originally developed for the encapsulation of water-insoluble drugs has been extensively used to date and its use with polylactide polymers was reviewed in detail (Jalil & Nixon, 1989, 1990 a &b). The method involves the preparation of a solution of a wall forming polymer in a water-immiscible organic solvent into which the drug is dissolved directly or with the aid of a cosolvent or dispersed in a fine state. This is then added in a controlled fashion into an aqueous solution of an emulsifying agent under intense agitation. This procedure usually yields a microsphere morphology and was applied to the encapsulation of a few water-insoluble peptides such as salmon calcitonin (Mehta et al, 1994, 1996, Li et al, 1995, Jeyanthi et al, 1996, 1997)) and cyclosporine (Chacon et al, 1999). It is generally not applicable to the encapsulation of highly water-soluble peptides within hydrophobic polymers

because upon emulsification of the dispersion of the drug-organic polymer solution/dispersion into the external aqueous phase, most of the peptide partitions out into the external phase resulting into negligible entrapment in the microspheres.

In 1970 a multiple emulsion solvent evaporation microencapsulation procedure was patented by Vrancken and Claeys and further by DeJaeger and Tavernier in 1971. In brief, an aqueous solution of the drug substance was emulsified under high-speed homogenization or sonication into a solution of polymer in an organic solvent. This emulsion, known as the primary emulsion, was then poured under constant stirring into an external aqueous phase containing a suitable emulsifier. This procedure was further modified to enable the encapsulation of highly water-soluble peptides (Ogawa et al,1988a; Okada et al, 1995).

For the successful development of a microencapsulation procedure it is essential to have an excellent understanding and control on the polymer and its chemistry, the microencapsulation procedure in terms of its engineering and various parameters which affect the product and the stability of the peptide. Each of these are considered in detail in the forthcoming sections.

1.3.1 The Polymer

One of the preliminary requirements in the successful development of a microencapsulation procedure and in achieving a product of reproducible quality in terms of microencapsulation efficiency, yield, scale-up performance, and finally, drug release characteristics is the selection of a suitable polymer as the coating material and the complete characterization of the polymer.

The requirements for a parenterally acceptable biodegradable polymer for drug delivery include controlled biodegradation rate, production of nontoxic degradation products and metabolites, reproducible and economically viable manufacturing process for large scale manufacture, non-immunogenicity, absence of impurities such as residual solvents, catalysts, monomers, stabilizers, etc., and ease of processing.

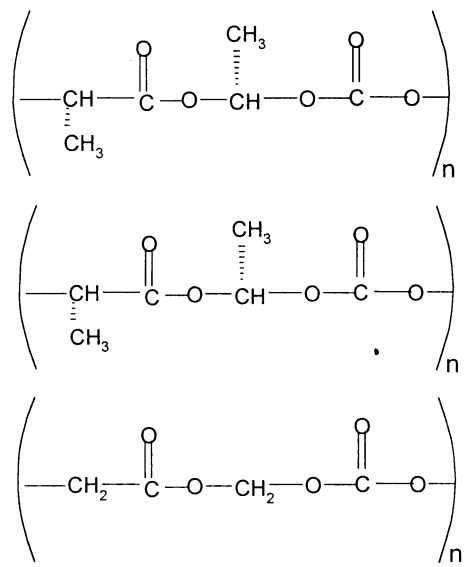

Fig 1.1: Structures of different lactide and glycolide homopolymers (Reproduced from DeLuca et al, 1993, with permission)

To meet these requirements it is important to understand the chemistry of the biodegradable polymers. The different classes of biodegradable polymers which have attracted attention for their use in drug delivery include natural polymers such as gelatin, albumin, caesin, etc. and synthetic polymers such as polyanhydrides, polyorthoesters, polyaminoacids, polyphosphazenes, polyhydroxy acids, etc. to name a few (Figure 1.1). The

synthetic polymers are generally more accepted because of the reproducibility in their manufacture through

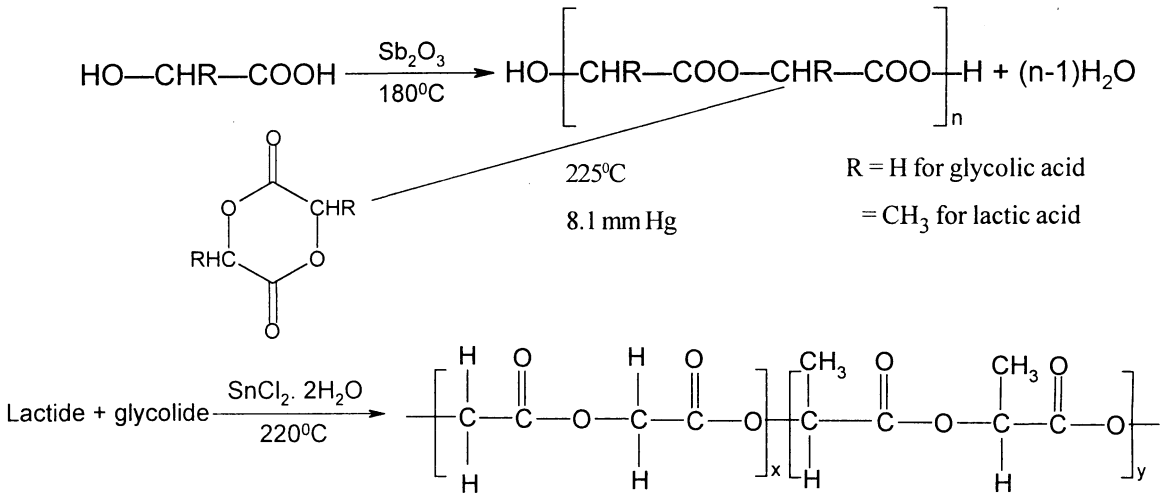

Fig 1.2: Methods of synthesis of the polylactide/polyglycolide polymer (Reproduced from DeLuca et al, 1993, with permission)

synthetic means, lack of immunogenicity, predictable biodegradation profiles, etc. Of these polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers in different ratios of lactic to glycolic acid (PLGA) are the only classes of polymers currently approved for clinical use in drug delivery (Figure 1.1). These polymers have established use as sutures, drug carriers and prostheses (Jain et al, 1998). The biodegradation profile and safety of these polymers and their biodegradation products, lactic and glycolic acid, are well characterized. In addition, it is possible to achieve a wide variety of release profiles simply by blending different PLAs and PLGAs together and even more so by changing the co-monomer ratios. This will be discussed in a subsequent section.

It is essential to differentiate between the terms polylactic acid or polyglycolic acid and polylactide or polyglycolide. These polymers are synthesized mainly by two methods (Fig 1.2):

1. The direct polycondensation of lactic and/or glycolic acid in the presence of a catalyst such as antimony trioxide, ion exchange resins or clays. This method usually produces polymers in the molecular weight range of less than 10,000 D. These polymers are called polylactic or polyglycolic acid or their copolymers (Gilding & Reed, 1979; Ogawa et al, 1997).
2. The ring-opening melt-condensation of the cyclic dimers of lactic and/or glycolic acid (lactide/glycolide) in the presence of catalysts such as antimony, tin, zinc, titanium, etc. This method produces polymers with a molecular weight greater than 10,000 D, which are called polylactides or polyglycolides (Gilding & Reed, 1979; Lewis, 1990).

In order to get reproducible results in microencapsulation experiments using polylactide polymers it is essential to characterize the polymer completely in terms of the following parameters (methods of characterization): molecular weight and polydispersity (gel permeation chromatography), whether it has free carboxylic acid end groups or whether it is end-capped, acid number (percentage of free carboxylic acid end groups, acid number through non-aqueous titrimetry), content of low molecular weight oligomers (water extractable residue through gravimetry), residual monomers (HPLC, GC), residual catalyst (HPLC, GC), residual solvent (GC), thermal properties (differential scanning calorimetry), co-monomer ratio (nuclear magnetic resonance), solubility in organic solvents (cloud point titration), inherent viscosity (capillary viscometry), etc.

For a more detailed description of some of these characterizations the reader is directed to an excellent review by Hausberger & DeLuca (1995). A change in one or more of these properties could result in a drastic change in the microencapsulation efficiency, the yield, the porosity, the burst effect, etc. Several researchers have noted a difference in different lots of polymer supplied by the same manufacturer and between polymers supplied by different manufacturers but claiming the same molecular weight and co-monomer ratio (Mehta et al, 1996).

1.3.2 Biodegradation

Once a polymer has been identified it is important to know how the polymer behaves in-vitro and in-vivo. We thus have to understant the definitions of the different terms used to define these polymers. "Biomaterials", "biodegradation", "biocompatible", "bioerosion", etc. are terms which are used interchangeably to describe the polymers used for microencapsulation. Biomaterials is a broad term which encompasses all materials which are introduced into the body for therapeutic or diagnostic purposes. Biocompatibility pertains to the lack of adverse response, immunogenicity, histocompatibility of the material upon injection. Biodegradation on the other hand relates to the break down of the polymer into its component monomers or oligomers upon coming into contact with the bodily fluids, enzymes, microbial flora, etc (DeLuca et al, 1993). For the purposes of this chapter the discussion focuses on the biodegradation of the polymers. The reader is directed to several excellent articles about the biocompatibility and in-vivo behavior of these polymers (Zaikov, 1985; Visscher et al, 1985, 1988; Yamaguchi & Anderson, 1993; Tabata & Ikada, 1988; Williams & Mort, 1997; Salthouse & Matlaga, 1975).

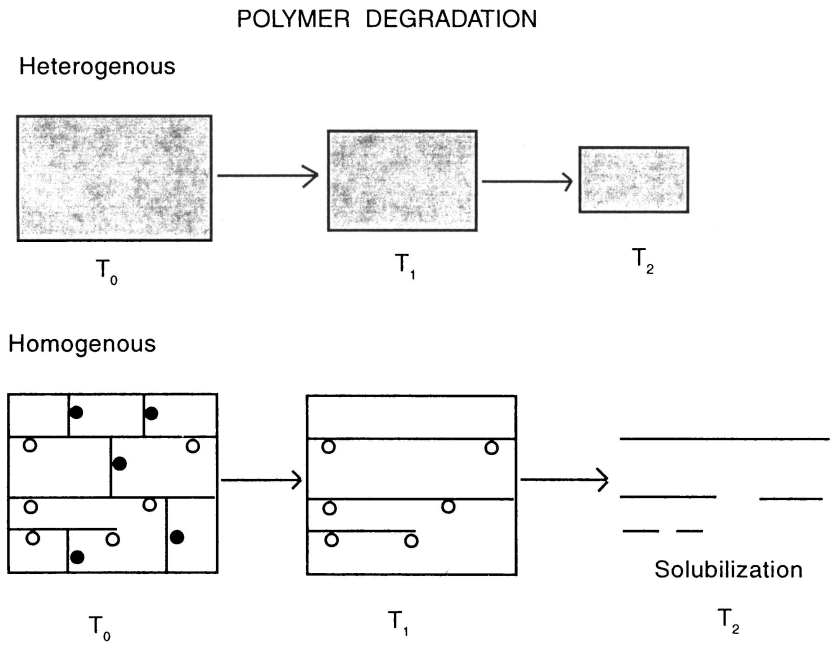

Fig 1.3: The mechanism of degradation of biodegradable polymers (Reproduced from DeLuca et al, 1993, with permission)

Biodegradation of the polymer is of two kinds; homogeneous and heterogeneous (Fig 1.3). Heterogeneous biodegradation starts at the surface of the microparticle and proceeds to the layers beneath, with the drug being released as the polymer degrades (assuming that biodegradation is the major release rate controlling process). The degradation rate is constant in this case with the undegraded carrier retaining its integrity throughout the process. Homogeneous degradation involves a random cleavage throughout the bulk of the polymer matrix. In this case the molecular weight of the polymer steadily decreases until a critical value of the

molecular weight is reached. Till this value is reached the carrier system retains its original shape, whereas beyond this value loss of mass and solubilization of the polymer commences.

The lactide-glycolide polymers degrade predominantly through a homogeneous degradation process. The different stages involved in the biodegradation of these polymers include:

1. Hydration of the polymer : There is initial uptake of the physiologic medium, swelling of the polymer and thus change in physical dimensions
2. Cleavage of covalent bonds throughout the matrix
3. Mass loss through step 2 and through solubilization of low molecular weight species

A critical molecular weight of approximately 1000 D is required to be reached before the polymer goes into solution. This process is thus associated with an uptake of the release medium and swelling of the polymer, a reduction in molecular weight, a decrease in the pH of the release medium as the low molecular weight species (lactic and glycolic acid) are released into the medium and finally complete disappearance of the pellet.

The rate of biodegradation of lactide/glycolide polymers depends strongly on the molecular weight, co-monomer ratio, the crystallinity of the polymer, porosity of the microsphere matrix, drug loading, additives, etc. A higher molecular weight of the polymer results in a significantly slower rate of degradation. The homopolymers poly(L-lactide) and polyglycolide are crystalline in nature and hence require longer degradation times when compared with their copolymers. In addition, lactic acid which has an asymmetric carbon gives rise to L and DL classes of polymers. Poly(L-lactide) is crystalline in nature and so degrades at a much slower rate when compared with poly-DL-lactide which is amorphous. Similarly, the copolymers of lactic and glycolic acid show different degradation properties as shown in Fig 1. 4 and Table 1.2. Starting with a polymer of DL-lactide, as the percentage of glycolide increases in the copolymer the degradation rate increases until a molar ratio of 50:50 %. Any further increase in the glycolide content results in a decrease in biodegradation rate. As the percentage of either the glycolide or the lactide increases the crystallinity of the polymer and hence its melting point also increases significantly (Fig 1.5). Table 1.2 demonstrates the properties of different polylactide and polyglycolide polymers.

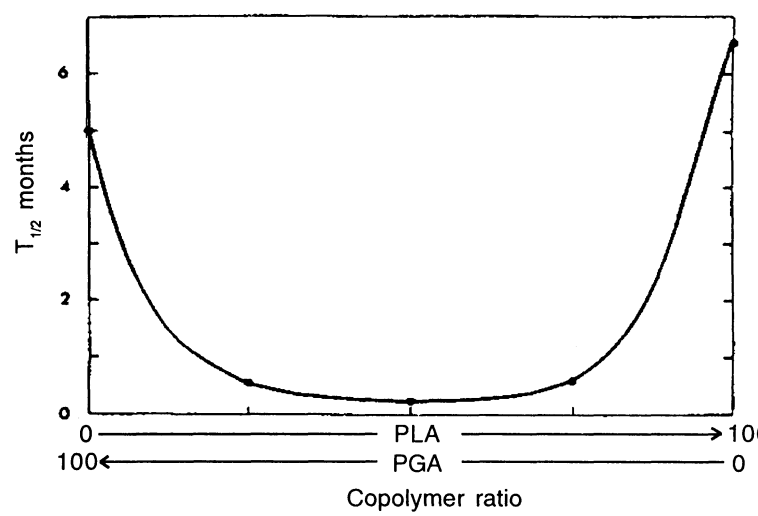

Fig. 1.4 : Schematic representation of the effect of the lactide and glycolide content on the biodegradation time of biodegradable polymers (reproduced from DeLuca et al, 1993, with permission)

The rate of biodegradation of the polymers is also affected by the nature of their endgroups. The original PLA/PGA polymers used as sutures, ligatures, etc. were endcapped polymers, where at the end of the polymerization reaction the terminal carboxylic group was blocked with some hydrophobic group. Many researchers observed that it would require months or years for these PLA/PGA polymers to disappear completely from the injection site because of their high hydrophobicity. To enhance the rate of biodegradation the hydrophilicity of the polymers was increased by having non-end-capped polymers with free terminal carboxylic groups.

The molecular weight of the polymer determines to a great extent the entrapment of the drug, the rate of biodegradation and so the rate and duration of drug release, and the final disappearance of the implanted pellet

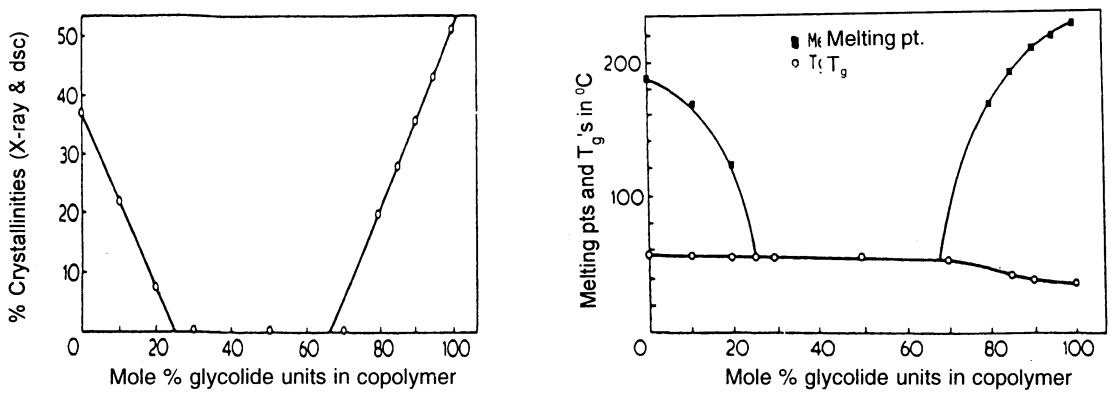

Fig 1.5 : The effect of the ration of the lactide/glycolide monomer content on the properties of biodegradabel polymers (reproduced from DeLuca et al, 1993 with permission)

from the site of injection. For development of products for parenteral controlled release it is essential to choose a polymer which will degrade slowly while providing release of the drug substance over the required time period. Subsequently, the implanted pellet should disappear completely from the site of injection. Generally, polymers with different molecular weights and comonomer ratios have to be tried before a suitable polymer is identified. Such a series of experiments demonstrated that a suitable polymer for a 1 month release formulation, for example, should have a molecular weight of 10,000-14,000 D and a lactide : glycolide comonomer ratio of 75 : 25 mole % (Ogawa et al, 1988a, b, c). This polymer provided a continuous release of leuprolide acetate over one month and the implanted pellet disappeared completely from the injection site after approximately 6 weeks.

Table 1.2 : Properties of some LA/GA homo and copolymers

Polymer	MW, D	Tg (°C)	Tm, (°C)
Poly (L-lactic acid)	2,000	40	140
Poly (L-lactide)	100,000	60	180
Poly (DL-lactide)	---	52	None
PLGA 85:15	232,000	49	None
PLGA 75:25	63,000	48	None
PLGA 50:50	12,000	40	None
PLGA 50:50	98,000	47	None
Poly (glycolide)	36,000	36	210-220

Adapted from DeLuca et al (1993) and modified

1.3.2. The Organic Solvent

In addition to the choice of the proper polymer for microencapsulation it is also essential to determine the appropriate solvent for the preparation of the primary emulsion. The selection of the solvent and the external continuous phase determine the microsphere formation and the entrapment efficiencies. A good solvent for microencapsulation should have the following properties:

1. Good solvency for the polymer: A highly concentrated polymer solution would precipitate rapidly on secondary emulsification with enhanced drug entrapment.
2. Poor solvency for the drug: A low drug solubility prohibits partitioning of the drug into the external aqueous phase during secondary emulsification with enhanced drug entrapment.
3. Low boiling point: A boiling point lower than that of water enables easier removal of the solvent without the use of harsh conditions such as high temperatures.
4. Should be immiscible with water yet should have a finite solubility in it : A high miscibility with water would result in the formation of a film or precipitation and not encapsulation, yet a minimum solubility is required for the solvent evaporation to occur.
5. Should not cause the degradation of the drug substance.
6. Should be acceptable for human use.

Methylene chloride is by far the most widely used solvent for microencapsulation using the solvent evaporation technique. It suffers from the drawback of being carcinogenic and has a low solubility in water. Other solvents with a lower toxicity than methylene chloride such as ethyl acetate have also been used in microencapsulation. Ethyl acetate suffers from the disadvantage of being a poor solubilizer for higher molecular weight polymers and those with a co-monomer ratio of 50: 50 mole % (lactide : glycolide) (Cleland, 1997).

1.3.3 The External Phase

The external phase in a solvent evaporation encapsulation method should be inexpensive, high boiling, non-toxic and immiscible with the organic solvent used. Water is the only medium, which fulfills all these requirements. Other external environments such as oils (cottonseed, mineral, olive, etc.), organic solvents, etc. have also been used to enhance entrapment efficiencies but they suffer from the disadvantage of residual levels in the finished product (Jain et al, 1998).

The external phase should also contain an emulsifier. As the solvent evaporation proceeds to a completion the droplets generated initially shrink in size as the organic solvent evaporates. During this early evaporation stage the droplets tend to coalesce and form agglomerates. A good emulsifier is required for the stabilization of the droplets to prevent coalescence by the formation of a thin film. As the evaporation proceeds, the emulsifier film helps to maintain the spherical shape of the droplets till such time as the droplets are hardened enough to be harvested. The emulsifier can then be washed off. Polyvinyl alcohol is by far the most commonly used emulsifier in solvent evaporation procedures. Other emulsifiers which have been used include poly(vinylpyrrolidone), gelatin, alginate, methylcellulose, polysorbates, hydroxypropylmethylcellulose, sodium lauryl sulfate, etc. (Jain et al, 1998).

1.3.4 The Process

Fig 1. 6 describes the different stages of the microencapsulation procedure and the different parameters, which need to be controlled to get a reproducible product.

1.3.4.1 Primary emulsification

The first step in the development of a successful microencapsulation procedure is the formation of a stable primary emulsion. Generally, during the preparation of the primary emulsion an aqueous solution of the peptide is emulsified under high shear into an oily phase consisting of a solution of the polymer in an organic solvent,

with or without an emulsifier. The finer the internal droplet size the more stable is the primary emulsion. Peptides and proteins by their amphiphillic nature act to stabilise the primary emulsions (Hermann & Bodmeier, 1995). But, a higher drug loading is detrimental to the primary emulsion stability depending on the volume of internal water used for dissolving the drug.

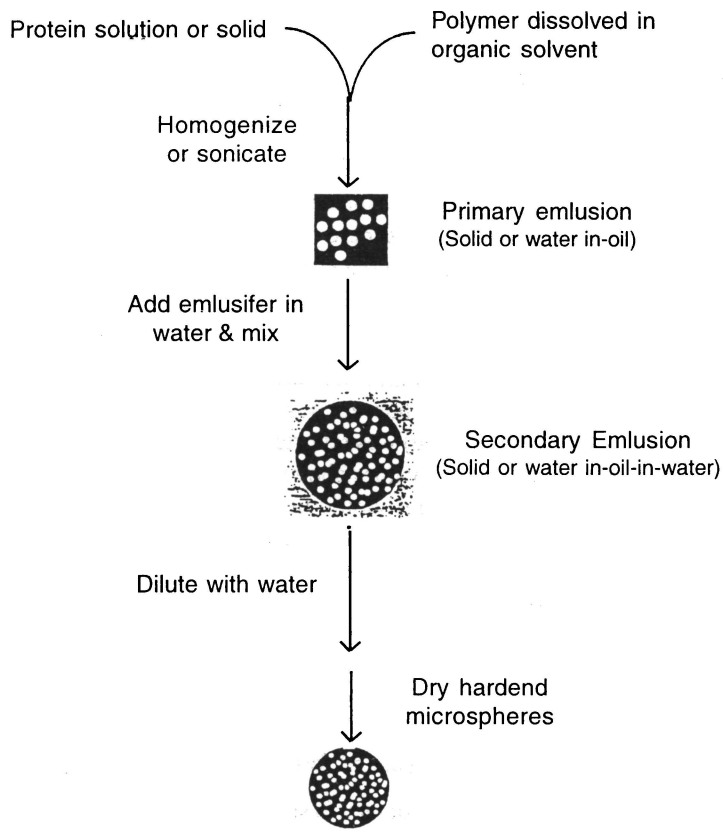

Fig 1.6 : Schematic representation of the water-in-oil-in-water microencapsulation procedure

Several modes of mixing have been used for primary emulsification including high speed homogenization, microfluidization, probe sonication, vortexing, static mixers, etc. A fine droplet size in the primary emulsion leads to a more dense structure of the final microsphere product. Vortex mixing generated larger droplet sizes and larger cavities in the microspheres than products using probe sonication (Hermann & Bodmeier, 1995). Probe sonication generates fine primary emulsions through the concept of cavitation but its use suffers from two major drawbacks. Degradation and reduction in molecular weights of PLGA polymers was observed during probe sonication probably due to the high localized increase in temperatures. Also, higher energy is required at the tip to sonicate solutions with a higher viscosity. In addition, it is difficult to use probe sonicators for higher volumes because of their localized action resulting in the inability to scale-up the product. High speed homogenization is by far the most popular method because of the different configurations of rotors, stators and their combinations available, the ease of scale-up when compared with other procedures, versatile application and their availability for use from volumes as low as 1 ml to production size batches. As the homogenization speed increases in rotor-stator homogenization the droplet size decreases until an equilibrium droplet size is achieved whereas the homogenization time does not have a major impact on the equilibrium droplet size (Maa & Hsu, 1996).

The stability of the primary emulsion has a profound impact on the surface and cross-sectional morphology, the entrapment and particle size of the final microsphere product ultimately also affecting the drug release characteristics (Nihant et al, 1994, Schugens et al., 1994, Maa & Hsu, 1997). Various formulation factors that affect the formation and stability of the primary emulsion include drug loading, polymer concentration, droplet size, viscosity, volume and pH of internal aqueous phase etc.

The volume of the internal aqueous phase affects the microsphere morphology, the drug entrapment and the rate of drug release. As the volume of the internal aqueous phase increases the phase volume ratio between the internal aqueous phase to the oil phase increases. This results in an insufficient volume of the oil phase and hence of the polymer being available for the entrapment of the internal aqueous droplets. Thus, upon secondary emulsification the internal aqueous phase is more prone to come in contact with the external aqueous phase thereby resulting in the loss of drug substance and hence a lower entrapment efficiency. Also, the internal water which gets entrapped in the microsphere matrix due to polymer precipitation diffuses out along with the solvent during solvent evaporation and creates pores and channels in the microsphere structure. The result is a microsphere system with a high porosity through which the drug substance is released at a faster rate (Hermann & Bodmeier, 1995).

The pH of the internal aqueous phase determines the state of ionization of the polymer and the drug substance which in turn determines the possible interactions between the polymer and the peptide. The dissociation constant of a poly(lactide-co-glycolide) was reported as approximately 4.0 (Makino et al, 1986). Thus, the polymer has a net negative charge at pH values above 4.0 as the carboxylic end groups are ionized. By adjusting the pH of the internal aqueous phase it should be possible to create an interaction between the negatively charged -COOH groups on the polymer and the positively charged groups on the amino acids from the peptide. Such an interaction is beneficial to enhance entrapment, to reduce the burst effect and to control the release rate of the drug as will be discussed in subsequent sections (Okada et al., 1995). This phenomenon was demonstrated for the encapsulation of somatostatin acetate within PLA microspheres (Hermann & Bodmeier, 1995) and for leuprolide acetate and thyrotropin within PLGA microspheres (Okada et al., 1995). This is the beneficial use of such a drug-polymer interaction. Other cases of drug-polymer interaction which result in the degradation of the drug or poor release profiles are also known (Cleland, 1997). It is thus essential to characterize such an interaction or the lack of it for microencapsulated formulations of peptide-protein drugs.

Generally, in the formulation of multiple emulsions w/o surfactants such as albumin or polyoxyethylene-polyoxypropylene block copolymers are needed to stabilize the primary emulsion. This may be detrimental to the final microsphere product as the primary surfactant could leach out into the external aqueous phase during secondary emulsification along with the diffusion of the internal water and organic solvent during solvent evaporation. This would probably generate a microsphere matrix which is more porous and would result in a large burst effect and a drug release which is governed more by diffusion through the movement of the dissolution medium into the pores and out rather than through the process of degradation of the polymer.

The overall viscosity of the primary emulsion influences the entrapment of the peptide, the particle size and the morphology. The viscosity can be increased through increasing the viscosity of the internal aqueous phase, adjusting the ratio of the internal aqueous phase to the polymer-organic solution phase, addition of a drug retaining substance, lowering of the temperature of the primary emulsion and increasing the concentration of the polymer in the organic solvent.

An increase in the concentration of the polymer in the organic solvent causes an increase in the viscosity of the polymer solution, the viscosity of the primary emulsion, the stability of the primary emulsion and also the rate at which the polymer precipitation occurs upon secondary emulsification. This results in a higher entrapment efficiency upon secondary emulsification. But, given the same processing conditions a much higher rate of shear is required to achieve the same particle size of the microparticulates.

The use of a drug retaining substance such as gelatin, albumin, pectin, etc., in the inner aqueous phase aids in achieving higher drug loading through an increased inner aqueous phase viscosity. Of course, the kind of drug retaining substance, its concentration, its molecular weight, etc. would also play a critical role. In addition, a reduction in the temperature of the primary emulsion aids in enhancing the entrapment ratio of the drug. But, as the overall viscosity of the primary emulsion increases the particle size also increases where above a certain viscosity the particles are actually deformed upon secondary emulsification (Okada et al., 1995).

1.3.4.2 Secondary emulsification

Once a stable primary emulsion is formulated the next step in the microencapsulation procedure is secondary emulsification (Fig 1. 6). In this step the primary emulsion is added into an external aqueous phase containing a suitable emulsifier, with intense agitation. The mode of addition of the primary emulsion during this step has an impact on the final particle size distribution. Several different modes of addition are practiced including direct pouring, slow addition with the use of a syringe or infusion pump, or through the use of a peristaltic pump, etc. Addition of the primary emulsion through a narrow bore tube or needle so that the emulsion is added at a slow controlled rate into the blades of the homogenizer/propeller usually provides the best results. A smaller droplet size to be subjected to secondary emulsification generates a finer final microparticulate product. A high speed of mixing during secondary emulsification usually leads to the formation of a smaller particle size. Also, the concentration of the emulsifier used helps to control the particle size of the final product.

It is essential at this point to understand the method of solvent evaporation. The solvent used in the procedure is usually immiscible with water. Upon secondary emulsification, the solvent from the surface of the droplet at the oil/water interface diffuses into the external aqueous phase, dissolves and then evaporates at the water to air interface (Fig 1. 7). The rate of evaporation is thus limited by the solubility of the solvent in the external aqueous phase. Methylene chloride has a solubility of approximately 1.32 %w/v in water at 25°C. This solubility may be further enhanced because of the presence of the surfactant. The rate can be modified by a rapid dilution of the external aqueous phase by gradual addition of a large excess of water. In addition, the temperature of the external aqueous phase could be raised suddenly or gradually in a programmed gradient to cause enhanced rates of solvent evaporation (Jeyanthi et al, 1996; Li et al, 1995). Another approach to enhance the rate of precipitation of the polymer is to add a water-miscible organic solvent such as isopropyl alcohol or acetone into the external aqueous phase. The presence of such a cosolvent results in the rapid extraction of the organic solvent from the droplets causing rapid hardening of the microspheres. It must be remembered that all of the techniques mentioned here for the enhancement of the rate of solvent evaporation also affect the microsphere morphology. It is important to characterize the effect that each such change has on the

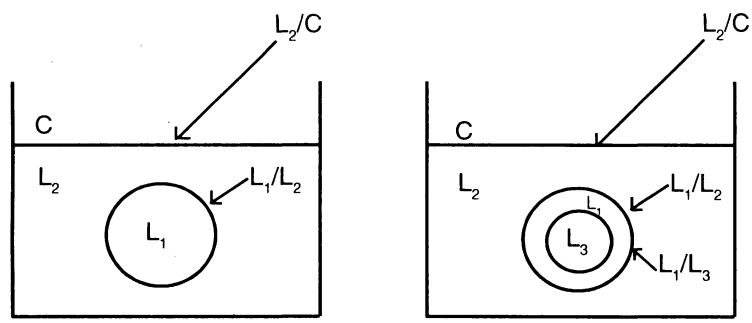

Fig 1.7: Schematic of the mode of solvent evaporation
(Reproduced from Benoit et al., 1996, with permission)

morphology of the final product. In addition, it is also essential to characterize the level of residual solvent in the microparticulates and the levels of residual emulsifier such as polyvinyl alcohol on their surface.

1.3.4.3 Hardening and Recovery

The microspheres in suspension have to be hardened by continuing the evaporation of the solvent. The hardened microspheres are recovered by filtration or centrifugation. The wet cake is then subjected to drying by one of various processes: heating, vacuum, lyophilization, etc. Lyophilization is the most preferred process because of the sensitivity of the peptide molecules. It must be remembered that raising the temperature of the product above the glass transition temperature of the polymer results in the agglomeration of the microspheres. So a careful control on the drying conditions is essential to achieve a product of reproducible quality.

1.3.4.5 Characterization of the microspheres

The microspheres formulated as described above have to be characterized thoroughly for the different properties. This is not the focus of the chapter and thus only a brief mention is made of the different properties and the methods by which this characterization should be carried out. The different properties include : peptide entrapment and entrapment efficiencies (HPLC), particle size (microscopy, sieve analysis, laser light scattering, coulter-counter, photon correlation spectroscopy), stability and activity of the peptide in the microspheres (HPLC and biological methods), yield of the process, bulk and tap density, porosity and specific surface area (mercury or helium intrusion porosimetry), drug release, thermal properties (differential scanning calorimetry, thermogravimetric analysis), moisture content (Karl Fischer titration, gravimetry, thermogravimetric analysis), surface and cross-sectional morphology (scanning electron microscopy, transmission electron microscopy, scanning probe microscopy, image analysis), biodegradation rate in-vitro (HPLC, GPC), residual organic solvent (GC, thermogravimetric analysis), surface and bulk sterility (microbiological methods), resuspendability, syringeability, etc.

Here a brief mention about the residual solvent levels has to be made. The residual organic solvent in the final microencapsulated product has to be characterized. The USFDA has allowed the product Lupron Depot to be marketed which has levels of less than 50 ppm residual methylene chloride. It is generally difficult to remove the organic solvents completely. Repeated lyophilization cycles or drying under vacuum at elevated temperatures may help reduce the residual levels. Temperatures in excess of 20°C may result in the agglomeration of the microspheres unless they are properly stabilized.

Mention of the use of alternate organic solvents such as ethyl acetate, methyl ethyl ketone, etc. for microencapsulation has been made earlier in this article. Approval for such use from the FDA for commercial manufacture would only be possible after establishing the residual solvent levels and the toxicity of such levels to human beings. Till then methylene chloride appears to be the solvent of choice for microencapsulation by solvent evaporation.

1.3.4.6 Drug release

One of the important aspects of the performance of the microencapsulated product is the drug release. The drug release profile from microencapsulated systems will differ depending upon whether it is a microsphere or a microcapsule system. The drug release profiles from biodegradable microspheres are governed by many properties, both of the polymer, the drug and the carrier system (Kissel et al, 1991; Washington, 1990). Polymer dependent factors include the molecular weight and its distribution, the comonomer ratio and the distribution of the monomers, the percentage of low molecular weight species, the crystallinity, whether the polymer is an end-capped or non-end-capped polymer, the percentage of free carboxylic end groups, etc. Drug dependent parameters include the molecular weight, the solubility in the dissolution medium and drug-polymer interactions. The carrier system parameters include the morphology, i.e. whether the product is a microcapsule or microsphere, the drug loading, the physical state of the drug in the polymer matrix, i.e. whether the drug is in the dispersed or dissolved state, the particle size and distribution, the porosity and the internal morphology of the microparticles depending upon the different parameters discussed above.

A general release profile from biodegradable microspheres is depicted in Fig 1.8. The drug release could be continuous acting through diffusion alone (as occurs for smaller molecules) or it could be triphasic (as occurs for most high molecular weight peptides and proteins). The triphasic profile also called the 'S' shaped profile is characterized by:

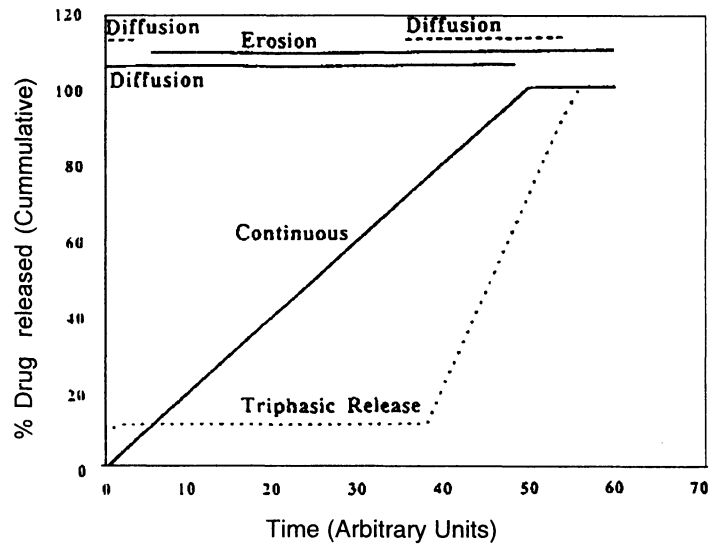

Fig 1.8 : A generalized release profile for drug substances from biodegradable microspheres (Reproduced from Cleland, 1997, with permission)

1. A burst effect which is a rapid initial release of the drug caused because of adsorbed or unentrapped drug
2. A lag time during which the polymer degrades and reaches the critical molecular weight where mass loss occurs
3. A controlled release of the drug substance through a combination of the degradation of the polymer and diffusion of the drug through newly generated pores and surfaces.

It is hoped to achieve a product with a minimum burst effect and which will release the drug in a zero order fashion. Several methods to control the burst effect have been used. The ionic interaction between the negatively charged polymer and the positively charged peptide was used to entrap leuprolide acetate within PLGA microspheres (Okada et al, 1995). The addition of a drug retaining substance into the internal aqueous phase helps to reduce the burst effect possibly through the formation of a barrier between the drug release medium and the drug substance (Okada et al, 1995). The presence of low molecular weight oligomeric species in the polymer also leads to an increased burst effect. Washing of polymer to reduce low molecular weight species helps to reduce this burst effect (Okada et al, 1995).

Additional medium related parameters which have to be controlled during drug release studies include volume, pH, osmolarity, buffer capacity, buffer species, ionic strength, additives, etc. Each of these has to be characterized in detail before a drug release method can be applied to a formulation. It is also essential to develop an accelerated dissolution method for the quick and reproducible characterization of the drug release from the product as an in-process test. This could be done through an increase in the temperature or a change in the medium or its pH, etc. For each such change it is essential to characterize the effect that the change has on the performance of the product.

1.3.4.7 Resuspendability and syringeability of the microspheres

The particle size of the microspheres is one of the important parameters which has to be finalized well in advance. The final particle size and the suspending vehicle for redispersion finally decide the syringeability of the suspension. The final pack for such a product would be either (a) a vial containing the microspheres, an

ampoule with a diluent and a syringe needle assembly, or (b) a dual chambered prefilled syringe containing the microspheres in one chamber and the diluent in the other. In both cases the microsphere powder is to be resuspended in the diluent vehicle before injection. The microsphere suspension is injected subcutaneously or preferably intramuscularly through a 18 gauge needle, though needles as fine as 22-23 gauge have also been used. An 18 gauge needle has an internal diameter of 838μm (Lee et al, 1991). Thus, a maximum particle size of 100μm should be formulated for injection through such a needle. Most of the marketed microencapsulated products have a particle size in the range of 5-100μm, with the largest percentage of the particles in the less than 40μm range. A particle size larger than 100μm may lead to interparticulate interactions and a possible eventual clogging of the needle during injection though this problem could be prevented by the inclusion of suitable surfactants such as Tween 80 in the vehicle.

Another approach to improve the syringeability is the use of extremely fine particles. Such small particles have an extremely high surface area and hence pose problems for proper wetting and dispersion during resuspension. Secondly, small particles also show agglomeration and development of static charge, which are both potential problems when it comes to flow of material during vial filling. Thirdly, small particles sometimes demonstrate non-Newtonian flow properties. Thus, a perfectly fluid, well dispersed suspension of the microparticles suddenly stops flowing as excess shear is developed at the tip of the needle. On relaxation of the shear the suspension becomes fluid again. This cycle continues causing problems for the smooth administration of the product.

The microcapsules along with the suspending vehicle form the final injectable suspension. The vehicle for suspension and its components should have the following properties: easy and complete wetting of the product, rapid resuspension, stability of the suspension adequate for injection, uniform dispersion, adequate viscosity to prevent sedimentation and agglomeration but not so high as to prevent flow, should cause no local irritation, nontoxic, and isotonic.

It is also essential to determine the concentration of microparticles in the final suspension so that the final product has adequate syringeability. Generally, a mock trial is carried out where the microsphere suspension is injected through the final syringe and needle combination into a matrix, which exerts sufficient back pressure as in the case of the actual injection into human beings. A similar study should also be carried out using a needle with a smaller bore than that to be actually used, as a more stringent challenge to the formulation.

1.3.4.8 Sterilization

The microspheres described in this chapter are intended for intramuscular or subcutaneous administration. It is therefore essential to develop a product which will be sterile and pyrogen free. Several methods of sterilization known include: autoclaving, dry heat, ethylene oxide, irradiation, aseptic processing. Most peptides and polypeptides are known to be thermolabile. Similarly, the polylactides degrade through simple hydrolysis and show a higher rate of degradation at elevated temperatures. PLA/PGA polymers and their copolymers degrade rapidly when subjected to irradiation sterilization as a function of the irradiation dose. The degradation is seen through a decrease in the molecular weight, reduction in the viscosity and mechanical properties. This is more predominant in the PLA and PLGA polymers than for PGA polymers (Sintzel et al., 1997). Thus, for the preparation of the microspheres aseptic processing is the most acceptable method. The polymer solution in methylene chloride can be easily filtered through 0.22μm filters. Similarly, the internal aqueous phase, the external aqueous phase and the washing water can all be sterile filtered and the final compounding of the product can be carried out in a sterile environment. It is important to establish the sterility of the microspheres, not only on the surface but also internally by dissolving the microspheres in some mild nontoxic solvent such as DMA or DMSO and further subjecting it to sterility testing.

1.4 CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

Microencapsulation of peptide or protein drugs is a challenging field for research. It can be seen from the above discussion that a variety of factors are responsible for achieving the correct microsphere characteristics of particle size, entrapment efficiency and drug release. In addition, other factors such as syringeability, sterility, residual solvent concerns, etc. have also been highlighted. The major challenges facing the pharmaceutical scientists include, scale-up problems, use of alternative solvents to replace methylene chloride, application of this and other techniques for the encapsulation of higher molecular weight proteins, to overcome the problems of peptide/protein degradation during microencapsulation and within the microspheres on stability and most important; the use of alternative biodegradable polymers to the polylactides/glycolides.

REFERENCES

Benoit J. P.; Herve M.; Rolland H. and Velde V.V. (1996) "Biodegradable microspheres : Advances in production technology", In : "Microencapsulation : Methods and industrial applications." Simon Benita (Eds), Marcel Dekker, New York, pg 35-72.

Bittner B.; Morlock M.; Koll H.; Winter G. and Kissel T. (1998) "Recombinant human erythropoietin (rhEPO loaded poly(lactide-co-glycolide) microspheres : influence of the encapsulation technique and polymer purity on microsphere characteristics." Eur. J. Pharm. Biopharm., 45(3) : 295-305

Chacon M.; Molpeceres J.; Berges L.; Guzman M. and Aberturas M.R. (1999) "Stability and freeze-drying of cyclosporine loaded poly(D,L lactide-glycolide) carriers." Eur. J. Pharm. Sci., 8(2) : 99-107

Cleland J. (1997) "Protein delivery from biodegradable microspheres" In : "Protein delivery : Physical systems." Pharmaceutical Biotechnology Series, Volume 10, Sanders L.M. and Hendren R. W. (Eds); Plenum Press, New York.

Cohen S.; Yoshioka T.; Lucarelli M.; Hwang L.H. and Langer R. (1991) "Controlled delivery systems for proteins based on poly(lactid-glycolic acid) microspheres." Pharm. Res., 8 : 713-720

Csernus V.J.; Szende B. and Schally, A.V. (1990) "Release of peptides from sustained delivery systems(microcapsules and microparticles) in vivo." Int. J. Peptide. Protein. Res., 35 : 557-565

Dejaeger N. C. and B. H. Tavernier (1971) British Patent, 1,405,108

DeLuca P.P.; Mehta R.C.; Hausberger A.G. and Thanoo B.C. (1993) "Biodegradable polyesters for drug and polypeptide delivery." In : Polymeric delivery systems, El-Nokaly,M.A., Piatt,D.M. and Charpentier, B.A. (eds.), pp. 53-79, American Chemical Society, Washington, DC.

Dunn R.L.; English J. P.; Cowsar D.R. and Vanderbilt D.P. (1994) "Biodegradable insitu forming implants and the method of producing the same." US Patent No. 5,278,201,

Gilding D.K. and Reed A.M. (1979) "Biodegradable polymers for use in surgery - polyglycolic / polylactic acid homo- and copolymers : I." Polymer, 20 : 1459-1464

Hausberger A.G. and DeLuca P.P. (1995) "Characterization of biodegradable poly(dl-lactide-co-glycolide) polymers and microspheres." Journal of Pharmaceutical and Biomedical Analysis, 13(6) : 747-760

Hermann J. and Bodmeier R. (1995) "Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on w/o/w-multiple emulsions." Int. J. Pharm., 126 : 129-138

Heya T.; Okada H.; Tanigawara Y.; Ogawa Y. and Toguchi H. (1991) "Effect of counteranion of TRH and loading amount on control of TRH release from copoly(dl-lactic-glycolic acid) microspheres prepared by an in-water drying method." Int. J. Pharm., 69 : 69-75

Jain R.; Shah N.H.; Waseem Malick A. and Rhodes C.T. (1998) "Controlled drug delivery by biodegradable poly(ester) devices : Different preparative approaches." Drug Dev. Ind. Pharm., 24(8) : 703-727

Jalil R. and Nixon J.R. (1989) "Microencapsulation using poly(L-lactic acid). I : Microcapsule properties affected by the preparative technique." J. Microencapsul., 6(4) : 473-484

Jalil R. and Nixon J.R. (1990a) "Microencapsulation using poly(L-lactic acid). II : Preparative variables affecting microcapsule properties." J. Microencapsul., 7(1) : 25-39

Jalil R. and Nixon J.R. (1990b) "Microencapsulation using poly(L-lactic acid). III : Effect on polymer molecular weight on microcapsule properties." J. Microencapsul., 7(1) : 41-52

Jalil R. and Nixon J.R. (1990c) "Microencapsulation using poly(L-lactic acid). IV : Release properties of microcapsules containing phenobarbitone." J. Microencapsul., 7(1) : 53-66

Jalil R. and Nixon J.R. (1990d) "Microencapsulation using poly(DL-lactic acid). I: Effect of preparative variables on the microcapsule characteristics and release kinetics." J. Microencapsul., 7(2) : 229-44

Jalil R. and Nixon J.R. (1990e) "Microencapsulation using poly (DL-lactic acid). II : Effect of polymer molecular weight on the microcapsule properties." J. Microencapsul., 7(2) : 245-54

Jeyanthi R.; Thanoo B.C.; Mehta R.C. and DeLuca P.P. (1996) "Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery." J. Control. Rel., 38 : 235-244.

Jeyanthi R.; Mehta R.C.; Thanoo B.C. and DeLuca P.P. (1997) "Effect of processing parameters on the properties of peptide-containing PLGA microspheres." J. Microencapsul., 14(2) : 163-174

Kissel T.; Birch Z.; Bantle S.; Lancranjan I.; Nimmerfall F. and Vit F. (1991) "Parenteral depot systems on the basis of biodegradable polymers" J. Control. Rel., 16 : 27-42

Lee V. H. (1991) "Peptide and protein drug delivery." Marcel Dekker, New York

Lewis D.H. (1990) In : Biodegradable polymers as drug delivery systems. Chasin, M., Langer,R. (eds), Marcel Dekker Inc: New York, NY, pp. 1-41

Li W-I.; Anderson K.W.; Mehta R.C. and DeLuca P.P. (1995) "Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/evaporation method." J. Control. Rel., 37 : 199-214

Maa Y-F. and Hsu C. (1996) "Liquid-liquid emulsification by rotor / stator homogenization." J. Control. Rel., 38 :219-228

Maa Y-F. and Hsu C. (1997) "Effect of primary emulsions on microsphere size and protein-loading in the double emulsion process." J. Microencapsulation, 14(2) : 225-241

Makino K.; Oshima H. and Kondo T. (1986) "Transfer of protons from bulk solution to the surface of poly(L-Lactide) microcapsules." J. Microencapsulation, 3 : 195-202

Mason-Garcia M.; Vaccarella M.; Horvath J.; Redding T.W.; Groot K.; Orsolini P. and Schally A.V. (1988) "Radioimmunoassay for octapeptide analogs of somatostatin : measurement of serum levels after administration of long-acting microcapsule formulations." Proc. Natl. Acad. Sci., USA, 85 : 5688-5692

Mehta R.C.; Jeyanthi R.; Calis S.; Thanoo B.C.; Burton K.W. and DeLuca P.P. (1994) "Biodegradable microspheres as depot system for parenteral delivery of peptide drugs." J. Control. Rel., 29 : 375-384

Mehta R.C.; Thanoo B.C. and DeLuca P.P. (1996) "Peptide containing microspheres from low molecular weight and hydrophilic poly(d,l-lactide-co-glycolide)." J. Control. Rel., 41 : 249-257

Nihant N.; Ch. Shugens, Ch. Grandfils, R. Jerome and Teyssie, Ph (1994) "Polylactide microparticles prepared by a double emulsion/evaporation technique. I. Effect of primary emulsion stability." Pharmaceutical Research 11(10) : 1479-1484

Niu C.H. and Chiu Y.Y. (1998) "FDA perspective on peptide formulation and stability issues." J. Pharm. Sci. 87(11) : 1331-1334

Ogawa Y.; Yamamoto M.; Okada H.; Yashiki T. and Shimamoto T. (1988a) "A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acids." Chem. Pharm. Bull. 36 : 1095-1103

Ogawa Y.; Yamamoto M.; Okada H.; Yashiki T. and Shimamoto T. (1988b) "Controlled release of leuprolide acetate from polylactic acid or copoly(lactic/glycolic) acid microcapsules : Influence of molecular weight and copolymer ratio of polymer." Chem. Pharm. Bull. 36 : 1502-1507

Ogawa Y.; Okada H.; Yamamoto M. and Shimamoto T. (1988c) "In-vivo release profiles of leuprolide acetate from microcapsules prepared with polylactic acids or copoly(lactic/glycolic acids) and in-vivo degradation of the polymers." Chem. Pharm. Bull., 36 : 2576-2581

Ogawa Y. (1997) "Injectable microcapsules prepared with biodegradable poly(alpha-hydroxy)acids for prolonged release of drugs." J. Biomater. Sci. Polym. Ed., 8(5) : 391-409

Okada H. and Toguchi H. (1995) "Biodegradable microspheres in drug delivery." Critical Reviews in Therapeutic Drug Carrier Systems, 12(1) : 1-99

Okada H.; Ogawa Y. and Yashiki T. (1987) "Prolonged release microcapsule and its production." US Patent 4,652,441

Ruiz J.M.; Tissier B. and Benoit J.P. (1989) "Microencapsulation of peptide : a study of the phase separation of poly(DL-lactic acid-co-glycolic acid) copolymers by silicone oil." Int. J. Pharm., 49 : 69-77

Salthouse T.N. and Matlaga B.F. (1975) "Approach to the numerical quantitation of actue tissue response to biomaterials." Biomater, Med. Devices Artif Organs, 3 47-56

Sanders L.M.; Kell B.A.; McRae G.I., and Whitehead G.W. (1986) "Prolonged controlled-release of nafarelin, a leutenizing hormone-releasing hormone analogue, from biodegradable polymeric implants : influence of composition and molecular weight of polymer." J. Pharm. Sci., 75 : 356-360

Shugens Ch.; Laruelle N.; Nihant N.; Grandfils Ch.; Jerome R. and Teyssie Ph. (1994) "Effect of emulsion stability on the morphology and porosity of semicrystalline poly l-lactide microparticles prepared by w/o/w double emulsion-evaporation." J. Control. Rel., 32 : 161-176

Sintzel M.B.; Merkli A.; Tabatabay C. and Gurny R. (1997) "Influence of irradiation sterilization on polymers used as drug carriers - A review." Drug Dev. Ind. Pharm. 23(9) : 857-878

Tabata Y. and Ikada Y. (1988) "Macrophage phagocytosis of biodegradable microspheres composed of L-lactic acid/glycolic acid homo- and copolymers." Journal of Biomedical Materials Research, 22 : 837-858

Visscher G.E.; Pearson J.E.; Fong J.W.; Argentieri G.J.; Robison R.L.; Maulding H.V., and (1988) "Effect of particle size on the in vitro and in vivo degradation rates of the poly(DL-lactide-co-glycolide) microcapsules." Journal of Biomedical Materials Research, 22 : 733-746

Visscher G.E.; Robison R.L.; Maulding H.V.; Fong J.W.; Pearson J.E. and Argentieri G.J. (1985) "Biodegradation of and tissue reaction to 50:50 poly(DL-lactide-co-glycolide) microcapsules." Journal of Biomedical Materials Research, 9 : 349-365

Vrancken M. N. and D. A. Claeys (1970) US Patent 3,526,906

Washington C. (1990) "Drug release from microdisperse systems : A critical review." Int. J. Pharm., 58 : 1-12

Williams D.F. and Mort E. (1997) "Enzyme-accelerated hydrolysis of poly glycolic acid." J. Bioeng, 1 : 231-238

Yamaguchi K. and Anderson J. (1993) "In vivo biocompatibility studies of medisorb 65/35 D,L-lactide/glycolide copolymer microspheres." J. Control. Rel., 24 : 81-93

Zaikov G.E. (1985) "Quantitative aspects of polymer degradation in the living body." J. Marcromol. Sci. Rev. Macromol. Chem.Phys., C25 (4) : 551-597