1. Introduction

1.1. Parenteral controlled release systems

The development of novel technologies in the area of drug discovery such as genetic engineering, combinatorial chemistry, and high-throughput screening leads to numbers of drug candidates with high therapeutic potentials. However, majority of them have poor oral absorption or a short biological half-life. The emerging of these complex active ingredients has drawn considerable attention on development of novel techniques to deliver them in an effective and efficient way. Parenteral controlled release of drugs represents one of such approach. After one administration, these systems can maintain the drug in the desired therapeutic range for days, weeks, months, and for some products, even years. Compared to conventional oral dosage forms, they offer several advantages including:

i. Increase of bioavailability: Parenteral drug administration overcomes the absorption barrier and enzymatic barrier imposed by gastrointestinal tract.

ii. Long release period: The drugs are released over extended period and hence improve the patient's compliance and reduce the need for follow-up care.

iii. Constant drug plasma concentration: The drug levels are maintained within a desired range (Fig. 1) and total dose can be reduced.

iv. Localized delivery of drug: The product can be administrated directly at the site where drug action is needed and hence systemic exposure of the drug can be reduced.

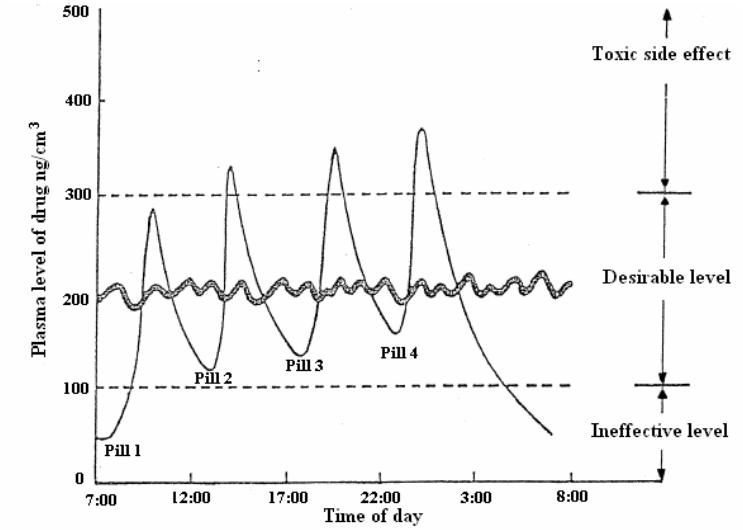


Fig. 1. Plasma drug concentration versus time profile of a drug when administered orally as compared to a parenteral controlled release drug delivery system (Graham, 1978).

The intravenous, subcutaneous, intramuscular, intraperitoneal, and intrathecal routs are examples of parenteral administration. However, the major administration routes of parenteral controlled release systems are subcutaneous and intramuscular, which results in products such as oil solutions (Larsen et al, 2002a,b), emulsions (Florence and Whitehill, 1982; Collins-Gold et al., 1990), liposomes (Sharma 1997), micelles (Alkan-Onyuksel, 1994), implants (Ueno et al., 1982), and microparticles (Herrmann and Bodmeier, 1995a).

The administration of these systems, in which drug is either dispersed or dissolved in vehicles, results in the formation of a depot at the site of injection. This depot acts as a drug reservoir that releases the drug molecules continuously at a rate determined by the characteristics of the formulation. The nature of the vehicle, the physicochemical properties of the drug, and the interaction of drug with vehicle and tissue fluid, determine the rate of drug absorption and hence the duration of therapeutic activity.

1.1.1. Polymeric controlled release systems

The development of polymeric controlled release system introduced a new concept in drug administration. These systems are less complicated and with high stability. Encapsulation in the polymer carrier eliminates the degradation of drugs; moreover, the release profile of the drugs can be controlled by properly choosing polymers.

Polymeric release systems can be classified into reservoir and matrix systems (Fig. 2). In reservoir systems the drug forms a core surrounded by polymer that forms a diffusion barrier. The drug release is by dissolution into the polymer and then diffusion through the polymer wall. In polymeric matrix systems the drug is dispersed or dissolved in a polymer. The drug release can be diffusion, swelling, and/or erosion controlled. Compared to reservoir systems, matrix systems are easier to be manufactured because they are homogeneous in nature and they are also safer since a mechanical defect of the reservoir device rather than matrix device may cause dose dumping. However, if polymer matrix is non-degradable, the constant release profile is difficult to be achieved with matrix system (Fung and Saltzman, 1997).

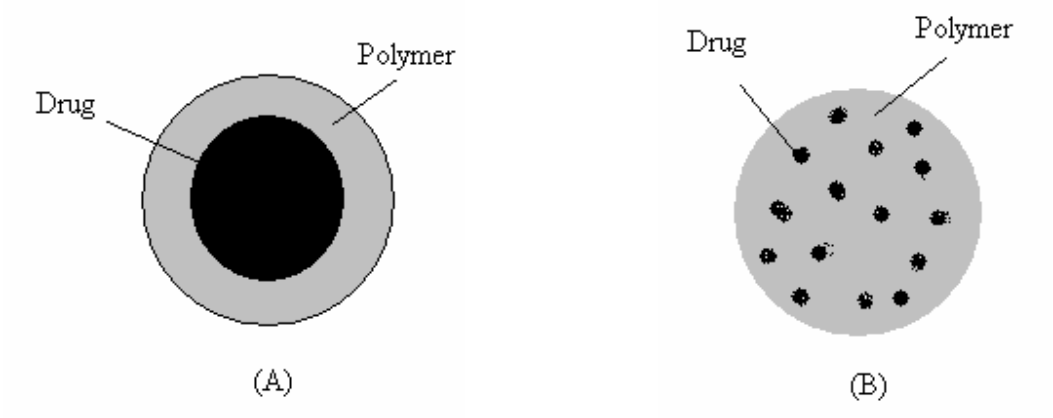


Fig. 2. Polymeric delivery systems; (A) Reservoir systems; (B) Matrix systems.

The polymer used in controlled release systems could be biodegradable or non-biodegradable. The first polymeric controlled release devices is a reservoir system based on non-biodegradable polymer silicone rubber (Folkman et al., 1966). The major disadvantages of such system lay in that the surgery is required to take these polymers out of the body once they are depleted of the drug. Biodegradable polymers alleviate this problem. These polymers used for the fabrication of delivery systems are eventually absorbed or excreted by the body. This avoids the need for surgical removal and thus improves the patient acceptance (Danckwerts and Fassihi, 1991).

1.1.2. Biodegradable polymers

A polymer based on the C-C backbone is non-biodegradable. Biodegradable polymers commonly contain chemical linkages such as anhydride, ester, or amide bonds. These polymers degrade in vivo either enzymatically or non-enzymatically to biocompatible and non-toxic byproducts. These can be further metabolized or excreted via normal physiological pathways. Biodegradable polymer not only have been extensively used in controlled delivery systems, but also extended to medical devices (Leenslag et al., 1987), wound dressing (Hubbell, 1996), and for fabricating scaffolds in tissue engineering (Shi et al., 1996). In addition to biocompatibility, biodegradable polymers also offer other advantages including thermoplasticity, high mechanical strength, and controlled degradation rate.

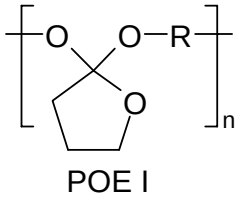
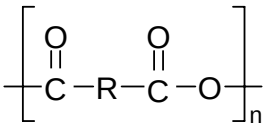
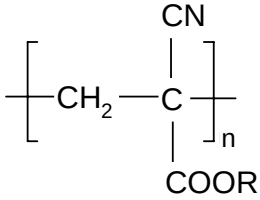
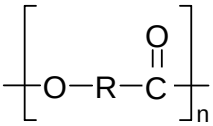
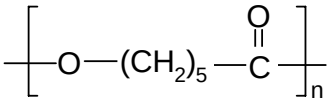
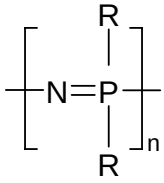
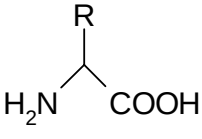
Biodegradable polymers are formed in nature or synthetic. The investigation of natural biodegradable polymer as drug carrier has been concentrated on proteins and polysaccharides (Table 1). Natural biodegradable polymers are attractive because they are natural products of living organisms, readily available, relatively inexpensive and capable of multitude of chemical modifications (Sinha and Trehan, 2003).

Table 1 Nature biodegradable polymers

Proteins	Globulin, Gelatin, Collagen, Casein, Bovine serum albumin, Human serum albumin
Polysaccharide	Starch, Cellulose, Chitosan, Dextran, Alginic acid

Synthetic biodegradable polymers have gained more popularity than natural biodegradable polymers. The major advantages of synthetic polymers include high purity of the product, more predictable lot-to-lot uniformity, and free of concerns of immunogenicity. In the past 30 years, there are numerous biodegradable polymers are synthesized. Most of these polymers contain labile linkages in their backbone such as esters, orthoesters, anhydrides, carbonates, amides, urethanes, etc. The synthesis, biodegradability, and application of these polymers have been well reviewed (Table 2).

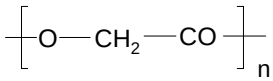
Table 2 Synthetic biodegradable polymers

General name	Structure	Literature
Polyorthoesters	 POE I	Heller et al., 2002
Polyanhydrides		Kumar et al., 2002
Polyalkylcyanoacrylates		Vauthier et al., 2003
Polyester (PLA and PLGA)		Jain, 2000d
Polycaprolactones		Sinha et al., 2004
Polyphosphazenes		Lakshmi et al., 2003
Pseudo-polyamino acids		Bourke and Kohn, 2003

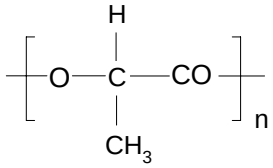
1.2. Polyester PLA and PLGA

Among the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) such as poly(lactide) (PLA) and its glycolic acid copolymer poly(lactide-co-glycolide) (PLGA) are most commonly used as drug carrier due to their excellent biocompatibility and biodegradability and mechanical strength (Anderson and Shive, 1997; Jain, 2000d; Athanasiou et al., 1996). They can degrade by non-enzymatic hydrolysis of the ester backbone in body fluid. The degradation products (i.e. lactic and glycolic acids) are metabolic compounds (Göpferich, 1996a). Most importantly, they have been approved by the United States Food and Drug Administration (FDA) for drug delivery.

PLG



PLA



PLGA

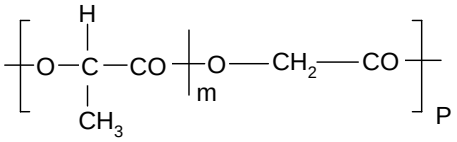


Fig. 3. Chemical structure of polyester PLA, PLG, and their copolymer PLGA

High molecular weight PLA and PLGA are obtained by the ring-opening polymerization of cyclic diesters, which are lactides and glycolides (Gilding and Reed, 1979). Comparing to the conventional step growth polymerization technique, this process could achieve higher molecular weight and easier control of the polydispersity and endgroup functionality of the PLGA (Jain, 2000d).

There are four established suppliers of GMP-grade PLA and PLGA: Purac (Purasorb); Birmingham Polymers (Lactel); Boehringer Ingelheim (Resomer); and Alkermes (Medisorb).

1.2.1. Degradation and erosion of the PLGA

The biodegradation of the PLGA occurs through random hydrolytic chain scissions of the swollen polymer. The cleavage of ester bond linkages yields carboxylic end groups and hydroxyl groups. The formed carboxylic groups then could catalyze and accelerate the hydrolysis of other ester bonds, a phenomenon referred as autocatalysis.

The polymer erosion in delivery devices is the degradation of polymers to water-soluble fragments, accompanied by a progressive weight loss of the matrix. Generally, the polymer erosion could be classified into two mechanisms, namely surface or bulk erosion (Göpferich, 1996b). In the case of surface erosion, the degradation is faster than the water diffusion. Thus the degradation and erosion take place on the surface of the matrix; in contrast, with bulk erosion, the water penetration is faster and the degradation and erosion affect all the polymer bulk (Fig. 1). PLGA are bulk erosion polymers. The weight loss of the polymer devices doesn't take place at the beginning of the degradation of the PLGA. Accompanying with the produced water-soluble oligomers, significant weight loss occurs when the molecular weight of the PLGA reaches certain threshold (Husmann et al., 2002).

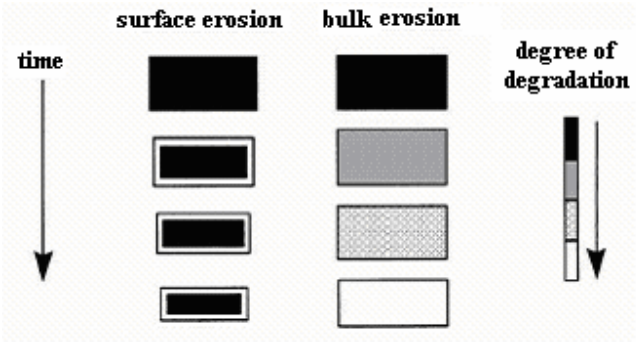


Fig. 4. Schematic illustration of the changes a polymer matrix undergoes during surface erosion and bulk erosion (Burkersrodaa et al., 2002).

The heterogeneous degradation of the large size PLGA devices has been reported recently (Okada, 1997; Vert et al., 1991; Li, 1999). It was found that after subcutaneously implantation,

the molecular weight of the outer phase of the polymer plate was higher than that of the inner phase. The outer phase was solid but the inner phase was sometimes semisolid (Okada, 1997). During the degradation of the polyester, the formed soluble acidic oligomers inside the matrix may not easily diffuse out, which may lead to a more acidic microenvironment inside the matrix. Therefore the autocatalysis is more prominent in the bulk than at the surface, which leads to the surface-interior differentiation.

1.2.2. Characterization of polyesters

The physical and chemical characteristics of PLGA such as molecular weight, glass transition temperature, and copolymer ratios are crucial to the biodegradation behavior of the polymers. At present, a numerous of analytical methodologies are introduced to characterize these properties, which then provide the potential clue to understand, predict and eventually modify the release behavior of the systems.

1.2.2.1. Molecular weight and polydispersity

The most commonly used method to analyze the molecular weight of PLGA is the size exclusion chromatograph (SEC).

SEC, commonly referred to as gel permeation chromatography (GPC), involves the separation of macromolecules according to their size. Using GPC, the molecular weight (number-average molecular weight M_n and weight-average molecular weight M_w) and polydispersity revealing the molecular weight distribution could be calculated by following equations:

$$M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{\sum w_i}{\sum w_i / M_i} \quad (1)$$

$$Mw = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum w_i M_i}{\sum w_i} \quad (2)$$

$$PD = M_w / M_n \quad (3)$$

Where n_i = number of moles of the *i*th component, M_i = the molecular weight of the *i*th component, w_i = weight of the *i*th component. The M_n is the simple arithmetic mean, representing the total weight of molecules present divided by the total number of the molecules. In M_w, it emphasizes the mass of the molecules so that the heavier molecules are more important. M_w is generally larger than M_n. Polydispersity reveals the molecular weight distribution. The higher the polydispersity, the wider the molecular weight distribution is (Hausberger and DeLuca, 1995).

1.2.2.2. Optical activity and crystallinity

Lactic acid contains an asymmetric carbon atom and has two optical isomers. PLA can exist in two stereo forms, optically active form (L-PLA) and optically inactive racemic form (D,L-PLA). 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. Hence the use of D,L-PLA is preferred over L-PLA as it enables more homogeneous dispersion of the drug in an optically inactive polymer matrix.

Crystallinity of the PLGA can be determined by DSC or X-ray diffraction. It is directly related to the molecular weight, type, and molar ratio of the copolymer component. PLGAs prepared from L-PLA and PGA are crystalline copolymers while those from D,L-PLA and PGA are amorphous in nature. It was reported that PLGAs containing less than 70% glycolide are amorphous in nature (Jain, 2000d).

1.2.2.3. Glass transition temperature (T_g)

T_g is the temperature at which the polymers change from glassy state to rubbery state. At this point, the mechanical behavior of the polymer changes from rigid and brittle to tough and leathery (plastic behavior). The T_g of PLGAs is commonly above the physiological temperature of 37 °C, which gives them enough mechanical strength to be fabricated into delivery devices. The determination of T_g is often performed by differential scanning calorimetry (DSC). The T_g of the PLGA decreases with decrease of lactic acid content in copolymer and with decrease in their molecular weight (Jamshidi, 1988).

1.2.2.4. Copolymer composition

Analysis of copolymer composition of PLGA can be accomplished by ^{13}C nuclear magnetic resonance spectroscopy (NMR). The lactic/glycolic acid ratio is determined from the integrated signal ratio between the methyl moiety of lactic acid and the methylene moiety of glycolic acid in NMR (Hausberger and DeLuca, 1995).

1.2.3. Influence of PLGA characters on polymer degradation

The biodegradable profiles of PLGA could be influenced by the physical and chemical properties of the polymer and the additives or encapsulated drugs in the polymer matrix. In general, the degradation rate of the PLGA decreases with the decrease of

- polymer molecular weight (Park, 1994; Jalil and Nixon, 1990a)
- initial crystallinity (Li, 1999)
- lactic/glycolic copolymer ratio (Ogawa et al., 1988)
- glass transition temperature (Omelczuk and McGinity, 1992)
- hydrophilicity of the polymer
- The degradation rate increase with incorporation of
- acidic or basic compounds (Bodmeier et al., 1989; Cha and Pitt, 1989; Mauduit et al., 1993; Li et al., 1996)

1.3. Biodegradable polymer devices

1.3.1. Implants

Implants are generally cylindrical devices injected into the subcutaneous tissue with a large bore needle (trocar). Comparing to other controlled delivery devices, these formulations have the advantages that they can be designed and prepared easily and with high uniformity. A major disadvantage is the need of a painful injection for their application (Göpferich, 1996). Implants are manufactured by standard techniques such as extrusion (Rothen-Weinhold, 1998), melt compression (Negrín et al., 2004) or injection molding (Rothen-Weinhold et al., 1999). In these cases the drug is distributed in a melt of biodegradable polymer and subsequently the device is

formed. For most biodegradable polyesters temperature between 80 and 175 °C is necessary for manufacturing implants. This high temperature may affect the polymer stability and encapsulated active ingredients especially for labile macromolecules (Rothen-Weinhold et al., 1999).

Despite these disadvantages, implants are useful tools for systemic and local drug delivery. For example, sustained systemic delivery of LH-RH agonists (Fukazaki et al., 1991), somatostatin analogue (Rothen-Weinhold, 1998), and sustained local delivery of anesthetics (Masters et al., 1993), antibiotics (Li et al., 2002) have been achieved using biodegradable polymer implant systems. PLGA based implant systems for controlled delivery of LH-RH agonists are available on the market under the brand names Zoladex® and Profact® for treatment of prostate cancer. They are manufactured by a melt extrusion method.

1.3.2. Microparticles

Microparticles based on biodegradable polymer have been extensively investigated as controlled release delivery system over the past three decades. In recent years, a continued interest in PLGA microparticles has been triggered by their application for the controlled release of macromolecular drugs. Microparticles are spherical particles with size ranging between 1 to 1000 μm. For injection purpose, microparticles smaller than 125 μm are preferred (Jain, 2000d). In contrast to implant, microparticles can be injected through normally used needle and thus alleviate the pain during injection.

Biodegradable microparticles can be prepared by several methods, but the most widely used techniques are phase separation (coacervation), spray drying, and solvent evaporation. The manufacturing method has much influence on the structure and release properties of the microparticles. General requirements for microparticle preparation include:

- Maintain the stability of the encapsulated active ingredient
- Obtain optimal drug loading, high encapsulation efficiency and yield
- Get desired drug release profiles and low initial release
- Produce microparticles with free flowability and syringeability
- Involve a simple, reproducible, and scaleable process

1.3.2.1. Phase separation (Coacervation)

Coacervation technique relies upon a decrease of the polymer solubility by addition of a non-solvent (Lapka et al., 1986; Ruiz and Benoît, 1991). At a certain point, two liquid phases are formed: a polymer rich coacervate and a supernatant liquid phase depleted in polymer. The drug dissolved or dispersed in polymer solution is thereby entrapped by coacervate. Microencapsulation by coacervation proceeds along three main steps: (i) phase separation of the polymer solution; (ii) adsorption of the coacervate around the drug particles; (iii) solidification of the microparticles (Nihant et al., 1995).

In a general procedure, biodegradable polymer is dissolved in an organic solvent (e.g. dichloromethane, ethyl acetate, or acetonitrile). Lipophilic drugs are dissolved or dispersed in the polymer solution; hydrophilic drugs are dissolved in water and then dispersed in the polymer solution (w/o emulsion) or are dispersed directly as solid powder. Coacervation is induced by gradually introducing an organic non-solvent (silicon oil is generally used). The phase separated system is then transferred to another organic non-solvent such as heptane to harden the microparticles. The microparticles are washed and harvested by filtration or centrifugation and dried (Fong, 1979; Sanders et al., 1984; Thomasin et al., 1997).

The major disadvantages of coacervation method include the difficulties in scaling-up and the use of large amount of organic solvent.

1.3.2.2. Spray drying

This method includes dissolving the biodegradable polymer in volatile organic solvents, such as dichloromethane or acetone. The drug is either dissolved or dispersed in polymer solution. This solution or dispersion is then atomized in a heated air. The solvent instantaneously evaporates resulting in the formation of solid microparticles (Pavanetto et al., 1993; Witschi and Doelker 1998; Berkland et al., 2001). In comparison phase separation method, spray drying is easily to be scaled up. However, there are large amount of product loss during process, which result in a low yield. Moreover, spray drying is also prone to produce agglomeration of microparticles.

A novel spraying into liquid nitrogen technique has been developed to encapsulate recombinant human growth hormone (rhGH) (Cleland and Jones 1996; Johnson et al, 1997). This so-called cryogenic process involved mixing the zinc stabilized rhGH with the PLGA/dichloromethane solution (Cunningham et al., 1991). The resulting suspension was then sprayed into a container filled with frozen ethanol that was overlaid with liquid nitrogen. Upon warming, solid ethanol liquefies and extracts the polymer solvent and hardens the microparticles (Fig. 5). This technique avoids the contact with water during the microencapsulation process, and thus avoiding the surface denaturation of the protein that happens at interface of organic and water (Cleland and Jones 1996).

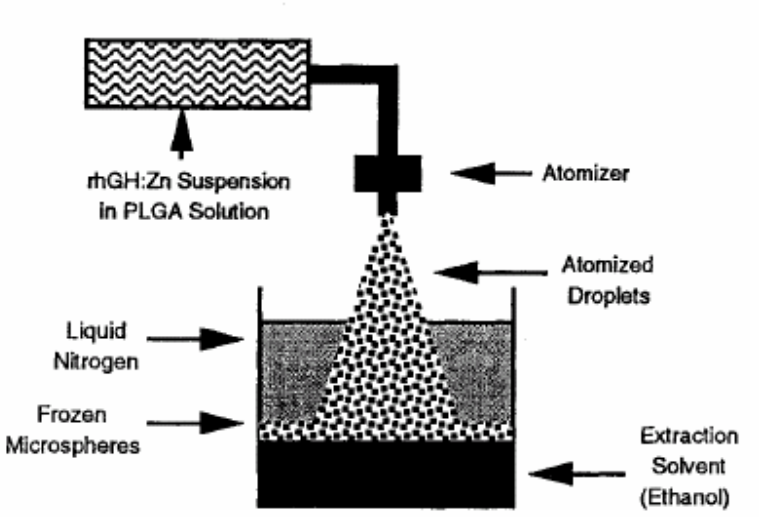


Fig. 5. Spray into liquid nitrogen technique (Johnson et al., 1997).

1.3.2.3. Solvent evaporation

Solvent evaporation method is the most popular technique of preparing microparticles (Bodmeier and McGinity 1987; Herrmann and Bodmeier, 1995b). It involves emulsifying a drug-containing organic polymer solution into a dispersion medium. Depending on the state of drug in the polymer solution and the dispersion medium, it can be further classified into oil in water (o/w), water in oil (w/o), and water in oil in water (w/o/w) double emulsion method (Fig. 6).

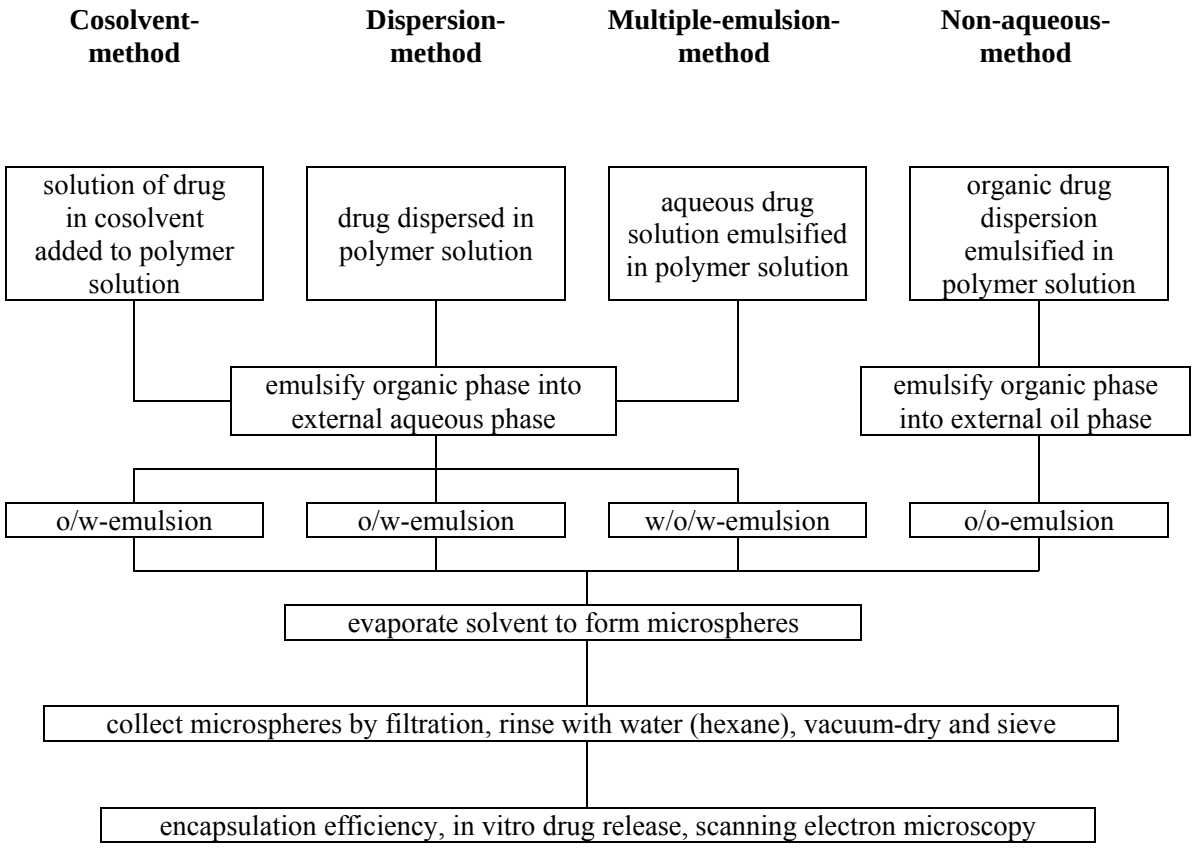


Fig. 6. Schematic diagram of the microparticle preparation with various types of solvent evaporation methods (Hermann and Bodmeier, 1998).

o/w method

In this technique, drug is dissolved or dispersed in a solution of the polymer in a water-immiscible and volatile organic solvent. This solution or dispersion is emulsified into an aqueous phase. The organic solvent then diffuses into the aqueous medium and finally evaporates into the air. After complete evaporation of the solvent, the solidified microparticles are obtained by filtration and drying (Bodmeier and McGinity 1987).

In solvent evaporation method, poly(vinyl alcohol) (PVA) is widely used as an emulsifier in the external aqueous phase. Dichloromethane is the most commonly used solvent to dissolve the polymer. Recently, ethyl acetate with low toxicity (class 2 comparing to dichloromethane class 4

in ICH guidelines) has been evaluated as an alternative (Witschi and Doelker, 1997). Due to the high water miscibility of the ethyl acetate, a pre-emulsification step is necessary to prepare microparticles (Freytag et al., 2000).

o/w solvent evaporation method has been successfully used to encapsulate lipophilic drugs into microparticles (Cavalier et al, 1986; Urata et al., 1990)

o/w cosolvent method

To encapsulate hydrophilic drugs, a dispersion of drug in polymer solution has to be used in o/w solvent evaporation method. Recently, a so-called cosolvent method has been developed to help dissolving hydrophilic drug in polymer solution. It uses a more polar cosolvent (methanol) mixed with dichloromethane to dissolve hydrophilic drugs followed by emulsification into aqueous medium (Herrmann and Bodmeier, 1998). Using this technique leuprolide acetate, a hydrophilic nona-peptide, has been incorporated into PLA and PLGA microparticles (Woo et al., 2001).

o/o method

In this method, drug and polymer are dissolved in a water miscible solvent (acetonitrile). The solution is emulsified into oily phase in presence of emulsifier (Span 80) to form oil in oil emulsion. The organic solvent is extracted by oil and microparticles are harvested by filtration. The existing oil on the microparticles is washed out by volatile solvents (*n*-hexane). This method is also referred as water-in-oil (w/o) method (Jalil and Nixon, 1990b). However, when hydrophilic drugs are encapsulated into microparticles with this method, a high initial release may happen.

w/o/w double emulsion method

An aqueous drug solution or dispersion is mixed with PLGA solution in an organic solvent (dichloromethane or ethyl acetate). This water in oil emulsion is further mixed with large amount of water containing an emulsifier (e.g. PVA) to form water in oil in water (w/o/w) emulsion, which is then subjected to solvent removal by extraction or/and evaporation. The solidified microparticles are collected by filtration or centrifugation followed by washing and drying. The

first mixing step in this technique is generally achieved by using homogenizer or sonicator, and second mixing could be carried by homogenization or high stirring.

Unlike w/o method, w/o/w double emulsion technique is suited to encapsulate water soluble drugs. The biodegradable microparticles loaded with peptide (Okada, 1997; Herrmann and Bodmeier, 1995a), protein (Esposito et al., 1996; Han et al., 2001; Li et al., 2001), DNA (Dunne et al., 2003), vaccine (Singh et al., 1995; Sah et al., 1995), and some small molecules (Erden and Celebi, 1996; Mandal and Tanjarla, 1996) have been prepared successfully with this method. The advantages of this technique include high yield and encapsulation efficiency; however, it involves complex and multi-step manufacturing procedures.

1.4. In situ forming devices

Because of various disadvantages of classical microencapsulation methods (e.g., complicated processes, difficult scale-up and encapsulation efficiencies), over the last decade, an increasing numbers of new in situ forming devices have been developed as alternatives. These systems consist of drug loaded biodegradable polymer semisolid, solution, or dispersion. After s.c. or i.m. injection, the polymer solidifies and generates solid depots in situ, which release drug in a controlled manner. In situ forming devices could be classified into in situ forming implants and in situ forming microparticles depending on the resulted depot.

In situ forming devices offer several advantages in comparison to conventional biodegradable polymeric controlled delivery devices. Firstly, the application of these formulations is less invasive and painful compared to implant, which improves the patient compliance. Secondly, from a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing cost (Packhaeuser et al., 2004).

The idea in situ forming devices should fulfil several requirements including:

- Low viscosity of the systems
- Loading with drug should be achievable by simple mixing
- Excipients used should be biodegradable and/or biocompatible
- Good drug stability in the system
- The system should present a minimum initial release of the drug.

1.4.1. In situ forming implants

In situ forming implants base on a drug-containing polymer semi-solid or solution, which after administration into the body undergo chemical or physical change to form a unit implant for the controlled drug delivery. The concept of in situ forming implants was originated by Dunn in the early 1980s (Dunn et al., 1990). They used injectable depot system loaded with antibiotics for local treatment of periodontal diseases. Thereafter, approval of Eligard® containing drug leuprolide acetate by FDA spurred interest in ISI system development. Numbers of ISI systems have been reported. According to different formation mechanism, ISI can be classified into 6 categories.

- Thermoplastic pastes
- In situ cross-linked polymer systems
- In situ forming cubosomes
- Thermally induced gelling systems
- pH induced gelling systems
- In situ polymer precipitation

1.4.1.1. Thermoplastic pastes

Polymers with low melting point could be injected into body as a melt and form depot upon cooling to the body temperature. The melting point or glass transition temperature of the polymers should range from 25-65 °C and the intrinsic viscosity of the polymers should range from 0.05 to 0.8 dl/g (25 °C) (Bezwada, 1995). Polymers with an intrinsic viscosity below 0.05 dl/g fail to release drug in a sustained manner, in contrast, the high intrinsic viscosity above 0.8 dl/g of polymer cause to bad injectability (Bezwada and Arnold, 1997). Before injection, the polymers are gently heated above their melting point. The drugs are mixed with molten polymers without application of solvents.

Original thermoplastic pastes are prepared from monomers such as D,L-lactide, glycolide, dioxanone, ε-caprolactone, trimethyl carbonate. For example polycaprolactone (Winternitz et al., 1996) and triblock copolymers PLA-PEG-PLA (Zhang et al., 1996) have been investigated as thermoplastic pastes for controlled delivery of antitumor agent paclitaxel. The disadvantage of

these polymers is the high injection temperature above 60 °C that may lead to a painful administration. Additionally, the drug release rate from these polymers is generally very slow.

The emerging of the bioerodible semi-solid polymer poly(orthoesters) (POE) has brought a solution to the problems. Low molecular POEs have low softening temperature in the range of 35 to 45 °C and they are semi-solid at room temperature (Packhaeuser, et al., 2004). The incorporation of drug in this system can be achieved by simple mixing at room temperature without using any organic solvent. Another feature of POEs is they degrade by surface erosion rather than bulk erosion in the case of PLGA. Thermoplastic pastes prepared with POEs own several advantages such as free of elevated temperature during injection, the low initial release, and faster drug release compared with original thermoplastic pastes (Schwach-Abdellaoui et al., 2002).

1.4.1.2. In situ cross-linked polymer systems

In these systems the formation of solid polymers or gels are achieved by in situ cross-link of the introduced macromers. The initiation of the reaction includes photon absorption or ionic interaction between multivalent cations and anionic macromers.

A photopolymerizable biodegradable hydrogel has been used for local drug delivery in the control of wound healing (Hubbell, 1996). This system consists of macromer, terminally diacrylated ABA block copolymers of lactic acid oligomers (A) and polyethylene glycol (PEG) (B). The introduction of PEG is to increase the water solubility of the macromer and the resistance to cell adhesion on the free surface. Oligomers of lactic acid are to provide nonenzymatic degradability of the systems. Acrylated polymerization was selected to provide for in situ photopolymerizability. 2,2-Dimethoxy-2-phenylacetophenone was dissolved in *N*-vinylpyrrolidinone (NVP) used as a photoinitiator. Exposing the aqueous solution of macromers and initiator to the light source resulted in a hydrogel network for local drug delivery (Sawhney et al., 1993).

The advantages of this system include that photoinitiated reactions provide rapid polymerization rates at physiological temperature; moreover, because the initial materials are liquid solutions, the systems are easily placed and subsequently reacted to form a polymer matrix of the required dimension (Hatefi and Amsden, 2002).

Alginates are natural block-copolymers of β-D-mannuronic acid and α-L-guluronic acid. They form 3-dimensional hydrogel matrix upon contact with divalent cations such as calcium ions. Calcium-crosslinked alginate gels have shown good mechanical properties even in low concentrated polymer solution and they can entrap drug physically and sustain their release. Because of the high $CaCl_2$ concentration in human eye, this hydrogel system has also been used in ophthalmic drug delivery (Cohen et al., 1997).

Despite these applications, the cross-linked polymer systems have certain disadvantages. The use of free radical initiator may cause the risk of tumor promotion and the concern of degradation of the therapeutic agents (Packhaeuser et al., 2004). In the case of calcium-crosslinked alginate gels, the potential immunogenicity and long term in vivo degradation requirement of the hydrogel limit its use (Suzuki et al., 1998).

1.4.1.3. In situ forming cubosomes

Acylglycerols (e.g. Glyceryl monooleate) are esters of glycerol and fatty acids. They are commonly kwon as amphiphilic lipids (polar lipid). In presence of water, these compounds form various liquid crystals. The appearance of the different phases is related to the structure properties of the lipid, temperature, incorporated drug, and the amount of water (Engstrom, Engstrom, 1992). For example, when the water content is about 0-5% (w/w), Glyceryl monooleate (GMO) forms reverse micellar phase, followed by a lamellar phase (~5-20% w/w water), and finally forms the cubic phase (35% w/w water) (Ganguly and Dash, 2004). This cubic phase consists of a three-dimensional lipid bilayer separated by water channels. It is gel with a high viscosity (Fig. 7).

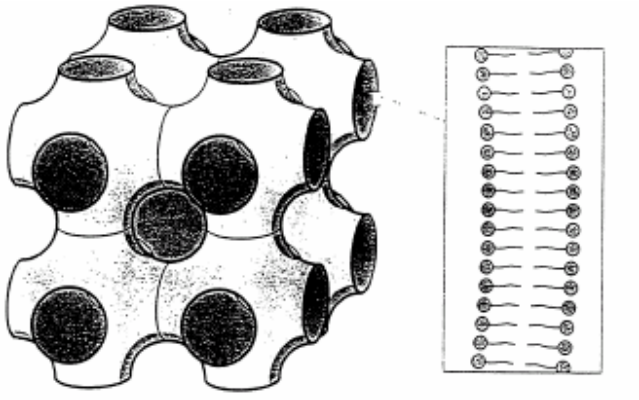


Fig. 7. Three-dimensional structure of glyceryl monooleate-water cubic phase with inset showing the lipid bilayer (Shah et al., 2001).

This gel formation has been employed to develop in situ forming systems for controlled delivery of both lipophilic drugs (e.g. levonorgestrel and thinly estradiol) (Gao et al., 1995a, b) and hydrophilic drugs (e.g. insulin) (Sadhale and Shah, 1999a, b). In situ forming cubosome system has been also used to reduce the initial release from microparticles and pH induced gelling systems (Ganguly and Dash, 2004).

The advantages of the system include the low cost of the lipid comparing to other synthetic polymers and the biodegradability of the formulation. Biodegradation occurs through the action of lipases. However, this approach also has certain disadvantages. The stability of the oils is a major issue that need to be addressed (Hatefi and Amsden, 2002). This system is not suitable for drugs which may interfere with the cubic phase such as lidocaine (Wyatt and Dorschel, 1992; He and Craig, 1999).

1.4.1.4. Thermally induced gelling systems

These systems base on polymers that undergo abrupt changes in solubility in response to the variation in environment temperature. One example of such polymer is Poloxamer 407, also known as Pluronic F 127 that is an ABA triblock polymer consisting of poly(oxyethylene) and poly(oxypropylene) units. It exhibits a rather sharp lower critical solution temperature (LCST) below 37 °C. Poloxamer 407 forms a liquid in an aqueous medium at room temperature but transforms into a reversible semi-solid gel-like structure at 37 °C above a critical concentration

of 20% (Krezanoski, 1980; Mortensen and Pedersen, 1993). Poloxamer 407 has been studied in series of papers for controlled delivery of several drugs such as mitomycin C (Miyazaki et al., 1992), and some peptides (Johnston et al., 1992; Bhardwaj and Blanchard, 1996). However, the application of high concentration of Poloxamer 407 solution leads to notable cytotoxicity (Müller et al., 1997).

Triblock copolymer PEG-PLA-PEG has been also used in thermally gelling system. They are claimed to have good biodegradability and biocompatibility (Bae and Kim, 1993; Youxin and Kissel, 1993). The aqueous solution of polymer exhibits low viscosity at room temperature but once inside the body, it turns into a gel with very high viscosity. One product OncoGel®, which contains paclitaxel and use PEG-PLA-PEG copolymer as drug carrier was launched by MacroMed for intratumoral injection, followed by a continuous release over 6 weeks (Jeong et al., 1997, 1999). The release mechanism of lipophilic drugs from PEG-PLA-PEG hydrogels is described as diffusion-controlled release followed by a degradation/diffusion release, but the hydrophilic drugs show a mainly diffusion-controlled release profile (Jeong et al., 2000). The advantage of this system includes the low viscosity of the formulation and the free of organic solvent. However, similar to other gel forming system, when a polymer system undergoes gelation, it contracts and reduces its volume dramatically. This may lead to diffusion of encapsulated drug out of the gel and hence a high initial release.

1.4.1.5. pH induced gelling systems

The abrupt change of polymer solubility in aqueous medium could also be achieved in response to the change in environmental pH.

Chitosan, poly(N-deacetyl glucosamine), is a biodegradable polycationic polymer with low toxicity (Ganguly and Dash, 2004). It is obtained by the alkaline deacetylation of chitin that is a polysaccharide found in the exoskeleton of crustaceans and insects (Romoren et al., 2002). Chitosan is insoluble at neutral and alkaline pH values. In acidic mediums, the amine groups in chitosan will be positively charged and its solubility increases. Acidic solutions of chitosan when subjected to alkaline pH form viscous gels. The in situ gel formation has been used for controlled delivery of several drugs via oral or parenteral routes (Miyazaki et al., 1988; Ganguly and Dash 2004).

A polymer complex of polyethylene (PEG) and polymethacrylic acid (PMA) or polyacrylic acid (PAA) has also been investigated as a pH sensitive gelling system (Joshi, 1993). The complex is barely soluble at low pH aqueous solution but forms a clear solution at the presence of ethanol. After administration of the drug-containing solution into physiological environment, ethanol diffusion and physiological fluid (neutral pH, at which polymer is insoluble) infusion lead to the gel formation. As time proceeds, the drug release from the gel with the dissociation of the complex into water soluble polymers. These polymers were expected to be excreted renally because of their low molecular weight and high water solubility. This system has been used for controlled delivery of small molecular drug (Hagelund et al., 1996) and macromolecular drug (Joshi et al., 1998). Ethanol is added to dissolve the polymer complex. At low ethanol concentration, the solution is turbid, and at high ethanol content, the solution is too viscous to be injected. 50% of ethanol was employed to compromise the complex solubility and viscosity of the formulation (Hagelund et al., 1996). NMP was also used to replace the ethanol which caused the local intolerance at high concentration (Joshi et al., 1998).

1.4.1.6. In situ solvent removal systems

The method of in situ solvent removal systems relies on the phenomena of solute precipitate from the solution by solvent removal. It could be further classified into three techniques. Atrigel® and Alzamer® techniques base on biodegradable PLA/PLGA polymer. Saber® technique uses non-polymer sucrose acetate isobutyrate as drug carrier.

Atrigel®

This technique was developed by Atrix laboratories and had been patented by Dunn and co-works in 1990 (Dunn et al., 1990; Dunn and Tipton, 1997). This in situ forming implant system is prepared by dissolving biodegradable polymer PLA/PLGA in biocompatible organic solvent. Therapeutics are either dissolved or suspended in PLGA solution. When liquid composition is injected intramuscularly or subcutaneously, the organic solvent diffuses into body fluid and water penetrates into organic solution. This leads to the phase separation and precipitation of polymer forming a solid polymeric implant at the site of injection. Based on Atrigel® technique, Eligard® containing the LH-RH agonist leuprolide acetate and PLGA dissolved in *N*-methyl-2-

pyrrolidone (NMP) was launched to the market for management of prostate cancer (Ravivarapu et al., 2000a, b; Chu et al., 2002; Dunn et al., 2003).

NMP is so far the most popular organic solvent used in preparation of PLGA in situ implants. It is classified as a Class 2 solvent in ICH guideline. However, the evaluation of injection of Atrigel® in rhesus monkeys showed only a mild local tissue responds without any visual inflammatory effects such as swelling, redness or irritation (Royals et al., 1999). Besides NMP other organic solvents such as dimethyl sulfoxide (DMSO) (Lambert and Peck, 1995), glycerol formal (Chern and Zingermann, 1999), have been also used in formation PLGA implants in situ.

A general problem relating to Atrigel technique is the high initial release of the drug during the first day after injection into body, which can lead to tissue irritation and systemic toxicity. This high initial release is mainly attributed to the high porosity of the formed polymer matrix. NMP the solvent used to dissolve PLGA is completely water-miscible. Upon contact with aqueous environment, NMP diffuses rapidly into water, which resulted in fast polymer precipitation and formation of porous polymer matrix (Graham, et al., 1999). High polymer concentration that retards the polymer precipitation could reduce the initial release of the formulation (Lambert and Peck, 1995).

Alzamer®

Alzamer® technique from ALZA cooperation differs from Atrigel technique at the use of organic solvents with low water miscibility rather than completely water-miscible solvents (NMP). Brodbect et al have demonstrated that solvent with water solubility less than 7% (e.g. benzyl benzoate) could significantly reduce the initial release (Brodbeck et al., 2000). In a comparison of in situ implants prepared with NMP, triacetin, and ethyl benzoate, partially water-miscible solvent such as triacetin and ethyl benzoate led to slower polymer precipitation with lower initial release due to the formation of denser matrix structure than complete water-miscible solvent NMP (Brodbeck et al., 1999a).

Saber®

This system consists of sucrose acetate isobutyrate (SAIB), organic solvent, and active ingredient. SAIB, a sucrose molecule esterified with two acetic acid and six isobutyric acid moieties, is a highly lipophilic, water insoluble sugar and exists as a very viscous liquid. It is orally non-toxic and is currently used to stabilize emulsions in food industry (Tipton, 1999). SAIB forms a low viscous solution when dissolved in organic solvents such as ethanol, NMP, triacetin, and propylene carbonate, which is mixed with active ingredient prior to administration. Once placed into the body, the solvent diffuses away and results in a highly viscous depot for controlled delivery of active ingredient. The release of active ingredient from the formulation could be influenced by the concentration of SAIB, type of solvent, and additives used.

The system has been used for controlled delivery of gonadotropin hormone (Burns et al., 2000) and other small molecules such as diclofenac, and theophylline (Tipton, 1999), The advantages of this technique over Atrigel include the small amount of solvent used in the system (approximately 15 to 35%), the low viscosity of the formulation, and the low manufacturing cost due to lack of expensive polymers (Matschke et al., 2002). However, the biodegradability and biocompatibility of SAIB in vivo needs more investigation.

1.4.2. In situ forming microparticles (ISM)

Despite the numbers of application of the ISI systems, they have several disadvantages:

- Generally ISI systems involve viscous semi solid polymer or polymer solution, which may lead to bad injectability of the formulation.
- After administration, they form in situ a single polymer implant. The presence of them may lead to intolerability of the patients.
- The morphology of the resulting implants is liable to the injection techniques and physiological condition of the injection site. The drug release behavior of the systems may be inconsistent and irreproducible.

To address these drawbacks, ISM systems are recently being developed. The systems are based on an emulsion of a biodegradable polymer solution and a continuous oil or aqueous phase. Once injected, polymer solution droplets solidify to form microparticles in situ. The advantages of ISM-systems include a lower viscosity of the emulsion when compared to the pure

polymer solution and thus a reduced pain during injection; a reduced initial rapid release because of the presence of an external oil phase; in addition, ISM are multiparticulates and could thus minimize the variation of single unit implant morphology and provide a more consistent and reproducible drug release.

So far there are two techniques to prepare ISM. One technique is based on preformed emulsion; the other is based on emulsification directly prior to injection.

1.4.2.1. Preformed emulsions

This technique was developed by Jain et al (Jain et al., 2000a, b, c). A solution of PLGA and active ingredient in the cosolvent of triacetin and PEG 400 and Tween 80 (oil phase 1) is added drop wise under homogenizing to Miglyol 812/Span 80 solution (Oil Phase 2), which forms an oil in oil (o/o) emulsion stabilized by Tween 80 and Span 80. After injection into the body, triacetin diffuse into body fluid, which leads to the PLGA precipitation and form the microparticles in situ.

This ISM system has been used for controlled delivery of proteins (Jain et al., 2000a). A controlled release of protein over 14 days was achieved. The drug release increased with decreasing PEG 400 concentration and increasing drug loading.

There are two problems related to the preformed emulsions technique. Firstly, the manufacturing procedure is relatively complex. Secondly, the stability of emulsion over long period of storage is questionable.

1.4.2.2. Emulsification directly prior to injection

Recently, a novel ISM technique based on emulsification directly prior to injection was developed by Bodmeier et al (Bodmeier, R., 1997, Kranz, H., 2001). This ISM system is comprised of a drug containing polymer solution (internal polymer phase) which is emulsified into an external continues phase (oily or aqueous) using two syringe system. The resulting emulsion is administrated immediately after the preparation. Upon contact with aqueous medium (body fluid or release medium), diffusion of the PLGA solvent into the aqueous environment causes the polymer precipitation and the formation of solid microparticles in situ. By using this

technique, the emulsions are prepared by simple mixing steps, and it is free of stability concerns of the emulsion during the storage.

Due to its well proved biodegradability, PLGA is a preferred polymer used in this technique. Depending on the different external phases, the ISM system could be further distinguished into oil in oil (o/o) and oil in water (o/w) ISM system. In o/o ISM system, PLGA is dissolved in a completely water-miscible solvent (NMP and DMSO) or a partial water-miscible solvent (triacetin), in which drugs are dissolved or dispersed. This solution is emulsified into an oily external phase such as sesame oil or peanut oil containing Span 80 as stabilizer to form an o/o emulsion. In o/w ISM system, PLGA is dissolved in a partial water-miscible solvent such as ethyl acetate, triacetin, or propylene carbonate, in which drug is mixed. This solution is emulsified into an aqueous external phase containing Lutrol F68 as stabilizer to form o/w emulsion. Once injected, the organic solvents diffuse directly or through the oil or aqueous barrier into the aqueous medium, which induces the PLGA precipitation and form microparticles in situ.

1.5. Controlled delivery of polypeptides

Due to the recent advance of recombinant DNA technology, a variety of polypeptide (proteins and peptides) have been produced and investigated for therapeutic applications. These molecules are an integral part of the body and carry out all important physiological process. With this new class of therapeutic agents, many diseases could be treated more effectively such as cancers, autoimmune diseases, memory impairment, mental disorders, hypertension and certain cardiovascular and metabolic diseases (Banga and Chien, 1988; Sinha and Trehan, 2003). Recent statistics show that the FDA approved 130 biotechnology derived protein medicines and vaccines, 70% of which were approved in the last 6 years. Currently, over 350 biopharmaceuticals are in clinical trials (Crommelin et al., 2003).

Despite the promising therapeutic efficiency, the formulation development for delivery of polypeptides is difficult and challenging. Polypeptides have very poor oral bioavailability as a result of their instability to protease in gastro-intestinal tract and the poor absorption due to their large size and hydrophilic nature. Other administration methods such as rectal, buccal, transdermal, nasal, and ocular also result in poor bioavailability mainly due to the poor permeability of polypeptides through the mucosa and skin (Ho, et al., 1992, Cullander, et al.,

1992, Okada, 1997). Parenteral injection is so far a preferred method to deliver polypeptides; however, due to their short plasma half-live, daily multiple injections are needed. This reduces the patient's compliance, increases the need for medical supervision, and causes the drug plasma concentration fluctuation.

The biodegradable polymer based parenteral controlled delivery systems meet the formulation challenge of the polypeptides. These systems could provide sustained release of macromolecules ranging from a few days to several months, which avoid the daily multiple injections. Furthermore, encapsulation in polymer matrix also protects such labile molecules from the degradation by enzymes. The application of these controlled delivery systems result in numerous products in the market (Table 3). These products all base on polyester PLGA due to the favorable regulatory status of the polymer. The most often used formulation is microparticles, but in situ forming devices are emerging currently. The indication of the products is generally for the treatment of hormone related diseases.

Table 3 PLGA depot formulations on the market

Product	Active ingredient	Distributor	Indication	Formulation
Lupron Depot®	Leuprolide acetate	TAP	Prostate cancer	Microparticles
Nutropin Depot®	Growth Hormone	Genetech	Pediatric growth hormone deficiency	Microparticles
Suprecur® MP	Buserelin acetate	Aventis	Prostate cancer	Microparticles
Decapeptyl®	Triptorelin pamoate	Ferring	Prostate cancer	Microparticles
Sandostatin LAR® Depot	Octreotide acetate	Novartis	Acromegaly	Microparticles
Somatuline® LA	Lanreotide	Ipsen	Acromegaly	Microparticles
Trelstar™ Depot	Triptorelin pamoate	Pfizer	Prostate cancer	Microparticles
Profact® Depot	Buserelin acetate	Aventis	Prostate cancer	Implant
Zoladex®	Goserelin acetate	Astrazeneca	Prostate cancer	Implant
Eligard®	Leuprolide acetate	Sanofi-Synthelabo	Prostate cancer	In situ forming implant

1.6. Difficulties in the controlled delivery of polypeptides

1.6.1. Protein instability

Unlike conventional small molecules, proteins are relatively large molecules with complex structures. They posse structure based on secondary structure (alfa-helices, beta-sheets and random coil areas), tertiary structure (folding of the secondary structure into complicated three-dimensional structure) and in some cases quaternary structure (where different monomer interact) structure (Crommelin et al., 2003) with labile bonds and side chain. The disruption of these structures or the labile bonds and side chain can cause the activity loss or immunogenicity.

The instability of proteins can be separated into chemical and physical models. The chemical instability refers to modifications involving covalent bonds within protein molecule. The cause of chemical instability includes deamidation, oxidation, and disulfide bond shuffling. The physical instability includes protein unfolding, undesirable adsorption to surfaces, and aggregation (Chi et al., 2003; Manning et al., 1989). The chemical and physical instability also interact with each other. The perturbation of secondary or tertiary structure can lead to exposure of previously buried amino acids and facilitating their chemical reactivity; alternatively, chemical changes can lead to loss of conformational stability.

The inactivation of protein in PLGA controlled release devices may happen during manufacture, storage, and release. The presence of water/organic solvent interface during the manufacture and acidic pH environment inside the polymer matrix due to trapped acid PLGA degradation products during release all could contribute to the inactivation of the proteins (Sah, 1999; Morlock et al, 1997; Zhu et al., 2000). To preserve the stability of protein, several strategies including addition of stabilizing additives or modification of fabrication process for delivery systems have been devised. It was reported the addition of protein such as bovine serum albumin (BSA) (Sah, 1999b), and Sugars (trehalose) (Cleland and Jones, 1996) could alleviate the inactivation of specified protein during emulsification. Inorganic bases or salts such as magnesium hydroxide (Zhu and Schwendeman, 2000), sodium bicarbonate (Agrawal and Athanasiou, 1997) have been proposed to inhibit acid-induced protein degradation during release.

1.6.2. Initial release

The drug release from controlled delivery systems can usually be divided into an initial release (burst) phase followed by a slower continuous release phase. The initial release, which plays an important role in the therapeutic efficacy and toxicity of formulations, is normally defined as the amount of drug released during the first 24 hours. Depending on the drug, a lower or higher initial release is required in order to initiate a pharmacological effect; an undesirable high initial release may exhaust the encapsulated drug from microparticles too rapidly and even cause toxicity problems. Thus, the proper control of the initial release phase is one of the key issues in the design of controlled delivery systems.

The initial release is commonly attributed to the release of drug located close to the surface of microparticles or to easily accessible drug, for example in the case of highly porous microparticles (Batycky et al, 1997; Cohen et al., 2002, Herrmann and Bodmeier, 1995b, Ravivarapu et al., 2000c). It is related to the microstructure (porosity) of the microparticles. A high porosity correlates with a large surface area and rapid penetration of the release medium and consequently a high initial release.

A popular method for the preparation of microparticles is the solvent evaporation method (Bodmeier and Chen, 1989). The drug is dissolved, dispersed or emulsified into an organic polymer solution. After emulsification of the polymer phase into an external (mostly aqueous) phase, the solvent diffuses into the external phase and evaporates; simultaneously, the external phase (nonsolvent) penetrates into the surface of the polymer droplets. The precipitation kinetics of the polymer droplets determines the microstructure of the solidified microparticles. In general, a rapid polymer precipitation causes the formation of porous microparticles because of a hardening of the droplets with still significant amount of solvent present, while a slower precipitation results in more concentrated polymer droplets and denser microparticles (Schlicher et al., 1997, Graham et al., 1999). Although having the same final composition, different microstructures of the particles with different release profiles can be obtained.

From a mechanistic point of view, many similarities exist between the formation of filtration membranes by phase inversion and microparticles by the solvent evaporation method. The polymer precipitation in ternary systems of polymer, solvent and nonsolvent in the formation of phase inversion membrane has been investigated in detail (Strathmann and Kock, 1977; Kimmerle and Strathmann, 1990; Wienk et al., 1996). The resulting membrane structure was

mainly dependent upon the velocity of the solvent/nonsolvent exchange. A fast solvent/nonsolvent exchange led to the formation of membranes with a thin surface skin and a highly porous finger-like inner structure; in contrast, a slow exchange resulted in a thicker surface skin and a denser spongy inner structure.

The PLGA precipitation kinetics in an in situ PLGA implant system was examined by McHugh et al., (Graham et al., 1999, Brodbeck et al., 1999a). Parameters leading to a faster PLGA precipitation (e.g., PVP or water addition to the PLGA solution or a decreasing polymer concentration) resulted in more porous implants and a high initial release. In contrast, a slower precipitation resulted in denser sponge-like implant with a low initial release.

1.6.3. Tri-phasic release

The drug release from PLGA microparticles commonly has a tri-phasic pattern in vitro (Ruiz and Benoît, 1991; Diwan and Park, 2001; Igartua et al., 1998) as well as in vivo (Cleland et al, 1997a). A fast initial release phase (burst) is followed by a second slow release phase lasting days or weeks and a third rapid release phase. Except for vaccine immunization (Cleland et al., 1997b), a tri-phasic drug release is generally not desirable for most drug therapies. Insufficient drug may be delivered to maintain the desired pharmacological effect in the slow release phase and toxicity problems may occur during the rapid initial and third release phases because of too high drug levels.

After the initial drug release, a diffusion-controlled slower release phase follows. Finally, when the molecular weight of PLGA approaches a certain lower threshold, the weight of the microparticles decreases rapidly and an erosion-controlled rapid release phase occurs (Husmann et al., 2002). Recently, the formation of a nonporous film around the microparticles after incubation in the release medium has been reported (Wang et al., 2002). The decreased surface porosity of the microparticles led to a reduced drug permeability and resulted in the slow release phase.

1.7. Leuprolide acetate

Prostate cancer is the most common cancer and the second leading cause in men in the United State (Boring, et al., 1993). For patients with advanced disease, treatment strategies focus on symptom amelioration and disease control to increase symptom free survival by months to years.

Leuprolide acetate is a luteinizing hormone-releasing hormone (LH-RH) agonist, which was firstly synthesized by Fujino et al (Fig. 8). It has been used for treatment of prostate cancer for more than 15 years. The administration of leuprolide acetate has a biphasic effect on the pituitary. It initially stimulates gonadotropin secretion by the pituitary and steroidogenesis in the genital organs; however, on long-term continuous administration it paradoxically produces antagonistic inhibitory effect and the testosterone in men or oestrogen in women drop sharply (Kochhar and Imanidis, 2004). These so called chemical castration effects are due to the downregulation of the receptors that are temporary and reversible. Besides prostate cancer, leuprolide acetate is also indicated for other hormone-dependent diseases such as breast cancer, endometriosis, uterine fibroids, central precocious puberty and adenomyosis (Lemay and Quesnel 1982; Okada, 1997).

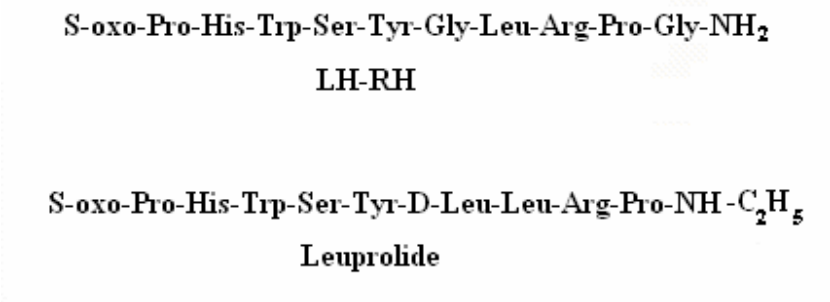


Fig. 8. Amino acid sequence of LH-RH and leuprolide.

As a nona-peptide with molecular weight of 1269, leuprolide acetate has very poor oral absorption and limited bioavailability through other administration routes such as transdermal (Kochhar and Imanidis, 2004), nasal (Adjei et al., 1992), and vaginal (Okada et al., 1982).

Leuprolide was first launched to the market as a daily subcutaneous injection (Sharifi and Soloway 1990). To avoid the daily injection and improve the patient compliance, several parenteral controlled release products appeared on the market including Viadur™, Lupron Depot®, and Eligard®.

Viadur™ (leuprolide acetate implant) is produced by ALZA Corporation utilizing the DUROS® platform. It is a sterile, non-biodegradable, single use system that is designed to deliver leuprolide acetate continuously over 1 year for the treatment of advanced prostate cancer. The implant consists of a cylindrical titanium alloy reservoir capped on one end by a rate-controlling membrane and at the other end by a diffusion moderator containing an orifice through which drug is released from the system. The system is inserted subcutaneously, after 1 year, the implant is removed and replaced with a new one (Wright et al., 2001). The advantage of this system is the long delivery period of the drug, but the insertion and replacement of the implants are painful procedures.

Lupron Depot® was marketed by TAP Pharmaceuticals. It is a sustained release intramascular formulation based on biodegradable polymer (PLGA) microparticles. One injection of the formulation is capable of sustaining required drug level for a period of 1, 3 or 4 months. Microparticles are prepared with modified w/o/w method (Okada 1987, 1991). Leuprolide acetate and gelatin are dissolved in water at about 60 °C. This solution is emulsified into PLGA solution in methylene chloride with a homogenizer. After cooling to 15-18 °C, the w/o emulsion is poured into PVA solution while stirring. The resulting w/o/w solution is stirred to evaporate the solvent and microparticles are filtered and redispersed in mannitol solution. The following lyophilization completes the removal of organic solvent and water. This depot formulation not only improves the patient's compliance but also reduces the needed dose to 1/4 to 1/8 of the injected aqueous solution by sustaining therapeutic drug levels at the target receptor sites (Okada and Toguchi, 1995). However, the multiple and complex manufacturing process leads to the high cost of the products.

Eligard® is injectable in situ forming implant (ISI) formulation based on biodegradable polymer PLGA. The 1, 3, and 4 months sustained release Eligard® formulations are marketed by Sanofi-Synthelabo Inc. utilizing the Atrigel® technique (Dunn et al., 1990, Dunn and Tipton, 1997). Eligard® is supplied in two separate syringes. One syringe contains the PLGA solution in NMP. The second syringe contains the leuprolide acetate. Two syringes are joined and single dose product is mixed until it is homogenous prior to administration (Ravivarapu, et al., 2000a,

b). After injection into the body, the solvent diffuses away and water penetrated into polymer solution, which leads to the precipitation of polymer and resulted in implant depot for sustained release of leuprolide acetate. This system avoids the complex preparation of the formulation and thus reduces the investment and cost; however, they also have some limitations. The high viscosity of the PLGA solution may lead to a painful injection; the surface area of the resulting implant, controlling the drug release, may be variable depending on the injection technique and site; in addition, a high initial release may occur because of the formation of highly porous implants.

1.8. Objectives

The objective of this work was to delivery therapeutic peptides in a controlled manner using biodegradable microparticles and in situ forming microparticles. Particular aims include

Microparticles prepared by the solvent evaporation (cosovlent) method

(i) The identification of key variables affecting the initial release and the encapsulation of the microparticles and to scale-up the lab size standard formulation.
(ii) The investigation of the parameters, which shift the release profile from the tri-phasic to a more continuous release profile.

In situ forming microparticles

(iii) The study of factors influencing the emulsion formation and morphology of the resulting microparticles.
(iv) The development of ISM systems for controlled delivery of leuprolide acetate with different delivery period e.g., 1, 4, and 6 months.
(v) The evaluation of influence of various formulation and processing parameters on the drug release.
(vi) Using a partial water-miscible cosolvent to reduce the initial release from ISM systems.
(vii) The investigation of influence of PLGA type (molecular weight and endgroup functionality) on the leuprolide release from in situ forming microparticle (ISM) systems.