1 Introduction

1.1 Biodegradable drug delivery systems

During the last two decades, significant advances have been made in the controlled release drug delivery of therapeutic agents. Drug release can be controlled by several means, such as diffusion through a rate-controlling membrane or a matrix, osmosis, ion exchange, or degradation of a matrix or a part of matrix [1].

Biodegradation for controlling drug delivery has the advantage that the drug delivery device may not have to be removed from the site of action after drug delivery is completed and the accumulation of polymers in the body during prolonged applications is avoided [2].

Polymers have been used extensively in controlled drug delivery systems. There are non-degradable polymers, those which are not hydrolytically or enzymatically cleaved in vivo, and biodegradable systems available [3].

Biodegradable polymers have been studied extensively for their biomedical applications such as temporary scaffold, temporary barrier for surgical adhesion, and matrices for drug delivery systems. Bioresorbable polymers, which degrade to low molecular weight fragments and are eliminated from the body are preferred for systemic applications [4]. A wide variety of natural and synthetic biodegradable polymers have been investigated for prolonged drug release. However, only a few have reached the stage of clinical experimentations due to the fact that being biodegradable is not sufficient. Many other prerequisites must be fulfilled for clinical use and commercialization as biocompatibility, biofunctionality and stability [5]. Synthetic polymers offer more advantages than natural materials, since they can be tailored to give a wider range of properties and more predictable lot-to-lot uniformity. The most popular and well understood class is the polyesters. Their commercial availability in a

variety of polymer ratios and molecular weights, make them desirable candidates for product development [6].

1.1.1 Poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA)

Aliphatic polyesters such as poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) belong to the most widely investigated polymers because of its biocompatibility, predictability of degradation kinetics, ease of fabrication and regulatory approval by the FDA [7-9]. They are synthesized by ring opening polymerization of corresponding cyclic dimmers, namely lactide diastereomers and glycolide, using Sn octanoate or Sn chloride as the initiator. Since degradation and bioresorption characteristics are very much dependent on chain characteristics and solid morphology, it is important to produce polymers with identical characteristics from batch to batch. Vert et. al founded that polymers initiated by Zn metal and Sn octanoate are different insofar as water uptake, degradation and fate of initiator residues are concerned [8].

The commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to their molecular weights [5, 10].

A broad spectrum of performance characteristics of these polymers can be obtained by careful manipulation of four key variables: monomer stereochemistry, comonomer ratio, polymer chain linearity and polymer molecular weight [7, 11].

The racemic poly(DL-lactide) DL-PLA is less crystalline and lower melting than the two stereoregular polymers, D-PLA and L-PLA. Further, the copolymers of lactide and glycolide are less crystalline than the two homopolymers of the two monomers. In addition, the lactic acid polymer, because of the methyl group, is more hydrophobic than the glycolide polymer [7]. The Tg (glass transition temperature) values range from 40 to 65°C. Poly(L-lactide) has the highest value and the Tg of PLGAs decrease with decrease of lactide content in the copolymer composition and with decrease in their molecular weight [5, 12, 13].

Their primary degradation route is hydrolysis. Degradation proceeds first by diffusion of water into the material (initially into the more amorphous zones) followed by random hydrolysis, fragmentation of the material, and finally a more extensive hydrolysis accompanied by phagocytosis, diffusion and metabolism. The hydrolysis is affected by the size and hydrophilicity of the particular polymer implant, the cristallinity of the polymer and the pH and temperature of the environment [14].

When the polymers are fabricated into controlled drug delivery systems, additional qualities such as surface area, bulk density, surface morphology and particle size are introduced, and many affect both degradation of and drug release from the polymeric system. Hausberger and DeLuca described a characterization program consisting of techniques that include, DSC for thermal analysis; ^{13}C nuclear magnetic resonance spectroscopy (NMR) to determine the comonomer ratio and sequence distribution of copolymers; size exclusion chromatography (SEC) for molecular weight; scanning electron microscopy (SEM) for surface morphology; gas adsorption for determination of specific surface area; tapped bulk volume measurements to determine bulk density of the microspheres; and in vitro analysis of degradation behavior [15, 16].

When the polymers are intended for parenteral administration, the final polymeric devices should meet the pharmacopoeial requirements of sterility. However, the physicochemical liability of the polymers limits most conventional methods to sterilize. Since sterilization by autoclaving induces a degradation of PLGA by hydrolysis and ethylene oxide lead to toxic residues, ionizing irradiation is the most used method [17, 18]. Nevertheless, the effects of γ-irradiation on PLGA showed controversial results, depending on the active ingredient used. Montanari et. al compared the effect obtained by β- and γ-irradiation performed on bupivacaine loaded PLGA microspheres. They founded that the microspheres were more sensitive to γ-irradiation, which caused an increase of the in vitro drug release [19].

1.2 Implantable drug delivery systems

Implantable drug delivery systems, although having the drawback that they need a small surgery to be administered, have been widely investigated because they offer the possibility of high drug levels and sustained release in areas that are usually inaccessible to peripherally administered drug, such as central nervous system, bone tissue and beyond the blood-retinal barrier. A variety of shapes (pellets, beads, rods or cylinders, disks and films) and sizes (from a few millimeters to several centimeters) of implantable drug delivery systems have been developed [20].

According to the mechanism of drug release, Bhagat and Lange classified them in six groups (Table 1). Processes used in manufacturing these devices depend on the type. Membrane-type polymeric devices may be prepared by coextrusion of the drug core and the polymeric membrane, as in the case of silicone capsules. Spherical membrane-coated devices are prepared with conventional pharmaceutical coating equipment. Matrix-type devices are simpler to prepare; techniques include compression under high pressure with or without heat, solvent casting of drug dispersion in polymer solution, melt extrusion and in situ polymerization [21].

Table 1.1 Mechanism of drug release from implantable systems.

Diffusion controlled	- Membrane-permeation controlled - Matrix controlled - Microreservoir dissolution
Chemically controlled	- Bioerosion - Pendent chain
Solvent activated	- Swelling controlled - Osmotically controlled
Externally regulated	- Magnetically controlled - Ultrasonically activated - Thermally activated - Electrically controlled
Self regulated	- Ionic strength / pH responsive - Glucose responsive - Urea responsive - Hapten – antibody interaction
Mechanically activated	- Pumps

Adapted from [21]

Many different implantable systems with biodegradable polymers have been developed by different manufacture techniques.

Compression-heat molding

Yamakawa et al. studied the insulin release from double-layered implants, which consisted of a PLA matrix containing insulin, fabricated by compression of the heated mixture (PLA and insulin, previously triturated and sieved) into a flat-faced disk, at a pressure of 200 Kg/cm^2. Removing an upper punch, PLA powder (heated at 40-50°C) were supplied into the die and re-compressed, to form a double-layered structure on one of the surfaces. In vitro release rates were controlled by changing the amount of PLA used in the layer. From the in vivo tests using diabetic Sprague-Dawley male rats, it was found that the double-layered implants provided a sustained release of insulin during 19 days after subcutaneous implantation [22].

Qian et al. developed a compression heat-molding procedure to fabricate PLGA controlled release drug delivery devices for the local treatment of tumors. The drug delivery devices were designed in the shape of cylindrical millirod (1.6 mm diameter, 10 mm length), which allows them to be implanted by a modified 14 gauge tissue biopsy needle into tumor tissues via image-guided interventional procedures. Trypan blue powder and PLGA microspheres were physically mixed by a vortex. The mixture was placed into a Teflon tube, which in turn was placed inside a stainless steel mold, and a stainless steel plunger was inserted into each end of the Teflon tube, to be compressed for 2 hours at different temperatures. The resulting PLGA millirods presented sufficient mechanical strength, homogeneous agent distribution and reproducible release profiles for controlled release applications [23]. In another study, Qian et al., developed doxorubicin-containing dual-release polymer millirods. Fabricated by applying two additional dip-coating layers on the monolithic millirods. The middle layer was formed by dipping the monolithic PLGA millirod into PEG/PLGA solution in CH_2Cl_2. The burst layer was formed by dipping the millirod into doxorubicin/PEO suspension. The in vitro release studies demonstrated the dual-release kinetics in which a burst release occurs within 2 hours followed by a sustained release over 7–10 days. Both the burst dose and the sustained release rate are independently adjustable by varying the structural composition of the outer and middle layers of the millirods, respectively [24].

Bodmer et al. studied the in vitro release of BSA (bovine serum albumin) from PLGA implants, prepared by mixing of the milled powders and posterior compression (80kN per disk) using a heated (60-70°C) press and flat punches (10 mm). The resulting implants had a height of approximately 2 mm and a weight of 200 mg. The release of BSA was found to increase with the increase in the molecular weight of the polymer, since low molecular weight polymers present a higher number of carboxylic end-groups per unit weight and therefore the interactions of the positively charged protein with the negatively charged cell wall polymer chain increased. Water uptake and pore formation of implants started as early as day one of exposure to phosphate buffer and increased slowly until approximately day 15, when fragmentation and erosion of the polymer were noticeable [25].

Melt extrusion

PLGA implants containing α-melanocyte stimulating hormone analog, Melanotan-I were developed by Bhardwaj et al. For the fabrication, a coarse mix of the peptide with fine granular PLGA (in the presence or absence of additives) was prepared manually a fed into a melt extruder. The operating temperature was maintained at 45–70°C depending upon the minimum temperature required for optimal extrusion of each formulation. The extrudate produced was rod-shaped and about 2 mm in diameter and 1 cm in length. After in vitro release, negligible loss of biological activity of the peptide was found and the profile was characterized by an initial rapid release followed by a secondary phase of slow release and then a tertiary phase of rapid release due to erosion of the polymer [26].

Sanders et al. studied the in vitro and in vivo release of nafarelin (luteinizing hormone-releasing hormone analogue) from PLGA implant prepared by melt extrusion, at 45–80°C. The rods were of approximately 3 mm in diameter; 5 mm length (in vivo), 1,7 mm length (in vitro) and 60 mg weight. The system provided a continuous efficacy in rat for longer than eight months, with partially effective levels of release of nafarelin continuing beyond 15 months [27].

The Durin™ biodegradable implant technology is based on the use of PLGA and PCL polymers. Typically, the drug and excipients are mixed together, and the mixture is formed into a fiber, rod, tablet or pellets by an extrusion or molding process. The implant can be placed by means of a needle or trochar. A commercially-successful biodegradable implant formulation is Zoladex®, which delivers goserelin acetate for the treatment of prostate cancer. Furr et. al founded, in the preclinical studies, a continuous release of the drug over at least 28 days, being highly efficient at inducing regression of sex hormone-responsive prostate and mammary tumors [28, 29].

Solvent casting method

Lu et al. investigated the in vitro degradation of thin PLGA films for application in retinal pigment epithelium transplantation and guided tissue regeneration. The films were manufactured by dissolving PLGA in chloroform. A certain amount of the solution was casted onto a glass coverslip and placed in the fume hood. The samples were air dried and subsequently the remaining solvent was removed under high vacuum. The films were formulated with varying copolymer ratios and thickness levels. Both parameters were seen to have a significant effect on the weight loss and molecular weight loss over 10 weeks of degradation on phosphate buffer. PLGA 50:50 degraded faster than 75:25 films of similar thickness. Increasing the thickness levels from 10 to 100 µm accelerated both the weight and the molecular weight loss over this time frame [30].

Lee et al. patented a biodegradable, locally administrable film for the treatment of periodontitis. The films were prepared by mixing drug-containing PLA or PLGA microspheres and polysaccharides with water to form a hydrogel where the microspheres were suspended. The hydrogel was then put and flattened on an board, covered with a polyester film and compress with a roller to even the thickness. The hydrogel was dried in the air after withdrawn of the polyester film. The resultant film could be cut into the proper size to insert it into the periodontal pocket. In order to increase the maintenance of the film in the

periodontal pocket, an additional coating with aqueous solution of cation salts is possible, either by soaking or by spraying [31].

Extrusion/compression

Hsu et al. Investigated the release of isoniazid (INH), a drug against tuberculosis, from PLGA cylindrical matrices, that were fabricated by two methods. The first method, the dry-mixing method involved the extrusion of micronized drug and polymer particles as rods. In the second technique, the low density polymeric foam method, PLGA solutions in acetic acid or benzene were freeze-dried, the foams were impregnated with aqueous solutions of INH, the water was removed by a second freeze drying and then compressing into matrices by compaction and high-pressure extrusion. In vitro, the dry-mixed matrices released INH more rapidly than the polymeric foam matrices. Matrices prepared by the dry-mixing method appear to segregate drug particles along polymer grain boundaries and thus have a pore diffusion mechanism, while matrices prepared by the foam method entrap drug within the porous structure of foams and thus display a lattice diffusion mechanism [32-34].

Dunn et al. patented a method to elaborate monolithic implants, where a flowable composition of biodegradable, thermoplastic polymer is dissolved or dispersed in a biocompatible solvent (with or without drug). Upon contact with an aqueous medium, the flowable composition solidifies. The different shapes may be obtained by use of a molding or extrusion device designed to provide such shapes as the flowable composition is contacted with the solidification bath [35].

Solid-liquid separation / particulate leaching

A new technique to produce scaffolds for tissue engineering was developed by Tu et. al. A known amount of PLA was dissolved in dioxane to form solutions with desired concentrations. Sieved NaCl powders (200–250 μm) were added, the slurries were maintained at 0°C for over 2 hours to induce the solid-liquid phase separation completely. After the solvent was removed by freeze drying for 3 days, the matrix was put into distilled water to leach out NaCl every 3

hours until no precipitation occurred when some drops of $AGNO_3$ solution were added. They founded that the polymer concentration played a vital effect on the porosity as well as on the mechanical property of the scaffold, and the pore morphology of the scaffold could be controlled by the quenching temperature [36].

1.3 Injectable drug delivery systems

Injectable drug delivery systems possess many advantages, which include ease of application, localized delivery for a site-specific action, prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort. Initial studies examined delivery systems such as:

Emulsions, are used extensively in parenteral products but usually not in long acting formulations because of the stability problems accompanying this dosage form. The possibility of dispersion breakdown or dissolution on the surrounding body fluid has made emulsions a poor choice for long acting formulations.

Liposomes, are not a promising dosage form for long acting formulations as well. Local retention of liposome-entrapped drugs is likely to be longer than that of free drugs, but it may not always be long enough to maintain local therapeutic drug levels, due in part to rapid clearance by macrophages and other cells. Other problems such as stability issues, sterilization problems and often-low drug entrapment, have played an important role in limiting the utility of liposomes.

Biodegradable microspheres, are easy to deliver to the site of action but they have several inherent disadvantages. These include the need for reconstitution before injection, a relatively complicated manufacturing procedure to produce a

sterile, stable and reproducible product, and the possibility of microspheres migration from the site of injection.

Micelles, which are also prone to migration, suffer from the fact that there are a large number of variables, which influence micelle properties. Controlling factors like core block length and corona outer shell length, which significantly influences drug loading and size distribution, at the same time is almost impossible. Furthermore the stability of micelles is highly dependent on their critical micelle concentration (CMC), which is the minimum polymer concentration required to micelle formation. The lower the value of the CMC, the greater the thermodynamic stability of micelles in dilute solutions. Once diluted below the CMC, micelles begin to spontaneously disassemble into single chains. Therefore dilution upon injection, as well as interaction with lipid components in the blood, may result in dose dumping [37].

As an alternative to overcome those problems, biodegradable sustained release injectables formed in situ have been developed.

1.3.1 In situ systems

They can be injected via a syringe into the body and once injected solidify to form a drug depot. This depot act as a drug reservoir that releases the drug molecules continuously at a rate determined to a large extent by the characteristics of the formulation, leading to the prolonged absorption of drug molecules from the formulation [38]. They can be delivered as a solution, which forms a monolithic system in the body (in situ forming implants) or a multiparticulate system (in situ forming microparticles).

1.3.1.1 In situ forming implants

The hardening of the polymer in the body after injection could be induced by solvent exchange, temperature or pH change.

1.3.1.1.1 Solvent exchange

Polymers are dissolved in an organic solvent in which the drug is either dissolved or dispersed, when the liquid composition is injected intramuscularly or subcutaneously, the solvent diffuses into the surrounding aqueous body fluids while water diffuses into the polymer. Since the polymer is water insoluble, it precipitates upon contact with water, resulting in a solid polymeric implant [37]. Many systems have been developed by using different solvents and/or polymers.

Systems with water soluble solvents

Dunn et al. developed a system (Atrigel®), in which PLA or PLGA is dissolved in an organic, water-miscible solvent, as NMP (N-methyl-2-pyrrolidone) or DMSO (dimethyl sulfoxide), in which the drug is either dissolved or dispersed. They stated that the degree of polarity of the solvent should be such that at least 10% solubility in water can be achieved [39-42]. Since this injectable implant system is administered as a liquid, it is reasonable to assume that there is a lag between the injection and the formation of the solid implant. During this lag time the initial burst of drug may exceed the plasma concentration achieved using conventional implant system. Thus, the drug release profile from these systems is characterized by a relatively high initial burst, followed by a more sustained drug release [43].

The polymer concentration and molecular weight, the solvent used, the drug loading and possible additives are factors that can control the burst effect [37].

To meet the requirements for excipients intended for parenteral applications, the solvents have to be biocompatible and non-toxic. In addition the polymers should show good solvent solubility. The viscosity of the resulting solution must be low enough to allow application by syringe. Thus, the search for suitable organic solvents for parenteral use has become a key issue in the pharmaceutical technology, as the number of highly hydrophobic drugs and biodegradable polymers for long term drug delivery increases [39].

Parameters affecting drug release

Dunn et al. founded different rates and duration of release depending upon polymer composition. The polymer solutions were prepared from D,L-lactide, 75:25 poly(D,L-lactide-co-glycolide) or 50:50 D,L-lactide/caprolactone with NMP as solvent, were the drug was dissolved by stirring. The release of doxycycline hyclate from poly(D,L-lactide) was typical of a first-order release and fairly rapid, from poly(D,L-lactide-co-glycolide) was biphasic with an initial burst followed by a very low rate of release until the polymer either hydrates or starts to degrade at which point a second burst effect is observed. The rate of release from the more hydrophobic lactide/caprolactone copolymer is much lower and more sustained [44]. Lambert and Peck also founded that for high molecular weight PLGA in NMP, the initial burst (which was found to be inversely proportional to polymer percent) is followed by zero-order release for up to 2 weeks. A significantly different release pattern is observed with DMSO. With low molecular weight PLGA, higher polymer concentrations in DMSO or NMP could be achieved and in consequence is possible to totally eliminate the initial burst, and have zero-order delivery for at least 1 week. They also stated that the burst effect rises with the capability of the solvent to dissolve the polymer [45]. Ravivarapu et al. described that polymer concentration (40-50%) and drug load (3-6%) did not affect the efficacy of the product, however, the use of a low molecular weight polymer gave a shorter duration of efficacy [46, 47]. Eliaz et al. described a system of BSA (bovine serum albumin) and/or sp55-R (recombinant human soluble p55 TNF receptor) and PLGA dissolved in glycofurol and they proposed that at the initial stages after polymer/protein solution injection into water, the protein and glycofurol diffuse out into the medium. The higher the polymer concentration and viscosity are, the slower the diffusion and the faster the solidification are, which results in a smaller burst. The release rates as well as the extent of the bursts can be adjusted by the co-monomers ratio in the copolymer. Since PLA, due to its additional methyl group, is more hydrophobic than PGA, thus the proportion chosen in the copolymerization is important for determining the crystallinity, solubility and water uptake of the final polymer [48]. They also founded no effect on the

release rate for lower loading (3%) suggesting a degradation dependent release while for loading of 10% and higher, diffusion in addition to degradation affects the release process [49].

In vivo studies

Ravivarapu et al. founded that a suspension system of 75:25 PLGA : NMP 45:55 with 3% leuprolide acetate formulation suppressed serum testosterone levels in rats to castration over a period of 3 months, and produced a similar efficacy in dogs [46, 47, 50]. Eliaz et al. evaluated the utility of the release of sp55-R (soluble form of human p55 TNF receptor) from PLGA and glycofurol implants as a means of protection against the pathological effects of chronic exposure to tumor necrosis factor (TNF). Nude mice bearing TNF-producing tumors exhibit severe cachexia, leading to death. Injection of PLGA formulations containing sp55-R into the tumor-bearing mice prevented body weight loss (body weight increases naturally) and mortality was prevented for the duration of the experiment. The animals also did not show any visible sign of discomfort or overt toxicity while the implant solidified [51].

Jarr et al. investigated the use of a local anaesthetic (lidocaine HCL) for the control of postoperative pain. They dissolved PLGA mixed with PEG-PLG in NMP and 10% lidocaine HCL and injected the formulation into Sprague-Dawley rats, showing a fairly constant release of drug out to 7 days, at which point the level begins to drop and finally tapers off at day 10. The same polymer formulation with 15% drug load also provided sustained serum levels for 7 days, but the area under the curve was 34% higher than for the formulation containing 10% drug [52].

Royals et al. studied the biocompatibility of formulations prepared from 75 :25 poly(D,L-lactide-co-caprolactone) dissolved in either NMP or DMSO when injected subcutaneously and intramuscularly in rhesus monkeys. Since the animals remained healthy and active throughout the study with no changes in food or water consumption, weight loss, or abnormal behavior observed. There were no differences between the two formulations in tissue response, and both

formulations were considered acceptable for use as injectable implant systems [53].

Systems with water insoluble / low soluble solvents

Brodbeck et al. founded that solvents that are very or relatively soluble in aqueous body fluids, promote rapid migration of water into the polymer composition, which results in a burst effect and premature polymer precipitation such that a hardened implant or one with a hardened skin is produced. The inner pores and much of the interior of the polymer containing drug are shut off from contact with the body fluids and a significant lag time. While when a solvent with a solubility in water of less than 7% by weight (e.g. benzyl benzoate) is present in the system, significantly reduce the water uptake. This results in slower release characteristics when compared to implants with NMP as the main solvent [39, 54, 55]. They also proposed the use of an emulsifying agent as water, ethanol, isopropyl alcohol and mixtures thereof, in order to decrease the viscosity of the system and improve the injectability [56].

Wang et al. studied the properties of PLGA / benzyl benzoate or NMP solutions and he founded that degradation occurs during storage at 25°C and 37°C in both solvents, probably due to the presence of moisture in the system [57].

Parameters affecting drug release

The release process is influenced by many factors, as nature of the polymer (molecular weight and viscosity), fabrication techniques, device and geometry. And the release rate are also influenced by polymer/water partition coefficients of the drug, drug diffusion coefficients in water and polymer, and drug solubility in aqueous medium and polymer, which in turn are dependent on the chemical structure and molecular weight of the drug [58].

Chen et al. studied the release of bupivacaine HCL from a system prepared from PLGA 50 :50 and benzyl benzoate (Alzamer ® depot™). It appeared that the higher the drug loading, the more sustained the release , when the same amount of drug was administered. They assumed that the early stage release mechanism might be predominantly diffusion, while at later stages, polymer

degradation might significantly contribute to the release [55]. Shah et al. postulated that when the drug is dispersed rather than dissolved, the release appears to occur not only through partitioning and diffusion through the polymer matrix but also through the porous network of tortuous channels created by the dissolution of dispersed drug. They also investigated the influence of formulation additives on the release, founding that the addition of mannitol appears to impart porosity to the matrix and therefore increases the release rate of myoglobin, this increase is directly related to the mannitol concentration. The addition of a hydrophobic material retarded the release of the hydrophilic protein, while water soluble solids such as sodium chloride or non-ionic surfactant such as poloxamer 188 (Pluronic F-68) enhanced the release. Since macromolecules are too large to diffuse through the polymer, the release occurs via diffusion through the interconnecting channels formed by macromolecule in the matrix. Increased loading provides simpler pathways, lower tortuosity and greater porosity for diffusion and facilitates the movement of water into and protein out of the matrix. Addition of co-diffusants such as mannitol or Pluronic F-68 can alter the structure of the polymer matrix and provide increased diffusion through interconnecting channels for release of the macromolecule [58]. Graham et al. reported that the exact way that the injected solution responds to its physiologic surroundings determines the final depot morphology and, consequently, its drug release characteristics. Conditions leading to rapid phase separation (e.g. increasing the affinity between the solvent and non-solvent) promote the formation of thin skins at the surface of the membrane and finger-like porous cavities in the body of the film. Conversely, changes leading to more delayed precipitation, promote thicker skins and more uniform, spongy sublayers in the body of the film. Initial drug release from injectable drug delivery systems occurs simultaneously with the formation of the device by phase inversion. Therefore, in order to control the drug release characteristics, it is important to quantify the dynamics of in vivo phase separation process. Fundamental parameters of the formation kinetics include the water influx rate and the gelation rate. They investigated the effect that formulation changes have on the phase inversion dynamics and in vitro release of chicken egg

lysozyme from a PLGA 50:50 and NMP or triacetin system, founding that the use of additives that accelerate the solution gelation rate at constant morphology result in high initial release rates, implying that drug diffusion through a tortuous two-phase gelled structure is faster than through a one-phase polymer solution. Conversely, additives that slow the rate of gelation dramatically reduce the initial drug release rate and lead to a more dense sponge like morphology [59, 60].

Brodbeck et al. studied the effect of solvent/non-solvent affinity on phase inversion dynamics, affirming that depots with low solvent/water affinity (PLGA in triacetin or ethyl benzoate) undergo much slower phase inversion than more water soluble solvents (NMP), resulting in a less porous, more fluid, two-phase structure that also releases protein more uniformly [61].

The effect of the cristallinity degree of the depot on the protein release kinetics was studied by DesNoyer et al. They stated that systems with high degrees of cristallinity are characterized by porous morphologies, the drug release profile consist of an initial lag period of slow release, followed by a short period of accelerated release. In contrast, depots with low degrees of cristallinity have dense morphologies and presented a more uniform drug release [60].

In vivo studies

Chandrashekar and Udupa evaluated the release of diclofenac sodium from microspheres and in situ system prepared with PLGA 50:50 and triacetin. The pharmacodynamic and pharmacokinetic studies in the adjuvant-induced arthritic rats showed that the microspheres offered steady therapeutic levels of the drug in the plasma for about 16 days following a single subcutaneous injection. However the in situ system provided a significantly higher maximum plasma concentration and increased inhibition of inflammation, maintained for about 10 days [62].

Brodbeck et al. studied the effect of altering the dynamics of phase inversion of a PLGA solution depot on the sustained release of human growth hormone (hGH) and the impact of adjusting the protein particle composition, by injecting formulations into space of normal rats and monitoring hGH serum levels over

the course of 1 month. The therapeutic protein levels, delivered from a benzyl benzoate/PLGA solution depot, were maintained for a period of 28 days with little or no burst on injection. Lowering the solvent's aqueous affinity slows the phase inversion rate, which in turn produces depot morphologies favorable to prolonged release. The use of an alkyl benzoate in the system substantially reduced injection burst and zinc complexation and particle densification both helped to eliminate the remaining protein burst effect, as a result of a decrease in the dissolution and the water absorption rate, respectively [63].

Sucrose acetate isobutyrate (SAIB)

The SABER Delivery System uses sucrose acetate isobutyrate (SAIB), a fully-esterified, water-insoluble sucrose derivative, as an excipient. It can be formulated as a low- to medium-viscosity liquid by the addition of small amounts of solvents such as ethanol, Miglyol®, ethyl lactate, propylene carbonate, or DMSO, resulting in an easily injectable formulation [64]. On administration, the composition is placed into the body or on a surface, and the solvent dissipates or diffuses away from the system, forming in-situ a highly viscous implant or composition that releases the substance over time. The composition optionally includes additives that modify the properties of the composition as desired. Cleland founded that the addition of PLA to the SAIB system has also shown utility in reducing the initial rate of protein release, perhaps by forming a shell on the surface of the gel [65]. In general, the less water soluble, i.e., more lipophilic, the additive, the more it will decrease the rate of release of the substrate, compared to the same composition without the additive. In one embodiment, it is desirable to use additives that increase properties such as the strength or porosity of the composition [66]. Toxicity, metabolism and pharmacokinetics have been extensively investigated in dogs following oral administration. Fully reversible changes in enzymatic function of the liver were reported indicating no toxicity. Thus SAIB was regarded to be of an acceptable tolerability in dogs. After oral administration to dog, radiolabeled SAIB was metabolized to carbon dioxide and various sucrose esters (urine). Non-metabolized SAIB was also detected in the faeces of the test animals.

Additional experiments concerning the exact mechanism of biodegradation for SAIB after subcutaneous/intramuscular applications are required. An advantage compared to injectable PLA/PLGA based implants and standard microspheres is the small amount of organic solvent (approximately between 15% and 35%) required during the manufacturing process. A lack of published data on the in vivo properties and reproducibility of drug release after subcutaneous/intramuscular injection means that final judgment on the usefulness of SAIB systems in comparison with PLA/PLGA based injectable implants and microspheres cannot yet be made. In addition, it should be realized that stability under applied storage conditions and suitable sterilization methods need further investigation [39].

1.3.1.1.2 Thermal induction

Some polymers undergo sol-gel transition upon injection into the body, as a response to temperature changes. This physical characteristics has been used to develop drug depots [37].

Poloxamer 407 / Pluronic 127

Poloxamer 407 is a biocompatible polymer of low toxicity and weak immunogenic properties. These copolymers are composed of hydrophilic poly(oxyethylene) blocks and hydrophobic poly(oxypropylene) blocks to produce a poly(oxyethylene)- poly(oxypropylene)- poly(oxyethylene) triblock polymer. They posses a reverse-phase thermal gelation property, being a liquid between 5-10°C and gels in situ at physiological temperatures [67]. Paavola et al. investigated the duration of analgesia, drug concentration in plasma and tissue irritability of lidocaine and ibuprofen from injectable poloxamer gel in vitro and after epidural injection to pigs [68]. The formulations reduced the systemic absorption of both drugs, but increased the epidural availability only of lidocaine. Only slight inflammatory changes in the epidural space tissue samples were observed [69].

Recombinant derived interleukin-2 (rIL-2) was also formulated with Pluronic F-127 and used in mice by intraperitoneal injection, providing a sustained drug delivery without systemic side effects [70].
However, the disadvantages are non-degradability of poloxamer polymers and the gel properties (water soluble gel) and drug release kinetics (very rapid) [71].

PEG-PLGA-PEG triblock copolymers

A thermo-responsive hydrogel of PEG-PLGA-PEG triblock copolymers was developed by Jeong et al. It aqueous solution formed a gel at body temperature when subcutaneous administered to rats. The integrity of the polymer remained for more than 1 month with little or no tissue irritation at the injected site even after 1 month [72]. The release rate of the drug can be adjusted by change in parameters such as, hydrophobic/hydrophilic component content, polymer concentration, molecular weight and polydispersity of the triblock copolymer [71]. No organic solvent is required for this system but it presents a high initial burst effect. The polymer biodegrades into non toxic products [37].

Crosslinkable polymers

Dunn et al. have also patented a thermosetting system, which comprises the synthesis of crosslinkable polymers which are biodegradable and which can be formed and cured in situ. The thermosetting system comprises reactive, liquid, oligomeric polymers, which contain no solvents and which cure in place to form solids, usually with the addition of a curing catalyst. The multifunctional polymers useful in the thermosetting system are first synthesized via copolymerization of either D,L-lactide or L-lactide with ε-caprolactone using a multifunctional polyol initiator and a catalyst to form polyol-terminated prepolymers and then converted to liquid acrylic-terminated prepolymers. The liquid prepolymer, when injected, will flow into the cavity or space in which it is placed and assume that shape when it solidifies after 5 to 30 min [40-42, 44, 73]. The advantage of this system is the easy syringeability, but there are some disadvantages, which have limited its application, as a burst effect due to the

lag time for solidification of the polymer which in turn leads to high drug concentration of drug at the site of action that result in the appearance of side effect of the drug. Furthermore, the crosslinking reaction is exothermic which can cause necrosis to surrounding tissues and additionally, the use of benzoyl peroxide as catalyst may induce tumor promotion [37].

1.3.2 In situ forming microparticles (ISM)

Bodmeier et al. developed an alternative method to in situ forming implants. The system consists of an internal, drug-containing polymer-solvent phase (polymer phase) dispersed into an external phase (for example an oil phase). Upon injection of this dispersion, the internal polymer phase releases the drug in a controlled release fashion. NMP, DMSO, 2-pyrrolydone or PEG 400 are solvents for the polymer and peanut oil, an oil for injection, was chosen as a biocompatible external oil phase. This system presents a reduced initial burst release and a lower viscosity, allowing an easier injectability. The preparation process for the system is simple.

Kranz et al. investigated the myotoxicity of in situ forming microparticles system, by measuring the cumulative release of creatine kinase from an isolated rat extensor digitorum longus muscle after injection of a test formulation, founding good muscle compatibility [74].

Jain et al. described another technique for the preparation of microglobules, which forms microspheres at the injection site. A solution of PLGA, triacetin, a model protein, PEG 400 and Tween 80 (Oil phase 1) is added dropwise with continuous homogenization to Miglyol 812-Span 80 solution (oil phase 2), thereby inducing phase separation (coacervation) of PLGA and forming PLGA-protein containing microglobules dispersed in the continuous phase. The system is a dispersion and has a viscous consistency, but is sufficiently syringeable. When the microglobules come in contact with the physiological fluid, they harden to form a solid matrix type microparticles [5, 75, 76]. If only the oil phase 1 is injected, in situ forming implants are obtained and if the total system is then added to a PVA solution, stirred, filter, washed and dried,

isolated microspheres are obtained. Jain compared the delivery of cytochrome c from the three devices, where the burst effect exhibited the following trend: in situ formed implant > in situ formed microspheres > isolated microspheres [77]. Unknown properties, such as stability characteristics at applied storage conditions, as well as the diameter of the particles and the consequent reproducibility of release profile after in vivo injection into companion animals still need to be investigated [39].

1.4 Freeze-drying

The process of freeze-drying or lyophilization is widely used for pharmaceuticals to improve the stability and long term storage stability of drugs that are unstable in solution. It is a drying process where the solvent, normally water, is first frozen and then removed by sublimation in a vacuum environment. Compared to spray-drying, freeze-drying is a low-temperature process and is normally regarded as being less destructive to the product, particularly for proteins. In addition control of sterility and foreign particulate matter is relatively straightforward. Thus, although inherently expensive from the viewpoints of plant cost and energy consumption, freeze-drying is often the processing method of choice for the production of parenteral products [78-80].

Non-aqueous solvents for freeze-drying

The choice of the drug solution to be lyophilized is usually determined by drug stability and solubility requirements and limitations. The majority of the products are lyophilized from aqueous solutions. But sometimes freeze-drying from organic solvents or mixtures of water and organic solvent may offer advantages, such as:

- increase in the rate of sublimation and decrease drying time
- increase chemical stability of the pre-dried bulk solution
- increases chemical stability of the dried product

- facilitates manufacture of bulk solution by increasing drug wettability and solubility in solution
- improves reconstitution characteristics (decrease reconstitution time)
- enhances sterility assurance for pre-dried bulk solution

However, it presents also potential disadvantages such as:

- toxicity concerns
- operator safety concerns due to high degree of flammability or explosion potential
- lack of compendia grades or monographs
- may require special manufacturing facilities/equipment or storage areas
- possess difficult handling properties
- requires high purity solvents with known impurities at low levels
- must reach acceptable residual solvent in final product
- high cost to use
- potential for splash/spattering of product in vial neck
- lack of regulatory familiarity [81]

One such example is a freeze-dried formulation of prostaglandin E-1 (Caverject, Pharmacia & Upjohn, Inc.) which employs a 20% tert-butyl alcohol (TBA)/water cosolvent system in the formulation prior to freeze-drying [82]. Koyama et. al used 5% w/w isopropyl alcohol/water to freeze-dried cefazolin sodium [83]. Porous collagen/chitosan complex sponges for tissue engineering were prepared by lyophilization of a 2% aqueous acetic acid solution [84]. Other solvents as ethanol, acetone, dimethyl sulfoxide, ethyl acetate, 1,4-dioxane or dichloromethane, among others, have used up to 20% as cosolvents in aqueous systems of different drugs [81, 85-87].
Although cosolvents usually improve both stability and solubility, they can cause incomplete freezing and drying stage that involves the simultaneous sublimation of ice and evaporation of liquid residue. The resultant dried product is often very dense and difficult to reconstitute. Tesconi et. al studied the use of organic

solvents that are solids at room temperature to lyophilize hydrophobic and water-sensitive compounds without conventional freeze-drying equipment. They found that the chlorobutanol hemihydrate/dimethyl sulfone eutectic was a suitable medium, since it was rapidly removed, the cakes produced contained less than 1% residual solvent and were rapidly reconstituted [88]. Deschamps et. al obtained highly porous sponges of by freeze-drying a 20% w/w solution of PEOT/PBT copolymers in 1,4-dioxane [89].

Since the quality of a freeze-dried product and the ease with which it can be reconstituted depend upon the morphology which develops during freezing and upon solvent sublimation. Meredith et. al studied the structural evolution during sublimation process of sponges prepared with PLGA 50:50 dissolved in 10% w/v of acetic acid by using cryoenviromental scanning electron microscopy and differential scanning calorimetry (DSC) [90].

Excipients considerations

For freeze-dried products, formulation and process are interrelated. The solid content and the components of the product determine its freezing and freeze-drying process. In addition to the active component or drug, excipients must be added for specific purposes. As an example, if the solid content is small than 2%, usually the structure of the dry product is mechanically not stable. During drying, particles of the product might not adhere to the matrix. The water vapor can move these loose particles and deposit them somewhere in the vial, on the stoppers or even transport them to the vacuum chamber. Therefore, the addition of bulking agents such as mannitol and dextran strengthen cake structure and enhance appearance. Buffers are frequently added for pH control, and salts may be added to yield an isotonic solution [91, 92]. Excipients may also be added to increase collapse temperature, enabling the sublimation process to be performed at as high a temperature as practicable. Collapse temperature can be modified by dextran (-10°C), gelatin (-8°C) and human serum albumin (-9.5°C) among others [79, 93]. In the case of proteins, lyoprotectants are frequently added to enhance stability during the freeze-drying process, as well as during storage. These stabilizing agents are mostly sugars

and polyols such as trehalose, sucrose and mannitol [94, 95]. Rapid freezing, combined with subsequent drying of those excipients, will maintain the excipients in the amorphous state, which will protect the conformational structure of the proteins and inhibit the unfolding and aggregation of such molecules. However, a sugar glass is thermodynamically unstable and will transform to a more stable, crystalline form, leading to destabilization of proteins. The addition of polymers, such as polyvinylpyrrolidone (PVP), inhibit the crystallization of amorphous sugars [96]. Many studies have been performed, in order to find the exact mechanism of protein stabilization. Taylor and Zografi investigated the hydrogen bonding interactions between a variety of glass-forming sugars and PVP [97]. Izutsu et. al founded a loss of β-galactosidase activity with crystal-forming additives [98].

1.5 Research objectives

In situ forming systems

PLGA solutions in biocompatible organic solvents, which are used for the preparation of in situ implant systems, are not stable at room temperature and are therefore stored refrigerated within a syringe. In addition, the drug is stored separately in dry form in a second syringe and is dissolved/dispersed in the polymer solution just prior to injection. With commercial products, this mixing process requires 30-100 cycles (the plungers of the two connected syringes are pushed forward and backward 30-100 times) [99-101].

The idea/question for the present study was: Is it possible to also store the PLGA polymer in solid rather than in dissolved form and then rapidly reconstitute (dissolve) it in the biocompatible organic solvent just prior to injection during the drug mixing step? This would eliminate the prior preparation of the PLGA solution and allow storage at room temperature rather than refrigerated (as presently required for the PLGA solutions).

Therefore, the influence of the following parameters on the dissolution rate of solid PLGA in organic solvents was investigated:

(i) Solvent type
(ii) Polymer type and concentration
(iii) Drug presence and type
(iv) PLGA particle size
(v) Freeze-dried PLGA
(vi) Mixing rate
(vii) Syringe size

Biodegradable implants

Implants are mostly produced by melt extrusion and melt molding in the form of cylindrical polymer rods with embedded drug. Low dose drugs could result in content uniformity problems and high temperatures during the processes could lead to degradation of the drug. Thus, the aim of this study was to develop and characterize an alternative method to prepare PLGA implants without the above mentioned problems. The method selected was based on the lyophilization of PLGA solutions, which resulted in sponge-like implants.