Studies of injectable in situ depot-forming systems for drug delivery

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2012

Liu, H. (2012). Studies of injectable in situ depot-forming systems for drug delivery.
Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/50488>

<https://doi.org/10.32657/10356/50488>

Studies of Injectable in situ Depot-forming Systems for Drug Delivery

**NANYANG
TECHNOLOGICAL
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NANYANG TECHNOLOGICAL UNIVERSITY

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**Studies of
Injectable in situ Depot-forming
Systems for Drug Delivery**

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A thesis submitted to the Nanyang Technological University in fulfillment of
the requirement for the degree of Doctor of Philosophy

2012

ACKNOWLEDGEMENTS

I wish first to express my sincere gratitude for the guidance and support offered by my supervisor, Professor Subbu S Venkatraman, through the course of this project. I especially appreciate his patience, kindness and encouragement.

I would like to thank my TAC members, Prof. Lu Xuehong, Prof. Loo Say Chye Joachim and Prof. Andrew Clive Grimsdale, for their suggestions and cooperation.

Thanks to my FYP students Shu Mei and Tiffany for their hard working and their contributions to this project. Thanks also to Yingying, Esther, Jay, Jen Fong, Xu Wen, James, Ma Zhen, Peng Yan, Wei Li and other friends in the lab for their helping during my project. Thanks to Xia Yun for her training on the cell culture and Averil for her help on the solvent cytotoxicity study. I would also like to extend my appreciation to Phyllis, Serene and Yuan Quan for their schedule meeting arrangement and technical support. Thanks to Siu Din, Yong Li, Vivian, Kek Koon, Boon Sheng, Sandy and Sharon for their help during these four years. I would like to offer my special thanks to Nelson for his generous sharing with me his technical knowledge and his patient guidance on my equipment operation.

I would like to thank my family for their constant support and care. I would also like to thank my boyfriend for his firm support in the past four years.

Finally, I would like to thank Seri funding for financial support.

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List of Abbreviations

PLA	Poly(_{D,L} -lactide)
PLGA	Poly(_{D,L} -lactide-co-glycolide)
PGA	Poly(glycolide)
PEG	Poly(ethylene glycol)
FDA	Food and Drug Administration
RES	Reticuloendothelial system
CMC	Critical Micelle Concentration
I.V.	Intrinsic Viscosity
POEs	Poly(ortho ester)s
Poly(NIPAAM)	Poly(N-isopropylacrylamide)
LCST	Lower Critical Solution Temperature
T _g	Glass Transition Temperature
PEO-PPO-PEO	Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)
DMSO	Dimethyl Sulfoxide
NMP	N-methyl-2-pyrrolidone
NG	Nucleation and Growth mechanism
SD	Spinodal Decomposition mechanism
ISM	<i>In situ</i> Microparticles-forming Drug Delivery System
BB	Benzyl Benzoate
BA	Benzyl Alcohol
EPR	Electron Paramagnetic Resonance Spectroscopy
BT-MRI	Applied Benchtop Magnetic Resonance Imaging

EC	Electrical Conductivity
Metosalt	Metoclopramide Monohydrochloride
Metobase	Metoclopramide Base
ACN	Acetonitrile
PVA	Polyvinyl Alcohol
PBS	Phosphate Buffer Saline
NMR	^1H Nuclear Magnetic Resonance
DCM	Dichloromethane
LA	Lactic Acid
GA	Glycolic Acid
Mw	Molar Mass
HPLC	High-performance Liquid Chromatography
GPC	Gel Permeation Chromatograph
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy

Abstract

In this project, injectable *in situ* depot-forming systems for drug delivery were studied. These systems are composed of a biodegradable polymer poly(_{D,L}-lactide) (PLA) or poly(_{D,L}-lactide-co-glycolide) (PLGA) and a low Mw model drug in an organic solvent. Polymer solution undergoes phase inversion when injected into an aqueous solution and forms depots *in situ*, with the drug trapped inside released over time. Several parameters were studied to understand their effects on the drug release dynamics, including polymer hydrophobicity and Mw, drug type and solvent type. These parameters also affect phase inversion dynamics, which was indirectly studied using solvent release kinetics and depot morphology. Furthermore, biocompatibility of the system was investigated. The results showed that some parameters, such as polymer hydrophobic property and high Mw as well as high affinity of solvent with water, lead to fast phase inversion and a high burst release; as the morphology of the depot formed is a porous solid. When polymers became hydrophilic or hydrophobic solvent was added into the formulation, phase inversion rate was slowed down, resulting in hollow depot structure formation; drug burst release was decreased. Through above studies, the drug release behavior in injectable *in situ* depot-forming systems can be better understood. The results of *in vitro* biocompatibility studies showed that DMSO was more biocompatible than NMP which was used in the commercial products. Based on the results from this work, we can make recommendations for reducing the high burst release and the toxicity of the organic solvent, which are two big problems in these systems.

Chapter 1 Introduction

1.1 Background

Since the appearance of synthetic polymers 70 years ago, people realized that these polymers have potential in therapeutic applications. A great many polymers have been determined to be potential biomaterials which cover a wide range of applications in medical sciences, owing to the great variety of their compositions, structures and properties [1]. In particular, a family of polymers that biodegrade in the body, yielding harmless end-products, has become popular in recent years. Among them, poly(glycolide) (PGA), poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) are well-accepted because of their reputation as safe suture materials and excellent biocompatible and biodegradable properties. They are widely used in drug delivery systems because no surgery is required for their removal since polymers can degrade into soluble, non-toxic oligomers or small molecular products which can then be metabolized from the body.

Of the various drug delivery systems, the field of injectable *in situ* depot-forming drug delivery systems based on water insoluble polymers has grown quickly, especially after the great development of protein therapeutics. Poly(esters) were first introduced into this system by Dunn et al. [2-4]. This system is attractive for parenteral application because it is relatively non-invasive, allows for sustained release of bioactive and may be administered away from a hospital setting.

Such an injectable drug delivery system is based on a mechanism of non-solvent induced

phase inversion. Briefly, a water insoluble biodegradable polymer, dissolved in a biocompatible solvent first along with the desired drug, will precipitate after subcutaneous injection upon contact with non-solvent water and form a solid depot *in situ*, which acts as a reservoir for the drug release for a long time.

1.2 Problem

Injectable drug delivery systems have shown great potential in the area of delivering lower molecular weight drugs and proteins and peptides. Currently, based on this technique, there are a series of EligardTM products (for delivery of leuprolide acetate for prostate cancer) on the market manufactured by Atrix Labs using NMP as solvent. However, successful exploitation of this technology beyond delivery of a few drugs [5-7] has not been reported to date. The limitation of this technique is due to the toxicity of the solvent and the inability to control the release of the bioactive compounds, in particular, the control of the burst release. On the other hand, the release behavior of low molecular weight drugs may be different from large molecular weight drugs, hence the study on the release of low molecular weight drugs in such system is of interest.

Drug release kinetics from such solutions is complicated and not extensively characterized. In particular, one of the biggest challenges in translating this system from the research stage to a clinical application is coping with the high burst release; therefore, the key to the success is to control the initial burst release. In this work, we have studied the effects of various depot variables on control of the burst release, including the study of the effect of the hydrophobicity of the depot-forming polymer, and the use of a co-solvent that mitigates the rate of phase inversion. Neither factor has been studied extensively or

rationalized to date. The choice of the co-solvent is also dictated by the toxicity of the main solvent used; with a co-solvent the range of drugs and of polymer types that may be used is expanded.

1.3 Hypotheses and Objective

We hypothesize that manipulation of depot characteristics (polymer hydrophobicity, Mw, solvent type and drug type) have significant effect on the overall drug release profiles and depot cytotoxicity. In particular, certain characteristics will also influence the so-called burst release from an injected depot.

In order to test these hypotheses, the relationships between the depot variables (as mentioned above) and the drug release kinetics were explored. Methods to monitor the drug release, as well as the cytotoxicity were developed and used for these studies.

1.4 Scope

To achieve the above objectives, the work undertaken is outlined as follows:

- 1) Polymer type: four polymers were selected based on their degree of hydrophobicity and molar mass. These were PdlLA, PLGA75/25, RG502 and RG502H. All were formulated with model drugs and in different solvents, and studied in an *in vitro* model.
- 2) Drug type: Two different forms of the same drug molecule were used to study the drug effects on release and on biodegradation. Specifically the salt and base forms of metoclopramide were used in these studies to evaluate their effects on release

kinetics.

3) Co-solvent effects: Addition of co-solvent to the depot has substantial effects on drug release kinetics, particularly the burst release, and on cytotoxicity. NMP, DMSO and triacetin were used as co-solvent mixtures.

4) Mechanistic studies: These were used to explain the observed effects of the depot variables on drug release profiles. Specifically, the release of solvent in conjunction with drug, depot morphology, swelling kinetics, polymer degradation and pH change in the medium were all measured to understand better the observed effects. Although attempts were made to actually observe and quantify the kinetics of phase inversion, this was not possible in this thesis.

Through the integration of these studies, we aim to understand the significant parameters that influence drug release overall, including the second burst release caused by depot degradation.

Chapter 2 Literature Review

The literature review is divided into the following sections:

2.1 Introduction to biomaterials and drug delivery

2.2 Injectable drug delivery systems

2.3 Injectable *in situ* depot-forming systems

2.4 The characterization of phase inversion

2.5 PLGA degradation and drug release

2.1 Introduction to biomaterials and drug delivery

During the past two decades great progress has been achieved at the field of the medical sciences from the development of degradable materials [8]. The term degradation describes the scission process of polymer chain during which smaller Mw materials oligomers or monomers were produced. Erosion designates the mass loss of polymer as a result of oligomers and monomers leaving the matrix. Generally, we define degradable materials as those that degrade/erode during their application. Non-degradable materials are those that need a much longer time to degrade/erode than the duration of the application [9].

The main drawback of non-degradable materials is that surgery is required to retrieve these polymers out of the body after they accomplish their missions. In contrast, degradable materials do not require surgical removal or further manipulation and their degraded products can be metabolized by the body. Therefore, degradable polymers are attractive candidates for developing therapeutic devices such as controlled drug delivery

vehicles, scaffolds for tissue engineering and temporary prostheses. Each of these applications requires different types of materials with specific properties to provide efficient therapy [8].

A variety of natural and synthetic polymers which can degrade by hydrolysis or enzyme mechanism have already been investigated for biomedical applications. Natural polymers include gelatin, collagen, chitosan and so on. They have well defined polymer structures, and they are monodisperse, biocompatible and biodegradable in some cases. However, their exact properties may vary from batch to batch. In contrast, the physicochemical properties of synthetic polymers are well characterized and can be produced in large quantities.

The most successful applied synthetic biodegradable polymers at present are polyesters and their copolyesters, such as poly(glycolide) (PGA), poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA). The main reasons for this are their reputation as safe suture materials and their ability to degrade to toxicologically acceptable glycolic and lactic acids that can be eventually eliminated from the body [1]. Meanwhile, they can be designed to fit the desired drug release profile by altering their structures in the drug delivery system. In addition, they have won the Food and Drug Administration (FDA) approval for use in therapeutic devices. Wide ranges of physical and hydrolytic properties make them preferred candidates for use in therapeutic products such as degradable sutures [10], controlled drug delivery matrices [11, 12] and temporary orthopedic fixtures [13].

Figure 2.1 Plasma drug concentration when administered orally as compared to a controlled drug delivery system.

Among these applications, controlled drug delivery matrices are particularly important, especially after the development of new protein drugs, which have higher specificity and activity. However, most peptide drugs show low oral activity and have to be administered by multiple injections through the parenteral routes. As we know, each drug has a therapeutic range below which it is ineffective and above which it is toxic, as shown in Figure 2.1 [14]. After injection of standard dosage forms in conventional drug therapies, the level of the drug in the blood rises, reaches the peak and then declines. Multiple injections may cause drug levels oscillating and thus cause ineffective or toxic periods. In contrast, controlled drug delivery systems can keep the drug in the desired therapeutic level in the blood for a long time after a single administration. Other potential advantages include tailoring the release to be at a required rate (controlled release), delivery to the required site (targeting to specific area or organ) and reducing the frequency of injection

(sustained release), all with the benefits of reduced danger of overdose and side effects [26].

2.2 Injectable drug delivery systems

Figure 2.2 Injectable particulate drug delivery systems, microparticles [15] (left), liposome (middle) [16] and micelle [17] (right).

The term drug delivery covers a variety of techniques used to deliver therapeutic agents (small molecule drug, DNA, protein, etc.) into the physiological environment. Among a variety of delivery systems, injectable drug delivery systems as the promising systems have received extensive attention over the past few years due to the advantages of these systems possessing, such as simple manipulation, localized delivery [18], prolonged delivery periods, decreased drug dosage associated with reduced possible side effects and improved patient comfort and compliance [19, 20]. Injectable drug delivery systems include injectable particulate systems and injectable *in situ* implant forming systems. Injectable particulate systems such as microparticles [21-23], liposomes [24, 25] and micelles [26, 27] have been studied for some time and have demonstrated some success uncertain applications. Injectable *in situ* implant forming systems form implants in the site injected and release drug in a slow and sustained manner. In the following section,

these drug delivery systems and their applications will be briefly described.

2.2.1 Injectable particulate drug delivery systems

2.2.1.1 Microparticles

Microparticles were first developed as carriers for vaccines and anticancer drugs. They have now been used to increase the efficiency of drug delivery, improve release profiles and drug targeting. The ability to target a particular site is an important aspect of any drug delivery system. Among the numerous drug delivery techniques reported, microparticles occupy a unique position because they tend to accumulate in inflamed areas of the body. Furthermore, microparticles can be delivered to the specific site of the body by injection due to its microsize, which also increases the chance of microparticles accumulation in the inflamed areas. Many methods were developed to prepare microparticles, such as emulsion cross-linking [28, 29], spray drying [30], coagulative precipitation [31, 32] and ionic cross-linking [33-35]. One product currently on the market which uses microparticles formulations to treat cancer is the Lupron[®] Depot from TAP Pharmaceuticals [36]. However, they have some intrinsic shortcomings, including the relatively complicated manufacturing procedure, the need for reconstitution before injection and the possibility of microparticles migration from the injection site as well as the inability to remove the dose once injected [37].

2.2.1.2 Liposomes

Liposomes mainly consist of phospholipids with or without the addition of cholesterol. Through mixing in suitable concentrations with water, phospholipids form vesicles

immediately [38]. The liposome membrane is made of a liquid bilayer in which the hydrophobic tails aggregate in the interior of the membrane, while the hydrophilic heads point to the external aqueous phase. The production of liposome has been developed from basic science into applications in pharmaceutical systems because they have potential to be used as drug delivery system. Hydrophilic drugs can be incorporated into the interior water phase, while hydrophobic drugs can be encapsulated into the phospholipid membrane. Liposomes can be absorbed into almost any cell type and release drugs when close to the adsorbing cell. *In vitro* studies have also shown that the incorporation of antibodies onto the surface of liposome can make them target at particular cells. Liposomes can apply to controllable drug release systems due to the restricted permeability of their membranes. Furthermore, they can be metabolized after administration and the toxicity is quite low [38]. However, they are not a promising system for long term drug delivery. Although the retention time of drugs entrapped by liposome may be longer than that of free drugs, it is unable to maintain local drug levels in body fluid for a long time, owing partially to the rapid degradation due to the reticuloendothelial system (RES) and the rapid clearance by macrophages and other cells [39, 40]. Apart from this, other issues, such as stability, low drug entrapment and sterilization problems, have limited the utility of liposomes [25].

2.2.1.3 Micelles

Copolymers, composed of hydrophobic and hydrophilic blocks, tend to form micelles in medium by hydrophobic interactions to reduce free energy. The core block or corona outer shell is hydrophobic or hydrophilic depending on the medium hydrophilicity [41]. A

typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions pointing to the surrounding aqueous medium, while the hydrophobic tail regions aggregate in the centre of the micelle. Such polymeric micelle systems have potentially useful properties as drug carriers [42]. The core of the micelles, which is usually the hydrophobic part of the nanoparticles, can contain molecules such as therapeutic drugs, while the hydrophilic shell protect the molecules from exposing to the aqueous environment and make the nanoparticles stable in the liquid. Micelles are smaller than typical blood cells, and hence may be injected into the blood. At the same time, the size of micelles can be adjusted by controlling the length of the hydrophobic and hydrophilic blocks. After intravenous injection, micelles may arrive at site-specific delivery and the drug incorporated in the micelles can be released after the uptake of the micelles by the cells of the target tissue. This system has many advantages, such as enhanced efficacy, reduced side effects and injection dosage. However, most micelles are cleared by the RES in a short time after intravenous injection, no matter what their chemical composition is [43]. On the other hand, micelles are also prone to migrate, suffering from numerous variables which influence micelle properties. Moreover, their stability depends highly on their critical micelle concentration (CMC), only above which micelles can form. A low CMC value results in better thermodynamic stability of micelles in dilute solutions. Once the concentration is below the CMC, micelles disassemble spontaneously to form single chains [44, 45]. Therefore, dilution after injection or interaction with lipid components in the blood may lead to micelles disassembling.

2.2.2 Injectable *in situ* implant forming systems

With these disadvantages in mind, *in situ* implant forming drug delivery systems were developed as alternative delivery systems. In such systems, a polymer solution was injected via a syringe into the body and solidified to form a solid depot when it comes in contact with the body fluid, based on a physical gelation mechanism or a chemical gelation mechanism (Figure 2.3). The drugs incorporated in the depot would be released at specific injection site [46-48]. *In situ* forming drug delivery systems are promising for use in parenteral applications for a number of reasons. First, administration of these systems is less invasive and painful than administration of non-injectable implants, which require surgery to implant into the body. Second, drug delivery can be achieved locally or systemically for desired periods, usually ranging from weeks to years. Third, they can reduce side effects by achieving constant drug level, which is very important for proteins delivery, which tend to have narrow therapeutic indices. Moreover, the manufacture of *in situ* forming systems is also relatively simple [49, 50]. Finally, in comparison to injectable micro-particulate systems, the *in situ* depots do not need reconstitution before injection and can be preserved in injectable solution form. Meanwhile, there is no migration or clearance after injection compared to microparticles [51, 52].

In situ forming drug delivery systems are divided into four categories based on the mechanism of depot formation: (1) thermoplastic pastes, (2) *in situ* cross-linking polymer systems, (3) thermally induced gelling systems and (4) *in situ* polymer precipitation [49, 50].

Figure 2.3 *In situ* forming drug delivery systems

2.2.2.1 Thermoplastic pastes

Thermoplastic pastes are polymer or polymer blend systems that have a low melting point, which can be injected into the body as a melt and then transformed into a semi-solid depot upon cooling to body temperature [53]. Drugs incorporated into these polymeric systems through simple mixing procedures were then released after injection. The requirements for polymers in such drug delivery systems include a low melting point in the range of 25-65°C and an intrinsic viscosity (I.V.) in the range of 0.05-0.8 dl/g at 25°C. Outside of this I.V. range, the polymeric system is viscous and loses its injectability, or diffuses fast and causes burst drug release. The major disadvantage associated with these systems is that the minimum temperature of the polymeric system at the time of injection is above their melting point (normally above 37°C but below 65°C) [54-56], which is very painful for the patient and may cause necrosis and scar tissue formation at the injection site. In addition, such high temperatures can easily reduce the activity of some drugs, especially protein and peptide drugs. Furthermore, the drug release rate from these formulations is very slow.

Until now, the most well-known formulation is based on PLGA copolymers. However,

obtaining a proper release rate and maintaining protein biological activity during injection has limited the development of PLGA systems. Poly(ortho ester)s (POEs) were found as a potential alternative to PLGA having suitable properties for the use as thermoplastic paste drug delivery systems [57, 58]. In these systems, drug can be loaded into the POEs through a simple mixing method without elevating temperatures or using an organic solvent. In addition, its degradation by surface erosion avoids the problem of the microenvironment becoming much more acidic.

2.2.2.2 *In situ* cross-linking polymer systems

Several patents have introduced a drug delivery approach where cross-linked polymer networks are formed *in situ* to get a solid polymer or depot. It usually consists of a liquid prepolymer with double bonds, a reaction initiator (e.g. free radical) and a bioactive agent. When such solution is injected into the organism, the prepolymer is cured by free radical reactions initiated by heat (thermosets) thereby forming a solid implant. However, the double bonds and free radicals are harmful to the organism and further to the bioactive agents; therefore protection of the drugs during the process of cross-linking is very important. The application of thermoset systems is limited by the quite stringent requirements of the environment, including the requirements for a non-toxic prepolymer/solvent and clinically suitable rates of polymerization [59].

In comparison to thermosets, the cross-linking process for photocrosslinked systems is much more rapid, in which polymers are photocured *in situ* by fiber optic cables [59]. Furthermore, the liquid property of the initial materials can make the systems form into different complex shapes as required. Hubbell et al., studied a photopolymerized

biodegradable hydrogel as a tissue material and drug release carrier [60]. The mixture of PEG-oligoglycolylacrylates and a photoinitiator undergoes rapid cross-linking and forms a network when exposed to the light source. These hydrogels can entrap hydrophilic drugs and enzymes and release them at a sustained rate during the application. To illustrate the drug delivery properties of this technique, the release rate of various proteins from photopolymerizable PEG-PLA hydrogels was studied and it was found that the release rate from such hydrogels was rather high, with drug exhausted within 5 days. Meanwhile, the release rate relied on the protein Mw, decreasing as Mw increased. Moreover, the release was converted from diffusion controlled to erosion controlled when the drug Mw is above the critical Mw. Therefore, this system is relatively suitable for large drug molecules in order to achieve prolonged release.

There is another *in situ* cross-linking system based on the ionic interaction between small cations and polymer anions, such as chitosan/phosphate ions and alginates/calcium ions [61, 62], which can be used directly as drug carrier. This system does not require additional cross-linking reagents, with short-term or no contact of the drug with organic solvents. Besides, it is less problematic to inject an aqueous suspension compared to viscous polymer solutions in organic solvents. However, the application of this system for *in vivo* use is impractical as the concentrations of the cations available under physiological conditions are usually not enough for cross-linking of the polymer anions. Only the calcium concentrations in the eye may lead to *in situ* formation of cross-linked alginates.

2.2.2.3 Thermally induced gelling systems

Many polymers show an abrupt change in solubility as a function of environmental temperature, so-called thermosensitive polymers. This physical characteristic has been used to form drug carriers by using such polymer systems, which undergo a gelation when transferring into the body temperature environment. Poly(N-isopropylacrylamide) [poly(NIPAAM)] is the prototype of a thermosensitive polymer. It exhibits a well defined lower critical solution temperature (LCST) of approximately 32°C [63], which can form poly(NIPAAM) based particles with salt and surfactants upon injection into the body . However, it does not suit for biomedical applications owing to its famous cytotoxicity [64] and non-biodegradation [65], which make it difficult to get FDA approval. In contrast, triblock poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) copolymers (PEO-PPO-PEO), known as Pluronics or poloxamers, which shows a sol-gel transition at body temperature when the concentration of the polymer solutions injected is more than 15% (w/w) [66, 67], has received great attention because of FDA approval. However, biocompatibility studies reveal that such high concentrations lead to severe cytotoxicity [68] and increases plasma cholesterol and triglycerol levels in rats after intraperitoneal injection [69].

Biodegradable ABA and BAB thermosensitive triblock copolymers, were introduced by MacroMed, and were composed of polyester (PLGA or PLA) as A blocks and poly(ethylene glycol) (PEG) as B blocks. Such polymers with low Mw are soluble in aqueous medium and show a temperature-dependent reversible gelation. The values of LCST are a function of block composition and block-lengths [70]. Both ABA and BAB

kinds of triblock copolymers can be used as drug carrier systems with sol-gel transition properties as a function of temperature [71, 72]. ReGel[®], one of MacroMed's proprietary drug delivery systems and composed of 23% (w/w) PLGA-PEG-PLGA in a buffer solution (pH 7.4), is already in the market as minimally invasive injectable solutions. OncoGel[®], which is ReGel[®] containing paclitaxel at a concentration of 6 mg/g, is designed as the MacroMed's lead product for the treatment of solid tumors. The liquid solution below room temperature spontaneously turns into a gel after injection in response to body temperature, followed by a continuous paclitaxel release from the gel locally over a period of 6 weeks, resulting in extremely low plasma levels of paclitaxel for a long time and fewer toxic side effects. The obvious advantage of this system is the ability to solubilize the hydrophobic drug like hydrophilic drug. In addition, protein/peptide drugs can be easily loaded into these systems by simple dissolution or suspending in aqueous medium. Moreover, these systems are composed of biocompatible polymers and water, thus the possibility of the organic solvent toxicity will be avoided.

2.2.2.4 *In situ* polymer precipitation

Dunn et al., have introduced an *in situ* polymer precipitation system for drug delivery [2-4], which is based on non-solvent induced phase inversion mechanism leading to polymer precipitation from solution upon contact with water. Briefly, the water insoluble polymer is dissolved in a water miscible organic solvent to form an injectable polymer solution, with the drug dissolving or being suspended in the polymer solution. After subcutaneous injection via a syringe, water, as non-solvent, penetrates into the polymer solution and the solvent diffuses out. The exchange of the organic solvent and non-solvent

leads to a decrease in the solubility of the polymer and eventually, a loose “depot” is formed, in which there is a polymer-rich phase and a polymer-poor phase. The depot formed *in situ* serves as a reservoir for the controlled release of the incorporated drug for a long time. Besides some common advantages other *in situ* forming drug delivery systems possess, these systems do not need other agents to initiate the transition, have high drug loading and are quite simple to manufacture that combines the advantages of microparticulate delivery and an implanted device [2, 4, 73].

Figure 2.4 Testosterone levels in dogs with Eligard (45 mg) 6-month depot. LA = leuprolide acetate.

Biodegradable polymers used in this system include polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyorthoesters, polyethylene glycol, and copolymers, terpolymers, or combinations of the above materials and so on. PLGA and PLA polymers are commonly used for their good biocompatibility and biodegradability. Solvents which can be used in this system include dimethyl sulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP), 2-pyrrolidone, glycofurol and triacetin, PEG 400 and acetone. The most preferred

are DMSO and NMP due to their pharmaceutical precedence [73]. In addition, NMP has been approved for use in an injectable system (EligardTM) manufactured by Atrix Labs. The limitation of these systems is the toxicity of the organic solvent as most organic solvents are poorly tolerated and some may cause pain at the injection site.

Figure 2.5 Eligard® 6 uses the Atrigel® delivery system.

AtrigelTM technology was developed based on this technique by Atrix Labs [74]. The more advanced product using AtrigelTM as a drug carrier, EligardTM (leuprolide acetate/PLGA/NMP system) which can suppress testosterone levels for up to 1-6 months (Atrigel[®] system) [75] (Figure 2.4), has been commercialized, as shown in Figure 2.5 [76]. A series of EligardTM products are listed in the table 1. Success with AtrigelTM inspired more researchers to study *in situ* depot forming systems for optimizing release profiles. However, only a few kinds of drugs has been commercialized by this technology to date, such as leuprolide acetate and human growth hormone [5-7]; this is at least partly due to

the inability of controlling the release of diverse bioactive compounds, especially burst release. In addition, the toxicity of organic solvent has been a great challenge until now. Therefore, reducing the burst release and looking for more biocompatible solvents is significant for the development of this technique.

Table 2.1 EligardTM products

Product	Recommended Dose	Leuprolide acetate	Polymer	Polymer Mw	Polymer/NMP ratio (w/w)	Leuprolide acetate c (w/w)
Eligard 1-Month	250 mg dose (0.25mL)	7.5 mg	PLG50/50H	23-45 kD	34/66	3%
Eligard 3-Month	375 mg dose (0.37 mL)	22.5 mg	PLG75/25OH	15-24 kD	45/55	6%
Eligard 4-Month	500 mg dose (0.50 mL)	30 mg	PLG75/25OH	15-24 kD	45/55	6%
Eligard 6-Month	375 mg dose (0.375 mL)	45 mg	PLG85/15OH	-	50/50	12%

2.3 Injectable *in situ* depot-forming systems

Among all *in situ* forming drug delivery systems, injectable *in situ* depot-forming systems based on the polymer precipitation mechanism are the most attractive due to ease of manufacture and high loading capacity. In this project, we will use this system to obtain an ideal controlled drug release behavior. In order to better understand this system, we describe its mechanism below and indicate some parameters which are important for drug release control.

2.3.1 Mechanism

Figure 2.6 Schematic of non-solvent (NS) induced phase separation process, polymer (P) and solvent (S), with dissolved or suspended drug (D) [77].

Figure 2.7 Schematic illustration of membrane formation/drug release in an injectable system. Morphologies in upper right show fast phase inversion (FPI) and slow phase inversion (SPI) hardened membrane structures [77].

In injectable *in situ* depot-forming systems, water insoluble polymer dissolved in the organic solvent was injected into the buffer solution, water as non-solvent penetrated into

the matrix while solvent diffused out resulting in the depot formed *in situ*, as shown in Figure 2.6 and Figure 2.7.

The exchange of non-solvent and solvent results in a ternary system within the matrix, composed of polymer, solvent and non-solvent, which can be schematically shown by a ternary phase diagram (Figure 2.8) [78]. In this diagram, the binodal curve means the separation of single-phase and two-phase regions, while the spinodal curve separates the metastable and unstable composition areas. These critical curves can be calculated based on the Flory-Huggins theory and its interaction parameters of the system determined by experiments [78-81]. The phase inverts by two mechanisms, the nucleation and growth (NG) mechanism which occurs in the metastable region between the binodal and spinodal curve, and the spinodal decomposition (SD) mechanism which occurs in the unstable region inside the spinodal curve. When the composition passed through the critical point (path B), the phase inversion occurs in the unstable region by the SD mechanism. In this case, the first stages of phase inversion consist of a co-continuous structure of the segregated phases from which the final pores are formed on further penetration into the miscibility gap. When the composition passed slowly in the metastable region as path A and C, the phase inversion occurs by the NG mechanism. In this case, nuclei formed in the matrix, which consist of the more dilute phase, grow during the phase inversion, forming the membrane's pores in the precipitated gel [78].

To know which phase inversion mechanism occurs under different conditions, the ternary phase diagram has to be calculated by theoretical and experimental methods. Generally, the spinodal decomposition gap is surrounded by the metastable region where the phase

inverts by the NG mechanism; since the process of latter is relatively fast, spinodal decomposition is less likely in polymer solutions [82]. When the polymer solution first reaches the binodal curve, it separates into two liquid phases, the polymer-rich phase and the polymer-lean phase. During the process of membrane formation, the polymer-rich phase grows in extent until it reaches the viscous effect region and finally forms the matrix of the membrane, while polymer-lean phase will form the pores of the membrane.

Figure 2.8 Schematic representation of a ternary phase diagram.

2.3.2 Parameters in this system

As we all know, drug release, especially the burst drug release, largely depends on the phase inversion dynamics and the resultant morphology of the implant formed after injection, so we will introduce some common parameters that affect the phase inversion process and implant morphology, such as polymer type or Mw [83], solvent hydrophilicity [84, 85], additives [3] and the medium (non-solvent) composition [84, 85].

2.3.2.1 Polymer property (polymer type and Mw)

The effect of polymer type on the protein release from PGA and PLA-injectable implant was studied, where the burst release decreased with increasing LA content of the implant [86]. They believed that hydrophobic polymer solution solidified faster when in contact with water, resulting in more proteins being trapped in the solid device yielding a lower burst release from the implant. The influence of end groups on the leuprolide release was studied in *in situ* microparticles-forming (ISM) drug delivery system [83]. PLGA with -COOH end groups (e.g. RG502H and RG503H) led to a lower leuprolide release than that with esterified end groups (e.g. RG502 and RG503). The terminal -COOH groups might interact with leuprolide acetate, resulting in a slower drug release. Furthermore, the more hydrophilic property of RG502H and RG503H may result in a higher affinity to hydrophilic solvent NMP, leading to a slower solvent release from the polymer droplets and thus a slower drug release [83].

Polymer Mw effect on the drug release is much more complicated in *in situ* forming implant systems, in which polymer solution precipitates gradually to form semi-solid or solid form after injection into the medium. The polymer Mw effect on the drug release was studied in ISM and implant drug delivery systems [83, 87]. PLGA formulations with a lower Mw resulted in a significantly lower burst release than in a higher Mw, which is attributed to the slower solvent diffusion from lower Mw PLGA into the release medium. It is explained that PLGA with a lower Mw has a higher solubility in water, i.e., higher affinity to water and stronger interactions with polar solvent NMP, compared to higher Mw PLGA, and hence requires more time to complete the phase inversion and release the

drug; therefore the burst drug release is lower [83, 87]. However, in another study, a lower initial release of leuprolide acetate from higher Mw polymer (RG504H) than that from lower Mw polymer (RG502H and RG503H) was reported [88, 89]. They observed that the lower Mw system solidifies faster than the higher Mw system. The polymer solution with a higher Mw tends to yield higher solution viscosity, thus decreasing the diffusion rate of peptide into the medium during the initial phase [86].

Until now very few studies have been conducted on the effect of polymer hydrophobicity on the dynamics of phase inversion and the release of low Mw hydrophilic drugs. In Chapter 4, four PLA/PLGA polymers with the similar low-molar mass, composed of different LA/GA ratio and different end groups, were chosen to study the effect of polymer hydrophobicity on the dynamics of phase inversion and the release of low Mw hydrophilic drug (metosalt) as they have been the most widely-used and studied polymers. The results found that the burst drug release increased with the LA component of the implant, which is opposite to the results of Eliaz's [86], and also found that the drug release from high Mw depots is faster than that from low Mw depots. These cannot be explained only from polymer hydrophobicity or polymer solution viscosity, drug Mw and drug solubility, water uptake and depot morphology should be considered to understand the whole system. We will clearly illuminate these in Chapter 4 and 5.

2.3.2.2 Solvent property

The solvent type is one of the most critical factors under both *in vitro* and *in vivo* conditions. Many papers have reported the influence of the solvent on the dynamics of phase inversion and the kinetics of the drug release. Brodbeck et al. demonstrated that

solution thermodynamics influenced protein release kinetics, e.g., the solvent strength and water miscibility [84, 85]. They studied the ternary phase systems of NMP, triacetin and ethyl benzoate with PLGA and water. NMP, being hydrophilic, dissipates into surrounding aqueous medium quickly after injection upon contact with water and thus causes the polymer solution to exhibit fast phase inversion, with a high burst release and formation of a porous, solid depot structure. In contrast, triacetin and ethyl benzoate, both more hydrophobic solvents, leave the “depot” very slowly and lead to slower phase inversion and form a semi-fluid structure, resulting in a slow gelation and significant reduction in the burst release [84, 85]. It is hypothesized that using a mixture of a hydrophilic solvent and a hydrophobic solvent wherein the required solvent miscibility with water can be adjusted by varying the mixing ratio will tune the rate of water migration into the polymer matrix and thus the rate of the burst release [90].

Glycerol and ethyl heptanoate was mixed into the polymer solution to study the effects of their addition on the drug release and solvent release from PLGA injectable implants [91]. The results show that the addition of each of these resulted in the decrease in the *in vitro* drug release, which is ascribed to the low affinity of solvent to non-solvent brought by their addition, leading to a more dense structure formed and the burst release is reduced. Benzyl benzoate (BB) as a hydrophobic solvent and benzyl alcohol (BA) as a hydrophilic solvent have been used widely as the mixed solvents to study the effect of the solvent characteristics on the drug delivery [90, 92-94]. However, some of the results were conflicting with respect to drug release. Higher burst drug release was found in formulations with greater hydrophilic component BA as reported by Singh et al. [92-94], while the release of the drug was slowed when the BA content was increased as reported

by Prabhu et al [90]. Prabhu et al. deduced that the drug they used diffuses out of the hydrophilic component (BA), causing less drug to be trapped within the hydrophobic implant, resulting in a lower release rate. But the burst release as a result of drug diffusing into the aqueous medium was not obvious [90]. Others thought the hydrophilic solvent part leaves the gel when contacting with water, resulting in a shell formed around the exterior of the gel, then the hydrophobic solvent part delays the water uptake and therefore reduce the drug release [92-94]. These observations were not sufficiently rationalized or reconciled, primarily due to lack of complementary experimentation.

To understand the effect of solvent hydrophobicity on the drug release in this system, the effect of mixed solvent of hydrophilic solvent and hydrophobic solvent at different ratio on the drug release was studied and discussed in Chapter 6. NMP was chosen as the hydrophilic solvent because of its miscibility with water and reasonable biocompatibility. DMSO was chosen as the other hydrophilic solvent because of its much higher LD50 (oral, rat: 14500 mg/kg, based on MSDS) than that of NMP (3914 mg/kg, based on MSDS) and lower cytotoxicity as reported by Kranz et al. [95]. Similarly, triacetin as hydrophobic co-solvent is preferred over BB (oral LD50 (rat): 1700 mg/kg, based on MSDS) because of its relatively lower systemic toxicity (oral LD50 (rat): 3000 mg/kg, based on MSDS). We not only found the reduced burst release after the addition of triacetin, but the swelling and porous structure were also found, rather than the dense structure formed as reported by Bakhshi et al. [91]. The basic reason is due to the difference in the polymer used. The polymer they used was PLGA50/50, while the polymer we used was PdlLA. The difference in the hydrophobicity of the polymer may lead to different reaction of the depot to the co-solvent addition. So we studied the effect

of triacetin on the drug release from polymers with different hydrophobicity. PLGA50/50 with low Mw was also chosen in our research to compare polymer type on the role of co-solvent addition on the reduction of the drug release from polymers with different polymer hydrophobicity [96].

2.3.2.3 Medium property

The role of non-solvent components on the dynamics of phase inversion and the kinetics of *in vitro* protein release from PLGA solutions has been investigated by a dark ground imaging technique [85]. A short chain triglyceride (triacetin) was added into the aqueous receptor bath. Results clearly show that the phase inversion dynamics and drug release of NMP-based depot are insensitive to the presence of the hydrophobic solvent (triacetin) in the medium. In contrast, the behavior of the ethyl benzoated-based depot shows that both of them are clearly increased by the presence of triacetin in the medium [7]. Another study shows that the finger morphology becomes shorter and even disappears when the amount of solvent that was added to the coagulation bath was increasing [97]. Since rapid precipitation results in finger morphology and delay precipitation leads to a spongy formation, it implies a transition from instantaneous precipitation to delayed precipitation when the solvent was added into the medium. In fact, the instantaneous precipitation and delay precipitation are concomitant in *in situ* depot-forming systems. On the surface, the polymer solution undergoes instantaneous precipitation. But in the deeper of the polymer solutions the solvent concentration at the coagulation front is much higher, which was as if the solvent was added into the medium, hence causing a transition from the instantaneous precipitation process taking place globally to a delayed precipitation taking

place locally [97]. In our research (data not published), we found that the finger morphology appeared near the surface of RG504 depots which is believed to be caused by the instantaneous precipitation, while the cellular structure or honeycomb morphology appeared in the deep-layer which is attributed to the delayed precipitation.

2.3.2.4 Additives

Another important factor is the incorporation of additives in the polymer solution, such as rate modifying agents and pore-forming agents [3], which can be added to the formulations based on the requirement of the drug release. Rate modifying agents are preferably more hydrophobic than the organic solvent of choice for that polymer such as dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin and hexanediol. Pore-forming agents can be added into the polymer solution to produce pores in the implant especially when the polymer solution is too impervious to water, and more particularly for the pores generated in the skin. They are capable of dissolving, diffusing or dispersing out of the polymer matrix and then form more pores and channels in the matrix [3].

In addition to above additives, the understanding of the effect of incorporated drug on the process of phase inversion and property of drug delivery systems is also important as they are essential in the drug delivery systems. In *in situ* depot-forming systems, the presence of organic solvent makes the system different from other implant formulations. Both hydrophilic drug and hydrophobic drug can dissolve in the polymer solution with the help of the solvent, which can eliminate the effect of solubility of the drug on the initial release. However, when drug has catalytic effect on the matrix degradation, the phase inversion dynamics may be influenced since the Mw of polymer has changed.

Until now, the effect of drug type on the phase inversion dynamics and drug release in injectable *in situ* depot-forming systems has less been discussed. In this project, metosalt, such as a hydrophilic drug and metobase as a hydrophobic drug were studied to understand the drug type effect on the phase inversion dynamics and drug release rate. When the polymer used is a high Mw PLGA50/50 polymer, metosalt release was faster than metobase. When the PLGA50/50 polymer used has a low Mw, the results switched. The results can be explained from the phase inversion dynamics and the morphology formed, which will be discussed in Chapter 5.

2.4 The characterization of phase inversion process

As drug release is strongly related to phase inversion dynamics, to quantify and visualize the phase inversion process is of particular importance. Currently, some techniques have been developed to monitor phase inversion process, in the following session we will introduce them.

2.4.1 Dark ground imaging

McHugh et al. [84, 85] introduced a dark ground imaging apparatus to quantitatively study the *in situ* imaging of the phase inversion process (Figure 2.9). Several collimating lenses focus the filtered laser beam onto a quartz cell containing the polymer solution was contained. The initial polymer solution was separated from the water bath by a thin metal foil which was quickly removed when activating the experiment. Water penetration into the polymer solution causes the concentration gradient, which is recorded by a dark ground imaging optical system. The region of striations shown in the dark ground image

is composed of interference fringes whose position is the water diffusion front (diffusion fringes). Other than diffusion fringes, reflected light is used in combination with the laser to illuminate the portion of the solution that has undergone liquid-liquid phase inversion to produce the two-phase structure (gel region). For fast phase inversion systems, diffusion fringes and gel solidification occur at the same time, while for slow phase inversion systems, a time lag exists between them [98]. Squares of the positions of both diffusion fringes and the liquid-liquid gelation front are plotted as a function of time and the slopes of these lines are equal to the water influx rate and the gelation rate, respectively [98].

Figure 2.9 Schematic of the dark ground optical system.

This technique has been useful in evaluating the phase inversion dynamics to facilitate the understanding of the drug release behavior in injectable *in situ* forming drug delivery systems, which is limited to *in vitro* experiments and cannot be used for the implant with

long term duration due to the short available operation time (minutes to few hours) [84, 85, 98].

2.4.2 EPR and BT-MRI

Figure 2.10 Chemical structure of tempolbenzoate (TB) and schematic illustration of the EPR mechanism during the implant formation process [99].

Electron paramagnetic resonance spectroscopy (EPR) has been developed as a suitable online non-invasive approach to detect phase inversion process and implant formation *in vitro* and *in vivo* in recent years [99]. This method investigates the interaction of paramagnetic compounds, composed of unpaired electrons in the polymer solution, obtaining unique information on the change of micropolarity and microviscosity inside the implant. The hyperfine coupling parameter ($2a_N$) and the rotational correlation time (t_c) are two useful parameters determined in this method [99]. The value of $2a_N$ depends on the polarity of the nitroxide environment. When the object has high polarity (e.g. water), $2a_N$ is higher, compared to the object with low polarity (e.g. oil). Another parameter of the microenvironment, microviscosity, highly affects the tumbling behavior of the

radicals. When the viscosity of the media is low, they can freely tumble, leading to symmetric spectra with narrow lines. When the viscosity of the media increases, the tumbling rate of the molecules decreases as their motion was restricted, resulting in a broad spectra and a decrease of the signal amplitude as well as an increase in t_c [100, 101].

Kempe et al. have used EPR to monitor the *in vitro* and *in vivo* diffusion rate of NMP from the implant [99]. The lipophilic stable nitroxyl radical tempolbenzoate was incorporated in the PLGA/NMP solution as spin probes, as shown in Figure 2.10 [99]. By means of EPR spectroscopy, the *in vitro* and *in vivo* exchange rate of the organic solvent NMP and water was detectable. Both the kinetics of water/NMP exchange and polymer solidification showed good *in vitro-in vivo* correlation. However, EPR spectroscopy could not provide direct information on the images of the implants *in vivo*, such as the size and shape of the implant after injection and its degradation in the injection site. To solve these limitations, Kempe et al. applied benchtop magnetic resonance imaging (BT-MRI) as a complementary non-invasive method to EPR spectroscopy without using contrast agents [102]. *In vivo* MRI images of the distribution and disappearance of PEG 400 or PEG 400 released from PLGA/PEG 400 implant after injection were obtained clearly, which is a proof that MRI has the potential to lead to insights in the *in vivo* fate of implants formed *in situ* as well as parenteral formulations. The limitation of this imaging technique is the requirement about the T1 relaxation time of the solvent in order to get the vivid images. On the other hand, it requires using the small molecular weight paramagnetic spin probes.

2.4.3 Diagnostic ultrasound image analysis

Solorio et al. introduced diagnostic ultrasound to visualize and quantify the phase inversion dynamics both *in vitro* and *in vivo* [103]. In a diagnostic ultrasound analytic system, electrical energy was converted into mechanical pressure waves that propagate through a material, by a piezoelectric transducer. Since *in situ* implants undergo transition from the liquid solution to the semi-solid or solid implant form, the change in phase alters the implant impedance, thus visualization of the phase inversion process is possible. The backscattered signal that arises from the mechanical interactions of the pressure waves with the material was collected during this process and form ultrasound images.

Solorio et al. evaluated the relationship between the phase inversion processes of the implants *in vitro* and *in vivo* to the drug release profiles [103], and found that the drug release both *in vivo* and *in vitro* are directly related to the percent of precipitation. Patel et al. [104] studied the injection site effect on the *in situ* implant formation as well as drug release, and found that the drug release from implant formed *in situ* is strongly related to the local compressive mechanical forces and interstitial fluid pressure, especially in the case of polymer implants with low Mw, since they have a greater tendency to swell and are prone to precipitate slower.

The use of diagnostic ultrasound imaging method to monitor changes during depot forming process has several advantages. First, this method is nondestructive, so the release of drug and solvent and the swelling of the depot can be obtained from the same implant when studying the implant formation dynamics. Meanwhile, the same implants can be imaged throughout their lifespan providing unprecedented information on the

immediate formation associated with implant degradation. Finally, the evaluation can be performed *in vitro* as well as *in vivo* to provide an exact representation of implant properties in an undisturbed physiological surrounding. However, the application of this technique is limited by the low resolution of the images, which makes this technique suitable for analyzing the macro-scale behavior of the implants.

2.4.4 Solvent release and water uptake

Another approach to trace the implant formation dynamics is to monitor the non-solvent/solvent exchange process by detecting the rate of solvent released from the matrix and water uptake as the mechanism of the *in situ* implant formation is non-solvent induced phase inversion. Briefly, a non-solvent like water penetrates into the polymer solution and the solvent diffuses out during the process of implant formation. So the amount of solvent released from the depot and the data of water uptake can directly disclose the implant formation dynamics. Wang et al. developed a novel electrochemical method, electrical conductivity (EC), to measure the amount of solvent released from the organogel to simulate gelling procedures [105]. EC measures the total amount of ions dissolved in the solution. More ions in the solution will generate higher conductivity, thereby causing a higher electrical current in the solution [105]. NMP release was studied by testing the change of the EC of the medium after putting the buffer medium around the polymer solution. EC decreases as the amount of NMP increases. Maybe the nitrogen atoms in NMP molecules attract ions in the solution and reduce the number of ions in the buffer solution, hence resulting in the decrease of EC. Other approaches to test the solvent release rate widely used in our lab are high-performance liquid chromatography (HPLC)

and UV-Vis spectrophotometry [49, 106], which are more convenient and efficient than EC testing. The solvent release data combined with water uptake data clearly shows the non-solvent/solvent exchange process and thus gives some useful information about the implant formation process.

The methods used to measure the solvent release rate and water uptake to monitor exchange process cannot visualize the process of implant formation but can quantify this process easily and effectively. In this project, we usually detect the solvent release by the UV method, but sometimes using HPLC when the solvent like triacetin is insensitive to UV light.

2.5 PLGA degradation and drug release

In general, drug release from the matrix system is usually controlled by both diffusion of drugs within the matrix and the degradation/erosion of the matrix. In *in situ* depot-forming systems, the initial burst release highly depends on the phase inversion dynamics and the following release is closely related to the degradation of the matrix.

The degradation mechanism of biodegradable polyester is considered to be a hydrolytic process, following a three-phase mechanism [107, 108]: (1) random cleavage of an ester bond; (2) cleavage of the last unit; and (3) complete solubilization of degraded products. The observed decrease in Mw is assumed to be the hydrolytic cleavage of G-G and L-G as well as L-L ester bonds in PLA and PLGA backbone. The scission of an ester bond produces a -COOH and a -OH end group. The formed -COOH end groups are able to catalyze cleavage of other ester bonds via autocatalysis [109]. During the random

cleavage of the chain, PLGA polymer incurs a rapid loss of Mw but its polydispersity and LA/GA ratio does not change. Then LA/GA ratio increases and its polydispersity changes in phase 2. Finally the soluble degraded products dissolve in the external medium [110]. Factors that influence degradation, include material composition [111, 112], crystallinity [113-115], additives [116, 117], morphology [118-120] and medium [121, 122], will be discussed in the following section.

2.5.1 Chemical composition

Polymer composition is one of the most important factors influencing the matrix degradation because the composition determines the hydrophilic property of the matrix which is highly related to degradation. PLA are not preferred to be used in *in situ* forming implant systems because it can take years to degrade. PGA and its copolymer are preferred materials because their degradation can be tailored by varying the polymer Mw and composition. The degradation of porous implants made from amorphous polyesters was studied, and the results show that PLGA75/25 degrades faster than PLGA85/15, while PdlLA exhibited a minimal degradation rate, indicating that an increase of GA ratio in the polymer chain accelerates the degradation rate, which is in good agreement with the results reported for non-porous PLGA samples [112]. The amount of GA and LA released from PLGA were determined [123, 124] and it was found that GA left the devices almost twice as fast as LA. This result further indicates that the scission prefers to occur on G-G and G-L due to the higher hydrophilic property of GA unit which facilitated the absorption of water and thus accelerated the hydrolysis in progress. Methyl side groups on L-L make the polymer more steric and hydrophobic, resulting in the reduction on the

water susceptibility of ester bonds [125] and thus much longer time required to observe its degradation [124].

Polymer end groups and end group modifications also influenced the degradation [111, 117, 126]. Aliphatic polymers can be functionalized via the introduction of side groups at the end of the polymer chains to increase the polymer hydrophilicity and shorten their degradation time. In general, the presence of -COOH end groups of the polymer will catalyze the hydrolytic degradation of polyesters [127-130], which can be explained by the much greater initial water uptake brought by the carboxyl end groups and thus accelerate the hydrolysis of ester bonds in the polymer backbone.

2.5.2 Configurational structure

The configurations of polymers are usually classified into two types, crystallized or amorphous polymers. The degree of crystallinity typically ranges between 10 and 80% as no polymer can completely organize into a fully crystalline material and there are still amorphous regions in crystalline polymers, thus crystallized polymers are often called "semicrystalline" [131]. The regular repeating units of semicrystalline polymers can allow the chains to fold into dense regions, called crystallites, which act as cross-links, resulting in the higher tensile strengths and higher modulus properties in comparison to an amorphous analog.

Lactides are chiral molecules that have two optically active forms: *L*-lactide or *D*-lactide. Copolymerization of both forms generates racemic, intrinsically amorphous PDLA ($T_g \sim 55-60^\circ\text{C}$) with a random distribution of both isomeric forms of lactic acid, and

homopolymerization of L-lactide forms semicrystalline PLLA (~37% crystallinity) [8]. PGA is crystalline polymer with a high melting point [8]. PLGA copolymers can be either amorphous or semicrystalline depending on the type of lactide group (D,L-lactide or L-lactide) incorporated. For the L-lactide block, the copolymer is amorphous in a range of composition between 25 and 70% GA, and this range is extended from 0 to 70% if D,L-lactide is used instead of L-lactide [132, 133].

Generally, an amorphous region is more susceptible to hydrolysis due to the ease of permeation of water, especially in semicrystalline polymers, which makes the hydrolytic degradation rate decrease with the increase of crystallinity [134]. However, studies on the crystallinity effect on the degradation rate are still being conducted as the actual degradation is complicated when the crystallized polymer is involved.

Nakamura et al [135] have studied the Mw change for both amorphous and crystallized PLLA after *in vivo* injection for three months. The Mw for the amorphous samples decreased by 30-40% and its bending strength was retained over 90% of the original value, while for crystallized PLLA the Mw decreased by 70-80% and more than 50% of its bending strength was lost during the same period of time. The higher degradation rate of the crystallized PLLA may be due to the high processing temperature (200°C) during crystallization, which results in extensive degradation when the processing temperature is higher than the Tg since PLLA is very sensitive to the temperature. PLGA may undergo complicated recrystallization during the degradation process which slows down the degradation. This may be explained through three mechanisms. The first possible mechanism is that the chains in the amorphous region of the polymer were broken down

first during the degradation process and then occurred in the crystalline region [136]. The preferred mass loss to the amorphous component results in the crystallinity of the matrix increasing. The second mechanism is that the short chains rearranged to form crystallites during the degradation process, since the chain molecules have greater chain mobility when the chain length decreases upon hydrolysis and then facilitate them in forming new crystallites [137]. Another possibility is preferential hydrolysis of glycolide in the PLGA polymer system. This generates richer lactide units left in the residual polymer and the remaining chain crystallizes [115].

2.5.3 Additives

Additives incorporated in the polymer matrix, such as drugs, monomers, surfactants, plasticizers and catalysts, are also important factors. Thus the study of the additives effect on the matrix degradation is very important.

The addition of the plasticizer could affect drug diffusion, because it can decrease the T_g and make the matrix transform into a rubbery state quickly which has more free chain mobility, and also generate more water-diffusion channels, which facilitates water uptake into the polymer and then causes a faster release of drugs. For the polyesters, the presence of water can act as a plasticizer, lowering T_g and accelerating degradation [131]. Adding PEG into PLLA polymers could suppress the burst effect of heparin due to the hydrophilic property of PEG. However, its addition did not change the drug release profile from PLLGA80/20 polymer significantly. Maybe the alteration of hydrophilicity brought about by the addition of PEG is insignificant since PLLGA80/20 is more hydrophilic compared to PLLA [124].

The residual surfactant is another potential factor affecting the drug release, especially in nano- and microparticle systems. Polyvinyl alcohol (PVA) which was used as surfactant during the process of particle preparation could form a barrier to prevent the penetration of water into the particle, resulting in a lower degradation [116]. However, it could also act as a barrier to the release of degraded products, which have a catalytic effect on polymer degradation [138], causing enhanced degradation. Furthermore, large hydrophilic molecules PVA could swell in the medium [139] and then reduce the availability of water molecules for hydrolytic cleavage of PLGA chains and thus reduce polymer degradation [138].

In addition to the above additives, it is also worth noticing that the incorporated drug effect on the matrix degradation and property of drug delivery systems is also important since these are essential in drug delivery systems. Drug distribution not only affects the rate of the drug release [140], but the physico-chemical properties of the drug may also influence it. Hydrophilic drugs are expected to produce high osmotic pressure and lead to more water uptake, thus enhance the matrix degradation, which will in turn facilitate the drug release. In contrast, hydrophobic drugs can reduce the penetration of water into the matrix, which slow down the degradation of the polymer and decrease the release of the drug. However, when either of these kinds of drugs has an additional catalytic effect on matrix degradation, the system becomes complicated [141-143], such as acidic and basic drugs, which sometimes represent different types of drugs with various water solubility. Acidic drugs can accelerate the degradation of the polymer, since the drug located in the matrix can be ionized quickly and increase the acidity inside the matrix, resulting in a rapid degradation of the matrix. In contrast, the system is more complicated in case of

basic drugs because two effects may intervene simultaneously: catalysis of ester bond cleavage and neutralization of carboxyl end groups of polymer chains. Whether the degradation of the matrix is accelerated or slowed down is dependent on the relative importance of these two effects [142, 144, 145]. In fact, besides these effects, drug loading, device size, implant porosity and incorporated compounds' morphology should also be considered together to understand the drugs effect on the degradation of PLGA polymers and on the drug release [146].

An additional study shows that the presence of large amounts of coral granules produces coral/polymer interfaces that facilitated ionic exchanges between the interior of the blend specimens and the external medium [144], causing the acidic end groups to be neutralized and the catalytic effect to be eliminated thereby causing the specimens to degrade uniformly. Moreover, polymers which blended with an acidic component were found to degrade faster than the original material in an acidic aqueous medium [117], which implies that carboxylic acid modifications are more significant on the degradation of the polymer than the acidity of the external aqueous medium [126].

2.5.4 Matrix morphology

If the Mw decreases exponentially over the time, indicating almost the same degradation rate in the interior and at the surface of the materials, which is termed bulk degradation mechanism [147]. However, many studies have reported that degradations in the internal part and at the surface are not in the same rate. The polymer in the interior degraded quickly than at the surface, which is called heterogeneous degradation [118, 148]. The shape and size of device can affect drug diffusion and hydrolysis process. Degradation

starts with the penetration of water, followed by the chain scission. In the initial degradation phase, scission occurs preferentially at the surface of the sample due to the priority of water contact. This priority disappears after a short time because of relatively rapid water diffusion compared to polymer chain degradation [149]. When the degradation occurs at the internal of the matrix, the following degradation route will depend on the sample size. For the matrix with a large size, only acidic products that are near the surface can escape whereas those located in the interior of the matrix are difficult to diffuse from the matrix. As a result, the concentration of acidic products is higher in the interior than at the surface thereby the degradation rate in the center becomes faster and a surface-center differentiation is formed. Meanwhile, buffer ions from the external medium penetrate into the matrix, neutralize the carboxyl groups near the surface and reduce the surface acidity. Therefore, the autocatalysis is more severe inside than at the surface, and thus such differentiation is reinforced [146]. If the matrix size is as small as microparticles, thin films or slim fibers, soluble oligomers can escape and acidic products can be neutralized and thus the possibility of heterogeneous degradation is reduced [118].

When the scaffold is porous, the degradation rate will be lower than that of a solid scaffold because of the presence of autocatalytic effect in solid polymers [118, 150, 151]. The pH inside the pores of eroding poly(lactic acid) is as low as 1.8, far less than the pH of the external pH 7.4 medium, which can be attributed to the higher solubility and the low pKa of the monomers. However, if the thickness of the solid scaffold is near to the wall thickness of the porous one, their degradation rate will be close [112, 120, 152].

2.5.5 Medium

The release of therapeutic agent and the degradation of the implant may be influenced by the physiological surrounding of the injection site. Lots of potential reactions maybe occur between the polymer solution and the physiological environment, such as acidic and basic substances around in the injection site, which are well-known to have significant effects on PLGA degradation rates. It is well-known that the pH of gastric juice in the stomach is in the range from 0.9 to 1.5, while pancreatic juice in the duodenums can be as high as pH 8.2 and the urinary pH often ranges from 4.5-8.0 [153]. These characteristics of different body parts indicate the importance of studying the degradation under different pH values.

Generally, studies of pH influence on the hydrolysis reaction can be done under acidic, neutral and alkaline conditions [121, 123, 153, 154]. Chu found that PGA hydrolytically degrades faster in a highly alkaline medium (pH=10.09) than in a slightly alkaline or an acidic buffer (pH=5.25 and 7.44) [122]. The difference in this degradation behavior can be explained in terms of the cage effect in the crystalline region and the pH effect on the hydrogen bond. The chance of the recombining of two chain ends being located in the amorphous region resulting from a hydrolytic cleavage is too small to be significant. But two ends in the crystalline lattice might have higher chance to recombine owing to the greater restricted mobility of them. This is the so-called “cage effect”. Hydrogen bonds exist between two adjacent chain segments due to stereoregularity of the ester groups of PGA. They are intact in acidic and neutral media while they are destroyed at a higher pH level. Strong hydrogen bonds in acidic and neutral mediums could generate a relatively

more rigid and compact amorphous structure which blocks water penetration into the matrix and thus reduces the degradation. The fewer hydrogen bonds in higher pH medium makes the amorphous structure of PGA relatively open, which results in PGA degrading faster than that in a lower pH medium due to the better accessibility of the hydrolytic species. Another paper of this author reported that the degradation of absorbable suture material can be catalyzed by both acid and base [154].

The buffer capacity of the medium also impacts the degradation behavior. PGA degraded faster and lost more tensile strength in the buffered solution than in the un-buffered [155], which may be due to the presence of Na_2HPO_4 , which might remove the degradation products and make the reaction shift toward increased hydrolysis and accelerate the tensile strength loss [155].

When the acidic products were released upon degradation, the acidic products in the medium will decrease the pH of the aqueous medium and start to affect the whole polymer degradation. pH changes in the medium over time can be determined to confirm the degradation rate. This determination also could provide some information about the total amount of acidic products. The larger the pH decrease, the more the acidic products formation [118].

In summary, polymer degradation and drug release are enhanced by more hydrophilicity, less crystallinity and bigger size of the polymer. Therefore, for the release duration required less than one month, the choice of an amorphous polymer with rather high hydrophilicity would be appropriate. If the required release duration is longer, an amorphous polymer with a high Mw is suggested. When the release period is more than

half of year, a semi-crystalline polymer can be considered [141]. Nevertheless, one must remember that the degradation behavior of a polymer can be varied, depending on various intrinsic and extrinsic factors.

Chapter 3 Materials and Experiments

3.1 Materials

Biodegradable polymers were purchased from different companies and properties are listed in the Table 3.1 below. The following chemicals and devices were used as received: N-methyl-2-pyrrolidone (NMP) (Tedia), dimethyl sulfoxide (DMSO) (Fluka), triacetin (Sigma-Aldrich), metoclopramide monohydrochloride (metosalt) (Sigma-Aldrich) ($pK_a=9.71$ and 0.42) (solubility: 1428.6 mg/ml at 25°C), chloroform (Tedia), acetonitrile (ACN) (Tedia), tween 80, pluronic F-68 (PF-68), span 80, polyvinyl alcohol (PVA) (87.0 - 89.0% partially hydrolyzed, Mw: $11,000$ - $31,000$ Da), peanut oil, human dermal fibroblasts (L929) (ATCC), cell counting kit-8 (cck-8) (DoJinDo laboratories), DMEM + GlutaMAX (high glucose $1X$) w D-Glucose & Na Pyruvate (Gibco), foetal bovine serum 'Gold' - Defined FBS (EU Approved) (PAA Laboratories), HEPES (buffer solution, 1 M) (PAA Laboratories), trypsin (0.25% , $1x$, with 1 mM EDTA.4Na) (Gibco), penicillin-streptomycin liquid ($10,000$ U/ml penicillin, $10,000$ $\mu\text{g/ml}$ streptomycin) (Gibco), PBS Dulbecco's ($1X$, without Ca & Mg) (used for cell culture, PAA Laboratories), dialysis bag (Mw cut off $12,000$ - $14,000$ Da) and netwell insert (Corning incorporated). Metoclopramide base (metobase) (solubility: 0.2 mg/ml at 25°C) was prepared in the lab by reacting metosalt with NaOH solution. Phosphate buffer saline (PBS) ($\text{pH } 7.4$) was prepared in the lab. Solubility parameters of some solvents used in this project are listed in Table 3.2. The chemical structures of three main solvents and two drugs are listed in the Figure 3.1 and Figure 3.2 respectively.

Table 3.1 Copolymers and homopolymers used for preparation of formulations

Polymer	L/G ratio	L/G* ratio	End group	Manufacturer	Mw ⁺ (Da)	I.V. [#] (dl/g)
PdILA	100/0	100/0	Hydroxyl end	BioInvigor	16,700	0.18
PLGA 75/25	75/25	75/25	Hydroxyl end	BioInvigor	16,500	0.17
RG502	50/50	51/49	Esterified end	Boehringer Ingelheim	17,400	0.19(0.22)
RG502H	50/50	50/50	Carboxylic end	Boehringer Ingelheim	15,800	0.16
PdILA02	100/0	100/0	Hydroxyl end	PURAC	-	0.2
PDLG5002	50/50	52/48	Hydroxyl end	PURAC	-	0.16-0.24
PLGA50/50	50/50	48/52	Hydroxyl end	BioInvigor	36,700	0.33
RG504H	50/50	49/51	Carboxylic end	Boehringer Ingelheim	46,300	0.38
RG504	50/50	50/50	Esterified end	Boehringer Ingelheim	68,100	0.58

* L/G ratio determined by NMR in lab.

+ Molecular weight determined by GPC in lab.

Intrinsic viscosity as specified by manufacturer certified analysis.

Table 3.2 Solubility parameters of each solvent

Solvent	Solubility parameter	
	$(\text{J}/\text{cm}^3)^{1/2}$	$(\text{Cal}/\text{cm}^3)^{1/2}$
DMSO	26.7	12.9
NMP	23.2	11.3
triacetin	21.0	10.3
H ₂ O	47.8	23.4

Figure 3.1 Structure of solvent DMSO (left), NMP (middle) and co-solvent triacetin (right) respectively. **Figure 3.2** Structure of drug metosalt (left) and metobase (right).

3.2 Experimental procedures

3.2.1 Microstructure analysis by ^1H nuclear magnetic resonance (NMR)

^1H NMR spectra of the homopolymers and copolymers were recorded on a Bruker DRX

400MHz nuclear magnetic resonance spectrometer. Samples were prepared by dissolving ~9 mg of polymer in ~0.6 ml deuterated chloroform (CDCl₃). All measurements were carried out at 25 °C.

¹H NMR spectra were used to determine lactic acid/glycolic acid ratio (LA/GA) of copolymers, which is based on the distinct behavior of hydrogen atoms in a magnetic resonance. The molar fraction of LA to GA was calculated from the integrated peaks at 4.8 ppm and 5.2 ppm, which arise from hydrogens in the -CH₂- and -CH- groups. The ratio of LA and GA equals to the ratio of the peak area of LA and one-half of the peak area of GA.

Figure 3.3 Structures of GA and LA.

3.2.2 Preparation of formulations

Polymer solutions were prepared by dissolving each polymer and drug with the solvent together until clear solutions were formed. The concentration of the polymer solution and drug was all prepared in weight percentage. If a mixed solvent was used, co-solvent (e.g. triacetin) was mixed with the main solvent (e.g. DMSO or NMP) at various ratios (w/w of DMSO/triacetin or NMP/triacetin, 95/5, 90/10, 80/20 and 70/30) before the addition of drug and polymer.

In injectable *in situ* depot-forming systems, the polymer solution was put into a 65°C oven for 10 min to remove bubbles before injecting about 0.5 g of polymer solution (with or without drug) into 10 ml of PBS (pH 7.4) in a 15 ml glass bottle to form a drug incorporated depot, which was kept in a 37 °C incubator shaking at 50 rpm.

In injectable *in situ* microparticles-forming systems, 20% of tween 80 and 1% of PF-68 (both based on the weight of the polymer solution) was added to the polymer solution and magnetically stirred overnight to form the final polymer solution. Peanut oil with 2% of span 80 (based on the weight of peanut oil) was prepared to be the oil phase. The polymer solution was emulsified into the oil phase at a 1:1 ratio to form a uniform emulsion using an Ultra-Turrax homogenizer. After 1 min of emulsification, 1 ml of the emulsion was injected into a dialysis bag, which had been wet and placed into 10 ml of PBS (pH 7.4) contained in a 15ml capacity glass bottle for *in vitro* drug release studies. 0.02% w/v PVA was added into the release medium PBS as an anti-adherent.

3.2.3 Drug and solvent release

In both injectable *in situ* depot-forming drug delivery systems and injectable *in situ* microparticles-forming drug delivery systems, 1 ml of the release medium was removed at predetermined times to characterize the drug and solvent release and replaced with 1 ml of fresh buffer to maintain a constant total medium volume. For the experiments on drug and solvent release, the concentrations in the diluted solutions were determined by UV-Vis spectrophotometry (UV-2501, SHIMADZU) or HPLC (Agilent Technologies, 1200 series). The experiments for drug and solvent release were repeated at least 3 times and the mean and standard deviation were calculated, except where otherwise specified.

3.2.3.1 Release tested by UV

Figure 3.4 Calibration curves of metosalt and metobase in diluted (1/51) pH7.4 PBS at wavelength 309 nm, measured by UV-Vis spectrophotometry.

Samples were diluted using deionized water before being tested by UV-Vis spectrophotometry. Readings of absorbance at 309 nm were recorded to measure drug release, which is the characteristic absorbance of metosalt and metobase unaffected by solvents, degraded PLGA monomers and oligomers diffused out of the depot. The calibration curves were obtained using a few standard drug solutions in the same diluted buffer with concentrations ranging from ~0.001 to ~0.05 mg/ml, and a fresh diluted buffer was used as a reference, as shown in Figure 3.4. Readings of absorbance at 220 nm and 207 nm were recorded to calculate NMP and DMSO solvent concentration in the medium. Solvent calibration curves were obtained using standard solvent solutions in the same diluted buffer with concentrations ranging from ~0.001 to ~0.25 mg/ml, as shown in

Figure 3.5. The cumulative release amounts of drug or solvent were calculated based on calibration curves.

Figure 3.5 Calibration curves of NMP and DMSO in diluted pH7.4 PBS at wavelength 220 nm and 270 nm respectively, measured by UV-Vis spectrophotometry.

3.2.3.2 Release tested by HPLC

The amount of each solvent (DMSO, NMP or triacetin) released from depots without drug loading was quantified at 235 nm wavelength by HPLC. The chromatographic analysis was managed on an instrument equipped with a UV detector and a ZORBAX 300 SB-C18 column. The operating conditions were as follows: sample volume 10 μ l, ACN/water (v/v, 40/60) as mobile phase with 1.0 ml/min flow rate. Sample was filtered into a 2 ml glass vial through a 0.22 μ m disposable syringe filter and placed in an auto-sampler tray. Quantification was carried out by integration of the peak areas (DMSO: peaks at $\sim$ 2.6 min, NMP: peaks at $\sim$ 2.8 min, triacetin: peaks at $\sim$ 3.7 min). Solvent

calibration curves were obtained using standard solvent solutions in the buffer with concentrations ranging from ~0.025 to ~3.5 mg/ml, as shown in Figure 3.6. The cumulative release amounts of solvent were calculated based on the calibration curves.

The amount of triacetin left in the depots can also be detected by HPLC. At predetermined time intervals, depots were removed from the buffer solution and wiped lightly with a tissue to remove the surface water and then placed in a 15 ml centrifuge tube. 4 ml ACN was added to dissolve the depot to obtain a clear solution before 6 ml ultrapure water was added to precipitate polymer and form a suspension. The solution was centrifuged at 10000 rpm for 10 min to obtain the supernatant, which was filtered into a 2 ml glass vial through a 0.22 μm disposable syringe filter and placed in an auto-sampler tray to be measured by HPLC.

Figure 3.6 Calibration curves of NMP, DMSO and triacetin in diluted (1/11) PBS (pH 7.4) at wavelength 235 nm, measured by HPLC.

3.2.4 Contact angle measurement

A 30% polymer solution with dichloromethane (DCM) as solvent was coated on a clean Si slide using a spin-coating machine at 3000 rpm for 30 seconds. Water contact angles in the air were measured with a KSV-CAM200 contact angle meter with a PC-controlled motorized syringe with a precision of 0.5 °. Equilibrium contact angles were determined after the release of the drops from the needle, and only the initial values were reported as the equilibrium contact angle.

3.2.5 Degradation studies

For *in vitro* degradation, the samples collected at various time points were washed three times with deionized water to remove the deposited salts from the buffer and then freeze-dried. The remaining polymer mass was calculated based on the dried sample amount and the initial polymer amount.

The molar mass (M_w) of samples were determined using a gel permeation chromatography (GPC) system (SHIMADZU) equipped with a LC-20AD solvent delivery module, a SIL-20AC autosampler and a RID-10A differential refractometric detector. Dried sample was dissolved in chloroform at a concentration of ~5 mg/ml, filtered into a 2 ml glass vial through a 0.22 μm disposable syringe filter and placed in an auto-sampler tray. All measurements were carried out with two PL gel 5 μm mixed-C and one PL gel 5 μm mixed-E columns connected in series with chloroform as the mobile phase maintained at a flow rate of 1 ml/min. Twelve polystyrene standards with narrow M_w distribution in the range of 162-60,000 Da were used for calibration.

3.2.6 Swelling ratio or water uptake

Freshly collected wet samples were wiped with a tissue to remove the surface water and then weighed. In single solvent systems, the amount of drug and solvent released can be measured accurately. Meanwhile, there was no polymer mass loss in the early stage based on polymer mass remaining [156], so the water uptake could be calculated using the following formula based on the solvent release data:

$$\text{Water uptake\%} = \frac{W - W_p - W_s}{W_p + W_s} \times 100\% ,$$

where W represents the weight of the wet depot, W_p is the polymer weight in the depot and W_s is the weight of the solvent left in the depot. In mixed solvent systems, the amount of triacetin released is very difficult to measure in the presence of drugs, so swelling ratio is calculated using the following formula instead:

$$\text{Swelling ratio \%} = \frac{W}{W_o} \times 100\%$$

where W_o refers to the weight of the original depot. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

3.2.7 Viscosity studies

A Physica MCR501 Rheometer (Anton Paar) with temperature control was used to measure the steady shear viscosity and the dynamic flow properties of the PLGA solutions. The dynamic flow properties of the polymer solutions were measured by their oscillatory frequency “sweep”. The experiments were performed on a cone-and-plate

fixture with a diameter of 50 mm and a cone angle of 1 degree. The linear visco-elastic range of each sample was first determined by an oscillatory stress sweep, within which the strain amplitude ($\gamma=2\%$) was kept low enough so that structures were not disrupted by measurement. The range of the angular frequency (ω) was from 0.1 to 100 rad/s. The experiments were repeated 3 times, and the average and standard deviation were calculated.

3.2.8 pH measurement

pH measurements were conducted at room temperature using a Seven Easy bench-top pH meter (Mettler Toledo, Singapore) equipped with an Inlab Expert Pro electrode. The pH of the sample polymer solutions in PBS buffer were measured at predetermined time intervals to determine any changes in the pH. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

3.2.9 Morphology studies

At predetermined time point, depots were removed and cut into half and the water on the surface of the cross-section of the depots was first wiped off before the samples were freeze dried. Freeze-dried samples were fixed on an aluminum stub with conductive adhesive tape and then coated with gold for 50 seconds using an SPI-ModulateTM sputter coater to improve their electrical conductivity. The cross-sectional morphology of the depot was studied using a JEOL JSM-5410 LV scanning electron microscope (SEM) at 5 kV.

3.2.10 *In vitro* cytotoxicity studies of solvents

3.2.10.1 *In vitro* cytotoxicity studies of solvent by cck-8-based assay

Human dermal fibroblasts (L929) were used as cells. They were grown and expanded in DMEM medium consisting of 10% (v/v) FBS, supplemented with 100 U/ml of penicillin and 100 µg/ml of streptomycin in a 75 cm² tissue-culture flasks. After reaching ~90% confluence, cells (passage 9) were rinsed with 4 ml HEPES, afterwards detached from flask by a brief treatment with 4 ml 0.25% trypsin/EDTA and then neutralized by the same volume of fresh medium. The entire suspension was centrifuged at 220 G at 37°C for 5 min to collect cells by discarding the supernatant. Then, cells were counted after suspension in 1 ml of fresh medium. Afterwards, a certain volume of cell suspension, based on the requirement of the cells number in the whole 96-well polystyrene plate, was diluted into 10 ml of medium and then 100 µl cell suspensions with 8,000 cells were seeded into each well of the 96-well plate. The 96-well plate was incubated in an incubator in humidified air containing 5% CO₂ at 37°C for around 40 hours. Then the medium was replaced with solvent sample which was prepared in DMEM medium filtered through 0.22 µm sterilized filter before being added to each well of the 96-well plate. After a predetermined time point, the medium was discarded and washed by PBS (×1), then a 100 µl mixture of DMEM medium and cck-8 with a ratio of 10:1 was added into the well. After incubation for 4 hours in the incubator, the absorbance of each well was recorded at a wavelength of 450 nm with a wavelength of 650 nm as a reference by a microplate reader (Bio-Rad model 550). Each formulation has three samples and the data were expressed as mean ± standard deviation.

3.2.10.2 *In vitro* cytotoxicity studies of solvent by xCelligence analysis system

50 μ l medium was added into each well of E-plate 96 (Roche Applied Science and ACEA Biosciences). Then it was connected to the system and checked in the cell culture incubator for proper electrical-contacts and the background impedance was measured during 60 seconds. After a brief treatment with 0.25% trypsin/EDTA and neutralization by the same volume of fresh medium as well as centrifugation, the cell suspension was prepared in a DMEM medium at a certain concentration after counting cells number. Then, 100 μ l cell suspensions with 4000 cells were added to each well of the E-plate 96. After 30 min of incubation at room temperature, the E-plate was put back into the plate holder and left for around two days. Then, the medium was removed and 100 μ l solvent solution prepared in DMSM was added into each well to observe the change of the cell index for 10 days. Each formulation has three samples and the data were expressed as mean $\pm$ standard deviation.

3.2.10.3 *In vitro* cytotoxicity studies of injectable depots by cck-8-based assay

A 12-well plate with a netwell insert was used to perform an *in vitro* cytotoxicity study of injectable depots. A 1 ml cells suspension with 95000 cells were seeded in a 12-well plate overnight. Then, the medium was replaced with 4ml of fresh medium, and a netwell was inserted into each well, after sterilization by pure ethanol and washing by PBS ($\times 1$) as well as wetting by fresh medium. Each of the sterilized syringes with ~ 0.2 ml polymer solution was prepared before injection. After the injection of ~ 0.2 ml polymer solution into the medium of each well, the depot formed was suspended on the cells and surrounded by the medium. At the same time, the total weight of the syringe and needle

set was measured to get the exact weight of the depot injected. After a certain time point, the medium associated with the depots and netwell inserts were removed and the plate was washed by PBS ($\times 1$), then 1 ml mixture of medium and cck-8 with a ratio of 10:1 was added into the well. After 4 hours of incubation, about 110 μl of the mixture was transferred to a 96-well plate for testing by microplate reader as *in vitro* cytotoxicity study of pure solvents. Each formulation has three samples and the data were expressed as mean $\pm$ standard deviation.

Chapter 4 Effect of Polymer Type on the Dynamics of Phase Inversion and Drug Release

In injectable *in situ* depot-forming systems, the polymer and the solvent are two critical components. For the solvent, it is preferable to use NMP due to its biocompatibility and approval for use in the injectable system (EligardTM) manufactured by Atrix Labs [5-7]. However, very few studies were conducted to study the effect of polymer hydrophobicity on the dynamics of phase inversion and drug release.

Previously, our group reported the structure formation in injectable PLGA depots [157, 158]. In this section, we extend our studies to investigate the effect of polymer type on depot formation dynamics as well as on drug release: are the details dependent on the nature of the polymer? In this work, drug release, depot morphology and water uptake of four low-molar mass PLGA polymers with different hydrophobicity were studied to understand the effect of polymer type on the dynamics of phase inversion, as PLGA has been the most widely-used and studied polymer. The degradation of these depots was monitored to understand details of the entire period of drug release. The results enable us to predict the release profiles and thus to choose the proper components of the depot for different drug types.

4.1 Results and Discussion

4.1.1 The characterization of polymer hydrophobicity

Figure 4.1 Water contact angles on polymer spin-coated Si slides. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. Based on a PAIR-WISE T-test, $P < 0.05$, indicates that the contact angles of the two polymers are significantly different.

In this section, four polymers with various hydrophobicity, PdILA, PLGA75/25, RG502 and RG502H, were studied to understand the effect of polymer hydrophobicity on the drug release in injectable *in situ* depot-forming systems. To characterize the polymer hydrophobicity, the water contact angle on polymer spin-coated Si slides was measured. Si slide has been cleaned by $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ mixture before test, resulting in hydrophilic SiO_2 formation on the Si slide surface, which makes the contact angle of Si slide very low (<10 degree) and difficult to measure. As shown in Figure 4.1, the contact angle increases along with polymer hydrophobicity in the order $\text{RG502H} < \text{RG502} < \text{PLGA75/25} <$

PdLLA, all being more than 63 degree, which means that these surfaces are all significantly different from that of Si slide.

4.1.2 Effect of polymer type on the drug release

4.1.2.1 Drug release

Figure 4.2 Release profiles of metosalt from injectable depots in PBS (pH 7.4), where the formulation is polymer/NMP/metosalt = 40/60/1 (drug loading is based on the polymer and NMP weight, unless otherwise specified). Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

A polymer skin was formed immediately when the polymer solution was injected into the buffer solution. Non-solvent/solvent exchange then occurred through this skin concurrent with drug efflux from the depot. Four different polymers were studied, and Figure 4.2 demonstrates that the drug release from each polymer exhibited a four-phasic release profile in pH 7.4 PBS: an initial burst release (phase I), an induction period (phase II), a

phase with increased release (phase III) and the final burst release (phase IV). For RG502H and to some degree for RG502, the final burst phase is submerged into the phase with the increased release.

The extent of initial burst release in the first 24 hours occurred in the following order: RG502 ~ PLGA75/25 ~ RG502H < PdlLA. These findings can be related to the polymer hydrophobicity, as will be explained later. At this stage of release (24 hours), there is solvent-water exchange occurring, with two consequences: the solvent leaches out and usually a polymer-rich “skin” is formed at the periphery of the depot. In the initial burst, the drug at the periphery of the depot leaches out quickly along with solvent. Once the depot is formed, there is usually a period in which very little drug efflux occurs (the so-called induction period), and the drug molecules try to diffuse through the now-thickened depot; release during this phase occurs predominantly through the skin. Based on the initial burst release data, which are directly related to the dynamics of phase inversion, we can conclude that PdlLA undergoes a faster phase inversion than the other three polymers. This is related to its high hydrophobicity compared to the other polymers, as explained later.

The release in later periods is controlled by the combined effects of diffusion and degradation. Of the polymers studied here, RG502H undergoes the fastest degradation, due to its relatively low molar mass, almost 50/50 PLGA composition and the presence of -COOH end groups, which serve to catalyze the hydrolysis, resulting in a very short induction period and the onset of obvious degradation-controlled release (phase III) occurring earlier than for RG502, which is terminated with esterified end groups. The 50%

glycolide component of RG502H and RG502 results in faster degradation kinetics overall, allowing for the release of most of drug within ~7 days for RG502H and ~18 days for RG502.

To explain the details of the entire release profile, further characterization was carried out, and these results will be used to explain the release profiles below.

4.1.2.2 Solvent release

Solvent release from different polymer depots without drug loading was investigated by UV-Vis spectrophotometry as shown in Figure 4.3, The solvent release profiles resemble the drug release profiles. The initial burst release of solvent in the first 24 hours occurs in the following order: RG502 < PLGA75/25 ~ RG502H ~ PdlLA. This trend mirrors the trend in the initial burst release of drug, and is believed to be driven partly at least by the trend in hydrophobicity.

The initial solvent burst release is much higher in terms of amount (up to ~55%) than the initial drug release (up to 28%), which was expected due to the relatively small molar mass of the solvent and the fact that the solvent ‘release’ is actually due to the rapid exchange with water as a miscible solvent. The rate of burst release of the solvent from RG502H is higher compared to that from the other two polymer depots (PLGA75/25 and RG502) and exceeds that from PdlLA after 12 hours. The higher solvent release from RG502H at a later time mirrors the increase of drug release during the same period. Thus, the solvent release is also controlled by both degradation and diffusion.

Figure 4.3 Release profiles of NMP solvent from injectable depots in PBS (pH 7.4), where the formulation is polymer/NMP = 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset depicts the initial burst release profiles.

4.1.2.3 Morphology

To confirm the phase inversion of the polymer solution after injection and understand the high burst release from the PdLLA depot compared with other depots, the cross-sectional morphologies of PdLLA and RG502 depots were compared (Figure 4.4). Due to the difficulty of removing all of the residual solvent from the depot and the viscous properties of the inner portion, the inner morphology may be somewhat altered during the drying process. Thus, depot morphology is usually studied using the casting film method or the disk preparation method instead of the standard depot formation method [84, 88]. In this project, we used the depot formation method to study depot morphology and confirm the

rate of phase inversion, as this method is more reliable than the film casting method. Only ~0.15 g of the depot was used to reduce the influence of the amount of solvent on morphology formation.

Figure 4.4 Morphological images of the depot with no drug loading (~0.15 g) for 3 days: (a) cross-section of PdILA depot, (b) a higher magnification of PdILA depot cross-section; (c) cross-section of RG502 depot, (d) a higher magnification of the interior of RG502 depot at the boundary between the porous morphology near the surface and the more fluid polymer matrix in the middle, (e) skin surface of RG502H and (f) a higher magnification of (e).

Figure 4.4 shows that a highly porous phase dispersed over the entire cross-section of the PdILA depot, while the cross-section of the RG502 depot is less porous. The PdILA precipitates rapidly upon contact of the solution with water (skin formation), as it is the most hydrophobic (and hence has the least solubility in water); this rapid precipitation is then followed by solvent efflux through the polymer skin, resulting in a highly porous matrix structure, compared to the other polymers that precipitate less rapidly forming a smoother and more continuous matrix.

From Figure 4.4(e,f), the smoother and denser skin morphology compared to the inner

structure is clearly seen. This outer solid skin formation is due to rapid diffusion of the solvent out from the surface of depot, which confirms the retarded drug release during the so-called induction period. Porous skins are generated due to both the swelling and the degradation of the depot, especially for RG502H, which has the fastest swelling and degradation rate among the four polymers (as shown later).

To confirm the above conclusion, the cross-sectional images of each kind of wet depot (~0.5 g) were observed (Figure 4.5). Figure 4.5 shows a fairly dense solid structure over the entire cross-section of the PdlLA depot and a similarly solid but more porous cross-sectional structure of RG502H depot, while the cross-section of the PLGA75/25 and RG502 depots present hollow structures.

The relatively dense structure of the PdlLA depot indicates the fast progress of the solidification front to the interior of the depot. The gradual increase in the size of the hollow part found in PLGA75/25 and RG502 depots shows that the polymer components have gradually increasing time to migrate and rearrange before solidification, revealing their relatively slower phase inversion. The more porous morphology of RG502H depot is attributed to faster water uptake after skin formation and rapid degradation of the polymer caused by the strong ionization of the carboxyl end groups, that is responsible for the higher water uptake due to osmotic reasons.

Figure 4.5 Cross-sectional images of wet depots (~0.5 g) for 3 days, where the formulation is polymer/NMP = 40/60.

These results clearly demonstrate that, for the same solvent, more hydrophobic polymers (e.g., PdlLA) undergo faster phase inversion, resulting in a solid-like depot (although porous) structure being formed and faster initial solvent and drug release, while more hydrophilic polymers (e.g., PLGA75/25, RG502 and RG502H) undergo a slower phase inversion, resulting in a hollow structure formation and slower initial solvent and drug release. Then, the end groups of the polymer come into play and influence the subsequent release process.

4.1.2.4 Water uptake

Figure 4.6 Water uptake of injectable depots as a function of time in PBS (pH 7.4), where the formulation is polymer/NMP = 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset depicts the initial water uptake profiles.

Water uptake is the important event that occurs when the polymer solution comes into contact with water. The porosity of the matrix can also be determined by the degree of water uptake: high water uptake leads to highly porous matrix. Figure 4.6 shows that RG502H has the highest initial rate of water uptake followed by RG502, PLGA75/25 and PdILA, the latter three having almost the same water uptake at 6 hours. The mechanism of high initial water uptake is different for PdILA and the other polymers, particularly RG502H. PdILA precipitates quickly upon coming into contact with the water, which results in the release of a large amount of solvent, and the water occupies the spaces that the solvent occupied previously. This behavior explains why PdILA maintains the lowest

volume change at 6 hours after immersion but still has similar initial water uptake comparable to PLGA75/25 and RG502. The relatively higher water uptake of RG502H is due to the strong ionization of the carboxyl end groups, that results in higher osmotic pressure. As indicated by the contact angle measurements, RG502H is also more hydrophilic. The corresponding high drug and solvent release from the RG502H depot (at least initially) are related to this water uptake/solvent exchange.

4.1.2.5 Degradation data and polymer mass remaining

Because release in the later stage is usually controlled by both diffusion within the matrix and matrix degradation, the degradation was studied by measuring the change in molar mass (M_w) (Figure 4.7(a)) and the mass of polymer remaining (Figure 4.7(b)). Figure 7 shows that M_w decreases with degradation time, and the rates are ranked as PdlLA < PLGA75/25 < RG502 < RG502H, in concordance with the polymer hydrophilicity, nature of end groups and the rate of hydrolysis of the ester bond involved. In general, PdlLA esters degrade slower than PLGA copolymers (as the GA unit is more susceptible to hydrolysis than the LA unit), and the acid end groups in PLGA copolymers accelerate degradation through acidic catalysis. In addition, the higher water uptake by RG502H is also responsible for accelerated degradation of this polymer.

Figure 4.7 M_w change (a) and polymer mass remaining (b) of injectable depots as a function of time in PBS (pH 7.4), where the formulation is polymer/NMP = 40/60.

The polymer mass remaining is almost constant for a long period until it begins to gradually decrease. The induction period is also related to the polymer type, with more

hydrophobic polymers having longer induction periods. Later, polymer mass loss mainly occurs due to erosion caused by acidic conditions after the collapse of the porous structure. Comparing the two figures demonstrates that the onset of mass loss occurs when M_w approaches ~6000 Da. For example, the M_w of RG502 decreased to ~6000 Da after 15 days, with no polymer mass loss for up to 15 days. At 17 days (when the M_w has dropped to below ~6000 Da), the RG502 mass was 95.3% left (mass loss of 4.7%), and after that the polymer mass began to decrease gradually. The point of onset of polymer mass loss is also related to the time of depot collapse, which will be shown later. The mechanical properties of the depot were sufficient to withstand the osmotic pressure between the external medium and the inner medium caused by the matrix degradation until the M_w decreased below ~6000 Da.

4.1.2.6 pH value

Figure 4.8 displays the pH change in the external medium versus immersion time for the four polymers. The medium pH change is related to the extent of fragmentation and mass loss of the hydrolyzed polymer. For all four polymers, the pH decreases very slowly for some time prior to a sudden drop. The onset times of this sudden drop occur at ~6 days, ~17 days, ~27 days and ~36 days for RG502H, RG502, PLGA75/25 and PdlLA, respectively. A study of D,L -lactide oligomers in pH 7.4 PBS showed that the local pH inside the material is lower than the exterior medium, since the carboxylic groups formed are unable to rapidly leach out of the bulk polymer and decrease the pH then [149, 159, 160]. In contrast, the pH at the surface is unchanged less due to the exchange with the exterior buffer as well as the buffering capacity. The sharp pH drop in the polymer occurs

due to the collapse of the hollow depot. The acidic oligomers and the residual drug that were previously trapped in the core of the depot are released and lead to the sudden pH drop, and the final stage burst release occurs, reflecting heterogeneous degradation.

Figure 4.8 Change in medium pH as a function of immersion time for different injectable depots, where the formulation is polymer/NMP = 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

4.1.2.7 Comparison

Figure 4.9 Comparison among solvent release, polymer mass remaining and pH change in the medium for different injectable depots, (a) PdLLA and (b) RG502, where the formulation is polymer/NMP = 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Solvent release, mass loss and pH value change were compared, as shown in Figure 4.9. The results clearly show that the final stage burst solvent release, the onset time of polymer mass loss and the sudden pH drop occur at nearly the same time. This proves that the second burst release of the solvent is due to the collapse of the depot, which releases the acidic products and decreases the pH in the medium immediately and leads to rapid polymer mass loss. Meanwhile, the decreased pH erases the matrix of the depot, accelerating the mass loss too.

4.1.2.8 Discussion of release profiles

All four polymers exhibit a four-phasic release profile for the hydrophilic drug, metosalt. There is an initial burst release, followed by an induction period whose duration varies with the polymer used, a continuous release period, and then finally an acceleration period until the depot disappears completely. Schematically, this is what we believe happens during the various stages of depot formation and complete erosion (Figure 4.10).

The early burst release is due to the quick efflux of the solvent (see Figure 4.3) which carries the drug with it. This exchange of solvent with water (a non-solvent) leads to skin formation. This skin is a polymer-rich phase and is not very permeable to drugs, or has a fairly low diffusion coefficient for most drugs. Once the polymer-rich “skin” is formed, when the polymer-rich phase grows in extent, the entire depot becomes highly viscous and attains a depot texture. The detail of each step that happens next depends on the hydrophilicity of the polymer, and that is reflected in the difference in release profiles. For PdLLA, which is more hydrophobic, there is rapid progress of the solidification front to the interior of the depot, without a substantial increase in volume. As discussed

previously, solid matrix formation is rapid for PdLLA, but the matrix itself is quite porous (Figure 4.4). This allows for rapid efflux of solvent (with dissolved drug), along with filling of the pores by water. For a more hydrophilic polymer, its formation is less rapid and the matrix structure is less porous especially for RG502 and PLGA75/25, resulting in the lowest burst release of solvent.

Figure 4.10 Schematic illustration of depot formation and degradation in an injectable system. Upon injection of polymer solution into PBS, a polymer skin is formed immediately, due to the penetration of PBS into the polymer solution with concurrent solvent and drug efflux. A porous depot is formed, followed by water uptake that swells this depot. More hydrophilic polymers swell faster/more. As degradation proceeds, wall thinning occurs. Eventually, the depot collapses when its mechanical properties would no longer withstand the osmotic pressure.

When the drug and solvent near the surface are released, very little drug efflux occurs, leading to the so-called incubation stage due to the diffusional and partitioning barrier created by the polymer-rich phase. It is possible that the polymer-rich phase has very low solubility (hence unfavourable partitioning) for the drug until the phase becomes porous enough to acquire water-filled pores. Subsequently, the polymer-rich phase swells and

allows diffusion; concurrently, degradation occurs inside the core and reduces the molar mass in the interior. Both of these enable the faster release of the drug in the third phase (phase III). As degradation continues from the core outwards, the matrix wall thins out until the M_w of the depot approaches ~6000 Da. Beyond this, due to the high osmotic pressure created by the water-soluble oligomers inside the core, the depot collapses, releasing the rest of the drug in the 4th accelerated phase.

For RG502H, the presence of the -COOH end groups results in catalytic acceleration of the degradation, and the swollen phase merges into the wall-thinning phase, and leads to depot collapse by day 6 without an appreciable phase II (induction period). For the others, the duration of the induction period depends on hydrophobicity of the polymer. For PdlLA, the induction period is short, as the polymer-rich phase grows quickly (due to the relatively high hydrophobicity). Once the polymer-rich phase covers most of the depot, the water-filled pores allow for degradation and drug release, and this leads to phase III for PdlLA at around 4 days. For RG502 and PLGA75/25, the growth of the polymer-rich phase is slower. Due to the relatively viscous structure, the drug needs some time to diffuse out of the matrix, and the onset of phase III occurs at around 6-7 days.

During phase III, drug efflux occurs through water-filled pores (for PdlLA) or through a water-swollen skin (RG502 and PLGA75/25). The interior also becomes less viscous due to degradation, facilitating diffusion of the drug from the core. These two effects (drug diffusion through a less viscous core and through swollen polymer) cannot be separated for these polymers, and proceed concurrently.

Finally, as the degradation proceeds, the M_w of each polymer in the depot reaches ~6000

Da, the depot collapses (shrinks), loses mass and then subsequently dissolves completely. This onset of collapse is seen at different times for each polymer: ~6 days for RG502H; ~17 days for RG502; ~27 days for PLGA75/25; and ~36 days for PdlLA. In RG502, PLGA75/25 and RG502H, the onset of the final phase (or the 2nd burst for RG502H) is coincident with the incipient collapse of the depot, and is determined entirely by degradation kinetics. For these three polymers, the depot mass loss (Figure 4.7(b)) is rapid beyond these timepoints. For PdlLA, however, depot mass loss beyond the onset is more gradual.

Upon injection of the polymer solution into PBS, a polymer skin is formed immediately, due to the penetration of PBS into the polymer solution with concurrent solvent and drug efflux. A porous depot is formed, followed by water uptake that swells this depot. More hydrophilic polymers swell faster/more. As degradation proceeds, wall thinning occurs. Eventually, the depot collapses when its mechanical properties can no longer withstand the osmotic pressure.

To sum up the observed differences among the four polymers:

- (a) All polymers exhibit a four-phasic release profile: an early burst (phase I) due to solvent efflux (carrying drug with it); an induction period (phase II) where very little drug is released through the skin; a phase III where diffusion accelerates through a swollen porous matrix; and a final phase IV where substantial degradation leads to depot collapse and releases of all the remaining drug.
- (b) PdlLA differs from the others in that the induction period (phase II) is

relatively short, followed by a long phase III where diffusion occurs through a swollen skin/matrix. This is due to its relatively higher hydrophobicity which allows for a more rapid spread of the solidification front (which also is more porous).

(c) RG502 and PLGA75/25 exhibit fairly similar profiles, except for the onset of phase IV which occurs later for PLGA75/25, in accordance with its slower degradation.

(d) For RG502H, phases III and IV merge, due to its relatively fast degradation.

4.1.3 Effect of polymer concentration on the drug release

PLGA75/25 and RG502 polymers were chosen to study the effect of polymer concentration on the drug release. Figure 4.11 shows that at the early stage, there is no significant difference between the three formulations with different polymer concentrations. Then the release from the depot with 30% polymer concentration increases faster than that with 35% and 40% polymer concentrations while the difference between the release from the depots with 35% and 40% polymer concentrations is insignificant.

Figure 4.11 Effect of polymer concentrations on the metosalt release from injectable depots in PBS (pH 7.4), (a) PLGA75/25 and (b) RG502, where the formulation is polymer/NMP/metosalt = 30/70/2, 35/65/2 or 40/60/2. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset depicts the initial burst release profiles.

The initial burst release of the drug at the early stage is from the surface of the depot and

the water-filled pathways (polymer-lean phase) produced by the phase inversion. The depots formed with higher polymer concentrations will have denser structure formation, which will prevent water penetration into the depot, thereby reducing the subsequent release of the drug. For depots with lower polymer concentrations (eg. 30%), more porous structure is formed, resulting in most of the drug being released during the early stage. After the burst release stage, the increasing release phase is due to the degradation of the polymer and the second burst release results from depot collapse when the depot cannot bear the increasing osmotic pressure due to the further development of the heterogeneous degradation, as explained in previous sections.

4.1.4 Effect of drug loading on the drug release

Figure 4.12 Effect of metosalt loadings on the drug release from injectable depots in PBS (pH 7.4), (a) PLGA75/25 and (b) RG502, where the formulation is polymer/NMP/metosalt = 40/60/1, 40/60/2 or 40/60/3. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset depicts the initial burst release profiles.

Based on the effect of the polymer concentration on the drug release, a 40% polymer concentration was chosen to study the effect of the drug loading on the release as a 40% polymer concentration leads to a long term drug release with a low burst release. Figure

4.12 shows that there are no obvious difference among formulations with different drug loadings, for all the polymers studied (data of PdILA and RG502H depots not shown), which means the effect of the drug loading on the drug release is negligible.

The critical parameter for drug release is the rate of non-solvent/solvent exchange as the solvent can carry dissolved drugs out during this process, while the rate of the solvent release was not influenced significantly by the amount of the metosalt addition since hydrophilic drug metosalts can dissolve in the matrix completely and thus the depot morphology was not altered much by drug addition. Therefore, drug release depends on the solvent release only if the drug loaded can dissolve in the solvent and has no additional effect on the depot structure. The relationship between drug release and solvent release will be clearly illuminated in Chapter 7.

4.1.5 Effect of injection dosage on the solvent release

Since different injection dosages may be required in different applications, the effect of injection dosage on drug release was studied. Based on the above discussion, drug release is mainly dependent on the solvent release, so the solvent release was studied instead of drug release. Various dosages of the polymer solution, 0.1 g, 0.2 g, 0.3 g, 0.4 g and 0.5 g, were injected into the buffer solution and the solvent release was measured by UV-Vis spectrophotometry. The results show that the rate of solvent release decreases with the increase of depot size, as shown in Figure 4.13. For depots of small size, complete solidification is fast, while for depots of larger size, the solidification from the surface to the core will take more time to complete. As the diffusion coefficient of a polymer-lean droplet ($D \sim 10^{-5}$) is much higher than that of a polymer-rich matrix (rather viscous polymer solution) ($D \sim 10^{-7}$) [84], the same distance of solidification front plays much

more important role in the solvent release process for depots of small size. For depots of large size, a larger percentage of the solvent was trapped inside at the same time point, which is why the burst release from depots of larger size looks lower than that from depots of small size.

The discussions above indicate that the change in the pH value in the release medium affects the degradation of the depot as the degraded products of the depot diffuse out to decrease the pH in the medium, so the pH value change in the medium was studied to understand the effect of the depot size on the degradation associated with the drug release. As shown in Figure 4.14, the sudden pH drop occurs earlier for the depots of larger size, and relatively later for depots of smaller size. As mentioned before, the sudden pH drop of the medium is ascribed to the collapse of the depot, that is, the depot of larger size collapses earlier than that of smaller size. Meanwhile, the pH in the interior of the depot is much lower than that in the external medium, especially for the depots of larger size, which proves the occurrence of heterogeneous degradation (data not shown).

Heterogeneous degradation is more obvious for depots of larger size as it is more difficult for the acidic products to diffuse out; these acidic products aggregate in the core of the depot instead, resulting in a severe autocatalytic effect in the core of the depot compared to the part near the surface. For depots of smaller size the degraded products diffuse out easier and the buffer materials penetrate more easily into the depot to neutralize the acidic environment at the same time, which also lessens the aggregation of the acidic degraded products in the depot, leading to a more delayed collapse of the depot. However, it is worth noticing that even for depots of 0.1 g, the heterogeneous degradation is still existent since there is a sudden pH drop occurred although the time is later compared to depots of larger size (e.g. ~0.5 g).

Figure 4.13 Effect of depot size on the solvent release from injectable depots in PBS (pH 7.4), (a) PdILA and (b) RG502, where the formulation is polymer/NMP = 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset depicts the initial burst release profiles.

Figure 4.14 Effect of depot size on the pH change in the medium for injectable depots, (a) PdlLA and (b) RG502, where the formulation is polymer/NMP = 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

4.2 Proposed mechanism

An *in situ* forming depot is produced by the polymer precipitating from its solvent as the non-solvent penetrates into the polymer solution. From the phenomena of the hollow structure and the sudden drop of pH in the medium, it can be concluded that PdlLA and PLGA all underwent heterogeneous degradation. Generally, the release profile shows a four-phasic release pattern. The first stage is the burst release, which is followed by a lag time, and finally the diffusion and the degradation controlled stage.

In the first stage, the aqueous medium is filtered into the polymer depot and the solid skin is formed. The initial penetration of water into the polymer matrix initiates the release of the solvent entrapped very close to the surface of the matrix. And drug release will benefit from the solvent release which could bring the drug out by the solvent exchange process. The initial burst release is significantly influenced by the hydrophobicity of the polymers. The solvent releases faster in more hydrophobic matrices, due to the strong repulsion of the matrix resulting from the interaction between the polymer and the water. For

hydrophilic polymers, the initial release is slower since its water solubility is better than that of hydrophobic polymers, in which just a little water can make the polymer precipitate.

After the initial burst release, the drug release depends on the depot swelling and polymer degradation, especially for RG502H, which almost has no induction period, actually, the drug release from RG502H is quickly enhanced by the strong ionization of the end carboxyl groups, which facilitates the water uptake and enhances the solvent and drug release. That is why the drug and solvent release faster from RG502H than from RG502, which is terminated with esterified end groups.

Then the buffer solution penetrated into the polymer solution begins to degrade the depots from the surface to the inside. The duration of this stage is relatively short as compared to the later autocatalysis effect stage inside, because the water diffusion through hydrated homogeneous amorphous polymer matrix is very rapid.

When the entire solid structure is formed, the degradation continues in the matrix, but proceeds at a very low rate. However, the drug entrapped inside still needs some time to diffuse to the surface, so it appears as though very little drug is released until the drug molecule overcomes the barrier and reaches the surface.

The degraded oligomers produced near the surface could easily diffuse into the external aqueous medium, while the oligomers produced from the interior were trapped and accumulated inside, forming a more acidic microenvironment which catalyzed the degradation proceed. The surface part degraded more slowly, due to the degraded

products' rapid migration outward, and formed a hard skin and prevented the oligomers from diffusing out. With the interior autocatalysis proceeding, viscous solutions formed inside and then diffused out gradually until the hollow structure formed. Finally, collapse occurred because the hollow structure could not withstand the osmotic pressure.

4.3 Conclusions

Four polymers were used for *in situ* depot formation by injection of the polymer solution into pH 7.4 PBS, resulting in the formation of a polymer "skin" around the depot, followed by drug and solvent release. All of the depots exhibited four-phasic release profiles. The initial burst release depends on the polymer composition, and the drug release in 24 hours is ranked as RG502 ~ PLGA75/25 ~ RG502H < PdlLA, while the solvent release is ranked as RG502 < PLGA75/25 < RG502H ~ PdlLA. The morphology and water uptake studies showed that the higher initial burst release from PdlLA is due to its relatively faster phase inversion (solvent exchange) and subsequent formation of a porous structure. The duration of the induction period depends on hydrophobicity and onset of degradation, with PdlLA having a relatively short induction period while the RG502 and PLGA75/25 have slightly longer periods. The carboxyl-terminated RG502H is anomalous due to its relatively rapid degradation and water uptake, and hence shows practically no induction period. The Phase III release is a combination of diffusion through the swollen skin and release through an increasingly fluid core, due to polymer degradation in the core. This phase terminates at depot collapse, which signals the onset of phase IV or the rapid release of the remaining drug.

Apart from the hydrophobicity of the polymer, polymer concentration and the depot size

also affects drug release. High polymer concentrations lead to a low burst release due to the dense structure formed. The initial burst release from depots of larger size is lower compared to those of smaller size, but the sudden pH drop comes earlier due to the more severe heterogeneous degradation occurring in the interior of the depot which enhances the catalytic effect in turn. However, drug loading does not influence the drug release when the loaded drug is water soluble or can dissolve in the solvent uniformly. The drug release only depends on the solvent release, and the relationship between drug release and solvent release is closely related to the depot morphology while which is not altered much by the drug loading.

This study shows that the release profile of a hydrophilic drug from an injectable depot is sensitive to the nature of the polymer used in the depot. A hydrophobic polymer leads to a fairly high burst but the subsequent release is well-modulated, with durations that depend on the hydrolysis kinetics. More hydrophilic polymers tend to suppress the burst, but with a consequent rapid release of drug and depot collapse, mostly caused by more rapid water intake (swelling) and onset of degradation. Thus, an understanding of how the various polymer characteristics (hydrophobicity, degradation rate) affect the dynamics of phase inversion and drug release from these *in situ* gelling systems will enable broader exploitation of this technology.

Chapter 5 Effect of Drug Characteristics and Polymer Mw on the Dynamics of Phase Inversion, Drug Release and Depot Morphology

Usually, hydrophobic and hydrophilic drug release from film and microsphere differ in details, while the injectable depot, although simple to prepare, offers further differences. In injectable *in situ* depot-forming systems, the presence of an organic solvent may make hydrophobic drugs release like hydrophilic drugs and therefore make these systems a promising form of hydrophobic drug delivery system. Until now, few papers have reported on this. In this work, both the salt and base forms of metoclopramide were selected as model drugs to study the effect of drug type on drug release and water absorption as well as matrix degradation.

From the previous chapter, we know that low Mw hydrophobic polymers (I.V. ~0.2) undergo faster phase inversion and result in a higher burst release, while relatively hydrophilic polymers with low Mw (I.V. ~0.2) undergo slower phase inversion and result in a lower burst release. One question is: what will happen if the Mw of the hydrophilic polymer is higher? Some researchers have discussed the Mw effect on drug release, but there is still a lack of experience and data to confirm this.

Because the incorporation of drug may change the polymer Mw even during the process of sample preparation, which will affect the initial drug and solvent release and then interfere with the effect of drug characteristics on the release. Thus, in this chapter, two hydrophilic polymers representing high and low Mw polymers were studied to confirm

the impact of drug type and hydrophilic polymer Mw on the phase inversion dynamics and drug release. In addition, the effectiveness of drug type on polymer degradation of different types of polymers was also studied.

5.1 Results and Discussion

5.1.1 Effect of drug type on the release from high Mw polymer

5.1.1.1 Drug and solvent release

Figure 5.1 Release profiles of metosalt and metobase as well as solvent from injectable RG504 depots in PBS (pH 7.4), where the formulation is RG504/NMP/drug = 35/65/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Two model drugs, metosalt and metobase, representing hydrophilic and hydrophobic drugs respectively, were used in this research to study the effect of the characteristics of the drug type on the release. PLGA50/50 polymers were chosen as matrix materials

owing to their hydrophilic properties and suitable degradation rate in injectable systems. RG504 with an I.V. of 0.58 represents high Mw hydrophilic polymers and RG502 with an I.V. of 0.19 represents low Mw ones. In this section, the effect of drug characteristics on the drug release from RG504 depots is discussed first. Solvent release was studied together with drug release as it plays a critical role in the drug release control.

As shown in Figure 5.1, the release profiles of metosalt formulation and metobase formulation from RG504 depots are similar, drug and solvent release from injectable depots all exhibit a three-phasic release profile: an initial burst release (phase I), an induction period (phase II) and an increasing release phase (phase III). Actually, injectable depots usually undergo four-phasic release process [156]. For PLGA50/50 hydrophilic polymers, final burst release phase (phase IV) formed by the depot collapse merges into increasing release phase (phase III), that is why it looks like three-phasic profile. The initial burst release of metobase from RG504 depots is lower than that of metosalt, followed by a shorter induction period (9 days) than that of metosalt formulation (15 days). After the induction period, both RG504 depots have an increasing release period, resulting, first, from depot degradation and then erosion. Using other high Mw PLGA50/50 polymers, such as, RG504H (I.V. 0.38) and PLGA50/50 (I.V. 0.33), the same results were obtained: the release rate of the metobase is always lower than that of metosalt (data not shown). During the entire period, solvent release was always higher than drug release as a result of its smaller molar mass as mentioned in our previous study [156].

Metosalt and metobase are neutral and basic types of drugs respectively. The phenomena

of faster release of basic drugs form from depots in benzyl benzoate solvent with the same drug metoclopramide or from films with lidocaine base [106, 141], compared to the corresponding neutral drugs, have also been observed in our laboratory, which was explained by the catalytic effect of basic drug on polymer degradation. The contrary lower metobase release from depots than metosalt release was never reported.

To explain the difference of the release rate between metosalt and metobase from RG504 depots, the amorphous/crystallization state of matrix, drug solubility in water, polymer Mw and the catalytic effect of the drug on the polymer degradation need to be considered in such complicated systems.

RG504 (PLGA50/50) polymer used here retained the amorphous state throughout the entire release process [161]. So, the possibility of the development of crystallization need not be considered in this research. Metosalt was expected to have a higher osmotic driving force for water uptake than metobase, since hydrophilic drug metosalt dissolve in water quickly and easily and thus the solubility of metosalt is not a problem in this system. Metobase is hydrophobic and much more difficult to dissolve in water, which may make drug release rate different. However, in this system, the presence of NMP organic solvent can enhance the dissolution of the metobase in the polymer solution. Although 1% metobase loading can dissolve in the polymer solution completely as expected, since clear polymer solution was formed when 1% metobase was added, it may not be equally solubilized in the interior part of the depot after injection. So, the effects of metobase loadings on the release and matrix degradation were studied to determine whether 1% metobase loading dissolves in the interior part of the depot as in the polymer solution, and

also to confirm whether the drug has catalytic effect on the polymer degradation and how to influence the drug release.

5.1.1.2 Effect of metobase concentration on the release

In order to determine whether the 1% metobase loading can be solubilized in the solid depot after injection as well as to detect the maximum solubilized metobase loading in the depot, a series of metobase loadings (0.2%, 1%, 5% and 10%) were studied, as shown in Figure 5.2. The results show that the initial burst release of drug (in 24 hours) from depots loaded with 0.2% metobase is the highest among all the formulations, followed by formulations loaded with 1% and 5% metobase, then 10% metobase. After the burst release, a slow-release phase named the induction period appears in each profile of the release, longer for depots with 0.2% metobase loading (17 days), shorter for depots with higher metobase loadings (≥ 1) (9 days). In the third phase, the drug release rate from formulations with 0.2% and 1% metobase loading constantly increases until the drug was exhausted. However, for formulations with 5% and 10% metobase loading, this phase was much shorter and the residual drug was released out completely in a few days. The trend of solvent release is similar to the drug release albeit with a higher rate but the difference of solvent release among the formulations with 1%, 5% and 10% metobase loading was negligible, which means that metobase loading does not influence the solvent release rate (except for the 0.2% case, see below).

The faster metobase release from depots with 0.2% metobase loading is believed to be the result of faster NMP release, which is due to the relatively faster phase inversion resulted from less degradation of the polymer during the process of polymer solution preparation

(the data of the effect of metobase loading on the polymer degradation will be shown later). The similar release rates of metobase from depots with 1% and 5% metobase loading are ascribed to similar release behaviors of the solvent. However, the similar release rate of solvent from depots with 10% metobase loading results in relatively lower burst release of metobase which is contrary to expectation. It is observed that polymer solutions were clear when the metobase loading was less than 5%, meaning that such amounts of metobase loading can completely dissolve in the polymer solution. When metobase loading was increased to 10%, the drug polymer solution becomes a suspension and it was necessary to heat the polymer solution to 37°C and even 65°C for a while to dissolve the metobase before injection, which means that 10% metobase exceeds the maximum soluble metobase loading in the polymer solution at 37°C. Furthermore, the same amount of NMP released from depots with 10% metobase loading in the early stage (such as ~35% NMP released in 12 hours) as other depots with relatively lower metobase loading (e.g. 1%, 5%), results in higher drug to NMP ratio in the depot, and may lead to the status of part of metobase transiting from soluble to precipitated and thus results in a lower drug release because the extra metobase needs to be dissolved first before diffusing out. Apart from the effect of organic solvent NMP on enhancing metobase dissolution in the depot, acidic condition in the core of the depot may also play an important role in metobase dissolution as the metobase may react with acidic products in the depot to form a water soluble metosalt, which may explain the release rate from depots with 10% metobase loading increasing to the same level as that from other depots during the period of 3 days to 9 days (the acidic condition is presented by pH change in the medium, which will be shown later).

Figure 5.2 Effect of metobase loadings on the drug (a) and solvent release (b) from RG504 depots in PBS (pH 7.4), where the formulation is RG504/NMP/metobase = 35/65/0, 35/65/0.2, 35/65/1, 35/65/5 or 35/65/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

The similar release rate of metobase and NMP from depots with 1% metobase loading as

that from depots with 5% metobase loading, implies that 1% metobase is solubilized in the interior of the depot as in the polymer solution with the help of NMP, and the external medium is also under unsaturated conditions for the drug release, thereby making the hydrophobic drug metobase diffusing out like the hydrophilic drug metosalt. Therefore, the possibility of drug solubility for the different release rates from RG504 depots with 1% metosalt and metobase loading is excluded.

The slowly increasing release during the induction period for depots with high metobase loading ($\geq 1\%$), especially for NMP release, is mainly resulted from the swelling of the depot, which is attributed to the catalytic effect of the metobase on polymer degradation (swelling and degradation data will be shown later).

In the third phase, the rate of drug release is constantly increasing, mainly due to the degradation of the polymer matrix first and then its erosion after depot collapse, which releases the trapped degraded products into the medium and causes the environment to become more acidic, leading to polymer mass loss along with the drug being released out until the polymer matrix becomes fragmented. For formulations with 5% and 10% metobase loading, the greater catalytic effect of metobase on polymer degradation as well as the more water uptake produce a much more acidic environment after depot collapses, resulting in the polymer mass being lost quickly and the residual drug being released in a short time, that is why phase III is shorter for these formulations. For formulations with 0.2% and 1% metobase loading, the catalytic effect of metobase and the water uptake is not severe enough to accelerate the depot degradation as depots with higher metobase loading, therefore the phase III is much longer.

5.1.1.3 Effect of drug loading on degradation of the matrix

The Mw change of the polymer material as a function of incubating time was studied to understand the effect of drug characteristics and drug loading on the degradation of the depot. The polymer Mw of the injectable solution was measured before injection as the starting point to examine polymer degradation during the process of the polymer solution preparation, and we found that the Mw of the polymer was reduced, compared to the Mw of the original polymer material (~68,000 Da), as shown in Figure 5.3. After injection, the depots with 5% and 10% metobase loading degraded quickly, followed by the formulation with 1% metobase loading. 0.2% metobase and 1% metosalt loading also affected the degradation of the polymer compared to the formulation without drug loading, although not too much.

Figure 5.3 Effect of drug type and drug loadings on the polymer Mw change of injectable RG504 depots after injection as a function of time in PBS (pH 7.4), where the formulation is RG504/NMP/drug = 35/65/0, 35/65/0.2, 35/65/1, 35/65/5 or 35/65/10.

The viscosity of the polymer solution was measured to verify the reduction of the polymer Mw before injection, as shown in Figure 5.4. The results show that the viscosity of RG504 solution in the case of 1% metobase is much lower than that in the case of 1% metosalt or no drug loading. Other two polymers show similar results, although not too much. These results are in good agreement with the results of decreasing of polymer Mw. It proves that metobase can catalyze polymer degradation and has already had a catalytic effect on the degradation of the polymer during the process of the polymer solution preparation.

Figure 5.4 Effect of drug type on the viscosity of polymer solution before injection, where the formulation is polymer/NMP/drug = 35/65/0 or 35/65/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The obviously reduced Mw of the polymer and decreased viscosity of the polymer solution, illustrate that 1% metobase is sufficient to catalyze polymer degradation. This effect is enhanced when the polymer solution was placed in a 65°C oven for 10 min

before injection to remove bubbles.

Generally, the degradation of the polymer should be enhanced proportionally by the amount of metobase loading. However, it is limited by the maximum soluble metobase loading in the polymer solution as well as in the depot since metobase cannot catalyze polymer degradation if they are insoluble, that is why 10% metobase loading has the same catalytic effect on the hydrolytic cleavage of polyesters as 5% metobase loading. Apart from the limitation of the solubility of metobase in the polymer solution, it is also supposed that metobase reacts with acidic products in the depot (e.g. carboxyl end group) to form metosalt, which has no catalytic effect on the degradation. Both the limited metobase solubility in the depot and the potential neutralization by acidic products lead to the polymer degradation being not proportional to the amount of metobase loading. Compared to the larger catalysis of 1% metobase on the polymer degradation, a highest burst release from formulation with 0.2% metobase loading is due to the lower catalytic effect of metobase and the great polymer Mw left, which will be further explained from the point of Mw effect on the burst release later.

The pH value change in the medium occurred simultaneously with the process of the degradation was studied. The results (Figure 5.5) show that pH decreases faster for depots loaded with metobase than that loaded with metosalt - the more metobase loaded, the earlier the pH drop dramatically. The sudden pH drop before a long period of no pH change has been explained in the previous chapter [156]. Briefly, the size of ~0.5 g of the depot determined that the degradation occurred in the depot belonged to heterogeneous degradation. The inner part of the depot degrades faster than the surface part as the

degraded products in the core are difficult to diffuse out, thereby resulting in a more acidic environment in the core and faster degradation there, and thus forming a hollow structure. When the polymer could not bear the osmotic pressure created by the water-soluble oligomers between the interior depot and the exterior medium, the hollow depot collapses and acidic oligomers and the residual drug releases, consequently leading to a sharp pH drop in the medium. The onset time of the sudden pH drop is in accordance with the starting time of the increasing drug release phase (phase III).

Figure 5.5 Effect of drug type and drug loadings on the pH change in the medium for injectable RG504 depots as a function of time in PBS (pH 7.4), where the formulation is RG504/NMP/drug = 35/65/0, 35/65/0.2, 35/65/1, 35/65/5 or 35/65/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

5.1.1.4 Effect of drug loading on the water uptake

To know the effects of drug characteristics and drug loading on the drug release, water

uptake (as an important parameter affecting degradation) was studied. Figure 5.6 shows that water uptake increases much faster when metobase loading is more than 1% ($\geq 1\%$), and the more the metobase loading, the faster the water uptake. For depots with 1% metosalt or 0.2% metobase loading, the water uptake increases slowly but is still more than that without drug loading. It indicates that metosalt and 0.2% metobase may produce more paths for non-solvent/solvent exchange and subsequent water uptake (in the case of the metosalt, osmotic pressure may also play a role).

Figure 5.6 Effect of drug type and drug loadings on the water uptake of injectable RG504 depots as a function of time in PBS (pH 7.4), where the formulation is RG504/NMP/drug = 35/65/0, 35/65/0.2, 35/65/1, 35/65/5 or 35/65/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Based on above discussion, metobase catalyzed polymer degradation during the process of the polymer solution preparation. Comparing the data of water uptake and degradation, it is believed that the decreased Mw of the polymer as a result of the catalytic effect of

metobase on the degradation before injection resulted in relatively slower phase inversion and a lower burst drug release as well. For RG504 depots with metosalt loading, the relatively intact Mw of the polymer makes it undergo faster phase inversion and produces a faster solvent and drug release, which will be discussed in the Section 5.1.3. In addition, the lower polymer Mw of the RG504 solution with metobase loading resulted in the subsequent fast depot swelling and high water uptake, which impacted degradation and drug release in turn.

5.1.2 Effect of drug type on the release from low Mw polymer

5.1.2.1 Effect of drug type on the drug and solvent release

Figure 5.7 Release profiles of metosalt and metobase as well as solvent from injectable depots in PBS (pH 7.4), (a) PdlLA, (b) PLGA75/25, (c) RG502 and (d) RG502H, where the formulation is polymer/NMP/drug = 40/60/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

As above-mentioned, the release behaviors of metosalt and metobase from hydrophilic RG504 depots are different. In this section, four polymers with low Mw and different hydrophobicities, PdLLA, PLGA75/25, RG502 and RG502H, were studied to check the response of different polymers on the catalytic effect of metobase. As shown in Figure 5.7, a comparison of drug and solvent release from the same polymer reveals that drug type has almost no effect on the release, except for the early stage of RG502 depot, in which the release rate of metobase was higher than that of metosalt. As discussed above, metobase catalyzes RG504 degradation during polymer solution preparation, which makes the Mw of RG504 with metobase loading lower than that with metosalt loading and thus influences the initial drug release behavior. So, the effect of the metosalt and metobase loading on the degradation of four polymers was also studied.

5.1.2.2 Effect of drug type on the degradation

Figure 5.8 shows us that metobase has no additional effect on the polymer degradation compared to metosalt, except for RG502 depots. It is believed that among four polymers only RG502 polymer is sensitive to the catalytic effect of metobase on the hydrolysis cleavage of polymer chains. From the point of the hydrophobic structure of PdLLA and PLGA75/25 compared to the relatively hydrophilic structure of RG502 and RG502H, it is expected that the catalytic effect of metobase plays a smaller role in their degradation. For RG502H, which has the same ratio of LA/GA as RG502, the catalytic effect of metobase is reduced by the presence of the COOH end groups which can neutralize part of metobase and thus decrease its catalytic effect. Furthermore, the rapid degradation of RG502H depots after contact with water increases the neutralization on the metobase

catalytic groups. The pH value change in the medium further proves this conclusion, as shown in Figure 5.9. The pH for the RG502 formulation with metobase is far lower than that with metosalt, but for other formulations, there is almost no difference between two formulations.

Figure 5.8 Effect of metosalt and metobase on the polymer Mw change of injectable depots in PBS (pH 7.4), (a) PdlLA, (b) PLGA75/25, (c) RG502 and (d) RG502H, where the formulation is polymer/NMP/drug = 40/60/1.

The viscosity of the polymer solution was also measured before injection to know the effect of metobase and metosalt on the degradation of the polymer during the process of polymer solution preparation (Figure 5.10). For RG502 solution with metobase loading, the lower viscosity as compared to the solution with metosalt loading means that the starting point of the Mw of RG502 is lower than that with metosalt as well as the original Mw of the polymer.

Figure 5.9 Effect of metosalt and metobase on the pH change in the medium for injectable depots in PBS (pH 7.4), (a) PdlLA, (b) PLGA75/25, (c) RG502 and (d) RG502H, where the formulation is polymer/NMP/drug = 40/60/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The faster release rate of metobase from RG502 depots compared to that of metosalt can be explained from the morphology formed. For RG502 depots with metobase loading, the reduced Mw by the catalytic effect of the metobase generated a much more porous structure compared to metosalt, resulting in a higher drug release, which will be described in the Section 5.1.3.

Figure 5.10 Effect of drug type on the viscosity of polymer solution before injection, where the formulation is polymer/NMP/drug = 40/60/1.

5.1.3 Comparison between high Mw and low Mw polymers

5.1.3.1 Comparison of the drug release

In injectable *in situ* depot-forming systems, the Mw of the polymer is an important parameter in the drug release, so the drug release from different Mw polymers was compared. As shown in Figure 5.11, the initial burst release (in 24 hours) from depots with higher Mw (RG504) is much higher (~50% metosalt, ~20% metobase) than that from depots with lower Mw (RG502) (~5% metosalt, ~10% metobase), no matter what the drug loaded was hydrophilic drug (metosalt) or hydrophobic drug (metobase), followed by a longer induction period for RG504 depots compared to a shorter induction period for RG502 depots. After the burst release, the latter release is affected strongly by the degradation or erosion of the polymer. The duration of the whole process for both

depots is similar, 20-25 days.

Figure 5.11 Release profiles of metosalt and metobase as well as solvent from injectable RG502 and RG504 depots in PBS (pH 7.4), (a) metosalt formulations and (b) metobase formulations, where the formulation is polymer/NMP/drug = 35/65/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Compared to the other polymer pair, RG504H (I.V. 0.38) and RG502H (I.V. 0.16), the release rate of the drug from low Mw polymers is always lower than that from high Mw polymers, no matter what kind of drug was loaded (data now shown). For the obviously different and contrary release rates of metosalt and metobase from two kinds of depots, the reasons should be found from aspects of drug characteristics and polymer Mw, basically, from the phase inversion dynamics and depot morphology. Note that in these two factors the final reason may be ascribed to the polymer Mw because polymer Mw will be changed if the drug has a catalytic effect on the polymer degradation.

5.1.3.2 Comparison of the depot morphology

Morphology as an important parameter which can directly reflect phase inversion rate of polymer solution after injection into the buffer solution was studied to understand the effect of drug characteristics and polymer Mw on the phase inversion dynamics. The cross-sectional images and SEM morphologies were taken to observe the polymer precipitation process visually and microscopically. Figure 5.12 shows that RG504 depots form solid and dense structures while RG502 depots form hollow structures, regardless of drug type. Both the diameter of the solid RG504 depot and the thickness of the shell of the hollow RG502 depot are relatively smaller in case of metosalt than that in case of metobase. SEM morphologies show that the cross-section of the shell of RG502 depots appears a honeycomb structure in the case of metosalt loading, and a more cellular structure in the case of metobase loading. RG504 depots have apparently different morphologies compared to RG502 depots. Finger morphology appears near the surface for both of RG504 depots; however, in the deep-layer of the depots, honeycomb appears

in the case of metosalt loading, while cellular structure appears in the case of metobase loading.

For either RG502 depot or RG504 depot in case of metobase, a slight increase in the size of the depots or the cellular structure of the morphology illustrates that metobase has a catalytic effect on the polymer degradation and leads the depot to form a more porous structure compared to metosalt and thus benefits water diffusion through the matrix, which explains the earlier development of the third phase in case of metobase.

The finger morphology near the surface appeared in only RG504 depots is believed to be caused by instantaneous precipitation when the polymer solution comes into contact with water. The deep-layer of the honeycomb structure is attributed to the delayed precipitation since, in the interior of the polymer solution, the solvent concentration at the water diffusion front is much higher, and we may expect a transition from the instantaneous precipitation process taking place globally to a delayed precipitation taking place locally [97]. This viewpoint was confirmed when the finger morphology became shorter and even disappeared when increasing amounts of solvent were added to the coagulation bath [97], in which a transition from instantaneous precipitation to delayed precipitation occurs.

Figure 5.12 Cross-sectional images of wet depots and SEM of dry depots at 2 days with metosalt and metobase drug loading respectively. The left row is the pictures of wet depots, the right two rows are the detailed SEM morphologies of dry depots. (a) and (b) are RG502 depots while (c) and (d) are RG504 depots. (a) and (c) are in the case of metosalt loading while (b) and (d) are in the case of metobase loading.

5.1.4 Discussion on the effect of polymer Mw on the drug release and depot morphology

Generally speaking, it is expected that higher Mw polymer leads to slower drug release since the higher Mw of the polymer causes more chain entanglements, and thus slows down the diffusion of drug within the polymer matrix [1, 89]. However, our results are opposite: drug release from depots with higher Mw was much faster than that from depots with lower Mw.

Although the effect of the polymer Mw on the initial burst drug release is related to the phase inversion dynamics of the polymer solution, for the entire release process the main reason should be finally attributed to the depot morphology formed, which also influences the burst release since the solid/hollow depot structure almost could be formed before 24 hours. For all the formulations, after the injection of polymer solution into the buffer solution, water penetrates into the polymer solution, resulting in the polymer solution near the interface forming a skin immediately, followed by solid particles formation from the skin to the core. For hydrophilic polymer with high Mw (e.g. RG504 polymer), which undergoes faster phase inversion upon contact with water, less water penetration makes the solid particles develop rapidly and there is no migration before solidification and thus a highly dispersed phase forms over the entire cross-section. The solid structure with porous interior results in a high burst release. But for low Mw hydrophilic polymer (e.g. RG502 polymer), the relatively slower phase inversion leads to a time lag between water diffusion front and gelation front. Solid particles developed in the diffusion front are much mobile before gelation, compared to viscous polymer solution. Meanwhile, more

solvent left in the depot further leads the solid particles difficult to develop, so they have time to migrate and concentrate around the inner skin, while the liquid phase (water and solvent mixture with drug inside) is contained in the core due to the barrier of the skin, thereby forming a hollow structure with most of the drug in the core. The hollow depot works like a drug reservoir providing constant drug release and long term delivery.

The inner part of RG504 depot is much more porous but the surface is much smoother and denser (Figure 5.13), which should be a great diffusion barrier to the drug release. However, two skins appeared in the hollow RG502 depots, an inner skin and an outer skin. As shown in Figure 5.14, both of them are very dense and smooth, resulting in drug and solvent having double difficulty to diffuse out, which should be the main reason for the slower and constant drug release from RG502 depots.

Figure 5.13 Surface morphologies of RG504 depots with metosalt and metobase drug loading respectively, (a, b) metosalt and (c, d) metobase.

Figure 5.14 Surface morphologies of outer skin and inner skin of RG502 depots with metosalt loading (the similar data for metobase loading not shown), (a, b, c) outer skin and (d, e, f) inner skin.

As reported by Dunn et al. [3], for the polymer system of injectable gelling systems, the release rate of the incorporated drug follows a “U” shaped curve with the increase of polymer intrinsic viscosity (I.V.), with the minimum point at I.V. ~ 0.2 . Due to the catalytic effect of the metobase, for RG504 depots (I.V. 0.58), the I.V. of the polymer with metobase loading before injection is lower than that with metosalt loading, but is still much higher than the minimum point of ~ 0.2 . For RG502 depots (I.V. 0.19), both the I.V. of the polymers with metosalt and metobase loading are lower than ~ 0.2 . So the I.V. of the polymer in the formulations is in this order: RG504/metosalt > RG504/metobase > 0.2 > RG502/metosalt > RG502/metobase, as shown in Figure 5.15. As above-discussed, the slower drug release from RG502 depots compared to that from RG504 depots is due to the slower phase inversion and the resultant hollow depot structure. For the slower metobase release than metosalt release from RG504 depots, which are both solid structures, the main reason should be due to the decreased polymer Mw before injection and the resultant slower phase inversion. However, for RG502 depots, which both

undergo slower phase inversion and form hollow structures, the higher metobase burst release than metosalt release is due to the cellular and much more porous structure formed caused by the decreased Mw due to the metobase catalyzation.

Figure 5.15 Burst release from injectable depots as a function of original I.V of the polymer.

5.2 Conclusions

In summary, the drug characteristics and polymer Mw have important effects on the phase inversion dynamics, depot morphology and drug release. For RG504 depots, as higher Mw polymers, the burst release of metobase is much lower than that of metosalt because metobase has a catalytic effect on polymer degradation and the decreased Mw makes the polymer solution undergo a relatively slower phase inversion and thus results in a lower burst release. For depots with a lower Mw, the situation is a little complicated. For RG502 depot with metobase, the catalytic effect of metobase makes the polymer Mw decreased, and the decreased Mw leads to the formation of a more porous structure and the

production of a higher burst release compared to depots with metosalt. For RG502H depot which is terminated with acidic end groups, the neutralization effect of the acidic groups with metobase leads to a reduction of the catalytic effect of metobase on the degradation, which results in the difference between metobase release and metosalt release being insignificant. When the polymer is more hydrophobic (e.g. PdlLA and PLGA75/25), the difference between the release of metosalt and metobase is insignificant as the metobase has less effect on degradation of these hydrophobic polymers. Comparing between high Mw and low Mw hydrophilic polymers, higher Mw polymers (e.g. RG504) undergo faster phase inversion, resulting in solid structures and higher burst release, while lower Mw polymers (e.g. RG502) undergo slower phase inversion leading to hollow structures and lower burst release.

Injectable *in situ* depot-forming systems can overcome the shortcoming of limited solubility of hydrophobic drug. However, the potential catalytic effect of such drugs should be carefully taken into account during the development of this type of advanced drug delivery systems. A slightly higher Mw of the polymer than required should be used in such a situation.

Chapter 6 Co-solvent Triacetin Effects on the Drug Release, Depot Swelling and Solvents Cytotoxicity Studies

From previous chapters, we know that the formation of the solid/semi-solid depot from the polymer solution is not instantaneous. Since this injectable implant system is managed as a liquid, it is unavoidable to have a time lag between the injection and the completion of formation of the solid implant. During this time lag, the initial drug burst release occurs, typically over a period of minutes to several hours, resulting in the release of a large amount of drug, especially when the drug is soluble in the solvent or water, and causing tissue irritation and sometimes systemic toxicity if the drug is particularly toxic [162]. Due to this shortcoming, this system is limited to drugs with a broad therapeutic index.

As the initial burst release is affected significantly by polymer phase inversion dynamics, many approaches related to manipulating the rate of phase inversion of the polymer solution were developed to control the burst release. Increasing the polymer concentration and adjusting the polymer molar mass are commonly used methods to control the burst release but limited because of the low viscosity requirement of the polymer solution during injection. To reduce the burst release while maintaining injectability, several methods were proposed recently, such as micronizing the drug with or without hydrophobic agents to form particulates [163], introducing carriers for the drug to form a mixture [164], adding a polymeric controlled release additive [162], or adjusting the solvent characteristics by mixing a hydrophilic solvent and a hydrophobic solvent at different ratios [165, 166]. Compression of the drug into tablets and subsequent grinding yields particulates of drug lower surface area to mass ratio than that formed by the

conventional methods, which finally provides a significant reduction in water uptake as compared to non-compressed particles in such a gel. If the drug is liquid, it may be incorporated into a porous solid particle, such as anhydrous calcium phosphate [163]. When a carrier is added into the system, the drug is isolated from the organic solvent and less likely to disperse into the surrounding aqueous medium along with the solvent. Instead, the drug is constrained within the delivery system as it solidifies to form a semi-solid implant. Consequently, the initial drug burst release may be suppressed [164]. The polymeric controlled release additive, preferably water insoluble, such as a poly(lactide-co-glycolide)/polyethylene glycol block copolymer (e.g., PLG/PEG-5000), can also be incorporated into the polymer solution to delay phase inversion so as to reduce the burst release [162].

In comparison to the complicated administration of compaction/grinding or adding a new component (carrier or additive) to the formulation, the simple administration benefit of mixed solvent system is particularly attractive. In contrast, the hydrophobicity of mixed solvents can be adjusted readily based on the requirement.

In the previous chapters, the effect of polymer hydrophobicity, the drug type and the polymer Mw on the phase inversion in pure NMP solvent system have been discussed. In this chapter, details on the influence of triacetin as co-solvent on the reduction of burst release were studied in a mixed solvent system [96]. The hydrophobic polymer PdlLA and the more hydrophilic polymer RG502 were chosen as representative polymers to study the effect of the amount of triacetin on the drug release from different types of polymer. The actual release of each solvent over time was also quantified to understand

the triacetin influence on the initial solvent burst release. The swelling ratio of the depot and the cross-sectional morphology were also investigated as parameters that could help to understand the details of drug release profile. In addition, pH and molar mass changes of these depots were tracked to further evaluate the effect of triacetin on degradation.

6.1 Results and Discussion

6.1.1 Effect of polymer concentration on the drug release

Figure 6.1 Effect of polymer concentrations on the metosalt release from injectable depots with DMSO solvent in PBS (pH 7.4), (a) PdlLA and (b) RG502, where the formulation is polymer/DMSO/metosalt = 30/70/1 or 40/60/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Dimethyl sulfoxide, DMSO, is known to possess much better biocompatibility than many organic solvents; as such its effect on the drug release is of interest. In the DMSO solvent system, polymer concentration as an important parameter was studied first. 30% and 40% (w/w) polymer concentrations were chosen to study the effect of the polymer concentration on the drug release using four different polymers. Figure 6.1 demonstrates that the drug release from each polymer also exhibited a four-phasic release profile in pH 7.4 PBS as in NMP system (data for PLGA75/25 and RG502H are not shown). The initial

rate of the drug release from the depot with a higher polymer concentration (40%) is lower than that with a lower polymer concentration (30%), no matter which kind of polymer is used. After the burst release, there is almost no drug release for a long time until the increasing release stage or the second burst release occurs, especially for PdlLA polymer. For the same polymer, the shape of the release profile is not affected by polymer concentration. In contrast, the onset time of the induction period and the increasing release as well as the final burst release is identical.

6.1.2 Effect of polymer concentration on the solvent release

To understand the effect of the polymer concentration on the drug release, the rate of the solvent release was studied, using 3 different polymer concentrations of 30%, 35% and 40%. Results show that the release profiles of the solvent resemble that of the drug in this solvent system. High polymer concentration results in a low DMSO initial release (Figure 6.2), which verifies the difference of the drug release brought by the concentration of the polymer. Almost no solvent release after the burst release explains the absence of drug release in the same period.

For the effect of polymer concentration on the drug release, two parameters compete with each other, namely phase inversion dynamics and chain entanglements. High polymer concentration results in relatively fast phase inversion since less water can make polymer precipitate, resulting in a higher burst release. However, high polymer concentration also increases the polymer chain entanglements, resulting in a denser structure and lower burst release as well. The result of the lower burst release from high polymer concentration indicates chains entanglements dominate the drug release in such condition.

Figure 6.2 Effect of polymer concentrations on the DMSO release from injectable depots in PBS (pH 7.4), (a) PdlLA and (b) RG502, where the formulation is polymer/DMSO = 30/70, 35/65 or 40/60. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

6.1.3 Effect of polymer type on the drug release

Figure 6.3 Release profiles of metosalt from different injectable depots with DMSO solvent in PBS (pH 7.4), where the formulation is polymer/DMSO/metosalt = 40/60/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

Comparing the releases from the four polymers, the profiles show the same four phases in

DMSO solvent systems as in NMP solvent systems (Figure 6.3 and Figure 4.2). For each polymer, all the burst releases in DMSO solvent systems are higher than those in NMP solvent systems, except for the RG502 polymer. At the same time, the induction period is much longer for depots in DMSO solvent systems, especially for the hydrophobic polymer PdlLA; there is almost no drug release after the burst release for a long time until depot collapse at 39 days.

For single solvent systems, especially for hydrophobic polymer PdlLA depots in the more hydrophilic solvent DMSO, the burst release is a significant shortcoming. In order to understand the effect of solvent type on the drug release and reduction of the high initial burst, hydrophilic solvent (DMSO or NMP) and a hydrophobic co-solvent (triacetin) are mixed as solvent to study the amount of co-solvent effect on the reduction of the burst release and depot swelling.

6.1.4 Effect of triacetin as co-solvent on the drug release

6.1.4.1 Effect of triacetin on the drug release

Figure 6.4 Effect of the amount of triacetin on the metosalt release from injectable PdILA depots in PBS (pH 7.4), where the formulation is PdILA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO/triacetin = 100/0, 95/5, 90/10, 80/20 or 70/30. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

The release of metosalt in mixed solvent systems was studied by UV-Vis spectrophotometry as described in the methods section. Figure 6.4 demonstrates that the rate of burst release of the drug from PdILA depots with a pure DMSO solvent is the highest among all the formulations. After the burst release, there is very little drug release for a long period until the depot collapses. By using triacetin as co-solvent, the high burst release is reduced significantly and the level of the reduction is proportional to the amount of triacetin, up to ~20% by weight of triacetin. The addition of 5% triacetin

reduces the burst release from ~67% to ~15%, while 10% and 20% triacetin reduces burst release to ~8%, and the trend is maintained within 30% triacetin (although subsequent release from the 30% triacetin is faster). After the burst release, sustained drug release is obtained from the depots with triacetin.

Figure 6.5 Effect of the amount of triacetin on the metosalt release from injectable PdLLA depots in PBS (pH 7.4), where the formulation is PdLLA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is NMP/triacetin = 100/0, 95/5, 90/10, 80/20 or 70/30. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

The initial burst release is related to the phase inversion dynamics of the polymer solution; the subsequent release is mainly controlled by the swelling of depot and degradation of the polymer material, which will be discussed later.

Figure 6.6 Effect of the amount of triacetin on the metosalt release from injectable RG52 depots in PBS (pH 7.4), (a) DMSO/triacetin and (b) NMP/triacetin, where the formulation is RG502/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO(NMP)/triacetin = 100/0, 95/5, or 90/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

In comparison to the higher burst release from PdlLA depots in pure DMSO solvent system, the burst release from PdlLA depots in pure NMP solvent is relatively lower, which can also be reduced by triacetin addition, but not significantly (Figure 6.5).

Triacetin can also be used as a co-solvent to reduce the initial burst release from the hydrophilic polymer RG502 depots, regardless of which solvent, DMSO or NMP, it is mixed with. As shown in Figure 6.6, the initial burst release is reduced when triacetin is added; the subsequent release kinetics is similar, with an acceleration of release noted for 10% triacetin addition. We attribute this to greater solvent retention caused by addition of the co-solvent triacetin (see discussion below), that in turn leads to a more “plasticized” core polymer allowing for faster diffusion rates.

The burst release of the drug from PdlLA depots is much higher than that from RG502 depots in the same solvent system. We have explained this observation in a previous Chapter. Briefly, the higher initial burst release from hydrophobic polymer PdlLA depot was due to its relatively faster phase inversion (solvent exchange) and subsequent formation of a highly porous structure, while the more hydrophilic polymer RG502 depots underwent a slower phase inversion resulting in lower initial solvent and drug efflux and a less porous and more fluid polymer matrix formation [156].

As shown in Chapter 7 (and briefly discussed in Section 6.1.4.2), the burst release is closely related to the solvent release, which in turn is related to phase inversion (non-solvent/solvent exchange) rates. Triacetin is comparatively hydrophobic, and its addition slows down this exchange, and decreases the solvent burst release. The effect is more dramatic for PdlLA than for RG502, which has a relatively low burst to begin with.

6.1.4.2 Effect of triacetin on the solvent release

As the hydrophilic drug is dissolved completely in the organic solvent used, solvent efflux from the depot will also transport the dissolved drug molecules. We therefore quantified the solvent release by HPLC to determine the effect of triacetin on the rate of non-solvent/solvent exchange. For the purpose of simplifying the system, solvent release from both the pure solvent systems and mixed solvent systems were measured without any drug loading to compare the variation of the solvent release. The mixture of triacetin and DMSO or NMP was used as a mixed solvent, and PdILA was used as the polymer material. As shown in Figure 6.7, the burst release of DMSO is faster than that of NMP in pure solvent systems. After the burst release, there is almost no DMSO release for a long period until the depot collapses, which mirrors the drug release behavior during the same period (Figure 6.4). After incorporation of triacetin in the polymer solution, not only is the burst release of either DMSO or NMP reduced significantly, but the subsequent release is also modulated, especially for DMSO solvent system.

The difference between the DMSO system and NMP system is supposed to be the difference in their solubility parameter as the solubility parameter of DMSO ($26.7 \text{ (J/cm}^3)^{1/2}$) is closer to water ($47.9 \text{ (J/cm}^3)^{1/2}$) compared to NMP ($23.2 \text{ (J/cm}^3)^{1/2}$), which means DMSO has a higher affinity to water and thus a higher driving force to cause faster phase inversion (non-solvent/solvent exchange) along with the denser depot structure formed for the same polymer. The faster phase inversion makes the burst release higher but the denser depot structure formed subsequently makes it difficult for water to influx

and swell the depot, resulting in almost no non-solvent/solvent exchange and consequently very little drug release until depot collapse starts to occur.

Figure 6.7 Effect of the amount of triacetin on the solvent release (DMSO or NMP) from injectable PdLLA depots in PBS (pH 7.4), where the formulation is PdLLA/mixed solvent = 40/60 and the mixed solvent is DMSO(NMP)/triacetin = 100/0, 95/5, or 80/20. Data are shown as the average of 3 repetitions, and error bars represent standard deviations. The inset shows the initial burst release profiles.

Triacetin is a relatively hydrophobic solvent as it has only 7% miscibility with water by weight, consistent with its lower solubility parameter ($21.0 \text{ (J/cm}^3)^{1/2}$). The increased hydrophobicity of the mixed solvent slows down the non-solvent/solvent exchange rate and thus reduces the initial solvent release rate along with initial drug release rate. A larger amount of triacetin (30%) may exceed the critical point at which depot formation transforms from a solid state to a rubbery state (as more solvent is retained and a “plasticized” polymer phase is obtained), which is deformed easily and results in high initial release.

When the polymer in the mixed solvent is injected into the buffer solution, the hydrophilic solvent DMSO or NMP near the surface leaves the polymer solution quickly to shape the depot almost immediately. The hydrophobic solvent triacetin restricts water ingress into the matrix, delays phase inversion and ultimately suppresses the burst release (by slowing down the release of the solvent). Thus the rate of solvent and drug release can be tailored by regulating the solvent miscibility with water through mixing of hydrophilic and hydrophobic solvent at various ratios. A combination of a hydrophilic and a hydrophobic solvent can overcome the shortcoming of rapid phase inversion caused by a pure hydrophilic solvent, and enhance the subsequent release by allowing the formation of a more porous structure because of depot swelling, which will be discussed later.

6.1.4.3 Triacetin release

As the addition of triacetin influences the release of drug and solvent significantly, its release characteristics are also of some interest. The triacetin release was quantified by HPLC along with DMSO or NMP release. Since triacetin is miscible with DMSO or NMP, the release profiles of triacetin were predicted to be similar to the profiles of hydrophilic part (DMSO or NMP) of mixed solvent. At the initial stage, as shown in Figure 6.8(a), the rate of the triacetin release is indeed similar to the rate of the DMSO release, which means that the triacetin diffuses out of the depot together with the DMSO. At 12th days, ~40% and ~20% triacetin are released from formulation composed of 20% and 5% triacetin respectively. However, beyond 12 days, there is hardly any triacetin release in the case of 20% triacetin loading and another burst release in the case of 5% triacetin loading. Cumulative triacetin release is only ~40% even after the depot collapses

and almost disappears due to the erosion. It is different from DMSO or NMP release which has complete release after depot collapse. Triacetin release from depots with 20% triacetin is faster than that with 5% triacetin and the second burst release only appears in depots with 5% triacetin, both of them can be explained from the different swelling ratio brought by different amount of triacetin, which will be discussed later.

To determine how much triacetin was lost at various durations, the residual triacetin amount left in the depot was measured by HPLC. Figure 6.8(b) shows that almost no triacetin is lost during the first day, while ~15%, ~30%, ~40% and ~50% of the triacetin is lost at 3rd, 6th, 12th and 18th days, respectively. The pH of the buffer medium decreases with the time. As is known, triacetin hydrolyzes to glycerol and acetic acid when it dissolves in the water. Apart from this, the heterogeneous degradation of the depot may also lead to a lowered pH inside the depot over time, which can then catalyze triacetin decomposition [167]. The results of the experiment of the decomposition of triacetin in the pH 7.4 buffer solution prove this point, as shown in Figure 6.9. For triacetin at a concentration of 1.0mg/ml, almost half of triacetin was lost at 3 days. When the concentration is increased, the decomposition rate decreased since the products were removed and neutralized slowly by the buffer solution. Therefore, triacetin plays an important role in delaying polymer phase inversion in the early stages and less in the later stages.

Figure 6.8 (a) Release profiles of triacetin from injectable PdlLA depots in PBS (pH 7.4), where the formulation is PdlLA/mixed solvent = 40/60 and the mixed solvent is DMSO(NMP)/triacetin = 95/5 or 80/20. The inset shows the initial burst release profiles. (b) Total triacetin amount left at different periods (including released triacetin in the medium and residual triacetin in the depot) and pH value change in the buffer medium, where the formulation is PdlLA/mixed solvent = 40/60 and the mixed solvent is DMSO/triacetin = 80/20. Release data and pH data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Figure 6.9 The amount of triacetin left as a function of incubation time. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

6.1.4.4 Swelling ratio

Since the drug release following the burst release is largely influenced by depot swelling and polymer degradation, the swelling ratio was investigated. Surprisingly, the swelling ratio results show that the water uptake ability increases by increasing the amount of triacetin up to about 20% by weight, as shown in Figure 6.10. After all, the co-solvent triacetin added is hydrophobic, which should decrease water uptake. The swelling ratio of PdlLA depots in a pure DMSO solvent system can reach only ~150% at 15th days. When triacetin is added as co-solvent, PdlLA depots with DMSO/triacetin (80/20 w/w) mixed solvent can swell as high as ~470% but the maximum swelling ratio of PdlLA depots with NMP/triacetin (80/20 w/w) mixed solvent is only ~250%. When triacetin amount is increased as high as 30%, the maximum swelling ratio of depots with DMSO/triacetin and NMP/triacetin mixed solvent is ~230% and ~220%, respectively.

Figure 6.10 Effect of the amount of triacetin on the swelling ratio of injectable PdLLA depots in PBS (pH 7.4), (a) DMSO/triacetin and (b) NMP/triacetin, where the formulation is PdLLA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO(NMP)/triacetin = 100/0, 95/5, 90/10, 80/20 or 70/30. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Without the addition of triacetin, the PdLLA polymer solution forms depots quickly upon contact with water and generates a dense structure, which resists further water ingress as well as depot swells. In contrast, the presence of triacetin restricts the water/solvent

exchange and delays the onset of phase inversion. On the other hand, for the fast phase inversion formulation, the polymer precipitates quickly and the depot cannot take in any more water (because the polymer is hydrophobic). When the phase inversion is slower, more solvent which retains in the polymer phase becomes the driving force for the following water/solvent exchange; it will take in more water and thus results in greater swelling leading to the formation of porous structure. In addition, less water uptake (less polymer precipitation) in the depot means a more rubbery structure is maintained when combined with the subsequent solvent exchange, results in the depot swelling.

The initial burst appears related to phase inversion kinetics/solvent release. As discussed in Chapter 7, addition of triacetin lowers the rate of phase inversion, and hence the initial burst (of both solvent and drug). The subsequent drug release (following burst) appears related to depot morphology and swelling ratios. At the beginning stage, 20% triacetin delays the phase inversion significantly, which means a slower non-solvent/solvent exchange and less solvent released. However, the more rubbery structure of the depot caused by more triacetin retention associated with a great amount of triacetin left allows for greater non-solvent/solvent exchange and thus results in water ingress/swelling. This is why the swelling ratio of depots with 20% triacetin increases faster, and thus leads to solvent release increasing and finally reaching the same release level as the formulation with less triacetin in 2 days. The increasing and lasting swelling caused by triacetin incorporation explains the sustained drug release after the burst release. Meanwhile, the greater and longer swelling of the depot with 20% triacetin explains its faster triacetin release compared to that with 5% triacetin (as shown in Figure 6.8) and also explains absence of second burst release for depot with 20% triacetin compared to 5% triacetin

formulation since more water uptake in depot with 20% triacetin degrade triacetin faster and more completely.

Triacetin addition at 5 and 10% makes the RG502 depot swell less compared to the PdlLA depot, especially in DMSO system, as shown in Figure 6.11. The reason for this difference is not clear, and in any case does not appear to be related to drug release. It is possible that the lower swelling of RG502 depots compared to PdlLA depots after triacetin addition is due to degradation. The mechanical strength of RG502 depots is decreased by the relatively faster degradation, resulting in the RG502 depots shrinking earlier and a lower swelling as well.

Figure 6.11 Effect of the amount of triacetin on the swelling ratio of injectable RG502 depots in PBS (pH 7.4), where the formulation is RG502/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO(NMP)/triacetin = 100/0, 95/5 or 90/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

6.1.4.5 Triacetin effect on the morphology

Figure 6.12 and Figure 6.13 show the cross-sectional images of PdILA depots in DMSO/triacetin mixed solvent and their SEM cross-sectional morphologies as a function of time. The cross-section of depots with pure DMSO solvent exhibits much denser structures even after 30 days, as shown macroscopically in Figure 6.12(a) and Figure 6.13(a). In contrast, when triacetin is added as a co-solvent, a porous structure is developed and more pronounced for depots with a higher triacetin amount which is in good agreement with the results of the swelling ratio. Even 5% triacetin can make the depot swell very well and have a much more porous structure.

Figure 6.12 Cross-sectional images of injectable PdILA depots as a function of time in PBS (pH 7.4), where the formulation is PdILA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO/triacetin = 100/0 (a), 95/5 (b), 90/10 (c), 80/20 (d) or 70/30 (e).

The porous structure facilitates the diffusion of the solvent and the degraded products

outward and thus reduces the aggregation of acidic products in the interior of the depot and thereby the drug release. However, when triacetin amount is as high as 30%, the depot presents a semi-fluid structure at 6 days. We postulate that addition of 30% triacetin delays phase inversion to such an extent that a certain amount of solvent associated with co-solvent triacetin, is trapped within the depot, and this solvent/co-solvent is not lost during the process of freeze-drying, leading to a semi-fluid depot forming in the bottle.

Figure 6.13 Cross-sectional morphology of injectable PdILA depots at 12 days, where the formulation is PdILA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO/triacetin = 100/0 (a) or 90/10 (b).

Although triacetin delays the PdILA polymer phase inversion, the cross-section of the depots are still solid as the relatively slower phase inversion caused by triacetin addition is still fast enough to allow the solid particles of the polymer component to precipitate quickly, and have insufficient time to migrate and rearrange to form a hollow structure.

6.1.4.6 pH value

Based on many previous studies, the change of pH value in the medium can influence the degradation of the depot. The measured pH values show a decrease over time, with the

extent of the decrease being dependent on the amount of triacetin added to the formulation, as shown in Figure 6.14. The pH value of the medium for the formulation without triacetin remains almost unchanged for 48 days. But for the formulation with triacetin, the pH value begins to decrease after a few days; the higher the amount of triacetin added, the lower the pH value dropped. For the RG502 depot, the pH change of the medium after triacetin addition is not as significant as the PdlLA depot, as shown in Figure 6.15. But the sudden drop point for the formulation with 10% triacetin is also earlier than that with 5% triacetin.

Based on the results of triacetin hydrolysis, it is clear that triacetin hydrolyzed with time. Part of released triacetin in the medium may hydrolyze into acidic acid, which will lead to the pH in the medium decreasing. Meanwhile, the porous structures brought by triacetin addition also enable the faster escape of low-molar mass oligomers, leading to a decrease in pH value of the medium. However, the effect of swelling on the degradation here is complex. Generally, water uptake brought by swelling is the precondition for degradation - the more water uptake, the higher the possibility of degradation, and the lower the pH value in the medium. On the other hand, water uptake causes the depot to swell and form a porous structure which will facilitate degraded products to diffuse out of the depot and increase the acidity of the medium. However, the reduced concentrations of degraded products (carboxylic end groups) in the interior of the depot in turn reduce the rate of auto-catalytic degradation and thus lessen the entire heterogeneous degradation of the depot; thereby the decrease in the Mw of the matrix may be slowed down. Therefore, a pH value change is not enough to exactly characterize the entire degradation of the polymer. The change of the Mw of the depot should be tracked to confirm the effect of

the swelling on the degradation.

Figure 6.14 Effect of the amount of triacetin on the pH change in the medium for injectable PdLLA depots as a function of immersion time, (a) DMSO/triacetin and (b) NMP/triacetin, where the formulation is PdLLA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO(NMP)/triacetin = 100/0, 95/5, 90/10, 80/20 or 70/30.

Figure 6.15 Effect of the amount of triacetin on the pH change in the medium for injectable RG502 depots as a function of immersion time, where the formulation is RG502/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO(NMP)/triacetin = 100/0, 95/5 or 90/10.

6.1.4.7 Degradation and mass loss

To understand the relationship between the degradation of depot and water uptake, the Mw change and the remaining mass of polymer in DMSO solvent system were presented to confirm the degradation process. The measured Mw change in general also shows a faster decrease with added triacetin. Figure 6.16(a) exhibits that a clear decrease of PdlLA Mw was observed only after 30 days for depots with pure DMSO, while for the depots with co-solvent triacetin, the obvious decrease of PdlLA Mw occurred after 15 days, except for the 5% triacetin, which does not fit the trend. Mass loss is not very different for different triacetin amounts in the first 36 days, as shown in Figure 6.16(b). There is less than 5% polymer mass loss up to 36 days for all formulations, which implies that the

decrease in the pH value is caused by very little polymer mass loss. The mass that leaches out for the higher triacetin loading before 36 days consists of relatively shorter oligomers, leading to a disproportionate reduction in pH. The degraded products released after the collapse of the depot reduces the pH value to yield an acidic condition that make the polymer mass lose gradually due to erosion until it disappeared.

As discussed above, water uptake and swelling have a complicated effect on the degradation of the polymer. The anomalous faster degradation of depots with 5% triacetin is likely due to the heterogeneous degradation occurring in the interior of the depot. In this case, the oligomers have not leached out quickly enough, and the resultant lowered pH inside the core, leads to acceleration of degradation. The depots with pure DMSO have a denser structure because of a faster phase inversion, resulting in less water uptake and slower degradation. For depots with added triacetin, the degradation is still heterogeneous in nature, but depends on triacetin levels. For depots with 10% and 20% triacetin, the higher swelling and more porous structure facilitate leaching of the degraded products, which does not allow for the auto-catalytic effect typically observed in these systems. At 5% triacetin, the depot structure is not porous enough for this to happen, and this, combined with higher water uptake (relative to pure DMSO depots,) leads to the observed results.

Figure 6.16 Effect of the amount of triacetin on the polymer M_w change (a) and polymer mass remaining (b) of injectable PdlLA depots as a function of immersion time in PBS (pH 7.4), where the formulation is PdlLA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO/triacetin = 100/0, 95/5, 90/10, 80/20 or 70/30.

Thus, our explanation for the degradation behavior may be summed up as follows:

- Addition of triacetin generally increases swelling (or co-solvent retention), thus facilitating escape of oligomers
- The level of swelling determines the extent of heterogeneous degradation and thus the rate of Mw decrease
- The leached oligomers decrease the pH and facilitate further degradation while the non-released polymer with high carboxylic acid content is expected to accelerate the polymer degradation
- In general, the mass that is leached out of high-triacetin loaded depots is quite small, thus leaving behind a greater concentration of acidic products in the depot catalyzing the chain hydrolysis, leading to the observed greater decrease of pH and thus Mw change.

6.1.5 Injectable *in situ* microparticles-forming systems (ISM)

NMP and DMSO are biocompatible solvents and the production by NMP has already become commercialized, but these injectable *in situ* depot-forming systems still cannot be used in the sensitive parts of the body due to the high viscosity of the polymer solution and the potential toxicity of organic solvent. Injectable *in situ* microparticles-forming (ISM) systems can overcome the disadvantage of high viscosity by adding a peanut oil phase into the polymer solution to form an emulsion. The reduced viscosity brought by the oil phase offers an easy injectability and is less painful. At the same time, the toxicity of the organic solvent can be lessened significantly by the presence of an external oil phase [168-170]. In addition, the preparation process of ISM is much simpler when

compared to classical techniques for the preparation of microparticles [95, 168].

In this section, two representative polymers PdILA02 and PDLG5002 in NMP solvent were studied to understand the effect of the type of polymer on the drug release. We also tried to reduce the biggest problem, the high initial burst release, by adding a hydrophobic co-solvent.

6.1.5.1 Effect of polymer type on the drug release

Figure 6.17 Release profiles of metosalt from PdILA/PDLG5002 microparticles formed *in situ* in PBS (pH 7.4), where the formulation is polymer/NMP/metosalt = 30/70/5 and the ratio of two phases is polymer solution/peanut oil = 1/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Figure 6.17 shows that the burst release from PdILA02 microparticles is much higher than that from PDLG5002 microparticles, while both of them are much higher than that from corresponding depots, especially for PdILA02 microparticles, the initial burst release can be as high as 83%. After the burst release, the drug release is much slower even no drug

release.

Figure 6.18 Morphology of microparticles formed *in situ* at 1 day, (a,b) PdLLA02 microparticles and (c,d) PDLG5002 microparticles, where the formulation is polymer/NMP/metosalt = 30/70/5 and the ratio of two phases is polymer solution/peanut oil = 1/1.

The micrometer droplets of polymer solution after emulsion solidified faster and more completely than the depot with much large size. The rapid and complete solidification offers more paths for non-solvent/solvent exchange and leads to a higher burst release since the diffusion coefficient in the polymer lean phase is much higher than that in the polymer rich phase. On the other hand, the solvent may diffuse out of the polymer droplets into the external oil phase associated with the drug during the process of the emulsion preparation since NMP is partially miscible with peanut oil, which leads to rapid diffusion of the drug into the medium after injection and directly results in a high burst release. The higher burst release from PdLLA microparticles than that from PDLG5002

microparticles can be ascribed to the relatively hydrophobic properties of PdlLA02, which has been described in the injectable *in situ* depot-forming systems.

Morphology study (Figure 6.18) shows that PdlLA02 microparticles are much more dispersed, while PDLG5002 microparticles are severely aggregated. This phenomenon further proves that the hydrophobic polymer solution undergoes faster phase inversion and forms microparticles quickly, while the hydrophilic polymer solution precipitates slowly and the polymer droplets easily aggregate before solidification. At the same time, the aggregated status makes the diffusion paths longer and thus decreases the drug release rate in turn.

6.1.5.2 Effect of triacetin as co-solvent on the drug release

The high burst release from ISM is supposed to be reduced by adding a hydrophobic co-solvent, so triacetin was chosen as such co-solvent to study its effect on the reduction of the burst release. As seen from Figure 6.19, for PdlLA02 microparticles the reduction of burst release is proportional to the amount of triacetin, as expected, 5% triacetin reduces the initial burst release from ~86% to ~71% in 24 hours, while 10% and 20% triacetin addition reduce it to ~60% and ~43%, respectively. Like injectable *in situ* depot-forming systems, addition of triacetin to the PDLG5002 system did not show a very significant reduction in the burst release, as shown in Figure 6.19(b), which can be explained as in injectable *in situ* depot-forming systems (Section 6.1.4.1).

Figure 6.19 Effect of the amount of triacetin on the metosalt release from microparticles formed *in situ* in PBS (pH 7.4), (a) PdILA02 microparticles and (b) PDLG5002 microparticles, where the formulation is polymer/mixed solvent/metosalt = 30/70/5, mixed solvent is NMP/triacetin = 100/0, 95/5, 90/10 or 80/20 and the ratio of two phases is polymer solution/peanut oil = 1/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

6.1.6 *In vitro* cytotoxicity studies of solvents

The main limitation of injectable *in situ* depot-forming systems is the potential toxicity of the organic solvent used. So the evaluation of the toxicity of the solvent is particularly significant in this field. MTT-based or CCK-8-based assays are common used methods to check cell proliferation, cytotoxicity and viability where a small quantity of cells is required. However, such conventional endpoint assays limited the time points and it was sometimes hard to get long term results. Also, the results depend on the technique of the operator to some degree. Recently, a new method called the xCelligence impedance analysis system was developed to check the cytoviability of cells when supplemented with the samples in real-time. This method offers great advantages over traditional assays and is ideal for dynamic monitoring of the biological status of the cells, such as cell number, viability, morphology and assessment of solvent (drug) interactions with cells. After adding the sample into the cells, no operation is needed which offers an undisturbed environment for cells and thus gives the exact information about the reaction of cells to the external stimuli. In this section, *in vitro* cytotoxicity study of the solvent was studied by these two methods, cck-8-based assay and the xCelligence impedance analysis system.

6.1.6.1 *In vitro* cytotoxicity studies of pure solvent by CCK-8-based assay

To assess the cytotoxicity of each solvent, *in vitro* culture of human fibroblasts was performed. Figure 6.20 compares the cytoviability of fibroblasts incubated in each solvent solution as a function of solvent concentration and incubation time. For each solvent or mixed solvent, the cytoviability of cells decreases with the increase of the solvent concentration and incubation time. At any time point, the cytoviability of cells incubated

in DMSO solvent is better than that in NMP and triacetin solvents. For example, when the NMP concentration was as high as 25 mg/ml, no cells were alive in the test after only 24 hour; when it decreased to 15 mg/ml, all the cells ceased activity around 48 hours; when it decreased to 5 mg/ml, the cells were still alive after 72 hours. But for DMSO, the fluctuation of the cytoviability of cells in different solvent concentrations and different incubation time was negligible in 72 hours. Triacetin is even more toxic than NMP since all the cells were dead at around 48 hours even the concentration is as low as 5 mg/ml. But the cytoviability of cells incubated in DMSO/Tri mixed solvent is better than pure NMP.

Figure 6.20 Cytoviability of fibroblasts measured by CCK-8 assay as a function of solvent concentration and incubation time. The solvent concentration is in a range of 5-25 mg/ml, and the incubation time is from 6 hour to 72 hours. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

After observing the morphology of cells under microscope, most of cells supplemented with DMSO solvent showed an elongated shape, which is typical for the fibroblasts, meaning that DMSO did not influence cell proliferation. But for cells incubated in NMP or triacetin solvent, the number of normal cells decreased and the number of round cells increased instead, which means that most of cells become inactive when coming in contact with NMP and triacetin solvent, implying the toxicity of such solvents.

6.1.6.2 *In vitro* cytotoxicity studies of pure solvent by xCelligence system

Figure 6.21 Scheme of impedance measurement [171].

To determine the biocompatibility of each solvent and each mixed solvent, the xCelligence impedance analysis system was used to study the cytotoxicity of cells in real-time when supplemented with samples. In this system, E-plate 96 with a gold cell sensor array in the bottom was used to monitor and assay cells inside each well. The presence of cells on top of the electrode surface of E-Plate 96 influences the local ionic environment, resulting in an increase in impedance of the sensor electrodes which were

measured to monitor and detect the physiological changes of the cells on the electrodes. When no cell attached on the electrode surface, the ion environment both at the electrode/solution interface and in the bulk solution mainly determines the electrode impedance. When cells attached to the electrode sensor surfaces, they will act as insulators and alter the local ion environment, inducing an increase in electrode impedance [171]. The more cells that are attached on the electrodes, the larger the increase in the electrode impedance. Moreover, the impedance is also related to the quality of the cell interaction with the electrodes; more cell spreading or extension will further increase the electrode impedance, as shown in Figure 6.21. Cell index (CI), a dimensionless parameter, is derived as a relative change in measured electrical impedance to represent cell status. High cell index means more cells attaching on the E-plate substrate and more cells being alive.

As shown in Figure 6.22, for NMP or triacetin, the value of CI increase with the incubation time first and then decreasing to zero, which means cells attached on the electrode surface first but soon detached. For NMP or triacetin, when the concentration was more than 15 mg/ml, no CI signal appeared throughout the entire culture period since the addition of the solvent, which means such high concentration of NMP or triacetin is very toxic to cells and no cells can be alive after contact with such kind of solvent solution (Figure 6.22). But for DMSO, cells keep growing even its concentration was as high as 25 mg/ml. Meanwhile, the CI value increased with time, implying that the number of live cells is increasing due to the attaching of the original cells and their growing on the E-plate.

Figure 6.22 Cytoviability of fibroblasts measured by xCelligence system as a function of incubation time, (a) NMP, (b) DMSO and (c) triacetin. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The results of mixed solvents also show some useful information. When the concentration of mixed NMP/triacetin is more than 15 mg/ml, all the cells are dead when in contact with such high concentration of mixed solvent. However, when DMSO was used as the main

solvent instead, cells incubated in high concentration solvent are still alive for some time. After 3 days or 2 days, the CI value decreased for cells incubated in 15 mg/ml or 25 mg/ml of DMSO/triacetin solutions respectively. These results are in good agreement with the results from the *in vitro* cytotoxicity study by cck-8 based assay.

Figure 6.23 Cytoviability of fibroblasts measured by xCelligence system as a function of incubation time, (a) NMP/triacetin (w/w 80/20) and (b) DMSO/triacetin (w/w 80/20). Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

In summary, the cytoviability of cells were influenced by NMP and triacetin more compared to the DMSO solvent, in other words, the DMSO solvent has better biocompatibility than the other two. Based on previous drug release studies in different solvent systems, pure DMSO systems produce a high burst release, however, a little bit of

triacetin addition can adjust the high burst release to the acceptable range, so such a combination seems like a good choice.

6.1.6.3 *In vitro* cytotoxicity studies of injectable depots by cck-8-based assay

Figure 6.24 Cytoviability of fibroblasts measured by CCK-8 assay as a function of incubation time, where the formulation is polymer/mixed solvent = 40/60 and mixed solvent is NMP(DMSO)/triacetin = 100/0 or 80/20. Blank control was made by culture medium. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Above two experiments were done with a diluted solvent with DMEM medium. In this section, the cytoviability of cells upon contact with the released solvent from depot formed *in situ* was studied. The RG502 polymer was chosen as the polymer material as the burst release from its depot is relatively low and the subsequent release is constant as well. About 0.2 g depot was injected into a 4 ml DMEM medium to check the cytotoxicity of the solvent which was released from depot contacting with the cells then.

The netwell insert made the depots formed *in situ* suspend in the medium and kept the cells in contact with a uniform solvent medium after being released from the depot. After a certain incubation time, the medium was removed and the cells adhering on the bottom of the well were counted.

The results show that (Figure 6.24) the number of cells left in wells supplemented with the DMSO solvent was slightly larger than that with NMP solvent after 6 hours. After 24 hours, the absorbance decreases for the NMP solvent and the NMP/triacetin mixed solvent. However, the absorbance greatly increased inversely for the DMSO solvent and DMSO/triacetin mixed solvent, even more than that of the control cells. It further proves that the DMSO solvent is more biocompatible than the NMP solvent.

6.2 Conclusion

In summary, the high burst release which is a disadvantage in injectable *in situ* depot-forming systems can be reduced by using a mixture of hydrophilic and hydrophobic solvents. In such mixed solvent systems, hydrophilic solvent (DMSO or NMP) leaves the depot quickly (and helps to shape the depot), while the hydrophobic solvent triacetin delays the phase inversion to reduce the subsequent solvent and drug release. The higher the amount of triacetin added, the more the reduction of the burst release (up to ~20% of triacetin loading by weight). Triacetin as co-solvent only affects the solvent release at the early stage as triacetin decomposed to other components by hydrolysis. The more “rubbery” (or permeable) structure of the depot caused by triacetin addition allows greater swelling to form a more porous structure, which significantly controls and affects the subsequent solvent and drug release as well as the degradation. The effect of triacetin on

the reduction of the burst release and on the subsequent release depends also on the polymer hydrophobicity: the effect is greater on the hydrophobic PdlLA polymer and less on the hydrophilic RG502 polymer, which was also proved in injectable *in situ* microparticles-forming systems. Therefore, a hydrophilic solvent can be chosen when a hydrophilic polymer is used and a hydrophobic solvent can be chosen when a hydrophobic polymer is involved, while a mixture of hydrophilic solvent and hydrophobic solvent is preferred in most conditions as it can satisfy most of the demands by changing their mixing ratios.

Whether for pure solvents or solvents released from depots, the cytoviability of cells supplemented with DMSO solvent is always better than that with NMP and triacetin, which means DMSO has the best cytoviability among them. For the different applications in the body, the cytotoxicity study in this section offers a good reference for rational choice. For some applications with strict requirements on solvent biocompatibility and the burst release, the mixture of DMSO and triacetin is a good choice. For some applications with low requirements on solvent biocompatibility and where the amount of depots injected into the body is limited, NMP or a mixture of NMP and triacetin can be considered, as the toxicity is acceptable in certain range.

Chapter 7 Relationship between Drug Release and Solvent Release

From previous chapters, the relationships between each parameter (e.g. polymer hydrophobicity, polymer Mw, solvent property and drug type) and drug release were studied and presented for injectable *in situ* depot-forming systems. In this context, an additional parameter, namely the solvent release rate is also of interest, particularly with respect to the overall mechanism of drug release. For drugs soluble in the solvent (and of appreciable solubility in aqueous medium), this parameter is related to drug release, and has not been studied much or reported in the literature. In this section, we attempt to quantify the relationship between drug release and solvent release and derive some mechanistic details from this relationship. We found that this relationship is dependent on the polymer type and polymer Mw. The drug property and solvent type also influence this relationship. In the final analysis, this relationship is related to the phase inversion dynamics of the polymer solution and the depot morphology. Ultimately, a new approach based on the relationship between drug and solvent release is developed to estimate the phase inversion dynamics and depot morphology change.

7.1 Results and Discussion

7.1.1 Drug release versus NMP release for high Mw hydrophilic polymer

Figure 7.1 Curves of metosalt or metobase released versus NMP released from injectable RG504 depots in PBS (pH 7.4) (left) and their linear fittings in the early stage (right), where the formulation is RG504/NMP/drug = 35/65/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

To evaluate the relationship between drug release and solvent release, formulations of hydrophilic polymers (PLGA50/50) with high Mw were investigated first. A curve using the data of NMP solvent released (on the x-axis) and drug metosalt or metobase released (on the y-axis) was drawn. Using RG504 as the representative of high Mw PLGA50/50 polymers, the comparison of such curves between metosalt formulation and metobase formulation is shown in Figure 7.1. Three stages can be defined in these curves: the first linear stage, the induction period and the second linear stage. For RG504 depots loaded

with 1% metosalt, in the first stage near ~86% NMP and ~64% metosalt released are related linearity for 11 days and the slope of linear fitting of drug release versus NMP release curve during this period is 0.84 (R^2 0.999). For RG504 depots loaded with 1% metobase, in the first stage only ~48% NMP and ~26% metobase released are in a linear relationship for 4 days and the slope is reduced to 0.71 (R^2 0.994). From the data in Chapter 5, we know that both RG504 depots with metosalt and metobase loading are solid structures. Other high Mw PLGA50/50 polymers (e.g. PLGA50/50, I.V. 0.33; RG504H, I.V. 0.38) also show similar results: the drug release and solvent release are related linearity in the early stage and the curves of drug release versus NMP release for formulations with metosalt loading are always above those with metobase loading, as shown in Figure 7.2.

Figure 7.2 Curves of metosalt or metobase released versus NMP released from injectable depots in PBS (pH 7.4), (a) PLGA50/50 and (b) RG504H, where the formulation is polymer/NMP/drug = 35/65/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The perfectly linear relationship between drug release and solvent release in the first stage implies that the rate of the drug release depends significantly on the rate of the solvent

release since it can carry the drug out in the process of non-solvent/solvent exchange. The initial solvent release rate is influenced by the non-solvent/solvent exchange rate, but the drug release/solvent release ratio (or the slope of drug release versus solvent release curve) is related to relative diffusion rate of drug to solvent during the exchange process. When the relationship is linear, and the coefficient close to unity ($Y=0.84X$ in the case of metosalt), then it implies that both solvent and drug release out at similar rates, and the skin formed is not acting as a barrier to these low-Mw species. When the coefficient decreases (as in the case of metobase in the linear region), it means the diffusion of drug within matrix becomes difficult compared to solvent.

In the induction period there is little drug release, especially for the formulations with metosalt loading, because the drug near the surface or paths was exhausted during the first stage while the drug in the core needed some time to diffuse out. This stage is not obvious for formulations with metobase loading since the catalytic effect of metobase on polymer degradation causes greater water uptake and more depot swelling, which facilitates drug and solvent release and also results in sustained release after the burst release. After that, the stage is mainly controlled by the degradation/erosion of the matrix as the environment becomes much more acidic as a result of the release of the degraded products, especially after depot collapse, leading to a polymer mass loss associated with drug and solvent being released out and thereby giving rise to the second linear relationship between drug release and solvent release.

In summary, we refer to the first stage (where drug release is linear with solvent release) as being diffusion-controlled, whereas the second linear stage is dominated by the

degradation/erosion of matrix.

Figure 7.3 Curves of metobase released versus NMP released from injectable RG504 depots in PBS (pH 7.4), where the formulation is RG504/NMP/metobase = 35/65/0.2 (a), 35/65/1 (b), 35/65/5 (c) or 35/65/10 (d). Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

For RG504 depots loaded with metobase, the first stage was completed earlier than that with metosalt, which means that the onset of the degradation of this formulation occurs earlier than that with metosalt since metobase can accelerate polymer degradation due to its catalytic effect on the clearance of ester bonds. The higher the metobase loading, the shorter the first linear stage. This viewpoint is supported by the shorter and shorter first linear stage for formulations with increasing metobase loading, 9 days, 4 days and 3 days for formulations with 0.2%, 1% and 5% metobase loading respectively, as shown in Figure 7.3. For depots with 10% metobase loading, there is no clear linear relationship

between drug release and solvent release since 10% metobase exceeds the maximum soluble metobase loading in the polymer solution, and results in a suspension. For suspensions, the rate is also affected by the rate of dissolution of the drug prior to release out of the depot.

7.1.2 Drug release versus NMP release for low Mw hydrophilic polymer

Figure 7.4 Curves of metosalt or metobase released versus NMP released from injectable RG502 depots in PBS (pH 7.4), where the formulation is RG502/NMP/drug = 35/65/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

For RG502 depots, a representative of low Mw PLGA50/50 polymers, there is no clear linear relationship between NMP release and drug release, no matter what kind of drug was loaded, as shown in Figure 7.4. The faster solvent release than the drug release from RG502 depots in the early stage implies that solvent molecules pass through the matrix and the skin of the depot more easily, while the passing for metosalt or metobase

molecules is relatively more difficult. After some time, the drug release becomes faster than the solvent release due to onset of degradation as well as to the decreasing solvent concentration in the depot. No clear linear relationship between drug release and solvent release means that no stage is controlled by a single parameter, diffusion and degradation intervened with each other and had a final effect on the drug release and solvent release instead.

For the same amount of solvent released from RG502 depots, the simultaneous metosalt release is a little lower than corresponding metobase release in contrast to what was observed from RG504 depots, which has been explained in terms of polymer Mw and phase inversion dynamics as well as depot morphology in Chapter 5. Comparing to RG504 depots, the stage dominated by diffusion (the first linear stage) is shorter and not very well-defined for RG502 depots, which means that degradation plays a role in drug release earlier for RG502 depots. In addition, the depot structure changed sooner as a result of the rapid degradation of RG502 polymer, resulting in changes in the diffusion paths and diffusion coefficient, and the relationship being altered then.

In comparison to the obviously linear relationship between drug release and solvent release for RG504 depots, unclear relationship for RG502 depots should be caused by low polymer Mw since both RG504 and RG502 have the same LA/GA ratios and similar end groups. From our studies, we know polymer Mw influences phase inversion dynamics of polymer solution and depot morphology formation after injection. High Mw hydrophilic polymer RG504 undergoes faster phase inversion and forms solid structures, while low Mw hydrophilic polymer RG502 undergoes slower phase inversion and forms hollow

structures. The difference in the phase inversion dynamics and the resultant morphology can help to explain different relationship between drug release and solvent release, which will be discussed in the Section 7.1.5.

7.1.3 Drug release versus NMP release for different polymers with low Mw

Figure 7.5 Curves of metosalt released versus NMP released from four injectable depots in PBS (pH 7.4), where the formulation is polymer/NMP/drug = 40/60/1. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The relationship between drug release and solvent release from different types of polymers was compared (Figure 7.5), and it was found that, for PdILA, the most hydrophobic polymer, the linear relationship between the drug release and solvent release is much longer compared to other relatively hydrophilic polymers. For relatively hydrophilic polymers, the greater the hydrophilicity of the polymer, the farther the curve of drug release versus solvent release being away from the diagonal. Meanwhile, the boundary between each stage for PdILA depots is not as clear as high Mw hydrophilic

polymer depots because the degradation for PdILA is slower, and therefore it plays a role in the drug release, resulting in diffusion controlling drug release over longer time duration. Moreover, the curve of drug release versus solvent release is very linear for PdILA depots in the entire process, which means that the drug and solvent are being released at similar rates, and that the diffusion barrier for solvent and drug is similar in this case.

7.1.4 Drug release versus solvent release in mixed solvent systems

Figure 7.6 Curves of metosalt released versus NMP released from injectable PdILA depots with mixed solvent in PBS (pH 7.4), where the formulation is polymer/mixed solvent/metosalt = 40/60/1 and the mixed solvent is NMP/triacetin = 100/0, 95/5 or 80/20. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The relationship between the drug release and solvent release in mixed solvent systems was also explored. The polymer used was PdILA and the main solvent used was NMP.

Since the drug release is mainly related to the main solvent release, based on Chapter 6

[96], so here the relationship between the drug (metosalt) release and the main solvent (NMP) release was studied, as shown in Figure 7.7. When a small amount of triacetin (5%) was added into the polymer solution, the slope of drug release versus solvent release curve during the first linear stage dropped from 0.82 in the case of single NMP solvent to 0.59 (NMP/triacetin = 95/5 w/w). When the amount of triacetin was increased to 20% (NMP/triacetin = 80/20 w/w), the linear relationship becomes unclear and the curve is far away from the diagonal.

Figure 7.7 Curves of metosalt released versus NMP released from injectable PdLLA depots with mixed solvent in PBS (pH 7.4), where the formulation is polymer/mixed solvent/metosalt = 40/60/1 and the mixed solvent is DMSO/triacetin = 100/0, 95/5 or 80/20. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

When DMSO was used as the main solvent instead of NMP in mixed solvent systems, the linear relationship between DMSO release and drug release was also obtained, as shown in Figure 7.8. Moreover, the slope of the initial linear stage for PdlLA depots with a single DMSO solvent is much higher (1.12), greater than that of drug release versus NMP release curve for the same polymer (0.82), reflective of a faster non-solvent/solvent exchange rate for DMSO and water, compared to NMP and water. But after a large initial release, no drug and solvent release was released until the depot collapsed as a result of the heterogeneous degradation. When 5% triacetin was added to the polymer solution, the slope decreased to 0.86 but the linear relationship lasts for a longer period even an entire release process. When triacetin amount was further increased to 20%, the linear relationship is still maintained in the early stage but the slope further decreased to 0.54, and then the relationship is still in a perfect linear profile, but with a higher slope. Even so, the curve is still near the diagonal compared to the corresponding NMP systems or the hydrophilic polymer systems. It should be noted that all of these PdlLA depots are solid structures.

The lack of drug and solvent release after the burst release in single DMSO solvent systems has been explained in the previous section [96]. Briefly, the hydrophobic property of PdlLA and the greatly hydrophilic property of DMSO make the polymer solution undergo faster phase inversion, thereby causing a great amount of drug and solvent to release out in a short time. The PdlLA being hydrophobic, and slower-degrading, did not degrade for some time and thus resulted in less drug release for a long time until degradation starts to dominate the system.

The PdlLA polymer in single solvent systems undergoes fast phase inversion, resulting in a high slope value of the drug release versus NMP release curve. In the case of a much more hydrophobic solvent (e.g. triacetin) being added to the hydrophobic polymer system, the phase inversion rate was reduced and the slope of such curve in the early stage was decreased, which means that drug release rate is reduced more than solvent release rate, most likely due to the formation of a diffusion barrier during this period. After injection of polymer solution into the medium, the depot swelling increases with time gradually due to triacetin retention in the depot, resulting in the structure of the depot becoming much more porous compared to depots with a single solvent. The porous structure leads to the diffusion coefficients for drug and solvent within the matrix being similar and thus the release rates of them being similar. Moreover, the slower phase inversion as a result of the presence of triacetin can help the drug and solvent release constant and sustaining, thereby resulting in the drug release and solvent release maintaining a long term linear relationship.

In summary, for PdlLA polymer with a single solvent system, the system undergoes fast phase inversion and the slope of drug release versus solvent release curve appears close to 1 or even more than 1. When hydrophobic solvent triacetin was added into this system, phase inversion dynamics was slowed down and drug release versus solvent release curve was below the diagonal, along with the slope being far away from 1.

7.1.5 Discussion on the slopes of curves of drug release versus solvent release

Table 7.1 Summary of the key results

Variable	Formulation	Slope
Drug type and Polymer Mw (NMP)	RG504/metosalt	0.84
	RG504/metobase	0.71
	RG502/drugs	-
Polymer hydrophobicity (NMP)	PdLLA	0.82
	PLGA75/25	-
	RG502	-
	RG502H	-
Mixed solvents (PdLLA)	DMSO	1.12
	DMSO/triacetin (95/5)	0.86
	DMSO/triacetin (80/20)	0.64
	NMP	0.82
	NMP/triacetin (95/5)	0.59
	NMP/triacetin (80/20)	-

As summary in Table 7.1, we found that for certain polymer/solvent systems, such as hydrophobic (slow-degrading) polymers or polymers with a high original Mw in pure solvents, drug release is linear with solvent release and the slope is close to unity, which implies that the high non-solvent/solvent exchange rate dominates the drug release, resulting in smaller difference between drug release rate and solvent release rate and a unity slope as a result. It also appears that the magnitude of the slope is an indication of the rate of non-solvent/solvent exchange (phase inversion kinetics), and decreases in the

following order: DMSO > NMP > triacetin.

In comparison with above situations, formulations with hydrophilic (faster-degrading) polymers or polymers with a low-Mw, the slope is much less than unity or even no linear relationship between drug release and solvent release. We should notice that the former formulations undergo faster phase inversion and have solid structures, while the later ones undergo slower phase inversion and form hollow structures. For systems that undergo relatively fast non-solvent/solvent exchange (e.g. for PdlLA and RG504), the depot as a whole “precipitates” rapidly concurrently releasing the solvent, simultaneous with the solid structure formation. The skin formed as a main barrier to the drug and solvent release, make the difference between drug release and solvent release negligible because of its minor ratio in the whole system. For other hydrophilic polymers with low Mw (e.g. RG502), the depot formation is slower, and leads to the hollow depot formation caused by the time lag between water diffusion front and gelation front, with the drug trapped inside the core, which has been clearly discussed in Chapter 5. The cross-sectional morphology of shell may be much denser compared to above solid structure, resulting in the slower drug release compared to solvent release and thus the lower slope of drug release versus solvent release curve. Even though the shell inside may be porous, however, the inner skin which is very smooth will block the release, with outer skin together. The entire shell then acts as a diffusion barrier to slow down release of drug (relative to solvent) in the following stage. Figure 7.9(a) shows the case of fast-inversion, and Figure 7.9(b) shows a slower inversion depot. Addition of a relatively hydrophobic solvent (e.g. triacetin) has the same effect.

Furthermore, since the degradation plays a role in the RG502 depot system earlier, the morphology is changed simultaneously with the commencement of the degradation, resulting in the release paths being changed and the structure being porous, so the slope of the drug release versus solvent release curve starts increasing.

Figure 7.8 Schematic illustrations of depot morphology formed at different phase inversion rate, (a) faster phase inversion and (b) slower phase inversion.

So, the initial slope of the solvent release - drug release curve may be used to estimate the phase inversion dynamics and the depot structure. Being near to 1 on the slope indicates a fast phase inversion and a solid and uniformly porous structure formation, while being far away from the diagonal means a relatively slower phase inversion and hollow core (which could be liquid)/skin structure formation.

The release of the drug and solvent was influenced by non-solvent/solvent exchange rate initially and then closely related to the resultant morphology. If the polymer and solvent are fixed in a system, the phase inversion dynamics and depot morphology will be fixed, which means that the relationship between drug release and solvent release is clear. When the solvent release was almost not influenced by the incorporated drugs if the drugs have no additional effect on the system while the diffusion coefficient only depends on the Mw

of drug, the drug release will be same for a series of drugs with similar Mw. Therefore, many kinds of hydrophilic drugs with a similar Mw or even hydrophobic drugs if only under the saturated condition can be incorporated into the depots and the drug release behavior will be expected, thereby saving lots of work in the exploration of injectable *in situ* depot-forming systems.

7.2 Conclusions

In summary, the relationship between drug and solvent release is dependent on the polymer type and Mw, solvent type and drug property, which ultimately is ascribed to the phase inversion dynamics and the resultant depot morphology. The initial slope of drug release and solvent release curve defines the phase inversion dynamics, and the change of the slope can reflect the change of the release paths brought about by the polymer degradation. Formulations composed of polymers with high Mw or great hydrophobicity, undergo faster phase inversion and form solid structures, leading to a linear relationship between drug release and solvent release in the early stage and the initial slope of drug release versus solvent release curve near to 1. For polymers with low Mw and great hydrophilicity, which undergo slower phase inversion and form hollow structures, the relationship is less well-defined. The initial slope is far away from 1 and such curve is below the diagonal. When the hydrophobic co-solvent is added into the hydrophobic polymer solution, the phase inversion rate is reduced due to the lowered affinity of solvent to water, showing the decreased slope of drug release versus solvent release curve, but the linear relationship is still maintained, since the depot structure is still solid and even more porous over the entire cross-section.

The above conclusion is based on the drug being a low Mw hydrophilic drug or hydrophobic drug whose loading is under the saturated condition. Under certain conditions, the relationship between the drug release and solvent release from injectable depots allow us to estimate the rate of the phase inversion of different polymer solutions after injection into the buffer and depot morphology, leading to a better understanding of the drug release behavior.

Chapter 8 Conclusions

In this project, the polymer hydrophobicity and Mw, drug type and solvent type have been studied in injectable *in situ* depot-forming drug delivery systems. Polyesters with different LA/GA ratios and end groups as polymers, DMSO or NMP as solvents and metosalt or metobase as drugs, were used for *in situ* depots formation by injection of the polymer solution into pH 7.4 PBS. The depots formed *in situ*, controlled the drug release in a sustained manner.

In Chapter 4, the effect of polymer type on the phase inversion and drug release was studied. The results found that the hydrophobic polymer PdlLA underwent a relatively faster phase inversion (solvent exchange), leading to a higher burst release, and subsequently formed a porous structure, which resulted in a well-modulated sustained drug release. For other relatively hydrophilic polymers, they underwent a relatively slower phase inversion, resulting in a lower burst release which was however followed by a rapid drug release. The water uptake, the degradation and the polymer mass loss were in this order: PdlLA < PLGA75/25 < RG502 < RG502H, which is consistent with the hydrophilicity of the polymer. This study shows that release profile of the drug is sensitive to the nature of the polymer used in the depot.

In Chapter 5, hydrophilic polymers with high Mw and the drug type have been studied to check their effects on the phase inversion dynamics of polymer solution and the rate of drug release. Hydrophilic polymers with high Mw resulted in fast phase inversion and a high burst release, leading to a solid structure formation, while lower Mw hydrophilic

polymers underwent slower phase inversion, resulting in a lower burst release and a hollow structure. The hydrophobic drug metobase dissolved in the polymer solution and as well as in the interior of the depot after injection, therefore it was expected to release like hydrophilic drug metosalt. However, metobase has a catalytic effect on the degradation, resulting in the Mw of the polymer decreasing during the process of the polymer solution preparation. For hydrophilic depots with a higher Mw, the burst release of metobase was much lower than that of metosalt because the decreased polymer Mw by the catalytic effect of metobase made the polymer solution underwent a relatively slower phase inversion and thus resulted in a lower burst release. For hydrophilic depots with a lower Mw, the catalytic effect of metobase made the polymer Mw lower and led to the more porous structure formation and caused the burst release of metobase to be inversely higher than that of metosalt. When the polymer is much more hydrophobic, the catalytic effect on the degradation before injection is less important and thus the burst drug release was influenced less.

In Chapter 6, the effect of the solvent hydrophobicity on the drug release was studied. The high burst release in injectable *in situ* depot-forming systems can be reduced by using a hydrophobic solvent (e.g. triacetin) as a co-solvent. In this way, the affinity of the solvent to water is reduced, thus the phase inversion of the polymer solution is slowed down and the burst release is reduced. In such systems, hydrophilic solvents (DMSO or NMP) leave the depot quickly to form the shell of the depot, while the hydrophobic solvent triacetin delays the subsequent water uptake and thus slows down the non-solvent/solvent exchange process thereby reducing the subsequent burst solvent and drug release. The effect of triacetin on the reduction of the burst release is more effective on the

hydrophobic PdlLA polymer and less on the hydrophilic RG502 polymer. The subsequent release was also influenced by the retention of triacetin, by altering the swelling of the depot and the degradation of the polymer, the higher the amount of triacetin added, the more the reduction of the burst release, the higher the swelling of the depot, and the faster the degradation, although triacetin decomposed at the later stage.

The solvent toxicity was also evaluated, and it provides useful information on the selecting of a solvent. In both the cytotoxicity study of pure solvent and the solvent released from the depot formed *in situ*, DMSO showed a better biocompatibility than NMP and triacetin. If the high burst release from depots with the DMSO solvent is solved, the DMSO solvent is a good choice in injectable *in situ* depot-forming systems. Injectable *in situ* microparticles-forming systems were studied briefly, and the effects of polymer type and co-solvent on the drug release are similar to depot systems.

In Chapter 7, the relationship between the drug release and solvent release was studied. For the loading of a hydrophilic drug or a hydrophobic drug with low Mw which can dissolve in the system completely, the relationship between the drug release and the solvent release were related to the phase inversion dynamics and depot morphology formed. For the formulations with fast phase inversion and solid depot structure, the solvent release - drug release curve was linear at the early stage (diffusion control stage) and its slope was near to 1. For the formulations with slow phase inversion and hollow depot structure, the relationship between drug release and solvent release is not well-defined, and the slope of the solvent release - drug release curve is far away from 1.

Based on the above conclusions, we recommend the following approaches to sustained-release formulations:

- (1) A hydrophilic polymer with low Mw (e.g. RG502) is preferred when the required duration of the application is less than one month.
- (2) When the duration for the application is more than one month, a more hydrophobic polymer (e.g. PdlLA) with hydrophilic solvent (e.g. DMSO) is recommended. The high burst release and the slowly subsequent drug release from such system can be tuned by adding a hydrophobic co-solvent (e.g. triacetin) into the polymer solution.
- (3) In terms of cytotoxicity, DMSO is the preferred solvent and NMP the less preferred.

It should however be noted that these predictions work well only for systems where the drug is completely soluble in the solvent system used.

Chapter 9 Future Suggestions

From above results, triacetin being a partial water miscible co-solvent can postpone phase inversion and reduce burst release when added to the solvent as a co-solvent; however, one concomitant deficiency of this technique is the high swelling after the addition of triacetin, especially for the hydrophobic polymer PdlLA, which may cause the patient discomfort. The other problem is the most worrying one, which is the toxicity of the solvent. Based on the *in vitro* cytotoxicity studies of the solvents, DMSO is much safer than NMP. However, the solvent used in the Eligard products is NMP. Therefore, the study on the animals to confirm above results is significant. The major suggestions for future research on injectable *in situ* depot-forming systems are summed up as follows:

- 1) Reducing depot swelling
- 2) *In vivo* toxicity study

9.1 Reducing depot swelling

9.1.1 Effect of peanut oil on the depots swelling

Assuming the swelling of the depot after injection is brought by the complete or partial solubility of the solvent or co-solvent (NMP/DMSO or triacetin) in water, peanut oil as a water insoluble additive but miscible with NMP, termed co-solvent herein, was chosen to explore the effects of a water insoluble additive on the reduction of the depot swelling and burst drug release in NMP solvent systems. Peanut oil was mixed with NMP first before the addition of the polymer.

The swelling of RG502 depots after adding 5% peanut oil as the additive was compared; the ranking of the depot swelling is as follows, $\text{Tri}(5\%) \& \text{Oil}(5\%) < \text{Tri}(5\%) \& \text{Tri}(5\%) < \text{Tri}(5\%) \& \text{NMP}(5\%)$, in which the miscibility of the mixed solvent with water increased, as shown in Figure 9.1, which means water can penetrate into the depot more easily and can also be kept in the depot when the affinity of the mixed solvent to water increases, thereby yielding much swelling.

Figure 9.1 Effect of peanut oil as co-solvent on the swelling ratio of injectable RG502 depots in PBS (pH 7.4), where the formulation is RG502/mixed solvent/metosalt = 40/60/1 and the mixed solvent is NMP/triacetin/oil = 95/5/0, 90/10/0 or 90/5/5. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The effect of peanut oil on the reduction of the PdlLA depots' swelling was studied too, as shown in Figure 9.2. Results show that when 5% triacetin was replaced by 5% peanut oil, the swelling does not change much, but when 10% triacetin was replaced by 10% peanut oil, the swelling decreased significantly.

Figure 9.2 Effect of peanut oil as co-solvent on the swelling ratio of injectable PdlLA depots in PBS (pH 7.4), where the formulation is PdlLA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is NMP/triacetin/oil = 80/20/0, 80/15/5 or 80/10/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

The swelling actually decreased after the addition of peanut oil as part of co-solvent but was not completely avoided. Peanut oil is insoluble in water but the main components of the solvent NMP is miscible with water, which may be the driving force behind the swelling in such systems. On the other hand, it is not practical to add too high proportion of peanut oil into the polymer solution as it is not good at the polymer complete dissolution.

9.1.2 Effect of peanut oil on the drug release

The above results show that peanut oil can reduce the swelling of the depot although the swelling cannot be completely avoided. Subsequently, the effect of peanut oil on the drug release should be studied to check whether it influences the drug release. As shown from

Figure 9.3, there are no significant differences between the drug release from RG502 formulations with NMP/triacetin/oil (90/5/5, w/w/w), NMP (100%) and NMP/triacetin (95/5, w/w). This means that using peanut oil as an additive does not influence the drug release.

Figure 9.3 Effect of peanut oil as co-solvent on the metosalt release from injectable RG502 depots in PBS (pH 7.4), where the formulation is RG502/mixed solvent/metosalt = 40/60/1 and the mixed solvent is NMP/triacetin/oil = 100/0/0, 95/5/0 or 90/5/5. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

Regarding the effect of peanut oil on the drug release from PdLLA depots, peanut oil as the only one co-solvent was added first into the polymer solution to check its effect on the drug release, as shown in Figure 9.4, the burst drug release can be reduced by using peanut oil as a co-solvent, but is followed by a very slow drug release of more than 39 days before depot collapse and the remaining drug and solvent trapped were released completely then.

Figure 9.4 Effect of peanut oil as co-solvent on the release of metosalt (a) or NMP from injectable PdlLA depots in PBS (pH 7.4), where the formulation is PdlLA/mixed solvent/metosalt = 40/60/1 and the mixed solvent is NMP/oil = 100/0, 95/5 or 90/10. Data are shown as the average of 3 repetitions, and error bars represent standard deviations.

After peanut oil addition, the rate of non-solvent/solvent exchange in the first 24 hours was slowed down as peanut oil may prevent water influx. After ~80% of NMP was released after 10 days, the drug release became slower in the later stages since there is less NMP to carry the drug through the oil barrier until depot collapse due to the severe internal heterogeneous degradation after 39 days.

When NMP component was reduced to 80% (w/w) and triacetin was also added into the polymer solution as one kind of co-solvent, like NMP/Tri/Oil (80/15/5 w/w/w), the drug release was out of control: there was no drug release more than 15 days for some depots, while there was burst behavior to release all of drugs at one moment for some depots (data now shown). However, the solvent release is much more sustained. The lack of drug release for a long time may be due to the non-uniform distribution of the oil in the depot after injection into the medium. It can reduce the swelling of the depot to some degree, but might disturb drug release severely. The sustained solvent release further proves that

NMP can pass through the oil barrier as NMP is partially miscible with oil, which is impossible for a hydrophilic drug.

Peanut oil can complete its mission to reduce the swelling ratio to a certain degree, but it may influence the drug release, especially after most of the hydrophilic solvent releases out from a hydrophobic polymer formulation (e.g. PdILA). Polymers with hydrophilic properties might be able to use peanut oil as an additive to reduce the swelling, but for polymers with hydrophobic properties, the hydrophilicity of the solvent cannot be reduced too much. This still needs further exploration to realize the reduction on the swelling of the depot without influencing the drug release.

In summary, triacetin as a co-solvent to reduce the burst release and control the later release is due to its partial miscibility with water, while peanut oil cannot modulate the release as triacetin because it cannot tune the non-solvent/solvent exchange rate efficiently. It demonstrates that co-solvent being used to modulate drug release in injectable *in situ* depot-forming drug delivery systems is necessary to consider the extent of its miscibility with water, especially for hydrophobic polymers.

9.2 *In vivo* toxicity study

From the results of *in vitro* cytotoxicity studies of each solvent or each mixed solvent in Chapter 6, we saw that the biocompatibility of DMSO is better than that of NMP and triacetin. However, the solvent used in the commercial product Eligard system is NMP. Thus an *in vivo* toxicity study is indispensable to confirm the above results. In an *in vivo* toxicity study, the following experiments are suggested:

- 1) A study of localized physiological responses of muscular tissue from the effect of solvent efflux from injectable depot formulation
- 2) A study of the systemic effects of solvent efflux from injectable depot formulation
- 3) A determination or estimate of a 'safe' solvent dosage that can be safely used in future *in vivo* studies
- 4) A comparison between single solvents and mixed solvents
- 5) A comparison between the depot swelling when adding various co-solvents with different water solubility

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