Biodegradable microparticles and *in situ* forming implants/microparticles containing drugs in different physical states

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Chenghao Zhang

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Berlin, den 17.10.2022

Chenghao Zhang

To My family

Acknowledgements

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1. General Introduction

1.1. Biodegradable polymers

Controlled release drug delivery aims to deliver specified amount of drug at a predetermined rate [1]. Early efforts to control drug release involved the use of slowly dissolving coatings, drug complexes, suspensions or compressed tablets [2]. However, such methods generally did not permit long term release and the amount of drug released was still strongly influenced by environmental variations. The idea of controlled release based on polymers dates back to the 1960s via the employment of silicone rubber and polyethylene [3]. When the drug is incorporated into these polymers, the drug release is determined by the properties of the polymer-drug system and is only weakly dependent on environmental factors. However, the lack of degradability in these polymeric materials requires eventual surgical removal and thus limits their applicability.

In contrast, biodegradable polymers can maintain their properties for a given time and then degrade into biocompatible byproducts through chemical or enzyme-catalyzed hydrolysis [4]. In the 1970s, biodegradable polymers were first investigated as appropriate controlled drug release materials avoiding the requirement of surgical removal [3]. Since then, biodegradable polymers are widely used in oral, parenteral and transdermal drug delivery, and also popular for ocular, nasal and pulmonary drug delivery.

1.1.1. Classification of biodegradable polymers

According to their origin, biodegradable polymers are divided into natural and synthetic. Natural polymers are attractive due to their excellent biocompatibility, but they have undesirable properties like immunogenicity, less controlled degradation, short release profile and batch-to-batch variation. On the other hand, synthetic origin polymers can tailor mechanical and physicochemical properties and alter the rate of degradation from 1 week to several months according to the need.

Natural biodegradable polymers, such as proteins (e.g. albumin, collagen, fibrin, gelatine), polysaccharides (e.g. alginates, cellulose, chitosan, dextran, hyaluronic acid and starch) and lipids, are from biological sources comprising of plant, animal and microorganism.

Synthetic biodegradable polymers are chemically synthesized. They include poly(amides), poly(amino acids), poly(anhydrides), poly(cyanoacrylates), poly(dioxanones), polyesters (e.g., poly(ε-caprolactones) (PCL), poly(carbonates), poly(glycolic acids) (PGA), poly(hydroxybutyrates), poly(lactic acids), poly(tartrates)), poly(ortho esters), poly(phosphazenes).

All synthetic polymers are wildly studied. However, only a few of them have led to marketed products. Specifically, the majority of approved controlled release formulations are based on the poly(lactic acid) and the copolymer poly(lactic acid-co-glycolic acid) (PLGA) [5-10].

1.1.2. PLGA

PLGA has emerged as the most popular biodegradable polymer because of its long clinical experience, favorable degradation characteristics and numerous application possibilities for drug delivery and tissue engineering. It is a series of FDA-approved biodegradable and biocompatible polymers and is available from several different manufacturers (e.g. Evonik Industries, Polysciences, and Corbion Purac) [6, 11].

1.1.2.1. **Synthesis**

PLGA can be synthesized in a wide range of molecular weight, ratio of lactide to glycolide and end group. Direct polycondensation of lactic acid and/or glycolic acid is used to produce low molecular weight (MW) PLGAs (Figure 1.1). High MW PLGAs are generally synthesized via ring-opening polymerization of the cyclic lactones under high vacuum in the presence of a catalyst such as stannous octoate [5]. PLGA is purified by dissolution in chloroform and precipitation in ethanol, followed by vacuum drying. Commercially, different grades of PLGA are

available depending upon their molecular weight, ratio of lactic acid to glycolic acid, and ester or acid as end group. Low molecular weight, low lactic acid ratio and uncapped polymers, such as PLGA Resomer® 502H, are more acidic and hydrophilic. High molecular weight, high lactic acid ratio and capped polymers, such as Resomer® 858 S, exhibit a more hydrophobic property.

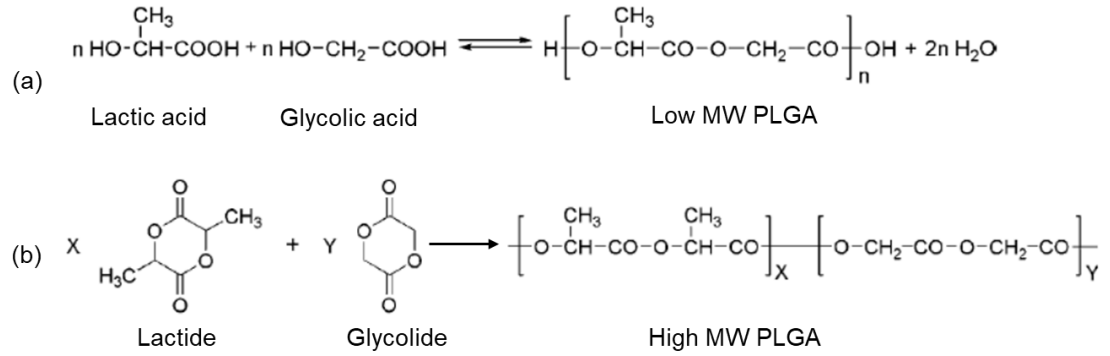


Figure 1.1. (a) polycondensation of lactic and glycolic acid, and (b) ring-opening polymerization of cyclic lactones to synthesize PLGA [12].

1.1.2.2. Degradation/erosion behavior

The term “degradation” refers to bond cleavage, whereas “erosion” refers to depletion of material (or mass loss) [13]. PLGAs degrade via random cleavage of the ester bonds through chemical hydrolysis (Figure 1.2). After the scission of long polymer chains, PLGA is degraded into shorter chain alcohols and acids. The pH inside the PLGA matrix would decrease significantly if the diffusion of acid degradation products is hindered. Thus, a decreased pH will further accelerate polymer degradation/erosion in the matrix core. It is known as the autocatalytic effect. Over the whole release period, the PLGA degradation/erosion can be divided into three stages [14]. In stage (1), the polymer matrix is just hydrated, and degradation is predominantly through non-catalytic hydrolysis homogeneously throughout the matrix while the concentration of carboxylic acid end groups is low. In stage (2), when the carboxylic acid end group concentration is sufficient, the autocatalytic hydrolysis reaction becomes

significant. The last stage involves dissolution of acid degradation products at a molecular weight of less than 1000 Dalton into the aqueous medium and significant mass loss. Generally, the degradation/erosion rate can be affected by many factors, e.g. polymer composition, polymer crystallinity, polymer molecular weight and release medium pH.

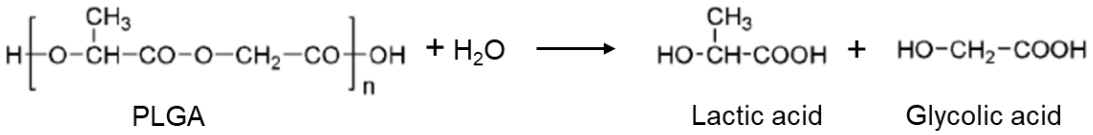


Figure 1.2. Hydrolysis of PLGA.

PLGAs eventually degrade into lactic acid and glycolic acid monomers. For human clinical use, these acids can be metabolized and excreted by the normal physiological pathway. Lactic acid is changed to pyruvic acid and is subsequently metabolized by the tricarboxylic acid cycle and excreted as water and carbon dioxide. Glycolic acid can be excreted directly through the urine or reacts to produce glycine, which is transformed into pyruvic acid and finally enters the tricarboxylic acid cycle [15].

1.2. PLGA based parenteral drug delivery systems

PLGA based parenteral drug delivery systems have been used to deliver small molecules and various macromolecules such as DNA, RNA, peptides and proteins for periods ranging from 1 week to 6 months. These systems have varied three dimensional geometry for the specific applications, e.g., implant rods, microparticles (> 10 μm) and films as local drug depots [15, 16], microparticles (1 - 10 μm) for vaccine, nanoparticles of < 200 nm for cancer therapy, or even moveable matrix such as self-anchoring implant.

1.2.1. PLGA formulations in clinical use

Table 1.1 lists PLGA based long-acting formulations in the order of the FDA approval date. These formulations have been approved as microparticles, solid implants, or *in situ* forming implants. Since the approval of Lupron® and Zoladex® Depot for the treatment of prostate cancer in 1989, the use of PLA/PLGA based delivery systems has enabled the development of controlled release formulations that can reduce dosing frequency. For example, Risperdal Consta®, the first long-acting injectable atypical antipsychotic approved by the FDA and indicated for the maintenance treatment of bipolar I disorder as monotherapy, has been shown to provide superior efficacy compared with oral atypical antipsychotics, with a low level of treatment-emergent side effects and favorable patient compliance [16]. Its prominent role in the treatment paradigm is largely attributed to improved compliance with treatment derived from the administration of the long-acting, injectable PLGA microparticles formulation every two weeks compared with self-administration of the oral formulation on a daily or every-other-day basis. Many of these PLGA based products have become leading medicines in their respective categories due to enhanced safety, efficacy and dosing profiles.

However, only about 20 different drug products are marketed in the last 30 years, indicating that the development of PLGA based formulations is challenging. Several intrinsic issues make it so difficult for these formulations: first, drugs, especially macromolecules, are unstable during manufacturing and storage, and even after application.; second, it is difficult to build *in vitro-in vivo* correlation and predict *in vivo* drug efficacy from *in vitro* release studies; third, many PLGA based formulations have a significant initial burst release and/or lag time, making the whole release profile not continuous. For example, in a clinical pharmacokinetic study of 15 patients, Trelstar® (triptorelin pamoate) had a maximum serum concentration of around 40 ng/mL only after 3 h administration, and it is followed by a relatively steady serum concentration of less than 1 ng/mL [17]. This initial high burst is unwanted and increases the potential risk.

Table 1.1. Examples of PLGA based formulations approved by FDA [18].

Product name	API[a]	Type	Duration	Approval
Lupron Depot®	Leuprolide acetate	Microparticle	1, 3, 4, 6 m	1989
Zoladex® Depot	Goserelin acetate	Implant	1, 3 m	1989
Sandostatin® LAR	Octreotide acetate	Microparticle	1 m	1998
Atridox®	Doxycycline hyclate	*In situ* implant	1 w	1998
Nutropin Depot®	Somatotropin	Microparticle	1 m	1999
Trelstar®	Triptorelin pamoate	Microparticle	1, 3, 6 m	2000
Somatuline® Depot	Lanreotide	Microparticle	1 m	2000
Arestin®	Minocycline HCl	Microparticle	2 w	2001
Eligard®	Leuprolide	*In situ* implant	1, 3, 4, 6 m	2002
Risperidal Consta®	Risperidone	Microparticle	2 w	2003
Vivitrol®	Naltrexone	Microparticle	1 m	2006
Ozurdex®	Dexamethasone	Implant	3 m	2009
Propel®	Mometasone furoate	Implant	1 m	2011
Bydureon®	Exenatide	Microparticle	1 w	2012
Lupaneta Pack™	Leuprolide acetate	Microparticle	3 m	2012
Signifor® LAR	Pasireotide	Microparticle	1 m	2014
Zilretta®	Triamcinolone acetoamide	Microparticle	3 m	2017
Sublocade™	Buprenorphine	*In situ* implant	1 m	2017
Perseris™	Risperidone	*In situ* implant	1 m	2018
Durysta®	Bimatoprost	Implant	6 m	2020

[a] Active pharmaceutical ingredient

1.2.2. Drug release from PLGA matrix

In most cases, drug release from PLGA matrix occurs via a combination of diffusion and polymer degradation/erosion. After hydration of PLGA matrix, drug located on or close to the surface dissolved and released, and this release is described as a burst release. Although the degradation started immediately with hydration process, the PLGA matrix stayed relatively stable for a period of time. During this period, drug diffused slowly either through the relatively dense

polymer or through a few existing pores. This slow diffusion may result in a lag phase over days to months. In this stage, drug may not permeate through a compact polymer matrix such as proteins may not further release until breakdown of the dense matrix. At a certain time point, scission of polymer resulted in sufficient pores that are larger than the size of the drug molecules, more water freely came into the porous matrix and the drug transport increased rapidly. A period of fast release happened at the same time (Figure 1.3).

However, drug release from a PLGA matrix is not necessarily correlated to the rate of polymer degradation. Some factors can let drug diffuse or leach out completely before the breakdown of matrices. Understanding the release mechanisms, as well as which factors that affect drug release, is important to be able to modify drug release. These factors include: polymer properties such as molecular weight, L:G ratio and end group; encapsulated substances such as characteristics of the drug (aqueous solubility, dissolution rate, molecular weight and drug-polymer interactions), drug loading and location, the characteristics of additives; polymer matrix characteristics such as size, porosity, density and shape; environmental conditions such as temperature, stirring, composition of the release medium, pH, osmolality, enzymes, lipids and immune responses [11, 19-22].

Drug release profiles can exhibit different shapes, such as zero-order, monophasic, biphasic, and triphasic, depending on the dynamics of the initial burst, diffusion through the polymer matrix and pores, and the stage of erosion. Although a zero-order release profile is mostly preferred, biphasic or more common triphasic release profiles are observed in most PLGA matrices. To establish a continuous release of such encapsulated compounds, many useful approaches have been found, e.g. a controlled induction of a defined level of porosity, employing surface eroding coating and a blend PLGA matrix [23].

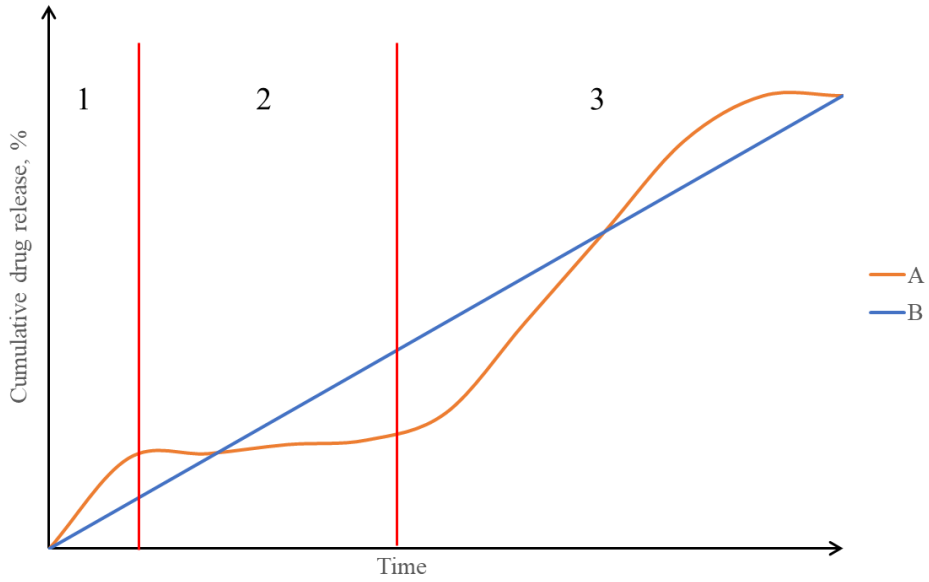


Figure 1.3. Release profile stages of (1) burst release, (2) lag phase and (3) erosion-controlled: A triphasic and B zero-order release.

1.3. PLGA microparticles

Among a variety of dosage forms have been used for PLGA based controlled release drug delivery, PLGA microparticles, sometimes also referred to as microspheres, are the most common type [24]. PLGA microparticles are free flowing powders having a particle size ranging from 1 to 1000 μm. However, parenteral administration of microparticles requires injectability through standard needles. Therefore, particle sizes below 100 μm are preferred. The idea of polymeric microparticles as drug delivery systems was reported in the 1960s and the concept of degradation was incorporated by Mason et al. (1976) through the employment of a degradable polymer coating [25, 26].

Degradable PLGA microparticles have shown great promise for the delivery of therapeutic agents due to their biocompatibility, ease of administration and capability for long-term sustained release without surgical procedures. In addition, PLGA microparticles are useful for delivering a variety of compounds, including small molecule drugs, protein therapeutics, vaccines and gene therapy agents. PLGA microparticles have been incorporated into a multitude of pharmaceutical

products used for the treatment of a wide array of indications, including cancer, psychiatric disorders, endocrine disorders and periodontal disease [27]. Disadvantages of PLGA microparticles include difficulty of large-scale manufacturing, maintaining drug stability and challenging control of drug release rates.

The past 30 years of research have seen exciting progress in the methods of fabrication, control of drug release rates and especially stabilization of encapsulated materials [28]. These improvements will ensure that microparticles play an important role in future applications. A few promising applications are briefly introduced here for which the use of PLGA microparticles as a minimally-invasive treatment has been studied extensively. PLGA microparticles can be used in various controlled drug delivery systems to achieve longer and better efficacy, and reduce administration frequency by many administration routes, including subcutaneous injection, intramuscular injection, oral administration, pulmonary administration, ocular administration, and so on. In particular, PLGA microparticles are hugely advantageous for encapsulation of fragile drugs such as nucleic acids and proteins by protecting biological entities that would otherwise be rapidly destroyed by the body [29, 30]. Over recent years much work has been conducted to develop and improve the use of PLGA microparticles for biomedical applications [31]. PLGA microparticles have been encapsulated into tissue regeneration scaffolds, since high interconnectivity enables the cells to seed more efficiently throughout the structure, as well as providing a large volume and surface area for nutrient transport/waste removal, and ultimately cell proliferation and differentiation [32, 33].

1.3.1. Preparation techniques

Many methods have been developed for preparing PLGA microparticles. The common fabrication technology is emulsification, e.g. oil/water (O/W), water/oil/water ($W_1/O/W_2$), water/oil/oil ($W/O_1/O_2$), or solid/oil/water (S/O/W). Besides these conventional methods, novel fabrication technologies such as membrane

emulsification and microfluidics have also been applied to prepare PLGA microparticles. The spraying technique is also briefly introduced below [34-36].

1.3.1.1. Aqueous emulsion solvent extraction/evaporation

Aqueous emulsion solvent extraction/evaporation is the most common method for creating PLGA microparticles. So far, this technique is most widely used by academic researchers. Both PLGA and drug are dissolved in an organic solvent and the resulted oil phase is emulsified under mechanical force into an aqueous phase that contains emulsifiers (Figure 1.4). Solvent extraction from the oil droplets starts immediately. Along solvent is extracted, dissolved PLGA molecules undergo coalescence first leading to formation of a shell (embryonic microparticles). After the specific amount of solvent has been extracted, the embryonic microparticles shrink and transform into final solid particles. To further encapsulate drugs that are poorly soluble in the organic solvent (commonly dichloromethane), a few co-solvents (e.g., dimethyl sulfoxide, acetone, acetonitrile or dimethylformamide) have been added to the oil phase to dissolve the drug completely. $W_1/O/W_2$ double emulsification is a practical method for encapsulating water-soluble drugs. In this method, drug is dissolved in an aqueous solution and the drug solution is dispersed in the oil phase which contains PLGA and organic solvent. The obtained W/O emulsion is subsequently emulsified into an emulsifier-containing external aqueous phase. The final solid PLGA microparticles are formed upon extraction and evaporation of the organic solvent. Additionally, solid drug powder can be dispersed into the oil phase forming a solid drug particle-containing oil phase (S/O). The S/O phase can further be emulsified into the water phase. In order to encapsulate drug particles into PLGA microparticles, the drug particles must be in the lower micrometer range and much smaller than the size of the corresponding microparticles.

There are several disadvantages of these methods that have limited their application. As these methods are inherently batch operations, the particle size distribution is wide and scale-up process is difficult. As the size of the particles

directly affects the drug release rate and syringe ability, it is important that size distribution is well controlled and ideally be relatively narrow. In addition, the presence of organic solvents and aqueous-organic interfaces may have adverse effects on encapsulated drugs, causing denaturation or aggregation of therapeutic proteins. Moreover, most commonly used organic solvents, which are toxic, are very difficult to remove completely.

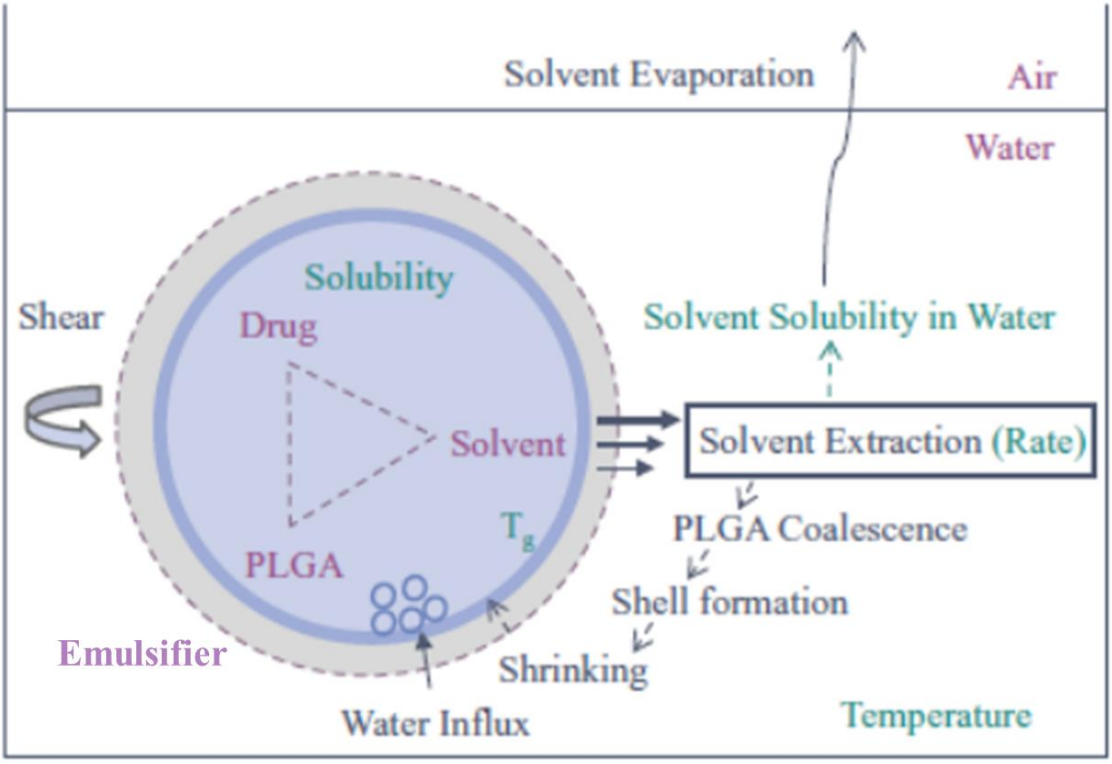


Figure 1.4. Microparticle formation by aqueous emulsion solvent extraction/evaporation [18].

1.3.1.2. Non-aqueous emulsification

When encapsulating hydrophilic and amphiphilic drugs, aqueous emulsification can be failed by a high amount of drug flux into the aqueous external phase during the emulsification and hardening process. To increase their encapsulation efficiencies, non-aqueous external phases have been explored to replace aqueous external phases. This method (O_1/O_2) is also referred to as organic

phase separation or coacervation. By the addition of a suitable non-solvent for the polymer, liquid-liquid phase separation is induced, causing the formation of a polymer-rich phase and then transferring into embryonic microparticles by emulsification. After extraction and evaporation of the inner organic solvent, the solidified microparticles were obtained. Sometimes, the embryonic microparticles are then transferred into a second non-solvent, and hardened PLGA microparticles are fabricated after extraction of the inner organic solvent. The non-solvent must minimize API solubilization and be miscible in the inner organic solvent. Examples of non-solvents that cause phase separation are silicone oil, vegetable oil, and low molecular weight methacrylic polymers [37]. Second non-solvents, used to solidify the polymer layer, can be hexane or petroleum ether. In $W/O_1/O_2$ emulsification method, an aqueous solution of drug mixed with the PLGA solution is emulsified into a non-mixable organic oil phase that may contain emulsifiers. $S/O_1/O_2$ method employs solid drug particles, similar to that described above for S/O/W preparation of PLGA microparticles. Nevertheless, this method is lack of process robustness, which easily resulted in agglomeration of microparticles. Additionally, this complex, multi-step batch process lacks scalability and transferability to industrial manufacturing processes.

1.3.1.3. Membrane emulsification

Compared to the previously described emulsification methods, membrane emulsification offers better control over particle size distribution and batch-to-batch reproducibility. In this technique, oil phase droplets are formed at the membrane pore openings and separated from the membrane surface by the movement of the continuous phase [36]. Generally, an emulsifier-containing water phase is flowing over the membrane through which the drug-containing oil phase is pumped, resulting in oil droplets of uniform size (Figure 1.5). However, as the organic phase is often forced through a narrow orifice at a relatively high velocity, the shear forces imparted may damage the encapsulated therapeutic agents.

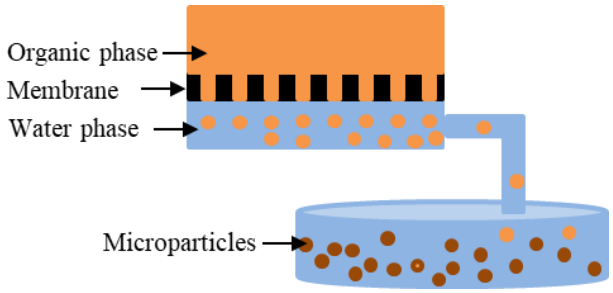


Figure 1.5. Schematic representation of membrane emulsification techniques for fabricating monodisperse microparticles.

1.3.1.4. Microfluidics

Microfluidic technologies have also been widely investigated to produce monodisperse PLGA microparticles. Among these microfluidic technologies, co-flow capillary, flow-focusing capillary and the combination of these two principles are the most popular approaches [38]. In co-flow capillary technique (Figure 1.6 A), the continuous phase is introduced into the external channel and the oil phase that contains drug and polymer is introduced into the central channel. By adjusting their pumping rates, monodisperse emulsion droplets can be continuously formed. In flow-focusing capillary method, the oil phase and continuous water phase are introduced from opposite directions into the microfluidic device (Figure 1.6 B). The internal organic phase is flow focused by the external aqueous fluid through the opening. But to scale up for a large batch by microfluidic is still challenging.

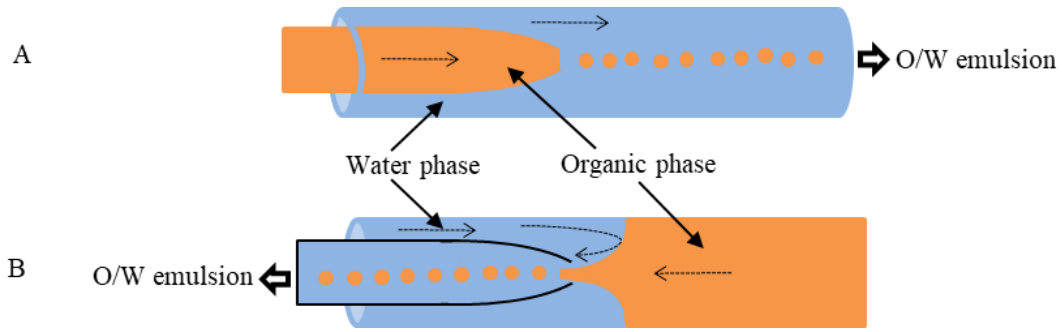


Figure 1.6. Schematic demonstration of microfluidic devices for fabricating monodisperse microparticles. A: co-flow capillary device and B: flow-focusing capillary device.

1.3.1.5. Spraying techniques

The spray drying or cryogenic spray-congealing method (also known as Alkermes' ProLease®), which has other principles and methodologies in droplet formation and drying, has evolved to manufacture PLGA microparticles [39]. Microdroplets of organic phase or W/O emulsions can be sprayed using an appropriate nozzle with a proper temperature. After solvent extraction (by cryogenic ethanol) or evaporation (by hot air), solid microparticles can be obtained. Spraying is a simple method for commercial production [40]. However, it has its drawbacks of the low yield for small batches. Moreover, a disadvantage of this technique is that the resulting PLGA microparticles display high polydispersity of particle size.

1.3.2. Porous PLGA microparticles

Compared with traditional nonporous microparticles, porous microparticles show unique drug absorption and drug release kinetics. Porous PLGA microparticles have external pores on the surface and/or internal pores in the core, and active substances can be absorbed on the surface or encapsulated in the core of the microparticles. The pores give porous microparticles different physiochemical properties such as large specific surface area and low density. The diameter, amount and structure of those pores are the critical factors affecting the properties of porous microparticles, to precisely control the pore structure is therefore of particular importance [41, 42]. Porous microparticles were identified as suitable tools for potential applications including as carriers for drug delivery and scaffolds for tissue regeneration.

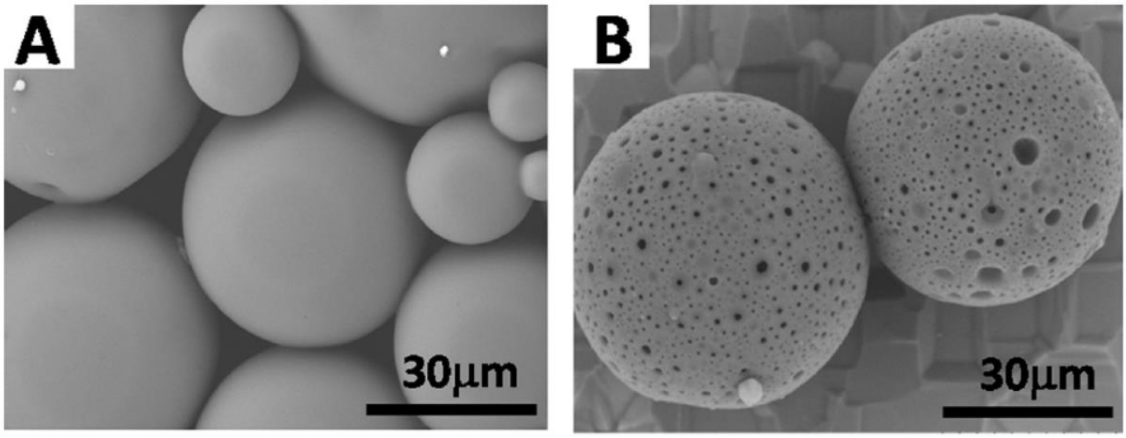


Figure 1.7. The scanning electron microscopy images of (A) traditional non-porous microparticles and (B) porous microparticles [43].

Both small chemical entities and large biomacromolecules can be encapsulated in porous structures or absorbed on the large porous surface [44-47]. Porous PLGA microparticles can provide a high encapsulation efficiency and a sustained release behavior [48, 49]. Their surface can further be functionalized by conjugation with ligand molecules, specific antibodies, and immune stimulators. These advantages make it a promising dosage for parenteral product development. In recent years, pulmonary applications gained much attention as a noninvasive alternative for drug delivery; drug can be absorbed efficiently in the large lung surface area of approximately 100 m^2, and then cross the thin pulmonary absorption barrier [50, 51]. In this case, porous PLGA microparticles can be adopted as a promising vehicle due to their low density and small aerodynamic diameter [52, 53]. Making the porous microparticles into an inhalable aerosol, the loaded drugs can be free from first-pass effects and realize a sustained release [54, 55].

Transferring porous PLGA microparticles into scaffolds in tissue engineering is widely reported. A highly porous structure with well-interconnected channels is required not only to achieve sufficient cell seeding, but also to facilitate the in- and out-transport of nutrients and oxygen for subsequent cell proliferation and differentiation [56, 57]. The sinter method is the most frequently used method to prepare porous PLGA microparticle scaffolds [58]. Usually, the porous PLGA

microparticles are heated just above their glass transition temperature to sinter into porous scaffolds. The porous scaffolds usually carry drugs like cell growth factors to assist the growth of cells during tissue regeneration.

Porous PLGA microparticles can be fabricated by similar manufacturing methods as conventional ones, including emulsion solvent extraction/evaporation, phase separation and spray drying [41, 42]. The most commonly used routine is the emulsion solvent extraction/evaporation technique. This process involves dissolving PLGA in a volatile organic solvent and emulsifying it into an aqueous phase containing a surfactant to form an oil-in-water emulsion, followed by solvent evaporation. Interestingly, by controlling the manufacturing process, the obtained particles can be modified to be porous structures [11]. To further increase the porosity, multi-emulsion solvent evaporation is commonly employed for the water exchange between the internal aqueous phase and external aqueous phase [59]. Usually, the internal water in the oil droplets can contribute to the formation of pores during drying. To obtain an optimum porosity, some additives like salts are often employed, which can generate osmatic pressure difference between internal and external water phase, then adjust the porosity and the diameter of the pores more easily. The generation of porous structure can also be achieved by traditional porogen leaching process. After a porogen is encapsulated into PLGA matrix, the porogen dissolves and leaches into a solvent which is a non-solvent for PLGA, resulting in a porous structure.

1.4. PLGA *in situ* forming microparticles/implants

The *in situ* forming systems are parenteral formulations in a liquid form that develop into a solid depot after being injected into the body. Several commercially available PLGA *in situ* forming systems were developed. Atridox® is the first PLGA *in situ* forming system available in the market after FDA approval in 1998. This product contains doxycycline licensed for treating chronic periodontal disease

over 3 weeks by injecting the drug into the periodontal pocket. Eligard® is a PLGA *in situ* forming system administered subcutaneously to deliver leuprolide acetate for treating advanced prostate cancer with variable dosing regimens for 1, 3, 4 or 6 months. Sandostatin LAR® is loaded with octreotide, a synthetic analog of somatostatin, and is commercially available as a monthly intramuscular PLGA *in situ* forming product. It is prescribed to treat acromegaly by controlling the growth hormone levels or to treat gastroenteropancreatic neuroendocrine tumors by controlling the gastrointestinal hormone secretions. Sublocade® is a recently approved buprenorphine-loaded PLGA *in situ* forming product prescribed for the treatment of opioid use disorder within 1 month period. The aforementioned products comprise two syringes in which the drug powder is filled in one syringe and the polymeric solution (PLGA/ N-Methyl-2-pyrrolidone) is present in the other syringe. Before injection, both syringes necessitate mixing several times to produce a homogeneous combination.

The *in situ* forming systems possess several benefits in comparison to the conventional pre-formed implant/microparticle. Due to their transient nature, implant placement is less invasive and less painful for patients thereby improving comfort and compliance [60, 61]. Besides, the manufacturing process of fabrication is proportionally mild and straightforward, allowing the *in situ* forming systems to be appropriate to deliver fragile protein and peptide therapies. However, the complications of the phase inversion process, poor *in vitro-in vivo* correlations, possible solvent toxicity and hurdles in controlling the initial burst drug release make these delivery systems less widespread in the market [62-64].

1.5. Drug physical state in PLGA formulations

Depending on the solubility of the drug in the polymer, and the amount in the formulation, the drug can exist in different physical states, such as in the dissolved state, the crystalline state, or the metastable state [65]. The physical state of the drug in the formulation can greatly affect its chemical stability, mechanical properties and *in vitro* and *in vivo* release characteristics [66, 67].

Besides the intrinsic properties of drug and formulation, the physical states of drug inside PLGA formulations are varied depending on the manufacturing process. If the drug is initially dispersed and remains in this form during the complete process, the situation is quite simple: the drug will be physically suspended in the polymer matrix (solid dispersion). However, if the drug is initially dissolved, three cases may occur (Figure 1.8): firstly the drug may be finally dissolved in the polymer, leading to a solid solution. Secondly, the drug may remain molecularly dispersed in the polymer, but with interactions between drug molecules and polymer chains too weak to lead to a stable state. This is a metastable molecular dispersion: the interactions between the drug molecules are strong and the molecules will diffuse through the polymer network and crystallize. The rate of diffusion of the molecules depends on the matrix viscosity and may take years. Thirdly, the drug may crystallize during the microparticle preparation: it will then be physically dispersed in the polymer matrix in the form of a crystalline dispersion. These three states are different in terms of storage stability and drug release characteristics, although they have the same chemical composition.

For example, dexamethasone is dissolved in a co-solvent system (e.g. dichloromethane/DMSO), followed by emulsification/hardening process to prepare microparticles, the encapsulated drug was in a dissolved state, a metastable molecular dispersion or a crystallized dispersed state as the drug loading increased or the manufacturing process varied [68, 69]. With the co-solvent process, it is difficult to control the dispersion state and particle size of dexamethasone during fabrication and storage [70]. Thus, the encapsulation of the active compound in microparticles in the dispersed state directly is expected to be a better method to ensure drug physical stability during fabrication and storage. Moreover, drug in the dispersed state is a proper way to achieve a high drug loading with good stability.

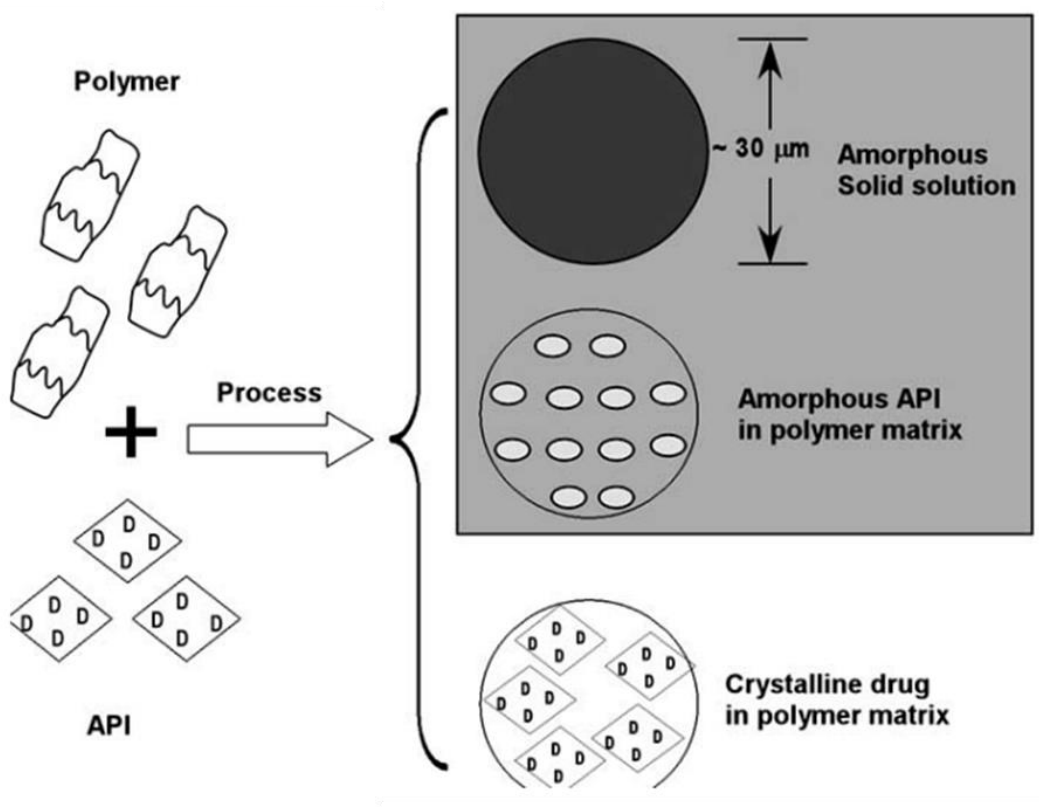


Figure 1.8. The API in polymer matrix can be in one of three states: dissolved (amorphous solid solution), metastable molecular dispersion and crystalline [71].

The crystalline drug also has significantly different physicochemical properties based on their particle sizes [72]. The physical states of drug are further divided into dispersion state and particle size and discussed separately below.

1.5.1. Drug dispersion state

The drug dispersion state can be classified into two major categories based on the order of drug molecular packing [73]. In the crystalline state (dispersed state), drug is in a regular order throughout the drug solid. This contrasts with amorphous state (or dissolved state) in which the regularity of structure is limited to the immediate neighbors of any particular molecule within the solid. Dispersed crystalline drugs can be further subdivided into polymorphs, which result from different crystalline forms of the same molecule, and multi-component crystals such as hydrates, solvates and co-crystals, which consist of more than one type of molecule. Dissolved drugs, because they exhibit a higher energy state than

dispersed drugs, are inherently less stable and have the potential for converting to the thermodynamically more stable crystalline form over time. In addition, because of their higher molecular mobility, they often show stronger chemical reactivity and hence a faster rate of chemical degradation. Nevertheless, the amorphous/dissolved form of a drug often has a higher solubility than its crystalline form and the use of the amorphous/dissolved form of a drug may provide an opportunity to enhance its bioavailability in the case of poorly water-soluble drugs [74].

Researchers in the pharmaceutical industry generally seek to deliver crystalline forms of their active compounds, mainly due to the inherent stability of crystalline materials and the well-established impact of crystallization processes on purification and isolation of chemical substances [75].

To encapsulate active compounds in different dispersion states, different PLGA microparticle preparation methods are used. To obtain the microparticles loaded with dissolved drug, the drug needs to dissolve in the oil phase (PLGA containing organic solvents) first and avoids any recrystallization over the whole manufacturing process and storage period. In this case, O/W or O/O methods are the most suitable techniques. It is worth mentioning that drug content should below the drug solubility in the PLGA matrix to keep the dissolved state.

Dispersed drug crystals can be encapsulated in PLGA microparticles using S/O/W or S/O/O techniques. This is a two steps solvent evaporation technique, in the first the active drug is dispersed in an oil phase along with solubilized PLGA. This organic phase is then added to the aqueous phase or another oil phase containing surfactant for emulsification followed by evaporation of the solvent. For S/O/W or S/O/O methods, the size of the solid drug particles for encapsulation should be significantly smaller than the final polymer microparticles, and if the solid drug powder is flocculated, appropriate mixing of the oil phase may become necessary. Microparticles containing dispersed drug crystals can also be produced by O/W or O/O methods by dissolving both polymer and drug in an appropriate solvent. During solvent removal in the preparation process, the

drug will crystallize out if its solubility in the polymer matrix is exceeded. However, the crystal shape and size are difficult to control precisely.

To embed drugs into PLGA implants, solvent solution methods or hot melt extrusion are commonly used [76]. All these techniques can obtain drugs with different dispersion states inside the matrix.

1.5.2. Drug particle size

Control of particle size is important in drug delivery. For example, only very fine particles penetrate the alveolar regions of the respiratory tract by pulmonary delivery. But, if the particle size is reduced too far, particles may be exhaled. Particle size also influences the uniformity of dosage of very potent drugs formulated as a solid dosage form, which is greater with smaller particles because of the larger number of particles. Particle size also has important effects on the bulk powder properties. Particles larger than about 250 μm are usually free-flowing, but flow problems are likely observed when the size is below 100 μm due to cohesion. Very fine particles (below 10 μm) are usually extremely cohesive and are not free-flowing.

Particle size of drug crystals has an important influence on dissolution rate. A larger surface exposed to the release medium increases the dissolution rate of smaller particles. Especially for drugs with very low solubility, the micronization approach can promote solubility and therefore drug bioavailability. Conventional mechanical technologies used to micronize particles are jet milling and ball milling. Very fine particles (below 10 μm) can be produced, but preparing smaller particles down to nanometer becomes difficult for these conventional methods because of fierce cohesive forces (arise from short-range van der Waals forces and electrostatic forces).

To further reduce the particle size, drug crystals down to nanometer size can be prepared by top-down and/or bottom-up techniques (Figure 1.9) [77]. Top-down

techniques comminute coarse drug powders by mechanical attrition through media milling or high-pressure homogenization [78], while bottom-up techniques grow crystals from solution [79, 80]. Top-down process is a universal technique to prepare crystalline nanoparticles [81] and is flexible in production scale [82]. Therefore, top-down techniques are wildly adopted by commercial products. However, it is generally time and energy consuming [83]. Contamination from grinding media can lead to unexpected side effects. Particle aggregation and growth due to wide particle size distribution and decreased crystallinity are other concerns. Conversely, bottom-up technique is less energy consuming, with superior crystallinity and narrow particle size distribution [79, 80]. Challenges remain in controlling nucleation and crystal growth and scale-up.

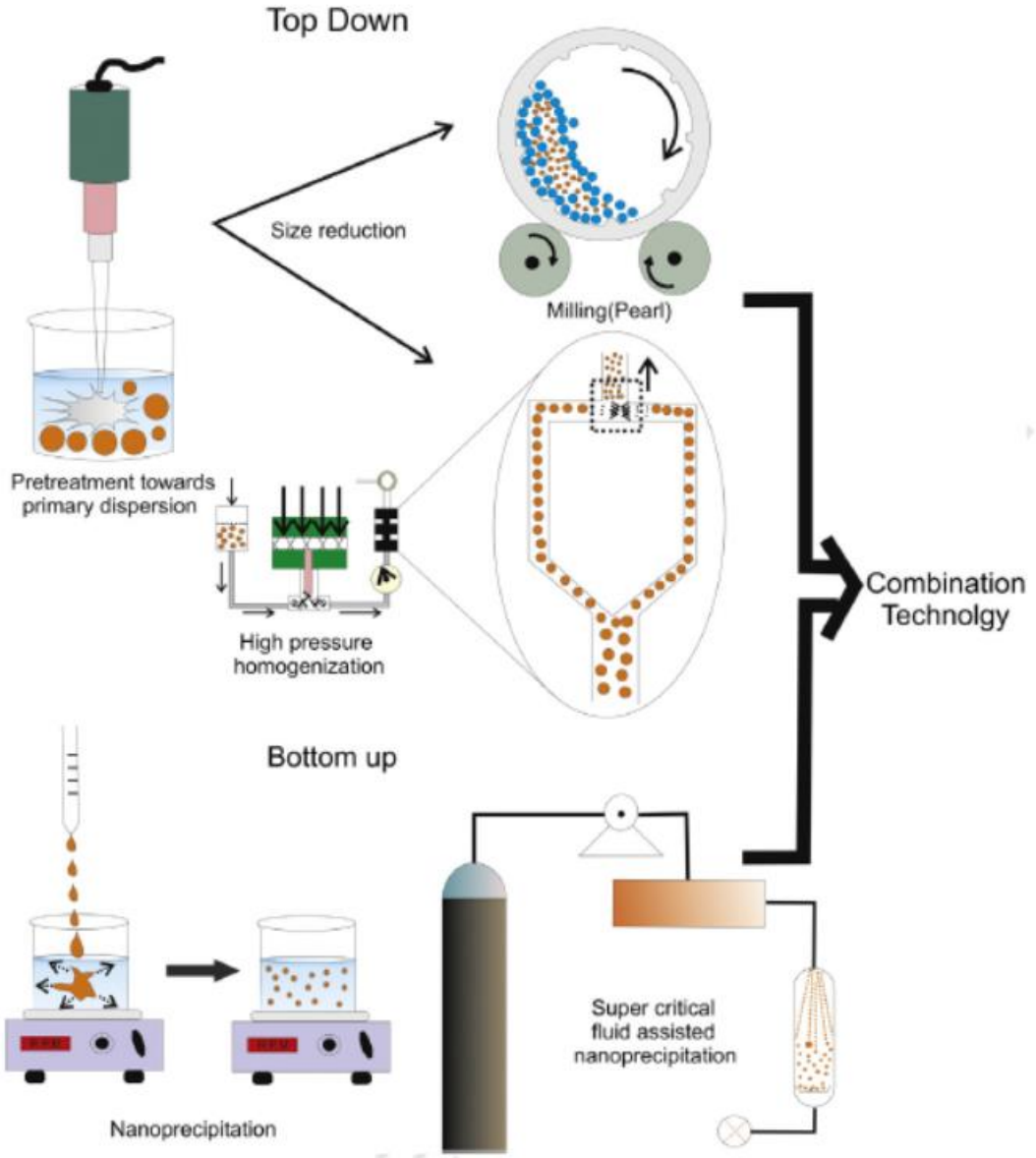


Figure 1.9. Manufacturing techniques employed for fabricating nanosized drug [84].

Nanosizing drug has been suggested as a useful technology to formulate drug molecules that fall into class II (low solubility and high permeability) and class IV (low solubility and low permeability) of the Biopharmaceutics Classification system (BCS) [85, 86]. The increased saturation solubility and dissolution rate are the most important features of nanosized drugs.

Increased saturation solubility of nanosized drugs is reported as “apparent” saturation solubility [87]. According to the Ostwald–Freundlich equation (1.1), the increased curvature of nanoparticles results in increased dissolution pressure

and, hence, drug solubility.

$$\log \frac{c_s}{c_\alpha} = \frac{2\sigma V}{2.303RT\rho\gamma} \qquad (1.1)$$

where c_s is the saturation solubility, c_α is the solubility of the solid consisting of large particles, σ is the interfacial tension of substance, V is the molar volume of the particle material, R is the gas constant, T is the absolute temperature, ρ is the density of the solid, and γ is the radius.

According to the Noyes–Whitney equation (1.2), the total surface area increases by nanosizing the particle size, resulting in a faster dissolution rate [88].

$$\frac{dc_t}{dt} = \frac{DA}{h}(c_s - c_t) \qquad (1.2)$$

In which, dc_t/dt is the dissolution rate; D is the diffusion coefficient; A is the available surface area; h is the thickness of diffusional layer; c_s is saturation solubility, c_d is the drug concentration in the bulk solution at time t.

Moreover, drug products containing nanosized drugs possess also increased adhesiveness to surface/cell membranes and reduced or even eliminated food effects [89]. Consequently, an improvement of oral absorption of poorly water-soluble drugs is observed, along with the increased saturation solubility and dissolution rate. All factors present a positive impact on the bioavailability of the drug.

Nanoparticles are much more unstable than microparticles because of the extra Gibbs free energy contribution. Addressing this problem is key to formulating pharmaceutical nanoparticles because they will tend to agglomerate. Various types of generally recognized as safe pharmaceutical excipients have been investigated and used as stabilizers. Such stabilizers could be polymers and surfactants, which are normally water-soluble [90, 91]. The type and concentration of stabilizer used have been found to strongly influence the size

reduction kinetics of the nanosuspension [78, 92]. In general, ionic surfactants are used to against the particles aggregation via electrostatic repulsion, while the non-ionic surfactants and polymeric stabilizers by a steric barrier.

Besides the nanosuspension, nanosized drugs are commonly dried to increase the stability and for further process. Due to a high surface area, they are coated with stabilizers to avoid aggregation. The reduced particle size and higher surface area are necessary for enhanced drug bioavailability [93]. For this reason, formation of irreversible aggregates is undesirable. In order to prepare solid dosage forms out of nanoparticles, the nanosuspension has to be dried and then processed further (into tablets or capsules) [94, 95]. Drying of nanoparticles can cause aggregation. For example, if the nanoparticles are coated with polymeric surfactants such as poloxamers, drying may lead to crystallization of the polymer, thereby compromising their ability to prevent aggregation. Drying can also create additional thermal stresses that may destabilize the particles. Due to these considerations, freeze-drying process was commonly used to prepare the dry powders [96].

After the dry powders were obtained, they were further dispersed in the oil phase to prepare the microparticles loaded with dispersed drug as described above. The input drug particle sizes may vary from micrometers to nanometers, and the output drug particle size inside the microparticle is normally kept constant.

1.6. Research objectives

This research has been designed to extensively investigate biodegradable microparticles and *in situ* forming implants/microparticles containing drugs in different physical states. The specific objectives of this project have been described in detail below:

- Prepare and evaluate PLGA microparticles loaded with micronized, nanosized and dissolved dexamethasone or hydrocortisone;
- Optimize parameters to fabricate dexamethasone, dexamethasone sodium phosphate and hydrocortisone nanocrystals by non-aqueous wet bead milling;
- Prepare and evaluate PLGA microparticles loaded with drug nanocrystals by solvent evaporation or coacervation method following the non-aqueous wet bead milling;
- Develop PLGA *in situ* forming system incorporating nanosized dexamethasone and thoroughly compare it *in vitro* with PLGA *in situ* forming systems loaded with micronized or dissolved dexamethasone;
- Optimize the parameters to fabricate sucrose, lactose and trehalose nanocrystals by non-aqueous wet bead milling;
- Explore the usage of nanosized/micronized sugar particles as porogen for the introduction of porosity within PLGA microparticles containing dexamethasone.

2. Materials and Methods

2.1. Materials

2.1.1. Drugs

Micronized dexamethasone and dexamethasone sodium phosphate (D_{90} < 15 µm; Caesar & Loretz GmbH, Hilden, Germany); micronized hydrocortisone (D_{90} < 15 µm; Tokyo Chemical Industry, Tokyo, Japan).

2.1.2. Polymers

PLGA (Resomer RG 502H, acid end groups, 0.16 - 0.24 dl/g inherent viscosity; Resomer RG 502, ester end groups, 0.16 - 0.24 dl/g inherent viscosity; Resomer RG 503H, acid end groups, 0.32 - 0.44 dl/g inherent viscosity; Resomer RG 752S, ester end groups, 0.16 - 0.24 dl/g inherent viscosity; Evonik Industries AG, Darmstadt, Germany); polyvinyl alcohol 4-88 (PVA) (Merck KGaA, Darmstadt, Germany); Poloxamer 188 (BASF SE, Ludwigshafen, Germany).

2.1.3. Reagents and others

Dichloromethane and triacetin (Merck KGaA, Darmstadt, Germany); dimethylsulfoxide (DMSO) (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany); polydimethylsiloxane (350 cP, Dow Corning Inc., Michigan, USA); sodium dodecyl sulfate (SDS), N-Methyl-2-pyrrolidone (NMP), acetonitrile, n-heptane, NaH_2PO_4, Na_2HPO_4, HCl, NaOH and sodium chloride (Carl Roth GmbH & Co. KG, Karlsruhe, Germany); sucrose (VWR International GmbH, Darmstadt, Germany); anhydrous D-(+)-Trehalose (Tokyo Chemical Industry, Tokyo, Japan); lactose (Merck KGaA, Darmstadt, Germany); Yttrium oxide-stabilized zirconia oxide beads (0.1 - 0.2 mm, Sigmund Lindner GmbH, Warmensteinach, Germany). Ultra purified water was purified by a Milli-Q-apparatus (Millipore GmbH, Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation

2.2.1.1. Preparation of nanosized drugs in water

10.0 g milling beads, 100.0 mg micronized dexamethasone or hydrocortisone and 10.0 g 0.05 % (w/v) SDS solution were added to a 15 mL Twist-Top-Vial (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Dual centrifugation was performed using a ZentriMix 380 R (0 °C and 1000 rpm; Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The resulted suspensions were separated from the beads by filtration through a 10 µm stainless sieve. Subsequently, they were centrifuged (7500 rpm and 60 min; Heraeus Biofuge Stratus Centrifuge, Thermo Fisher Scientific GmbH, Darmstadt, Germany) and the supernatant was replaced by distilled water. This washing procedure was performed three times and then the wet particles were frozen at – 80 °C followed by freeze-drying (– 30 °C and 0.37 mbar; Alfa® 2 - 4 LD Plus freeze-dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Goettingen, Germany).

2.2.1.2. Preparation of nanosized drugs in organic solvents

15.0 g milling beads, 250.0 mg micronized drugs, 5.0 g dichloromethane, 0 - 50.0 mg PLGA (or 12.5 mg poloxamer 188) and a magnetic stirrer were added in a 15 ml glass bottle with polypropylene cap. For every milling trial, the sealed glass bottle was put in an ice bath and the milling speed was set to 1500 rpm. Milling times, PLGA types and concentrations were varied to investigate their impact on the size of the drug particles.

10.0 g milling beads, 0.5 g dexamethasone and 10.0 g triacetin were added to a 15 mL Twist-Top-Vial (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Dual centrifugation was performed using a ZentriMix 380 R (0 °C, 1500 rpm and 2 h; Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany).

These final suspensions were obtained by removing the milling beads through a 10 µm stainless sieve.

2.2.1.3. Preparation of micronized and nanosized sugar particles

Micronized sucrose was prepared by jet milling sucrose powder using Spiral Jet Mill 50 AS (HOSOKAWA ALPINE AG, Augsburg, Germany) with 6 bar inlet pressure and constant feed rate of 0.5 g/min. Nanosized sugars were produced by non-aqueous wet bead milling. Add 15.0 g milling beads, 250.0 mg micronized sugars, 5.0 g dichloromethane and a magnetic stirrer in a 15 mL glass bottle with a polypropylene cap. Samples were milled (1500 rpm and 8 h) in an ice bath. The suspensions were separated from the beads by filtration through a 10 µm stainless sieve.

2.2.1.4. Preparation of microparticles

Microparticles loaded with micronized, nanosized and dissolved drug

O/W or S/O/W emulsion extraction/evaporation methods were used. PLGA was dissolved in dichloromethane or a mixture of DMSO and dichloromethane (Table 2.1), and micronized or nanosized drug powder was dispersed or dissolved into PLGA solution by sonication (2 min; Bandelin Sonorex RK512H, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) and homogenizing (1 min and 8500 rpm; IKA T 25 digital ultra-turrax homogenizer, IKA Works Inc., Wilmington, USA). Subsequently, the organic phase was emulsified into a 50 mL 0.25 % (w/v) PVA solution at 8500 rpm for 30 s and diluted in 150 mL 0.25 % (w/v) PVA solution and stirred at 300 rpm under the fume hood. After 4 h, hardened microparticles were passed through 50 µm and 20 µm sieves. 20 - 50 µm microparticles were transferred to 50 mL centrifuge tubes and then washed three times with deionized water (25 mL each time). During washing, microparticles were recollected using centrifugation (2500 rpm and 2 min). The washed microparticles were freeze-dried (- 30 °C and 0.37 mbar) and stored at 4 °C until further use. Blank microparticles were prepared under the same conditions.

Table 2.1. Composition of inner organic phase used for microparticle preparation.

Theoretical drug loading, %	Micronized		Nanosized		Dissolved	
	10	30	10	30	20	50
PLGA, mg	270	210	270	210	240	150
Drug, mg	30	90	30	90	60	150
Dichloromethane , g	1	1	1	1	0.7	0.7
DMSO, g	0	0	0	0	0.3	0.3

Microparticles loaded with drug nanocrystals by combining non-aqueous wet bead milling and encapsulation

S/O/W solvent evaporation method was used to encapsulate both water-soluble and water-insoluble nanosized drugs, while S/O/O coacervation method was only used to encapsulate water-soluble nanosized drugs.

In the S/O/W solvent evaporation technique, PLGA was dissolved in the drug dichloromethane nanosuspension obtained in section 2.2.1.2 with or without adding extra dichloromethane as listed in Table 2.2. Then, this PLGA nanosuspension was mixed (8000 rpm and 1 min). Subsequently, the suspension was homogenized into a 5 mL 1.0 % (w/v) PVA solution (8000 rpm and 30 s). The emulsion was diluted in 200 mL 0.25 % (w/v) PVA solution and stirred at 300 rpm under a fume hood. After 4 h, hardened microparticles were passed through 71 µm, 50 µm, 20 µm and 5 µm sieves. To prepare 0 - 5 µm microparticles, homogenizing speed was increased from 8000 rpm to 13500 rpm and hardened microparticles were passed through 5 µm sieves. Different fractions were transferred to 50 ml centrifuge tubes and washed three times with deionized water (25 mL each time), recollected using centrifugation (2500 rpm and 5 min) and dried via freeze-drying (- 30 °C and 0.37 mbar). The dried microparticles were stored at 4 °C until further use. Blank microparticles were prepared under the same conditions.

In the S/O/O coacervation method, 270 mg PLGA was dissolved in 4.4 g dichloromethane and mixed with 0.63 g dichloromethane nanosuspension. After adding 1 g of the non-solvent polydimethylsiloxane under homogenizing (8000 rpm and 30 s) to induce phase separation. The emulsion was injected under

stirring with a propeller stirrer (300 rpm) into the hardening bath containing 100 g n-heptane and stirred at room temperature (300 rpm and 1 h). The microparticles were passed through 50 µm and 20 µm sieves. 20 - 50 µm microparticles were transferred to 50 mL centrifuge tubes and then washed three times with n-heptane (25 mL each time), recollected using centrifugation (2500 rpm and 5 min) and dried via freeze-drying (- 30 °C and 0.37 mbar).

Table 2.2. Composition of inner oil phase used for microparticle preparation.

Theoretical drug loading, %	5	10	15	20	30
PLGA, g	0.285	0.270	0.255	0.200	0.117
Dichloromethane, g	0.700	0.400	0.100	-	-
Nanosuspension, g	0.315	0.630	0.945	1.050	1.050

Porous PLGA microparticles containing dexamethasone

The S/O/W emulsion solvent evaporation technique was accepted to fabricate porous microparticle. In this method, 180 mg PLGA was dissolved in 1 g dichloromethane with 0 %, 1 %, 2.5 %, 5 %, 10 % or 30 % (w/w, based on the weight of PLGA and dexamethasone) nanosized or micronized sugar (i.e. 0, 2, 5, 10, 20 or 60 mg). The 20 mg dexamethasone powder was added to the PLGA solution. Then, this PLGA solution was mixed (1 min and 8000 rpm). Subsequently, the suspension was homogenized into a 10 mL 1.0 % (w/v) PVA solution (8000 rpm and 30 s). The emulsion was diluted in 200 mL 0.25 % (w/v) PVA solution and stirred at 300 rpm. After 4 h, hardened microparticles were passed through 50 µm and 20 µm sieves. Gained fractions were transferred to 50 mL centrifuge tubes and washed three times with deionized water (50 mL each time), recollected using centrifugation (2500 rpm and 5 min) and dried using a vacuum oven (room temperature and 48 h; Heraeus VT 5042 EKP, Heraeus Germany GmbH & Co. KG, Hanau, Germany). The prepared microparticles were stored at 4 °C until further use.

2.2.1.5. Preparation of PLGA *in situ* forming systems

The *in situ* forming implants (ISIs) were prepared by mixing appropriate amounts of PLGA with the dexamethasone triacetin nanosuspension or micronized dexamethasone triacetin suspension under stirring (500 rpm and 24 h) to form a uniform polymer solution. Appropriate amounts of PLGA and micronized dexamethasone powder were dissolved in NMP or a mixture of NMP and triacetin (20:80) under stirring (500 rpm and 30 min). Afterward, these samples were kept without stirring for 24 h at room temperature to remove air bubbles. They were stored at 2 - 8 °C, and allowed to reach room temperature before use. For all ISIs, the polymer concentration was based on the amount of solvent varied from 20 % to 40 % (w/w), and dexamethasone loading was based on the amount of polymer varied from 10 % to 25 % (w/w).

The *in situ* forming microparticles (ISMs) were prepared as described previously [63, 97]. Briefly, a polymer solution was emulsified into an aqueous external phase of 2.0 % PVA at the ratio of 1:4 (w/w) in a two-syringe system (1 mL Luer-Lok™ Tip syringe, Becton, Dickinson and Company, New Jersey, USA), which was connected by a fluid dispensing connector (B. Braun Medical Inc., Pennsylvania, USA). The emulsion droplets were achieved by 5, 10 and 20 mixing cycles (approximately 1 cycle/s).

2.2.2. Characterization

2.2.2.1. Solubility

Excess drugs were added to 5 mL water, dichloromethane or triacetin in 10 mL glass vials. The vials were incubated in an incubator shaker (25 °C, 80 rpm and 72 h; New Brunswick Scientific Co. Inc., Connecticut, USA). 1 mL of sample was withdrawn and filtered using 0.22 µm PTFE syringe filters. The filtered samples were diluted with water and analyzed using the UV method described below (n = 3).

2.2.2.2. Particle size analysis

The micronized drug powder and freeze-dried nanosized drug were first dispersed in a drug saturated 0.1 % (w/v) PVA solution. PLGA microparticles were dispersed in 0.1 % (w/v) PVA solution. Then their particle sizes were measured by laser diffraction (Mastersizer® 2000, Malvern Instruments Ltd., Malvern, UK). Samples were measured with a volume-based approach (n = 6).

After milling in solvent, the particle sizes of nanosized drugs (after diluting 100 times in drug saturated dichloromethane) and nanosized sugars (after diluting 100 times in 1 % PLGA 503H dichloromethane) were determined by photon correlation spectroscopy using a Zetasizer® Nano ZS (Malvern Instruments Ltd., Malvern, UK). The z-average and the polydispersity index (PDI) are displayed (n = 3).

Particle size and size distribution of micronized sucrose particles and porous PLGA microparticles were determined using an optical microscope (Axioskop, Carl Zeiss Microscopy GmbH, Jena, Germany). Diameters of at least 300 particles were measured using an image processing program (Image J 1.53a, National Institutes of Health, Maryland, USA).

The ISMs were observed and imaged immediately after emulsification under an optical microscope. Diameters of more than 200 microparticles were measured using an image processing.

2.2.2.3. Morphology

SEM (SU8030, Hitachi High-Technologies Europe GmbH, Krefeld, Germany) was used to image morphologies of micronized and nanosized sucrose and PLGA microparticles. To investigate the inner structure, the microparticles were dispersed in a solvent-free glue (UHU GmbH & Co. KG, Baden, Germany). After drying in a desiccator, the hardened matrix was cut with a razor blade. Samples were sputtered under an argon atmosphere with gold to a thickness of 5 nm (CCU-010 HV, Safematic GmbH, Zizers, Switzerland) and then observed.

TEM (FEI Tecnai G² 20 S-TWIN, FEI Company, Oregon, USA) was used to

examine the morphology of the nanosized dexamethasone encapsulated in microparticles. The microparticles were dispersed in a solvent-free glue UHU®. After drying, the hardened matrix was cut with a diamond knife. Samples were loaded into electron microscope with a field emission gun, operating at 200 kV.

2.2.2.4. Drug content and encapsulation efficiency

The total amount of drug in the nanocrystal formulation and microparticles were determined using a UV spectrophotometer (Agilent HP 8453, Agilent Technologies Inc., Santa Clara, USA) at 242 nm for dexamethasone and dexamethasone sodium phosphate, and 244 nm for hydrocortisone after dissolving them in water: acetonitrile (8:2). The actual drug loading (% DL) and encapsulation efficiency (% EE) were calculated according to the equations (2.1) and (2.2):

$$\% \text{ drug loading} = \frac{\text{Mass of drug in formulation}}{\text{Mass of total formulation}} \times 100\ \% \qquad (2.1)$$

$$\% \text{ encapsulation efficiency} = \frac{\text{DL}}{\text{Theoretical DL}} \times 100\ \% \qquad (2.2)$$

2.2.2.5. Differential scanning calorimetry

Micronized drug (~ 3 mg), nanosized drug (~ 3 mg), PLGA microparticles (~ 10 mg), nanosized sucrose (~ 0.1 mg), physical mixture of nanosized sucrose and PLGA 503H (1:99 and ~ 10 mg), physical mixture of nanosized sucrose, dexamethasone and PLGA 503H (5:10:85, ~ 10 mg) were accurately weighed in 50 µL aluminum pans with pierced lids. Thermograms were recorded using a DSC 6000 (5 °C/min; PerkinElmer Inc., Waltham, USA) or DSC 1 (10 °C/min; Mettler Toledo GmbH, Greifensee, Switzerland).

To prepare dried nanosized drug particles for differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD) studies, the dichloromethane nanosuspensions were centrifuged (15000 rpm and 60 min). After removing the supernatant, the wet particles were dried under the fume hood. The triacetin

nanosuspension was centrifuged (15000 rpm and 2 h). Subsequently, the supernatant was removed. Distilled water was used to wash wet crystals three times and then the wet particles were frozen at －80 ° C followed by freeze-drying (- 30 °C and 0.37 mbar).

2.2.2.6. X-ray powder diffraction

XRPD patterns were performed with Cu Kα radiation at a voltage of 40 kV and current of 40 mA, a scanning rate of 2.07 °/min over a 2θ range from 6.5 ° to 50 ° using a Panalytical Empyrean diffractometer (PANalytical B.V., Almelo, Netherlands).

2.2.2.7. Physical stability

Around 2 g ISIs were placed in 5 mL glass vials and stored for a period up to 3 months at room temperature. Samples were observed and images were taken with a light macroscope (Inteq® Informationstechnik GmbH, Berlin, Germany).

2.2.2.8. Viscosity measurement

Viscosity was measured using a controlled stress mode of the plate and cone device connected with a computer interface (20 ± 0.2 °C; Rheostress RS 100, Haake Meb-Technik GmbH, Karlsruhe, Germany). The viscosities of nanosuspensions and *in situ* forming systems were measured by the plate and cone of 20 mm diameter/ 4 ° angle with fixed shear stress (10 Pa) for a constant time (120 s).

2.2.2.9. Injectability test

A texture analyzer (TA.XT.Plus, Stable Micro Systems, Surrey, UK) was used to determine the injectability of the *in situ* forming systems. The texture analyzer setup was illustrated in our previous study [98]. An empty 1 mL syringe with a needle size of 23 G (1 $^{1}/_{4}$ inch, 0.60 × 30 mm) was filled with the 0.2 mL formulation and fixed to the holder of the apparatus. The cylindrical probe of the apparatus was used to move the piston of the syringe downward at a constant

speed of 1 mm/s, up to 10 mm. Force-distance profiles were recorded at room temperature and the maximum injection forces were listed.

2.2.2.10. *In vitro* release study

Microparticles were immersed in 50 mL of 10 mM phosphate buffer (pH 7.4) with 0.01 % NaN_3 and incubated in an incubator shaker (37 °C and 80 rpm). At pre-determined time points, 5 mL release media was removed and replenished. Sink conditions were maintained throughout. The samples were passed through 0.45 µm syringe filters and the concentration of drug in each sample was determined using the UV method described above.

Around 300 mg ISIs and ISMs were injected to the bottom of a 50 mL high-clarity polypropylene conical tubes using a standard syringe. 20 mL preheated (37 °C), pH 7.4 phosphate-buffered saline was carefully added using a pipette. The vials were incubated (37 °C and 80 rpm). At pre-determined time points, the bulk fluid was completely withdrawn and replaced with fresh medium. The amount of drug released in each sample was determined using a UV spectrophotometer at 260 nm.

2.2.2.11. Dynamic changes in the implants' mass and morphology

At pre-determined *in vitro* release time points, excess water was carefully removed using Kimtech Science precision wipes (Kimberly-Clark GmbH, Koblenz, Germany) and weighed. The mass change in percent was calculated using the following formula:

$$\text{mass change (\%) (t)} = \frac{\text{mass (t)} - \text{mass (t = 0)}}{\text{mass (t = 0)} - \text{mass (container)}} \times 100 \qquad (2.3)$$

where mass (container) is the weight of the container, mass (t = 0) is the initial weight of the formulation and container, and mass (t) refers to the weight of the wet implant and the container.

After weighing the implants' mass, top and side pictures were taken with a light

macroscope to show their morphology change along with time.

2.2.2.12. pH Measurement

pH measurement was conducted at room temperature using an 827 pH lab benchtop pH meter (Metrohm AG, Herisau, Switzerland) equipped with a primatrode electrode. The pH of the release medium was measured at pre-determined time intervals to determine any changes in the pH.

2.2.2.13. Swelling of microparticles

Approximately 1 mg microparticles were put into each well of a 96-well standard microplate (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), which was completely filled with 10 mM phosphate buffer (pH 7.4). The well plates were incubated in an incubator shaker (37 °C and 80 rpm). To avoid water evaporation, the well plates were sealed by a parafilm membrane (Bemis Company Inc., Wisconsin, USA). At predetermined time points, pictures were taken using an optical microscope fitted with an Axiocam 105 color camera and ZEN software (Carl Zeiss Microscopy GmbH, Jena, Germany) after removing the phosphate buffer, and then refreshed with new buffer. The mean diameters of swelled microparticles were determined by averaging over 200 microparticles for each time point.

2.2.2.14. Emulsion observation

After organic phases (200 mg PLGA 503H, 0 mg or 5 mg nanosized or micronized sucrose and 1 g dichloromethane) were homogenized into the outer water phases (10 mL 1.0 % PVA solution), O/W emulsions were put into ground edge single concave microscope slides (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The concave was covered immediately by a cover slide to avoid dichloromethane evaporation. Within 2 minutes, pictures were taken using an optical microscope.

2.2.2.15. Nitrogen sorption

Nitrogen sorption experiments were performed on a Quantachrome NOVA 4000e

(Quantachrome Corporation, Florida, USA) with 9 mm stem sample cells. All samples were vacuum degassed overnight at room temperature. The specific surface area was determined in the BET region of the adsorption isotherm. Measurements of relative pressure were acquired at P/Po values of 0.06, 0.14, 0.22, 0.30, 0.38, 0.48 and 0.56. Specific surface area was determined using the Quantachrome NovaWin software.

2.2.2.16. Data evaluation and presentation

Data were evaluated with the help of Microsoft® Excel® (Office 365, Microsoft Corporation, Washington, USA) and GraphPad (GraphPad Prism 6, California, USA). All the experimental results were depicted as the mean value ± standard deviation (SD) from at least three measurements (unless otherwise specified). Significance of difference was evaluated using one-way ANOVA at a probability level of 0.05.

3. Results and Discussion

3.1. A comparative study of PLGA microparticles properties loaded with micronized, nanosized and dissolved drug

3.1.1. Introduction

To obtain PLGA microparticles loaded with drug crystals, micronized drug particles were added to a PLGA solution to obtain a solid-in-oil dispersion. Subsequently, this dispersion was emulsified and microparticles were hardened [99-101]. This method resulted in a heterogeneous distribution of large drug crystals in the microparticle matrix, which in turn caused low drug loading and high burst release since the drug crystal size is close to the size of the microparticles [68]. A pronounced burst is not desired since a high drug plasma peak may lead to systemic toxicity and drug lost in the burst phase is not available for later release [11, 102]. To prepare PLGA microparticles loaded with dissolved drug, drug and PLGA were dissolved in a single solvent or co-solvent system first, following an emulsification/hardening process to prepare microparticles. The final encapsulated drug in the dried microparticles might be dissolved, amorphous or a mixture of amorphous and crystallized particles [68, 69]. However, it is difficult to achieve high encapsulation efficiency and the drug solid state is unstable during storage [70]. This unstable state may cause chemical degradation in the matrix during storage or even after *in vivo* administration. Moreover, uncontrolled recrystallization may occur during preparation and/or storage, changing the drug release [69, 103, 104]. Thus, the encapsulation of crystalline drug is more suitable to ensure physicochemical stability during fabrication and storage. Recently, encapsulating nanosized crystals into microparticles was also investigated to improve stability, and to achieve continuous release. Breviscapine nanocrystals were prepared by a precipitation/ultrasonication method and further loaded into PLGA microparticles. These microparticles improved encapsulation efficiency compared with microparticles prepared by the co-solvent method [105]. Although it is recognized that the drug dispersion state and particle sizes inside PLGA

microparticles significantly affect encapsulation efficiency, stability and drug release of PLGA microparticles [106], there has been no systematic investigation to study this effect.

Dexamethasone and hydrocortisone are potent glucocorticoids [107]. They are effective and safe in the treatment of a wide variety of inflammatory diseases and in suppressing the immune system. However, their half-life is very short and this typically requires frequent administrations, thus reducing patient compliance and causing therapeutic failure. PLGA microparticles can be designed to achieve long-term release. Moreover, dexamethasone and hydrocortisone are selected as model drugs due to their proper physicochemical properties, like chemical stability and low solubility in water and dichloromethane. They were widely investigated in the field of PLGA microparticle as model drugs and encapsulated in PLGA microparticles in both dissolved and crystalline states using different preparation methods [69, 108]. Besides the encapsulated drug, the drug release from PLGA microparticles is governed by various properties of PLGAs, including lactic to glycolic ratio, molecular weight, and acid or ester end group. Both hydrophilic (502H and 503H) and hydrophobic (502 and 752S) PLGAs were used to understand the impact of lactic to glycolic ratio, molecular weight, and end group on various differences of PLGA microparticles loaded with different drug dispersion states and particle sizes.

In this study, nanosized drugs were prepared by aqueous wet bead milling and encapsulated into PLGA microparticles. Furthermore, PLGA microparticles loaded with micronized and dissolved drug were prepared. These three microparticles were characterized and compared in terms of particle size, encapsulation efficiency, drug solid state, inner and outer morphology, *in vitro* release and swelling. It is hypothesized that PLGA microparticles loaded with micronized drug have a stable drug solid state and poor continuous release, and microparticles loaded with dissolved drug have unstable drug solid state and low encapsulation efficiency. In contrast, microparticles loaded with nanosized drug will overcome these drawbacks of loading with micronized and dissolved drug,

and additionally achieve a stable drug solid state and high encapsulation efficiency. Moreover, the burst will be reduced, the lag phase will be shortened or eliminated, which results in a continuous release.

3.1.2. Characterization of nanosized drug

The wet bead milling method was used to break up the micronized drug particles into nanosized particles with narrow size distributions. In the milling process, the type of stabilizer, its concentration, speed and time were considered as critical factors in determining the size of the final particles [109, 110]. Considering the high milling efficiency of SDS [111], SDS was chosen as stabilizer. The size of the micronized drug decreased efficiently in 0.05% (w/v) SDS from originally around 6000 nm to 200 nm (Table 3.1). The milling speed was 1000 rpm as this speed has been successfully used for the attrition of various drug crystals by dual centrifugation [112]. Subsequently, the milling time was optimized. Drug particles with mean sizes around 200 nm were obtained when dexamethasone and hydrocortisone were milled for 2 h and 1 h respectively. Additionally, a high drug content around 100 % of freeze-dried nanosized powders was achieved.

Table 3.1. Characterization of original micronized and freeze-dried nanosized dexamethasone (2 h milling time) and hydrocortisone (1 h milling time).

Drug		LD, µm			Drug content, %
		D10	D50	D90	
Dexamethasone	Micronized	2.78 ± 0.04	6.18 ± 0.06	12.72 ± 0.11	100.0 ± 0.1
	Nanosized	0.08 ± 0.01	0.18 ± 0.01	1.24 ± 0.01	98.3 ± 0.3
Hydrocortisone	Micronized	1.42 ± 0.03	4.03 ± 0.04	10.73 ± 0.04	100.0 ± 0.1
	Nanosized	0.07 ± 0.01	0.16 ± 0.01	0.38 ± 0.01	100.9 ± 0.2

The solid state of nanosized drug was evaluated by comparing DSC and XRPD results with the original micronized drug. Dexamethasone original powder showed an endothermal effect starting at around 260 °C, which is the melting point of dexamethasone [70, 101]. The melting peak of nanosized dexamethasone was observed at around 220 °C confirming that dexamethasone was in crystalline form (Figure 3.1). The melting temperature of nanosized

hydrocortisone also decreased from 220 °C [113] to 180 °C. The melting peaks of nanosized drugs still existed, but the melting temperatures decreased suggesting a decrease in the degree of crystallinity due to imperfections in the crystal lattice by milling and downstream processing [109, 114]. Moreover, nanocrystal melting temperature depends on nanocrystal radius and differs from those of the infinite crystal (microcrystal). Gibbs–Thomson equation clarifies the observation that small crystals melt at a lower temperature than large crystals [115]. The XRPD spectrum of nanosized dexamethasone further confirmed that dexamethasone is in crystalline form, which is discussed in the next section in detail together with microparticles loaded with dexamethasone.

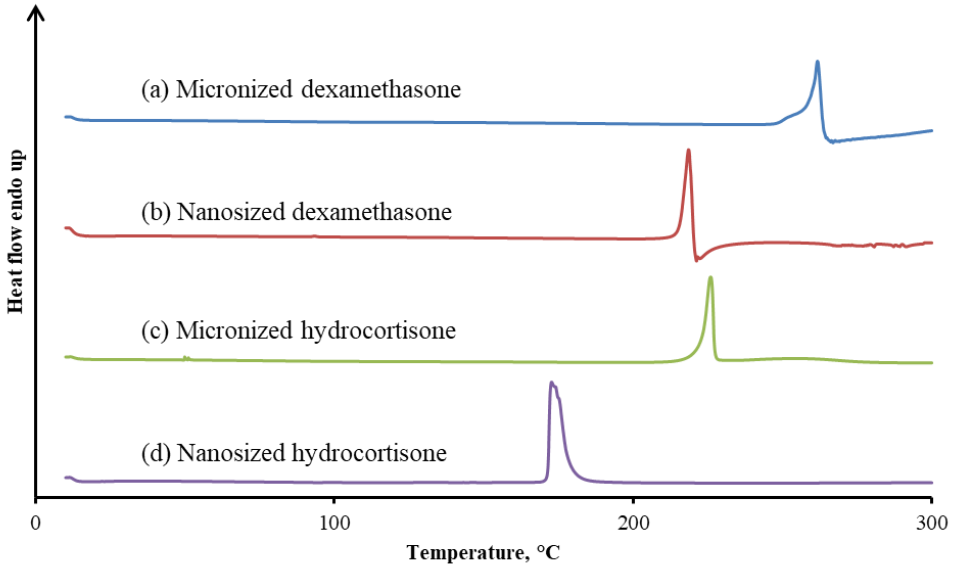


Figure 3.1. DSC thermograms of (a) original micronized dexamethasone, (b) freeze-dried nanosized dexamethasone, (c) original micronized hydrocortisone and (d) freeze-dried nanosized hydrocortisone.

3.1.3. Physical characterization of microparticles

Microparticles were characterized by their particle size, drug loading, encapsulation efficiency, drug solid state and morphology. The mean particle size and span of all microparticle formulations are quite similar and comparable (Table 3.2). The wet sieving process by 20 μm and 50 μm meshes before drying minimized the variation of particle size. The encapsulation efficiency is in the

range of 32 % - 99 %. The lowest encapsulation efficiency is caused by the co-solvent method to prepare microparticles loaded with dissolved drug, since drugs are dissolved in the co-solvent, and they diffuse out stronger during the emulsification process. Additionally, drug crystallized out during the hardening step. This results in heterogeneous pores at the surface and channels inside the particles. Therefore, drug particles have direct access to water. The slightly higher encapsulation efficiency of microparticles loaded with micronized drug compared with microparticles loaded with nanosized drug is due to a quicker dissolution rate in dichloromethane when the drug is nanosized, and therefore diffuses out faster into the outer aqueous phase during the emulsification process. PLGA 502 and 752S are end-capped resulting in a higher solubility in dichloromethane and precipitate slower than PLGA 502H and 503H. Slower hardening rates resulted in lower encapsulation efficiency [116]. The different molecular weights of PLGA 502H and 503H and the different lactic to glycolic ratios of PLGA 502 and 752S show no major impact on encapsulation efficiency. Compared to microparticles loaded with 10 % dissolved drug, microparticles loaded with 30 % dissolved drug have higher encapsulation efficiency. The smaller ratio of dexamethasone dissolved in dichloromethane solution and drug saturation effects of the outer aqueous phase are the major reasons for the higher encapsulation efficiency of 30 % actual drug loading microparticles. The encapsulation efficiencies of microparticles loaded with micronized, nanosized and dissolved hydrocortisone have a similar trend but are lower than corresponding dexamethasone microparticles (Table 3.2 and 3.3). A cause for the difference in drug loading and encapsulation efficiency can be attributed to the difference in solubilities of the drugs in the solvents. Hydrocortisone (1.7 ± 0.1 mg/g) with higher solubility in dichloromethane may diffuse towards the surface of the microparticles in a greater amount than dexamethasone with a lower solubility in dichloromethane (0.43 ± 0.05 mg/g). Another factor that may contribute to these drug loading trends is the aqueous solubility of the drug. While hydrocortisone (0.31 ± 0.02 mg/g) has a higher solubility in water compared to dexamethasone (0.08 ± 0.01

mg/g), the loss of surface-associated drug from microparticles may have been hindered by the lower aqueous solubility. The amount of drug loading is therefore determined by a combination of factors that involve drug properties, polymer properties, and drug-solvent interactions.

Table 3.2. Mean particle size, actual drug loading and encapsulation efficiency of the investigated dexamethasone microparticles.

PLGA	Dexa-methasone	Particle size, μm	Span*	Theoreti-cal DL, %	Actual DL, %		EE, %
502H	Dissolved	33.0 ± 0.9	0.70 ± 0.03	20	10.1 ± 0.2	10^{+}	50.5 ± 1.0†
	Nanosized	32.9 ± 0.5	0.98 ± 0.05	10	9.7 ± 0.1		97.2 ± 1.3
	Micronized	31.1 ± 0.8	0.70 ± 0.09	10	9.8 ± 0.1		98.0 ± 1.5
503H	Dissolved	35.7 ± 0.6	0.63 ± 0.01	20	12.1 ± 0.3	10^{+}	60.5 ± 1.3†
	Nanosized	36.3 ± 0.2	0.69 ± 0.01	10	9.2 ± 0.3		92.3 ±3.1
	Micronized	36.9 ± 0.2	0.85 ± 0.02	10	9.8 ± 0.1		98.1 ± 1.0
503H	Dissolved	30.8 ± 0.3	0.74 ± 0.04	50	31.6 ± 0.4	30^{+}	63.2 ± 0.7†
	Nanosized	37.0 ± 0.2	0.52 ± 0.01	30	26.4 ± 0.4		88.0 ± 1.3
	Micronized	38.1 ± 0.5	0.51 ± 0.02	30	27.2 ± 0.3		90.7 ± 1.0
502	Dissolved	31.6 ± 0.8	0.71 ± 0.03	20	8.4 ± 0.1	10^{+}	42.0 ± 0.7†
	Nanosized	33.5 ± 0.2	1.00 ± 0.05	10	8.7 ± 0.2		87.3 ± 1.5
	Micronized	34.9 ± 0.3	0.72 ± 0.02	10	9.2 ± 0.1		92.2 ± 1.1
502	Dissolved	33.0 ± 0.3	0.84 ± 0.06	50	23.0 ± 0.9	30^{+}	46.0 ± 1.8†
	Nanosized	35.7 ± 0.4	0.95 ± 0.07	30	26.0 ± 0.9		86.6 ± 2.9
	Micronized	32.8 ± 0.5	0.72 ± 0.03	30	28.9 ± 0.5		96.3 ± 1.6
752S	Dissolved	36.0 ± 0.6	0.56 ± 0.02	20	9.6 ± 0.3	10^{+}	48.0 ± 1.3†
	Nanosized	28.0 ± 0.2	0.91 ± 0.07	10	9.2 ± 0.2		92.1 ± 1.5
	Micronized	36.1 ± 0.3	0.74 ± 0.06	10	9.5 ± 0.1		95.0 ± 1.0

*Span = (D90 – D10)/D50

$^{+}$Approximate actual drug loadings which are used for the following text

†Significant difference in the mean values compared with microparticles loaded with micronized drug ($P < 0.05$)

Table 3.3. Mean particle size, actual drug loading and encapsulation efficiency of the investigated hydrocortisone microparticles.

PLGA	Hydro-cortisone	Particle size, µm	Span*	Theoretical DL, %	Actual DL, %		EE, %
503H	Dissolved	34.0 ± 0.1	0.65 ± 0.02	20	7.6 ± 0.2		38.0 ± 1.1†
	Nanosized	33.2 ± 1.0	0.62 ± 0.02	10	8.0 ± 0.1	10+	80.0 ± 1.2
	Micronized	31.0 ± 0.8	0.39 ± 0.01	10	9.9 ± 0.1		99.1 ± 0.7
502	Dissolved	33.5 ± 0.4	0.41 ± 0.01	20	6.4 ± 0.2		32.1 ± 0.8†
	Nanosized	32.2 ± 0.6	0.70 ± 0.01	10	6.8 ± 0.1	10+	68.0 ± 1.2†
	Micronized	34.0 ± 0.3	0.65 ± 0.02	10	9.7 ± 0.1		97.2 ± 1.2

*Span = (D90 – D10)/D50

+Approximate actual drug loadings which are used for the following text

†Significant difference in the mean values compared with microparticles loaded with micronized drug ($P < 0.05$)

The solubility of both drugs in dichloromethane is less than 2 mg/mL, and thus more than 95 % of the drug initially added during the fabrication was present in a suspended state. To prepare microparticles loaded with dissolved drug, DMSO as a co-solvent was used in the oil phase to dissolve all drug crystals. By varying input drug dispersion state and particle size in the oil phase in the solvent emulsion extraction/evaporation method, the resulting microparticles consisted of drug crystals and/or drug molecules and PLGA, forming matrix structures. DSC and XRPD were performed to understand the final drug dispersion states of drugs embedded in PLGA matrix after preparation.

DSC figures of PLGA 503H microparticle formulations were collected. Compared with blank microparticles (Figure 3.2 a), microparticles loaded with 10 % micronized drug and microparticles loaded with 10 % nanosized drug have an extra melting peak of dexamethasone, which indicates crystalline dexamethasone is embedded in the microparticles matrix (Figure 3.2 b and c). The decreased melting temperature of dexamethasone from 260 °C or 220 °C to around 200 °C was also found by several other researchers [101, 117, 118]. It is attributed to the interaction between crystals with melted PLGA. A recrystallization peak at around 90 °C was observed in microparticles loaded with 10 % dissolved drug, which shows dexamethasone is in the metastable molecular dispersion (Figure 3.2 d) [117]. For 30 % drug loading microparticles, they all

have a melting peak of dexamethasone (Figure 3.2 e, f and g). To prepare microparticles loaded with 30 % dissolved drug, dexamethasone is initially dissolved, but the drug concentration is higher than its solubility in the PLGA matrix, and interactions between the drug molecules and the polymer chains are too weak to lead to a stable state, causing dexamethasone crystallizing out during preparation. Unlike microparticles loaded with 10 % dissolved dexamethasone, completely recrystallized dexamethasone in microparticles loaded with 30 % dissolved dexamethasone shows only the melting peak (Figure 3.2 g). Melting temperatures of dexamethasone in 30 % drug loading microparticles are slightly higher than in 10 % drug loading microparticles since higher energy is required to melt a higher portion of crystalline drug. Hydrocortisone microparticles (Figure 3.2 h, i and j) have the same phenomena as dexamethasone microparticles, however the recrystallization peaks and melting peaks are at different temperatures. For all samples, the melting temperature of PLGA kept constant, indicating no plasticizer effect of these drugs. These observations also indicate that the glass transition phase of the polymer was not influenced by the preparation procedure.

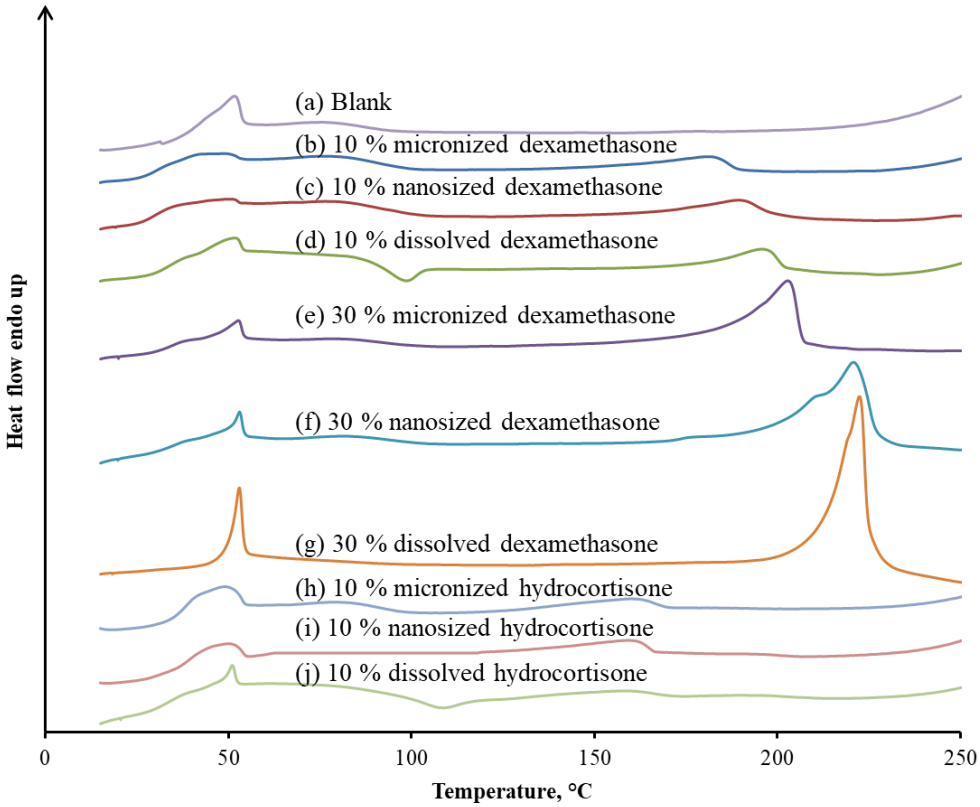


Figure 3.2. DSC graphs of PLGA 503H microparticles (a) blank, (b) loaded with 10 % micronized dexamethasone, (c) 10 % nanosized dexamethasone, (d) 10 % dissolved dexamethasone, (e) 30 % micronized dexamethasone, (f) 30 % nanosized dexamethasone, (g) 30 % dissolved dexamethasone, (h) 10 % micronized hydrocortisone, (i) 10 % nanosized hydrocortisone and (j) 10 % dissolved hydrocortisone.

To confirm the result of the DSC study and avoid high temperature, the crystallinity of drug in the PLGA microparticles was also investigated using XRPD (Figure 3.3). PLGA 503H microparticles loaded with 10 % micronized, nanosized and dissolved dexamethasone were selected as representatives of microparticles containing different drug dispersion states and particle sizes. PLGA 503H microparticles loaded with 30 % dissolved dexamethasone were also selected due to the absence of recrystallization peak in the DSC study. The diffraction spectrum of original micronized dexamethasone powder showed that the drug was in crystalline form A, exhibiting sharp peaks at 2θ equal to 7.9, 12.4, 13.5,

14.4, 14.9, 16.1, and 17.6 °(Figure 3.3 a) [70, 119]. The reduced intensity and broadening of the diffraction peaks of nanosized dexamethasone (Figure 3.3 b) may be attributed to the reduced degree of crystallinity, which further confirms the DSC studies in section 3.1.2 [109]. On the other hand, no peak was observed in blank microparticles (Figure 3.3 c). However, clear peaks originating from dexamethasone were detected in microparticles loaded with 10 % micronized drug, microparticles loaded with 10 % nanosized drug and microparticles loaded with 30 % dissolved drug (Figure 3.3 d, e and g), indicating that dexamethasone was incorporated in the polymeric particles in the crystalline state. Dexamethasone crystallized during preparation in microparticles loaded with 30 % dissolved dexamethasone and changed from polymorphism form A to B, exhibiting peaks at 2θ equal to 7.5, 13.7, 14.2, 15.1, 15.7, 17.0 and 18.6 °(Figure 3.3 g) [70]. No peaks were observed in PLGA 503H microparticles loaded with 10 % dissolved dexamethasone (Figure 3.3 f), which confirmed dexamethasone is in a dissolved (amorphous) state. Furthermore, this indicates that the recrystallization at 90 °C for this sample in the case of DSC, is an artifact of the DSC measurement (utilization of high temperature), which is not depicted in case of XRPD measurements which are performed at room temperature.

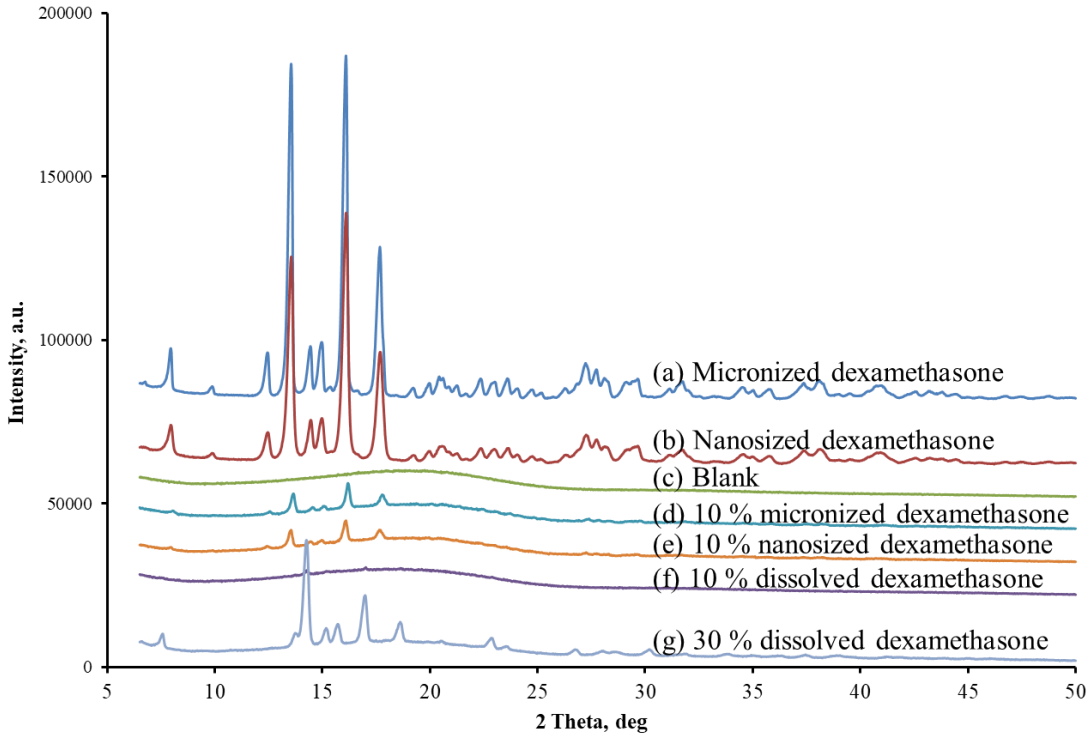


Figure 3.3. XRPD spectra of (a) original micronized dexamethasone, (b) freeze-dried nanosized dexamethasone, and PLGA 503H microparticles (c) blank, loaded with (d) 10 % micronized dexamethasone, (e) 10 % nanosized dexamethasone, (f) 10 % dissolved dexamethasone, and (g) 30 % dissolved dexamethasone.

Microparticles selected for XRD investigation were also chosen for further morphology observations. Uniform, spherical microparticles are observed by an optical microscope (Figure 3.4). The surface of the microparticles was smooth and homogeneous. The surface of microparticles loaded with nanosized drug is smoother than microparticles loaded with micronized drug and microparticles loaded with dissolved drug. There are a few cracks in microparticles loaded with micronized drug and dissolved drug, but it was not observed in microparticles loaded with nanosized drug. Cracks were formed by washing out the large crystals at the surface of microparticles loaded with micronized drug or by quick hardening of the co-solvent method to prepare microparticles loaded with dissolved drug. Cracks are more obvious at the surface of 30 % drug loading microparticles loaded with dissolved drug due to low PLGA concentration in oil phase and recrystallization of dexamethasone. Since crystals are in nanosize in

microparticles loaded with nanosized drug, the surface became smoother. In the cross-section of PLGA 503H microparticles, the microparticles loaded with 10 % micronized dexamethasone exhibited some rectangular drug crystals in the micrometer range, microparticles loaded with 10 % nanosized dexamethasone appeared as rectangular drug crystals in the nanometer range, microparticles loaded with 10 % dissolved dexamethasone showed no crystals, and microparticles loaded with 30 % dissolved dexamethasone emerged irregular crystals which is due to the recrystallization of dexamethasone in the micrometer range. More channels were observed in microparticles loaded with dissolved dexamethasone, as quick hardening of the co-solvent method resulted in a more porous structure of PLGA microparticles.

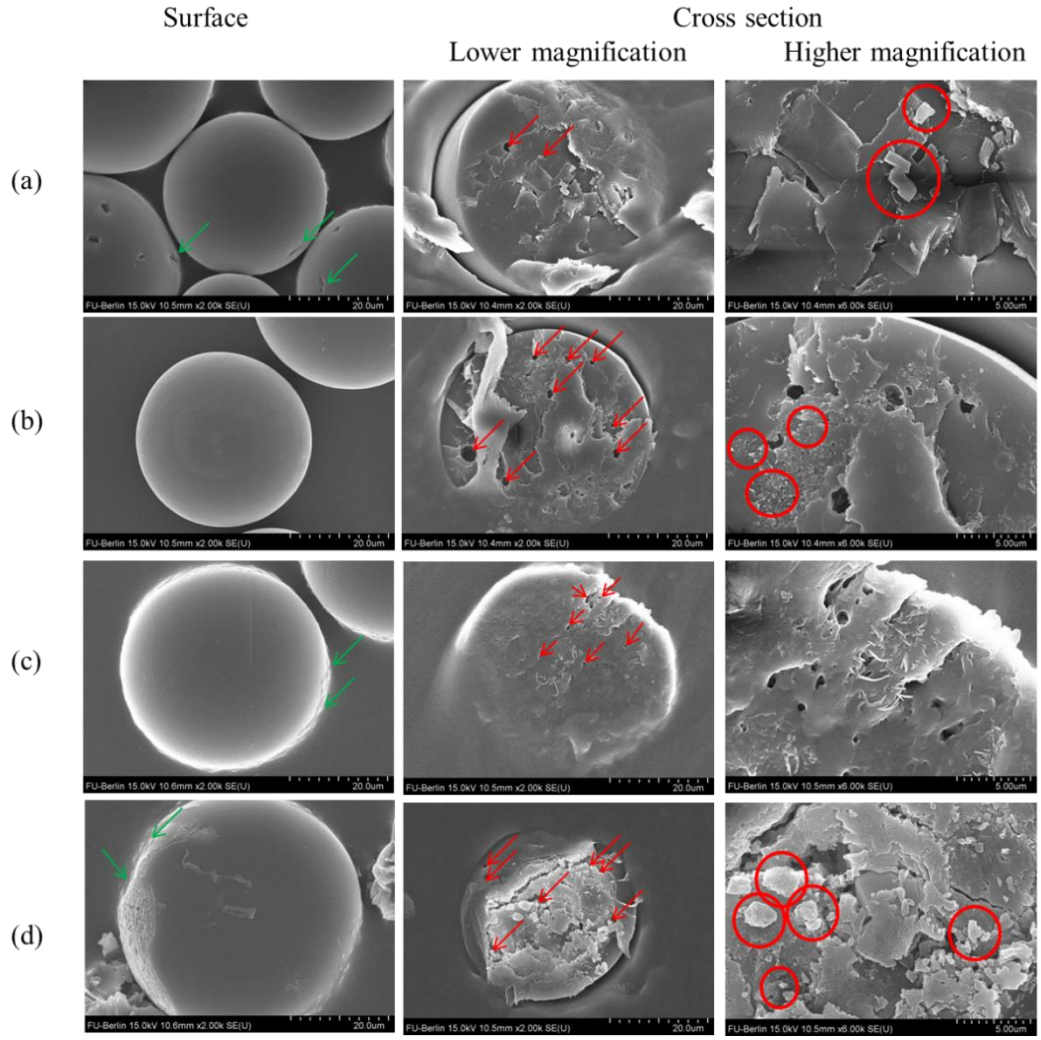


Figure 3.4. SEM pictures of surfaces and cross-sections (lower and higher magnification) of PLGA 503H microparticles loaded with (a) 10 % micronized dexamethasone, (b) 10 % nanosized dexamethasone, (c) 10 % dissolved dexamethasone, and (d) 30 % dissolved dexamethasone. Green arrow: cracks; red arrow: pores; red cycle: drug crystals.

3.1.4. *In vitro* release

There are lots of factors, like the manufacturing process and the chemical properties of the polymer and the drug affecting the drug release from the PLGA matrix [120]. As described above, similar manufacturing processes were used to prepare PLGA microparticles loaded with micronized, nanosized and dissolved drug to avoid the effect of a varied manufacturing process on drug release. Other critical factors, like particle size of microparticles and actual drug loading, were also kept similar for *in vitro* release comparison. Moreover, to understand the

effect of different drug dispersion states and particle sizes on the *in vitro* release, different PLAG types, drug loadings and drug types were included.

Mostly, biphasic or triphasic drug releases were observed from dexamethasone and hydrocortisone PLGA microparticles as reported by other groups [69, 108, 121-123]. A triphasic release profile includes a burst release, followed by a lag phase and finally a rapid release phase [99, 101, 124]. The biphasic drug release might happen when the drug is already completely released before the conditions for the following release phase are provided. In this study, the PLGA microparticles loaded with micronized, nanosized and dissolved drug have significantly different release profiles although microparticles have similar chemical composition, which indicates drug dispersion states and particle sizes affect the drug release at burst phase, lag phase and the final quick release phase.

3.1.4.1. Burst phase

The rapid release of drug during the first day is typically regarded as the burst phase. The burst release is likely due to the dissolution of the drug at microparticles' surface, which is directly in contact with the release medium. *In vitro* release profiles in this study indicated burst release is correlated with PLGA types: for hydrophilic PLGA 502H and 503H, the burst was in the order of Nanosized ＞ Dissolved ＞ Micronized (Figure 3.5 and Figure 3.7 a); For hydrophobic PLGA 502 and 752S, the burst was in the order of Dissolved ＞ Micronized ＞ Nanosized (Figure 3.6 and Figure 3.7 b). Regarding hydrophilic PLGA microparticles, quick wetting process and porous internal channels of microparticles loaded with nanosized and dissolved drug result in drugs are more accessible to the release medium and thus a higher burst is achieved. However, using hydrophobic PLGA, the highest burst release of microparticles loaded with dissolved drug was caused by recrystallization happened at the water and oil phase contact surface during preparation; the higher burst release of microparticles loaded with micronized drug is due to the poor miscibility of large drug crystals in the PLGA matrix [19]; the low burst release of microparticles

loaded with nanosized drugs is caused by a homogenous distribution of nanocrystals inside microparticles resulting in a decreased access to water. Additionally, the burst releases of hydrophilic PLGA (502H and 503H) microparticles were higher than hydrophobic PLGA (502 and 752S) microparticles. For hydrophilic PLGA microparticles, microparticles were quickly hardened due to the poor solubility in dichloromethane compared with hydrophobic PLGA 502 and 752S. The quick hardening rate resulted in more porous structures. Moreover, wetting of 502H and 503H microparticles is faster during the first day's release which results in higher burst release due to the hydrophilic properties of PLGA 502H and 503H. The burst of 30 % drug loading microparticles group was higher than 10 % drug loading microparticles, which is due to high drug loading and relatively lower PLGA amount. Moreover, more porous surfaces of 30 % drug loading microparticles facilitate water exchange inside the microparticles with the outer release medium to take the drug out quickly (as depicted before by SEM). Hydrocortisone microparticles have a smaller burst release (Figure 3.7) compared with dexamethasone microparticles since the higher solubility of hydrocortisone in water resulting in a more thorough washing process and less drug has direct access to the release medium. The mechanism of burst release is confirmed by dynamic changes in the diameter of microparticles. There are only minor increases in diameter of hydrophilic PLGA (502H and 503H) microparticles at the burst release stage (Figure 3.8 and 3.9) and no change in diameter of hydrophobic PLGA (502 and 752S) microparticles (Figure 3.10 and 3.11), which indicated the burst release is from the drug at the surface and not by the swelling of microparticles.

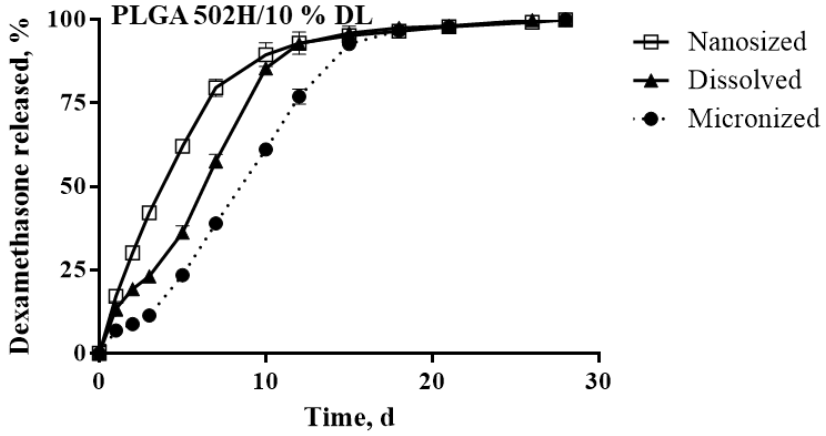
3.1.4.2. Lag phase

After the burst release, PLGA microparticles tend to have a very slow release period lasting for days to weeks, which is often referred as the lag phase. The lag phases of dexamethasone microparticles loaded with micronized, nanosized and dissolved are also correlated with PLGA types. For hydrophilic PLGA 502H and

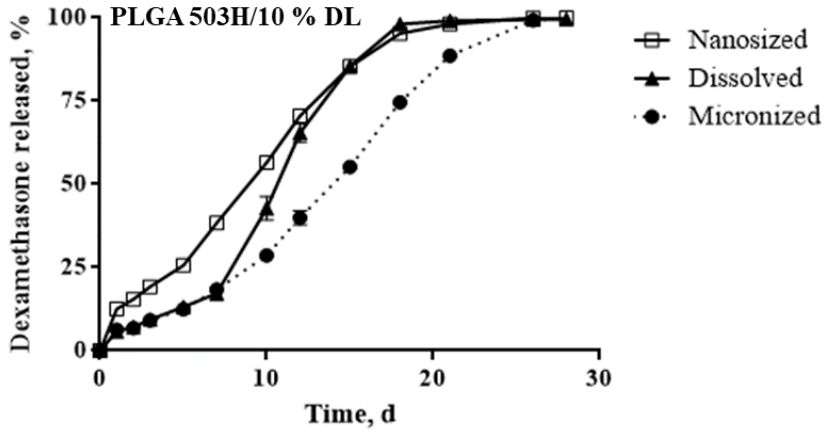
503H, the microparticles loaded with nanosized drug released faster than microparticles loaded with dissolved drug and microparticles loaded with micronized drug, making the whole release profile more continuous (Figure 3.5 and Figure 3.7 a). Homogenous distribution of nanocrystals and fast wetting of microparticles helped to form a uniform inner network. Therefore, more drug has access to water in this release phase. For microparticles loaded with dissolved drug and microparticles loaded with micronized drug, there is a lag phase after the initial burst release. The lag phase is due to micronized crystals or dissolved drug has limited access to water before the quick swelling of microparticles [69]. For hydrophobic PLGA 502 and 752S, the microparticles loaded with nanosized drug released faster than microparticles loaded with micronized drug but slower than microparticles loaded with dissolved drug, which is due to the different diffusion capacities of different drug dispersion states and particle sizes. The lag phase time was in the order of 752S $>$ 502 $>$ 503H $>$ 502H, which is consistent with the degradation rate of PLGA. The higher molecular weight and more hydrophobic PLGA ensure the strong structure of microparticles. The lag phase of 30 % drug loading microparticles is shorter than 10 % because the lower ratio of PLGA in the bulk resulted in a weaker structure for 30 % drug loading. Hydrocortisone microparticles have a higher release amount at the lag phase compared with dexamethasone microparticles, since hydrocortisone has a higher solubility in water. The mechanism of lag phase release is confirmed by dynamic changes in the diameter of microparticles. For hydrophilic PLGA 502H and 503H, the diameter of the microparticles loaded with nanosized drug increased more. Diffusion and limited swelling resulted in more drug being released at this stage (Figure 3.8 and 3.9). For hydrophobic PLGA 502 and 752S, there is no significant change in diameter at the lag phase (Figure 3.10 and 3.11), which indicates the release is only from diffusion through the polymeric barrier.

3.1.4.3. Final quick release phase

After the degradation of PLGA at the lag phase, the following rapid drug release phase started and was along with the quick swelling of microparticles due to the lower PLGA molecular weight after degradation and resulting weak microparticle structure (Figure 3.8, 3.9, 3.10 and 3.11). All release rates correlated with diffusion capacity, namely Dissolved $>$ Nanosized $>$ Micronized (Figure 3.5, 3.6 and 3.7). The final quick release time was also in the order of 752S $>$ 502 $>$ 503H $>$ 502H, which is consistent with the degradation rate of PLGA. The 10 % drug loading microparticles are released faster than 30 %. The more acid environment caused by less water exchange during the lag phase resulted in quicker degradation of PLGA, which is responsible for the faster final release of 10 % drug loading microparticles. Hydrocortisone microparticles have a faster release at the quick release stage compared with dexamethasone microparticles, since the higher solubility of hydrocortisone in water results in a higher diffusion capacity.



(a)



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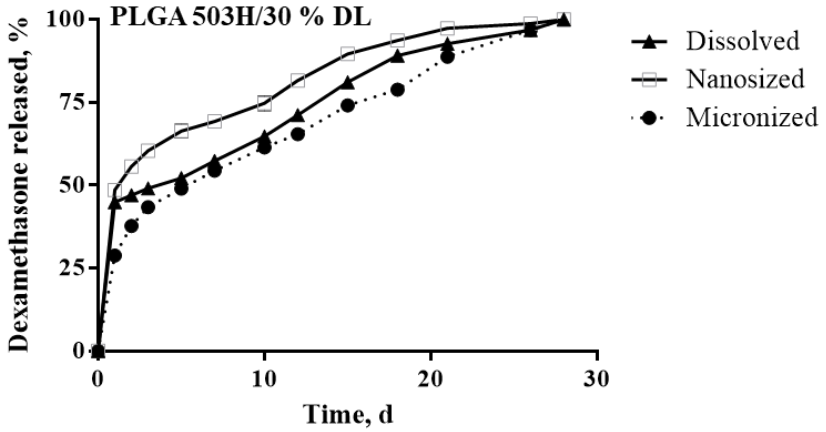
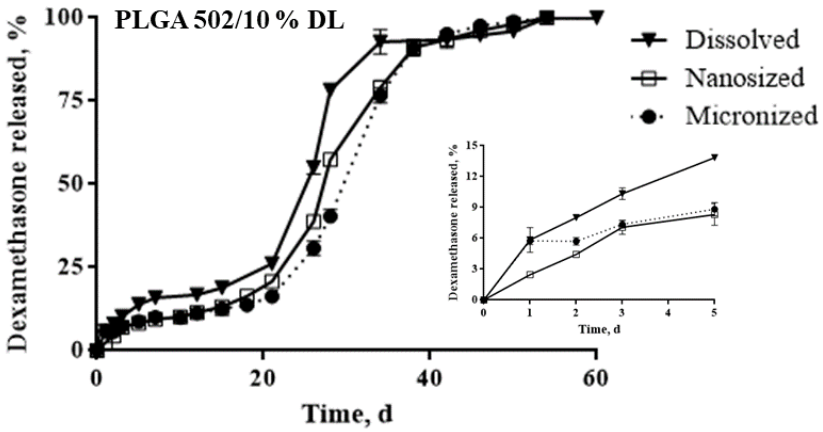


(c)

Figure 3.5. *In vitro* release data of dexamethasone from (a) PLGA 502H microparticles loaded with 10 % dexamethasone, (b) PLGA 503H microparticles loaded with 10 % dexamethasone, and (c) PLGA 503H microparticles loaded with 30 % dexamethasone.



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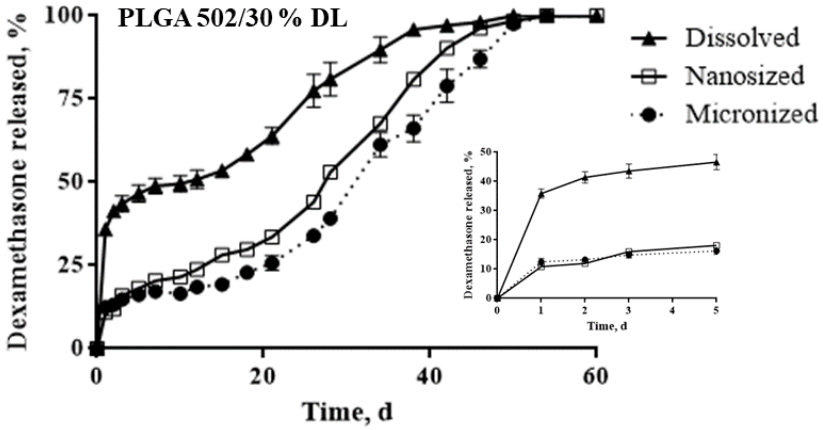

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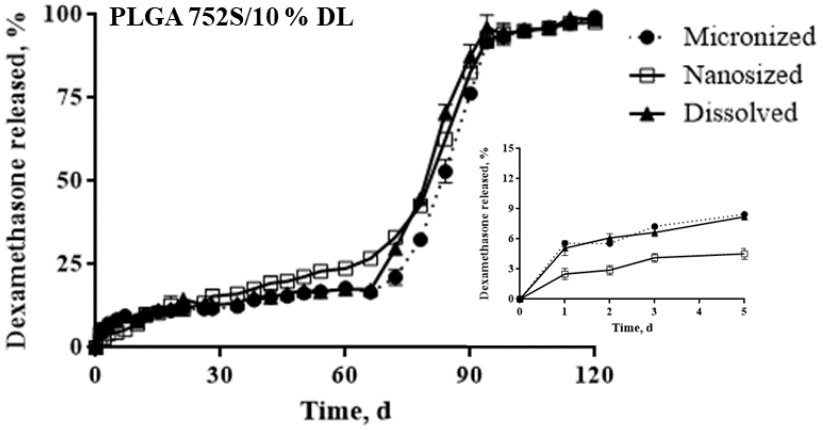
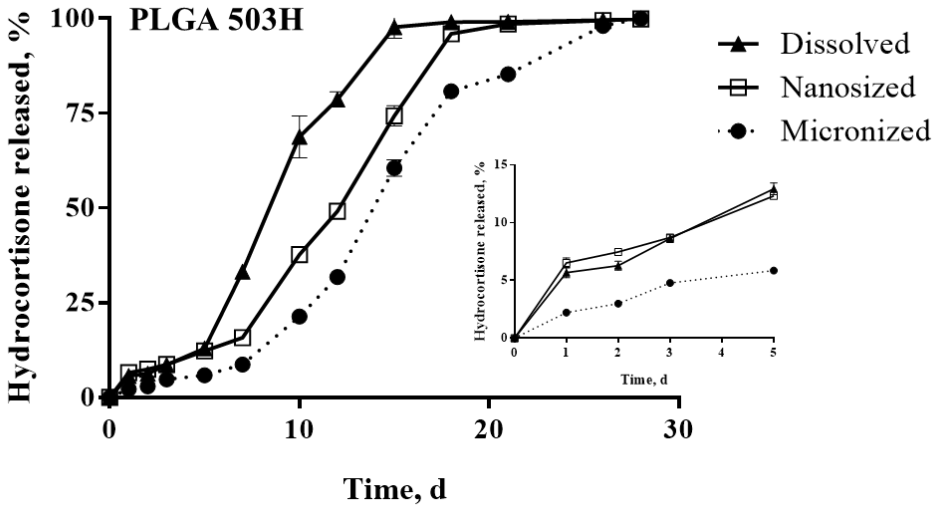
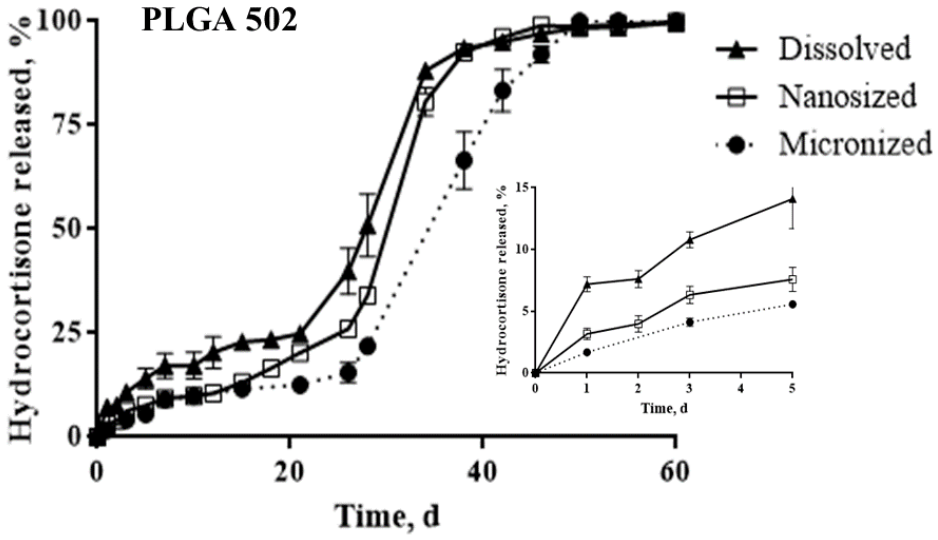


(c)

Figure 3.6. *In vitro* release data of dexamethasone from (a) PLGA 502 microparticles loaded with 10 % dexamethasone, (b) PLGA 502 microparticles loaded with 30 % dexamethasone, and (c) PLGA 752S microparticles loaded with 10 % dexamethasone.

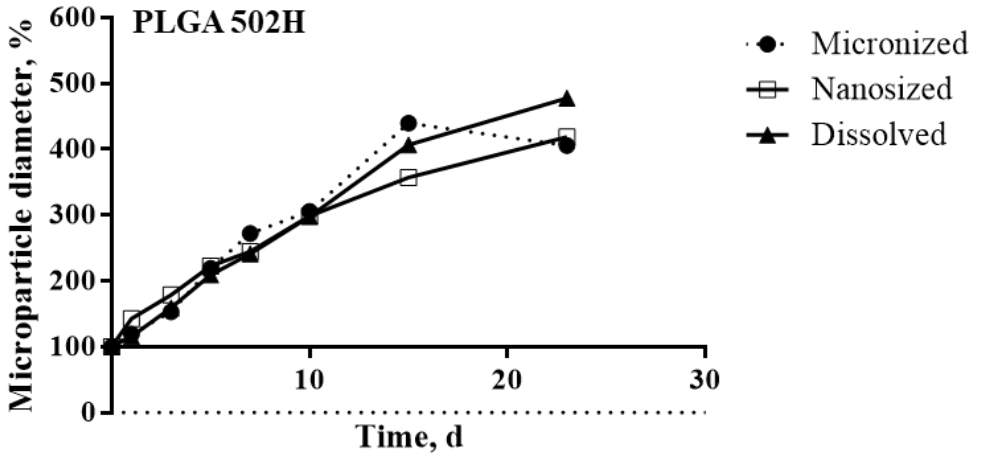


(a)

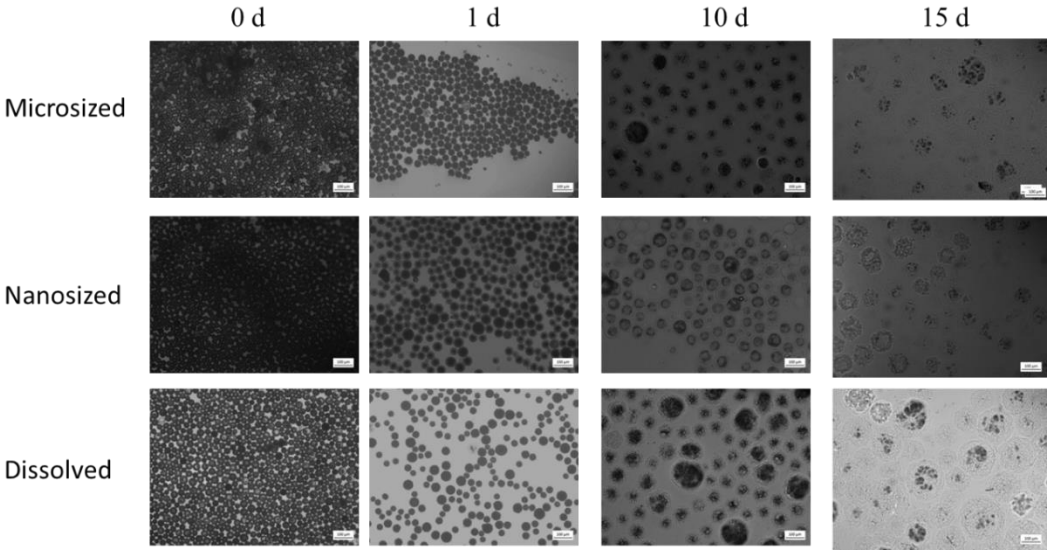


(b)

Figure 3.7. *In vitro* release data of hydrocortisone from (a) PLGA 503H microparticles and (b) PLGA 502 microparticles loaded with 10 % hydrocortisone.

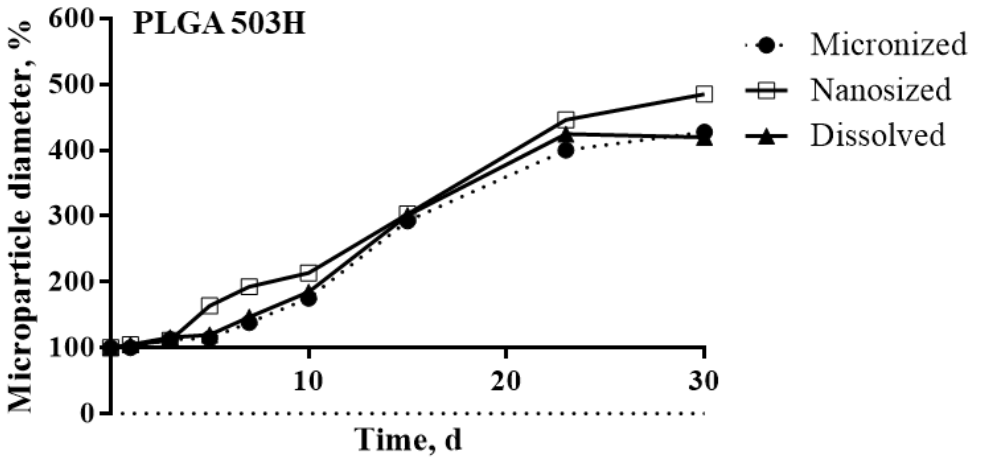


(a)



(b)

Figure 3.8. (a) dynamic changes in the diameter of PLGA 502H loaded with 10 % dexamethasone and (b) microscopic images of microparticles during swelling study.



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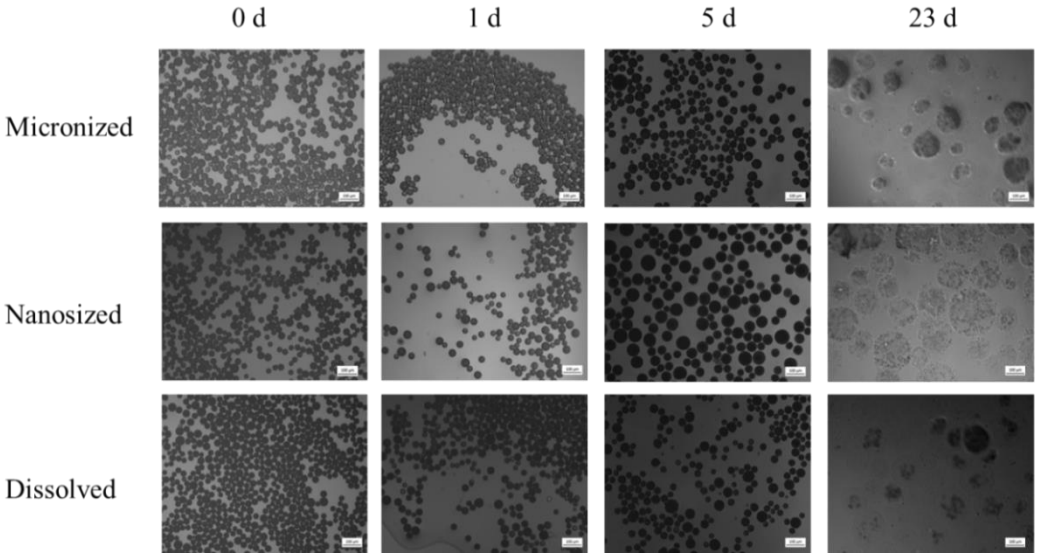
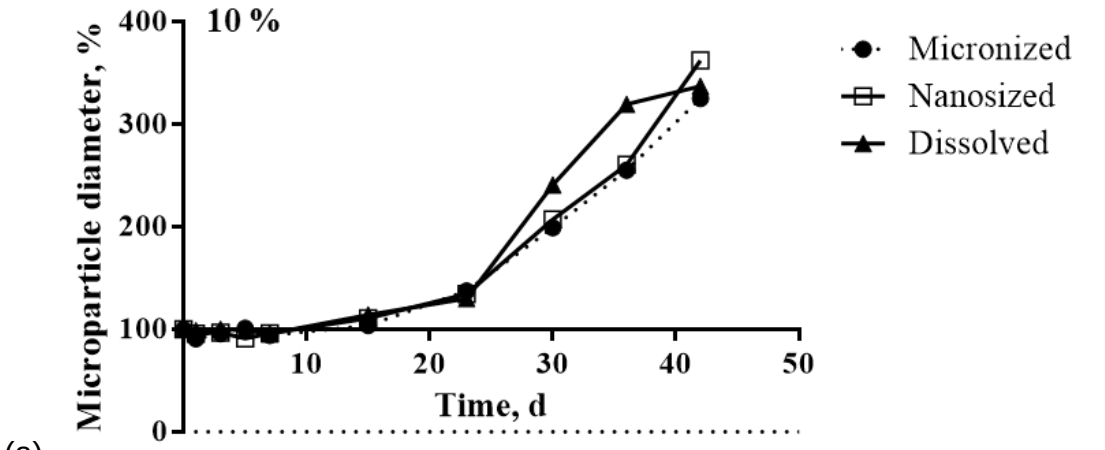
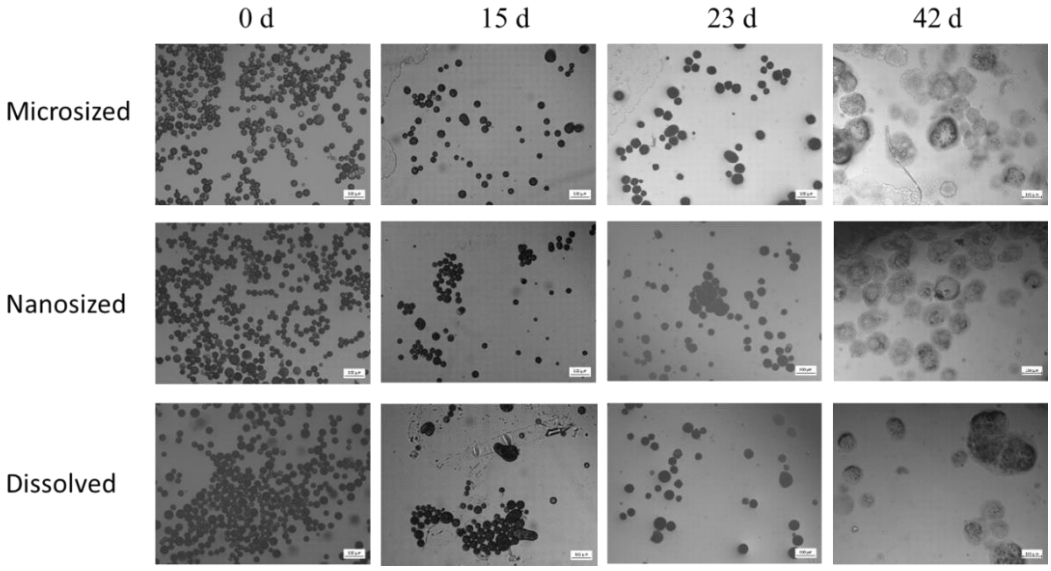


(b)

Figure 3.9. (a) dynamic changes in the diameter of PLGA 503H microparticles loaded with 10 % dexamethasone and (b) microscopic images of microparticles during swelling study.

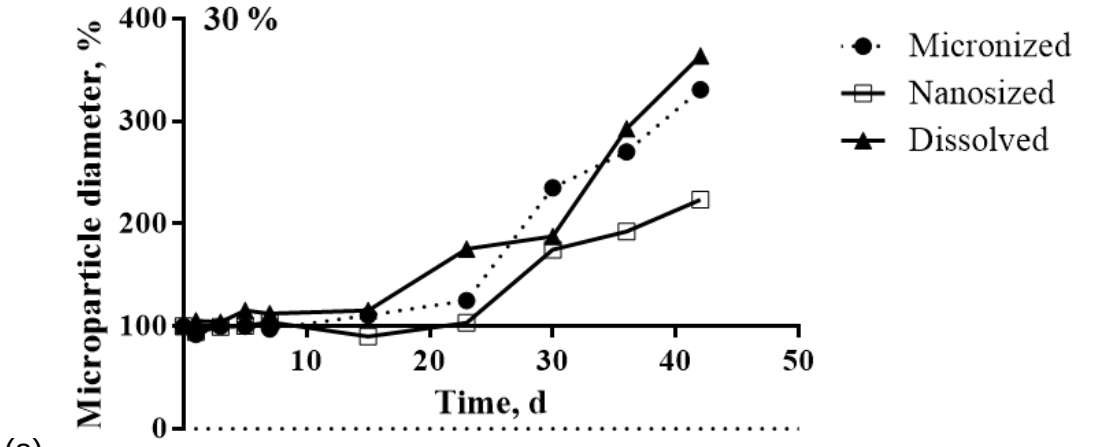


(a)

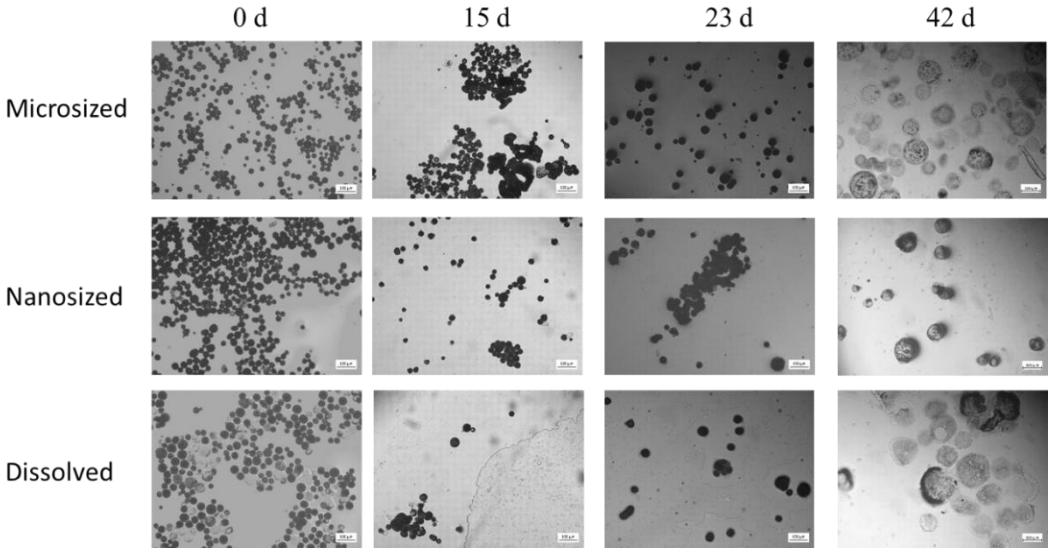


(b)

Figure 3.10. (a) dynamic changes in the diameter of PLGA 502 loaded with 10 % dexamethasone and (b) microscopic images of microparticles during swelling study.



(a)



(b)

Figure 3.11. (a) dynamic changes in the diameter of PLGA 502 loaded with 30 % dexamethasone and (b) microscopic images of microparticles during swelling study.

3.1.4.4. Continuous release

Consider all three release phases, by varying PLGA types and drug loadings, microparticles loaded with nanosized drug achieve a more continuous release profile: more drug release at the burst release and lag phase for hydrophilic PLGA; low burst and more drug release at lag phase for hydrophobic PLGA. The lag phase is problematic as the drug concentration at the site of action is too low to provide the required therapeutic effect. Therefore, microparticles loading with nanosized drug is a promising method towards a continuous release. Especially

when hydrophilic PLGA 502H and 503H encapsulate 10 % nanosized dexamethasone, quasi-linear releases were obtained (Figure 3.12). From day 0, dexamethasone was constantly released at an approximately zero order rate (more than 80 % cumulative drug release for 502H and around 100 % for 503H) with good linearity ($R^2 > 0.98$) during these release periods. Moreover, the deviation of microparticles loaded with nanosized drug in each sampling point is smaller than microparticles loaded with micronized drug and microparticles loaded with dissolved drug, which is also due to the poor distribution uniformity of drug in these two microparticle types.

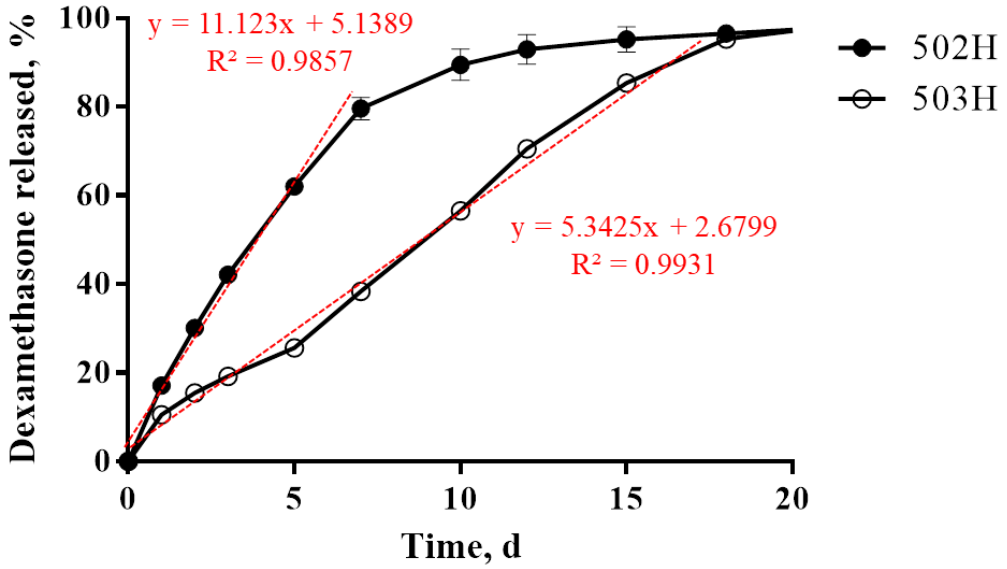


Figure 3.12. *In vitro* release data of dexamethasone from PLGA 502H and 503H microparticles loaded with 10 % nanosized dexamethasone. Linear regression was applied from day 0 to day 7 for PLGA 502H microparticles and from day 0 to day 18 for PLGA 503H microparticles.

3.1.5. Conclusion

PLGA microparticles loaded with micronized, nanosized and dissolved drug were effectively prepared by solvent extraction/evaporation method and thoroughly compared. It showed drug dispersion states and particle sizes indeed played an important role in the microparticle properties, i.e. encapsulation efficiency, drug solid state staility and *in vitro* release. PLGA microparticles loaded with

micronized drug had a poor continuous release in the lag phase and loading dissolved drug obtained a poor encapsulation efficiency and unstable drug solid state. Encapsulating nanosized drug into PLGA microparticles overcomes these drawbacks of loading micronized and dissolved drug and is a promising system to obtain a more continuous release.

3.2. Milling in PLGA solution facilitates the encapsulation of drug nanocrystals into PLGA microparticles

3.2.1. Introduction

The solvent evaporation technique is inexpensive, easy to perform at laboratory scale, and can be employed with nearly all APIs [22, 34]. The S/O/W solvent evaporation technique was used commonly, especially when the specific drug cannot be dissolved in a carrier solvent or solvent mixture, or extensive drug loss to the continuous phase can not be avoided [19, 125-127]. However, the S/O/W solvent evaporation method requires a small drug particle size to allow a complete encapsulation of the drug crystals. If large crystals were encapsulated, besides a low encapsulation efficiency, large voids in the surface of the microparticles may appear. Different particle sizes of theophylline were encapsulated into microparticles by S/O/W solvent evaporation method. Large drug particles resulted in a pronounced burst release and shorter release time [128]. Thus, it is important to diminutize drug particles for the following encapsulation process. However, with dry milling methods, drug particles less than 5 µm are difficult to obtain. Furthermore, due to high energy input during dry grinding solid state stability is decreased and a low recovery is obtained. Besides the necessity of small-sized drug particles using the S/O/W technique, the drug may show sedimentation (higher density than suspension medium) or flotation (low wettability) during the encapsulation process [19]. These problems are expected to worsen the manufacturing process and the release profile.

In chapter 3.1, we found that microparticles loaded with nanosized dexamethasone and hydrocortisone overcome two major problems. First, the burst release which may cause serious toxicity being a major hurdle for the development of microparticle products. Secondly, microparticles tend to have a very slow release period lasting for days to weeks after the initial burst period (lag phase). At this time the patient may not be effectively treated due to the lack of

sufficient drug release. Aqueous milling was used to prepare drug nanocrystals with the help of water-soluble stabilizers. However, a drying process and redispersing into oil phase are required in that S/O/W process. Moreover, a small amount of stabilizer covered the large surface of nanocrystals introduced into the microparticle matrix may impact the release profile and even toxicity for injectable drug delivery. A few studies also attempted to encapsulate drug nanocrystals into PLGA microparticles using bottom-up methods. However, the particle size of crystalline drug is very difficult to control [105]. Furthermore, the solid state stability of these crystals might be poor. Some studies tried sonication or non-aqueous wet bead milling to prepare small drug crystals and then encapsulated them into microparticles. L. Zhang et. al used the S/O spray-drying method to prepare polylactide microparticles with sustained release of a hydrophilic model drug isoniazid. High entrapment efficiency was obtained and the microparticles were spherical and smooth by spray drying [129]. Ciprofloxacin nanoparticles created by homogenization in dichloromethane, and then encapsulated into large porous PLGA particles resulted in a steady release [130]. Moreover, a polymorph with a decreased melting temperature was formed and the encapsulation efficiency of nanosized ciprofloxacin was quite low. However, in all these studies, detailed non-aqueous wet bead milling process was not investigated, neither the particle size of drug crystals nor the potential application of the stabilizers.

In this study, three model drugs dexamethasone, hydrocortisone and dexamethasone sodium phosphate were selected considering their varied water solubilities and many studies using S/O/W or S/O/O methods to investigate how to encapsulate them [101, 131, 132]. Model drugs were wet bead milled in organic solvent directly to prepare nanosized drug suspensions using PLGA as a stabilizer, which may not only reduce effectively the particle size but also enhance the wettability, and their successive microencapsulation. It is hypothesized that PLGA microparticles loaded with those nanosized drug particles achieve high encapsulation efficiency, a stable solid state, a low burst release and a quasi-linear release without lag phase utilizing a simplified preparation process.

3.2.2. Preparation and characterization of nanosuspensions

Wet bead milling is described in the literature as the most suitable technology for nanosizing drug particles on a small scale [133]. Dichloromethane was selected as the wet bead milling medium based on solubility measurement of model drugs and suitability for PLGA microparticle manufacturing. The low solubility of dexamethasone (0.43 ± 0.05 mg/g), hydrocortisone (1.7 ± 0.1 mg/g), and dexamethasone sodium phosphate (1.6 ± 0.1 mg/g) in dichloromethane (at 25 °C) ensured the formation of nanosized drug particles which will be suspended in the liquid medium. Moreover, avoiding water, hydration transition is not expected in dichloromethane.

The stabilizers that are used for screening should be accepted for iv administration based on the FDA's Inactive Ingredient Database. However, there are only a few known stabilizers that are accepted, i.e. polysorbate 80, polysorbate 20, poloxamer 188 and PEG 400. To increase the number of stabilizers and avoid other unrelevant stabilizers, it was investigated whether PLGA, approved by FDA as biodegradable and biocompatible [6], is capable to stabilize the nanoparticles in dichloromethane. The use of PLGA as a stabilizer, enhancing the size reduction of drug particles, has been reported previously [134]. However, in these earlier studies, dry grinding techniques were used and only micronized drug particles were obtained. The usage of PLGA as stabilizer for nanosuspensions has so far not been explored.

Wet bead milling of micronized dexamethasone in pure dichloromethane did not result in the production of nanosized particles, even after 4 h milling (Table 3.4). In contrast, wet bead milling of drugs in dichloromethane with PLGA or poloxamer 188 as stabilizer resulted in nanosized particles. Different PLGAs were tried and 502H was the most efficient stabilizer (Table 3.4). Due to the polar and hydrophobic functional groups PLGA or poloxamer 188 deposit between dichloromethane and apolar drug particle surface, and then improve the wetting process and stabilizes the drug particles. With carboxyl end PLGA, the

hydrophilic and hydrophobic differences became more pronounced, which causes enhanced stabilization. Furthermore, the carboxyl group may partially ionize in dichloromethane, which results in stronger electrostatic repulsion. The total concentration of dexamethasone was kept constant (5 %, w/w) and different dexamethasone to PLGA 502H mass ratios (i.e. 20:1, 10:1 and 5:1, which refer to 0.25, 0.5 and 1.0 %, w/w) were employed to elucidate the potential effect on the particle size reduction of dexamethasone. It has been shown by Van Eerdenbrugh et al. that steric stabilizers are commonly used at weight ratios between 10:1 and 1:0.8 (API to stabilizer) to hinder aggregation of the nanoparticles. Surface active stabilizers which decrease the interfacial tension like polysorbate 80 are commonly used at weight ratios between 20:1 and 2:1 [135]. Their concentration should be minimized to avoid solubilization of the API and to reduce undesired side effects. All investigated mass ratios reduced the particle size efficiently, thus lowest PLGA 502H concentration of 0.25 % was selected for further studies. PLGA 502H was also used as a milling stabilizer for nanosizing hydrocortisone and dexamethasone sodium phosphate. 200 - 300 nm particles with a low polydispersity index were obtained for all model drugs by varying milling times (Figure 3.13).

Table 3.4. Particle size (z-average, nm) and PDI of 5 % dexamethasone milled in dichloromethane with different PLGA types and PLGA concentrations.

Time,	Without	502H con., %			503H con., %		
h	PLGA	0.25	0.50	1.0	0.25	0.50	1.0
0	3538(0.91)	-	-	-	-	-	-
0.5	1573(0.38)	736(0.14)	803(0.18)	792(0.13)	1055(0.25)	894(0.21)	1500(0.22)
1	901(0.40)	388(0.24)	662(0.16)	568(0.16)	582(0.26)	918(0.34)	496(0.23)
2	773(0.32)	321(0.19)	324(0.21)	438(0.27)	290(0.20)	335(0.25)	286(0.19)
4	1135(0.33)	271(0.16)	278(0.18)	316(0.25)	280(0.18)	290(0.23)	281(0.16)

Table 3.4. continued

Time,	502 con., %			752S con., %		
h	0.25	0.50	1.0	0.25	0.50	1.0
0	-	-	-	-	-	-
0.5	1487(0.38)	1018(0.22)	999(0.22)	1270(0.17)	1139(0.18)	1293(0.33)
1	1225(0.38)	947(0.10)	920(0.19)	1152(0.24)	1180(0.19)	979(0.25)
2	653(0.43)	524(0.08)	380(0.13)	598(0.29)	448(0.20)	495(0.18)
4	1072(0.32)	878(0.040)	535(0.25)	1182(0.28)	1016(0.29)	1044(0.34)

"-": not measured

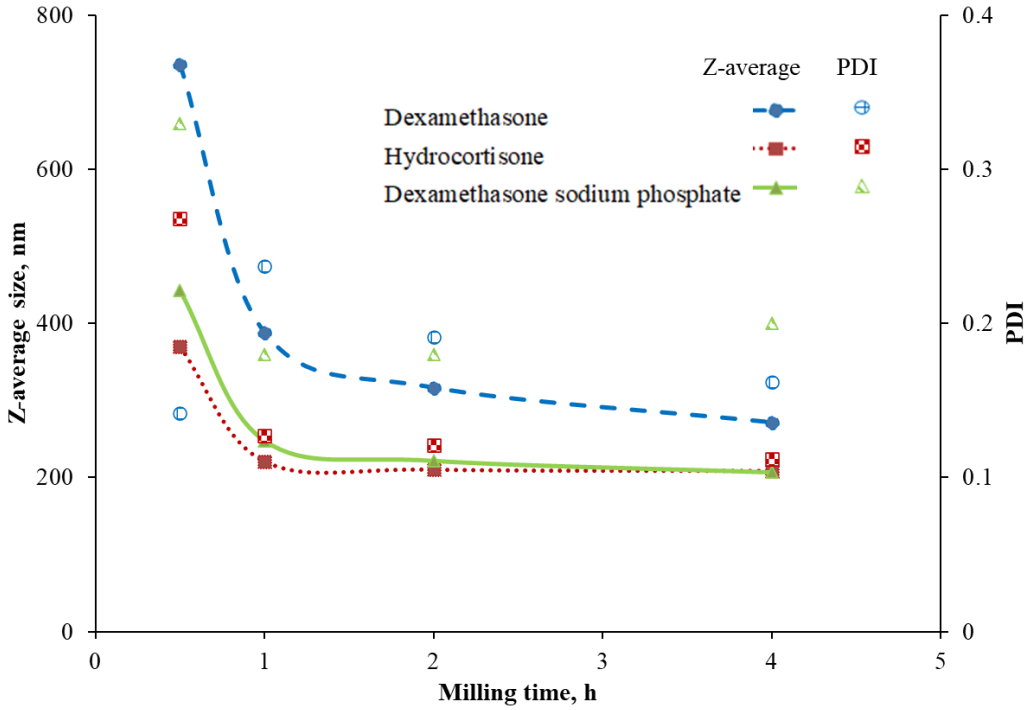


Figure 3.13. Particle size (z-average) and PDI of suspensions of dexamethasone, hydrocortisone and dexamethasone sodium phosphate with increasing milling time and using 0.25 % PLGA 502H as milling stabilizer.

Although nanosized drug particles were obtained by milling in dichloromethane with PLGA as stabilizer, there is the possibility of changing polymorphs and forming solvates. DSC indicated no desolvation of the dichloromethane for all model drugs (Figure 3.14). The melting peaks of nanosized drugs still existed, but the melting temperatures decreased suggesting a decrease in the degree of crystallinity due to imperfections in the crystal lattice by milling and downstream

processing [109, 114]. Moreover, nanocrystal melting temperature depends on nanocrystal radius and small crystals melt at a lower temperature than large crystals [115]. The exothermal degradation peak (Figure 3.14 a) of micronized dexamethasone just following its melting peak around 260 °C also moved to a lower temperature at 240 °C and separated with the melting peak of nanosized dexamethasone (Figure 3.14 b), which is also due to a decrease in the degree of crystallinity which accelerates the degradation. The melting peak of nanosized dexamethasone sodium phosphate at 210 °C (Figure 3.14 f) is not as significant as micronized dexamethasone sodium phosphate at 240 °C (Figure 3.14 e), indicating the milling process easier reduces its degree of crystallinity compared with dexamethasone. The nanosized hydrocortisone has a similar phenomenon. This is due to the hardness of hydrocortisone and dexamethasone sodium phosphate crystals is lower than dexamethasone crystals, which is consistent with the order of particle size getting smaller during milling.

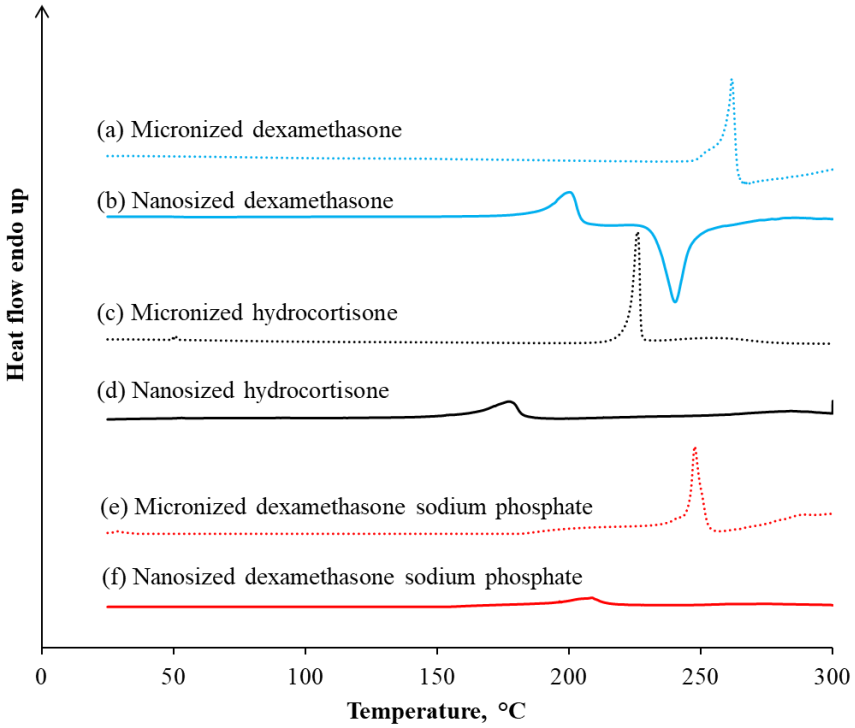


Figure 3.14. DSC thermograms of (a) original micronized dexamethasone, (b) air-dried nanosized dexamethasone, (c) original micronized hydrocortisone, (d) air-dried nanosized hydrocortisone, (e) original micronized dexamethasone sodium phosphate and (f) air-dried nanosized dexamethasone sodium phosphate.

The diffraction spectrum of original micronized dexamethasone powder showed that the drug was in crystalline form A, exhibiting sharp peaks at 2θ equal to 13.7, 14.1, 15.6, 17.1, and 22.3 ° (Figure 3.15 a). However, nanosized dexamethasone also showed characteristic peaks of polymorphism B, exhibiting peaks at 2θ equal to 7.5, 13.7, 14.2, 15.1, 15.7, 17.0 and 18.6 ° (Figure 3.15 b). The XRPD results indicated that dexamethasone changed its polymorph form from A to a mixture of A and B [70], but there is no polymorph form change for hydrocortisone, and dexamethasone sodium phosphate (Figure 3.15 c, d, e and f). Compared with micronized hydrocortisone and dexamethasone, micronized dexamethasone sodium phosphate shows the lowest peak intensity, which confirmed its low crystallinity. Similarly, characteristic peaks of nanosized dexamethasone sodium phosphate still existed but were very small (Figure 3.15 f), which indicates a further significant decrease of crystallinity.

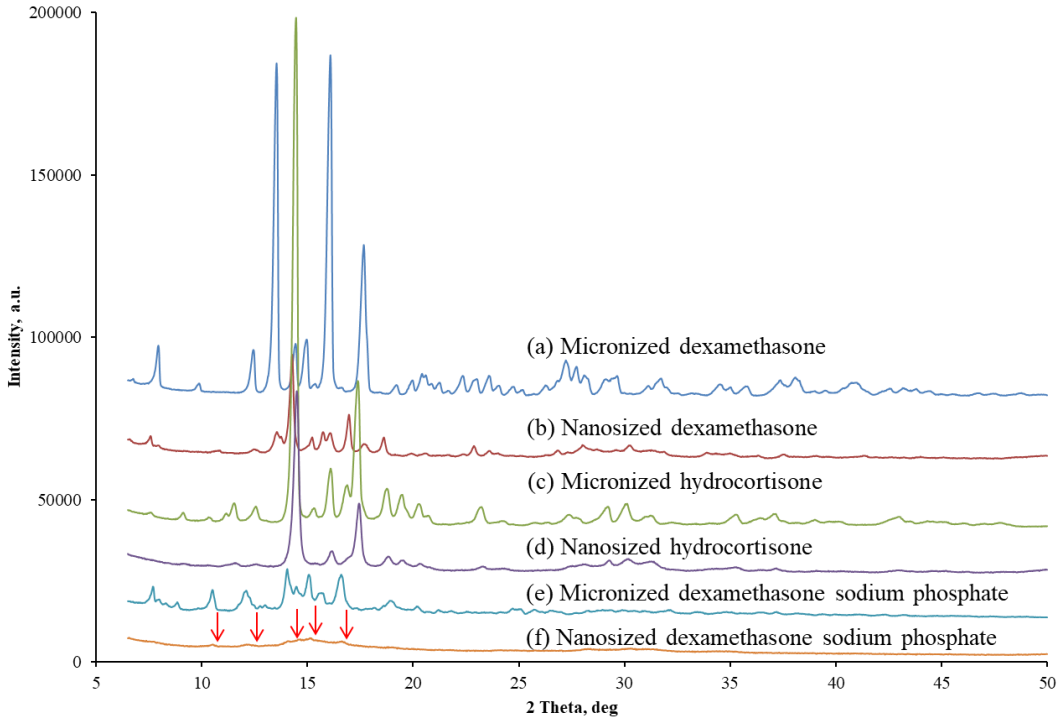


Figure 3.15. XRPD spectra of (a) original micronized dexamethasone, (b) air-dried nanosized dexamethasone, (c) original micronized hydrocortisone, (d) air-dried nanosized hydrocortisone, (e) original micronized dexamethasone sodium phosphate and (f) air-dried nanosized dexamethasone sodium phosphate.

Nanosized dexamethasone, hydrocortisone and dexamethasone sodium

phosphate were fabricated by milling in organic solvent using PLGA as a stabilizer. In this milling study, a small amount of PLGA in dichloromethane was proved as a good stabilizer to increase milling efficiency. After separation of nanosuspension and beads, further PLGA was added to prepare PLGA nanosuspension as inner oil phase. These nanosized particles were then encapsulated into PLGA microparticles directly by S/O/W solvent evaporation method or S/O/O coacervation method. This successive encapsulation process saved time and energy compared with the traditional aqueous milling, which still needs drying and redispersing in dichloromethane. Moreover, stabilizers like sodium lauryl sulfate used in the traditional aqueous milling process were avoided, which may impact the release profile and even toxicity for injectable drug delivery. By using this new preparation method, the microparticles only contain drugs and PLGA, minimizing other formulation impact factors. In sum, this new preparation method is faster and avoided additional hazards. However, it still needs clarification that these PLGA microparticles loaded with drug nanocrystals prepared by this new improved method also have the desired properties (e.g. linear release), which will be conducted in the following sections.

3.2.3. Characterization of PLGA microparticles

Tables 3.5, 3.6 and 3.7 list the microparticle size statistics and loadings for the various formulations. Microparticles smaller than 71 µm were successfully separated into different fractions: 0 - 5, 5 - 20, 20 - 50 and 50 - 71 µm. Microparticles with theoretical loading from 5 % to 30 % was prepared and had similar particle size around 35 µm. Additional, different PLGA types, PLGA blends and model drugs were also investigated to fabricate microparticles containing nanosized drug, having similar particle size and drug loading. Most microparticles' encapsulation efficiencies were high (i.e. larger than 80 %), but the encapsulation efficiency decreased with reduced particle size, increased theoretical drug loading, elevated hydrophobicity of PLGA and increased water solubility of drugs.

Smaller microparticles have a larger surface area ratio making drug stronger diffuse to water phase during emulsifying and washing process. Increasing theoretical drug loading from 5 % to 15 % slightly decreased encapsulation efficiency, due to more drug particles at the surface of the PLGA matrix have access to the outer water phase. Moreover, regarding 20 % and 30 % theoretical drug loading microparticles, reducing PLGA amount in dichloromethane decreased the oil phase viscosity and eased nanosized drug to diffuse out. Hardening of hydrophobic PLGA 502 and 752S is slower than hydrophilic PLGA 502H and 503H, giving nanosized drugs more time to diffuse out during microparticle preparation and resulting in lower encapsulation efficiency. Encapsulation of hydrophobic drugs is easier than encapsulation of hydrophilic drugs due to the latter's greater affinity for the external aqueous phase, which results in a lower loading efficiency. It has also been previously suggested that the lower loading efficiency of hydrophilic drugs is not only because of diffusion of the drug into the aqueous phase but also because of poor interaction between the drug and the polymer. This poor interaction causes an increased diffusion of the drug from the oil phase to the aqueous phase during the production of the microparticles [136]. To improve the loading of water-soluble dexamethasone sodium phosphate within PLGA microparticles prepared by S/O/W method, S/O/O was introduced where n-heptane was used as the hardening phase, resulting in an increased encapsulation efficiency from 22 % to 91 %. By changing the outer phase from water to n-heptane, dexamethasone sodium phosphate transfer to the outer phase was hindered during preparation since it is not soluble in n-heptane, thus the encapsulation efficiency increased significantly.

Table 3.5. Mean size, actual drug loading and encapsulation efficiency of PLGA 502H and 503H microparticles with different particle sizes and drug loadings.

PLGA	Mean particle size, μm	Span*	Theoretical DL, %	Actual DL, %	EE, %
502H	1.8 ± 0.1	1.15 ± 0.11	10	6.6 ± 0.1	66.0 ± 1.1
	12.5 ± 0.3	0.89 ± 0.02		8.6 ± 0.1	85.9 ± 0.9
	29.3 ± 0.2	1.07 ± 0.01		8.7 ± 0.1	86.8 ± 0.8
	58.0 ± 0.6	0.33 ± 0.05		9.0 ± 0.1	90.1 ± 0.8
	38.2 ± 0.3	0.54 ± 0.04	5	4.3 ± 0.0	86.0 ± 0.3
	37.4 ± 0.3	0.51 ± 0.03	15	12.6 ± 0.1	84.0 ± 0.6
	36.1 ± 0.4	0.45 ± 0.04	20	16.9 ± 0.1	84.5 ± 0.5
	32.5 ± 0.2	0.59 ± 0.02	30	23.3 ± 0.2	77.6 ± 0.8
503H	1.5 ± 0.1	3.07 ± 0.55	10	5.8 ± 0.0	58.1 ± 0.2
	12.9 ± 0.2	0.78 ± 0.01		7.5 ± 0.1	75.3 ± 0.7
	34.8 ± 0.4	0.64 ± 0.01		8.5 ± 0.1	85.3 ± 0.9
	61.3 ± 0.7	0.36 ± 0.01		8.6 ± 0.0	86.1 ± 0.3
	37.9 ± 0.5	0.58 ± 0.03	5	5.0 ± 0.2	99.4 ± 2.2
	36.5 ± 0.1	0.50 ± 0.02	15	13.8 ± 0.1	92.0 ± 0.8
	37.8 ± 0.3	0.53 ± 0.04	20	16.5 ± 0.2	82.5 ± 1.2
	32.4 ± 0.3	0.67 ± 0.04	30	23.7 ± 0.2	79.0 ± 0.6

*Span = (D90 – D10)/D50

Table 3.6. Mean size, actual drug loading and encapsulation efficiency of PLGA microparticles using different PLGA types and blends.

PLGA	Mean particle size, μm	Span*	Actual DL, %	EE, %
502H	29.3 ± 0.2	1.07 ± 0.01	8.7 ± 0.1	86.8 ± 0.8
503H	34.8 ± 0.4	0.64 ± 0.01	8.5 ± 0.1	85.3 ± 0.9
502	37.4 ± 0.4	0.70 ± 0.00	7.9 ± 0.0	79.1 ± 0.1
752S	26.4 ± 0.2	0.98 ± 0.04	7.2 ± 0.0	72.3 ± 0.2
1:1 502H:503H	36.0 ± 0.2	0.71 ± 0.02	9.3 ± 0.0	93.0 ± 0.4
1:1 502H:502	34.7 ± 0.2	0.41 ± 0.01	9.0 ± 0.0	90.2 ± 0.1
1:1 503H:502	44.1 ± 0.5	0.53 ± 0.04	9.4 ± 0.1	94.0 ± 1.2
2:1 503H:502	29.8 ± 0.3	0.58 ± 0.03	24.4 ± 0.3	81.3 ± 0.9
1:2 503H:502	28.9 ± 0.1	0.55 ± 0.01	25.3 ± 0.2	84.3 ± 0.7

*Span = (D90 – D10)/D50

Table 3.7. Mean size, actual drug loading and encapsulation efficiency of PLGA 503H microparticles incorporating different model drugs.

Drug	Mean particle size, μm	Span*	Actual DL, %	EE, %
Dexamethasone	34.8 ± 0.4	0.64 ± 0.01	8.5 ± 0.1	85.3 ± 0.9
Hydrocortisone	34.6 ± 0.2	0.50 ± 0.03	7.8 ± 0.0	78.2 ± 0.1
Dexamethasone sodium phosphate	30.1 ± 0.4	0.78 ± 0.01	2.2 ± 0.0	22.1 ± 0.1†
	37.5 ± 0.3	0.49 ± 0.04	9.1 ± 0.2	91.1 ± 0.7+

*Span = (D90 – D10)/D50

†Significant difference in the mean values compared with microparticles prepared by S/O/O method ($P < 0.05$)

+Microparticles prepared by S/O/O method

Figure 3.16 (a), (b) and (c) are SEM images of external and internal morphology of 20 - 50 μm PLGA 503H microparticles loaded with 10 % nanosized dexamethasone. Uniform, spherical microparticles are observed and the surface of the microparticles was smooth and homogeneous. The higher magnification image of cross-sectioned microparticles allows visualization of some of the discrete nanosized dexamethasone particles and pores in the nanometer range residing in the microparticle. At further magnification increase for more detailed observation, the electron beam destroyed the sample and the picture was not obtainable. Therefore, TEM with higher magnification was used to observe nanosized dexamethasone particles and pores in the nanometer range. The TEM image of a cut microparticle slide shows the distribution of nanosized dexamethasone in the microparticle. Nanosized dexamethasone particles are visible as relatively discrete dark regions in Figure 3.16 (d). The image shows a homogenous crystal distribution and the dexamethasone particle size was consistent with PCS results. 0.5 – 1.5 μm pores were also clearly observed by TEM.

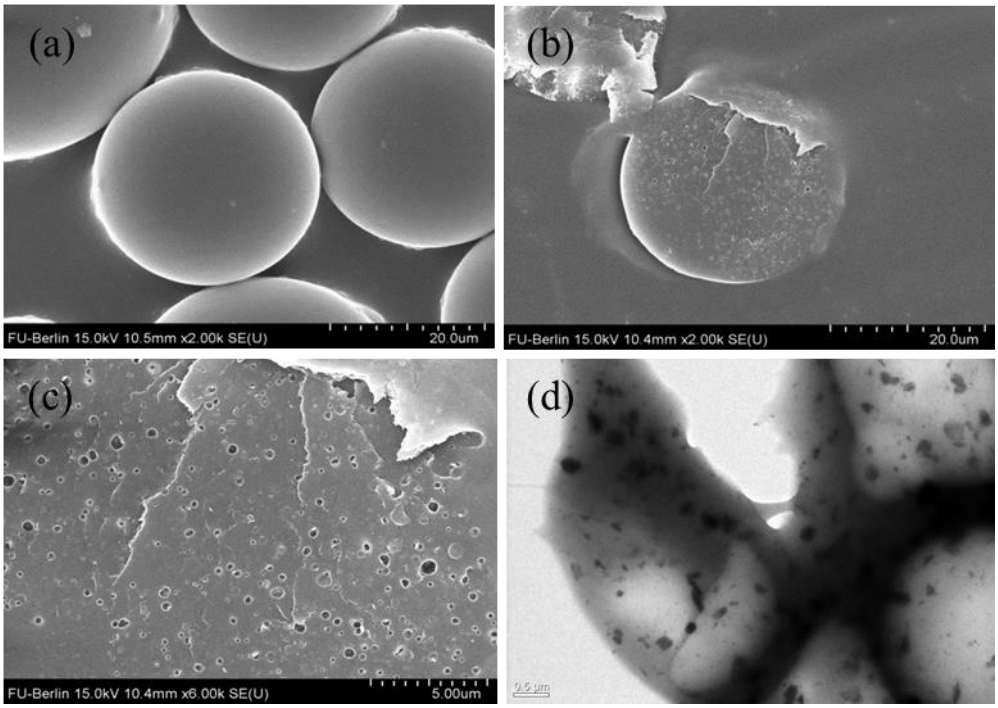


Figure 3.16. SEM pictures (a) surface, (b) cross-section, (c) higher magnification cross-section and (d) TEM picture of PLGA 503H microparticles loaded with 10 % nanosized dexamethasone.

DSC and XRPD were performed to understand the final solid state of drug embedded in the PLGA matrix. PLGA microparticles loaded with 10 % nanosized drug also have melting points of dexamethasone and hydrocortisone, which indicates crystalline drugs are embedded in the microparticles matrix (Figure 3.17). All melting temperatures of model drugs decreased, which was also found by several other researchers [101, 117, 118]. It can be attributed to the interaction between crystals with melted PLGA. The melting peak of dexamethasone sodium phosphate is disappeared due to the low crystallinity and dissolution in melted PLGA. For all samples, the melting temperature of PLGA kept constant, indicating no plasticizer effect of those drugs. These observations also indicate that the glass transition of the polymer was not influenced by the preparation procedure.

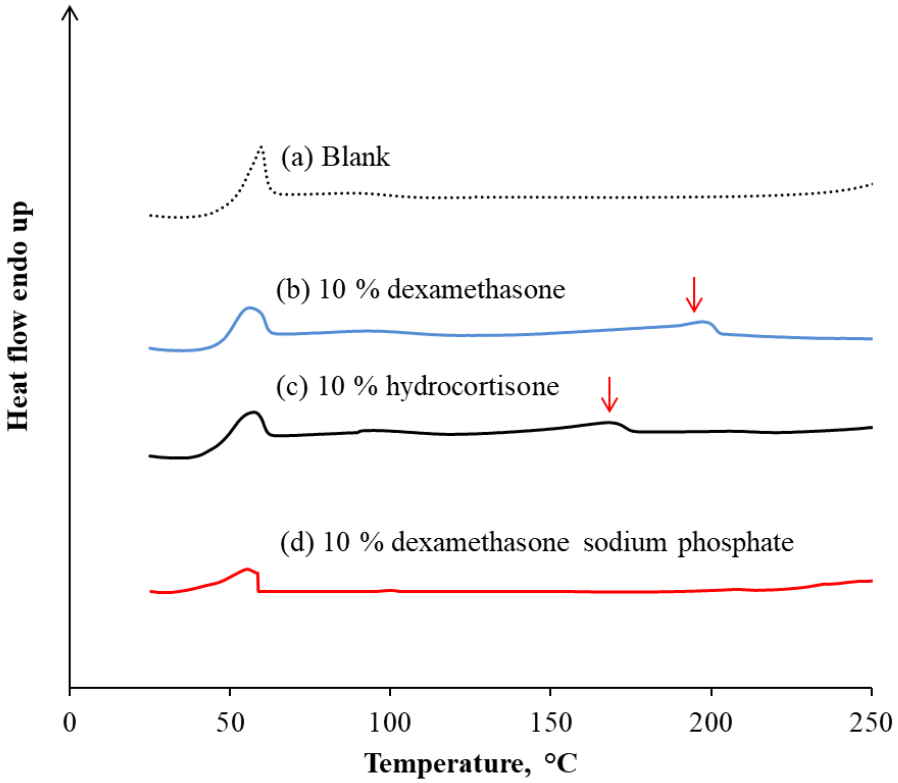


Figure 3.17. DSC graphs of PLGA 503H microparticles (a) blank, loaded with (b) 0 % nanosized dexamethasone, (c) 10 % nanosized hydrocortisone, and (d) 10 % nanosized dexamethasone sodium phosphate.

The XRPD results indicated that there is no further polymorphism change after encapsulating the nanosized dexamethasone, hydrocortisone, and dexamethasone sodium phosphate into PLGA microparticles (Figure 3.15 and Figure 3.18). However, the characteristic peaks of dexamethasone sodium phosphate are very small confirming its low crystallinity.

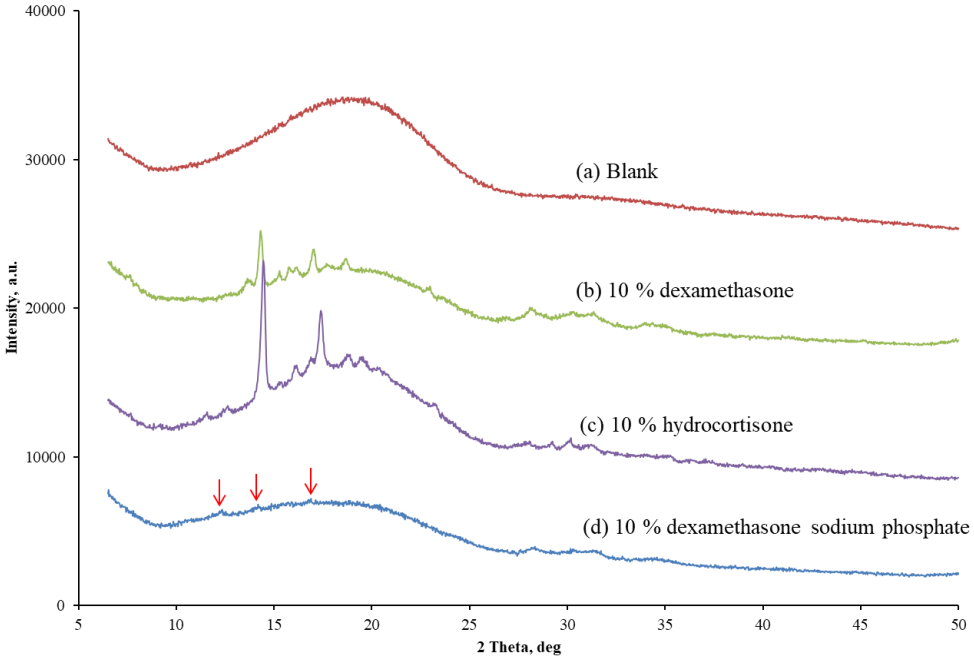


Figure 3.18. XRPD spectra of PLGA 503H microparticles (a) blank, loaded with (b) 10 % nanosized dexamethasone, (c) 10 % nanosized hydrocortisone, and (d) 10 % nanosized dexamethasone sodium phosphate.

3.2.4. *In vitro* release

The manufacturing process to prepare PLGA microparticles and chemical properties of polymer and drug affect the drug release from the PLGA matrix [120]. Similar manufacturing processes were used to prepare PLGA microparticles loaded with nanosized drug to avoid the effect of the varied manufacturing process on drug release. Other critical factors, like milling stabilizers, PLGA types, microparticle particle sizes, drug loading, PLGA blends and different model drugs were included in this study for investigation. A triphasic release profile includes a burst release, followed by a lag phase, and finally, a rapid release phase is often observed in PLGA microparticles. In the following sections, *in vitro* release profiles of the PLGA microparticles loaded with nanosized drug will be discussed in terms of these three phases, and how the factors mentioned above affect them.

3.2.4.1. The effect of milling stabilizers

Poloxamer 188 can also work as an efficient stablizer, which is similar to PLGA 502H. After 4 h milling, 295.4 nm (PDI = 0.20) dexamethasone nanoparticles were obtained. After encapsulating into PLGA 503H microparticles, 32 μm particles were fabricated with 9.0 % drug loading (89.5 % encapsulation efficiency). Indeed, poloxamer 188 as a milling stabilizer did not impact the drug encapsulation. Although poloxamer 188 is not biodegradable, it is meaningful to compare the *in vitro* release to using PLGA 502H as a milling stabilizer. However, it is obvious that 2.5 mg poloxamer 188 in 180 mg PLGA 503H matrix can significantly impact the release profile. More than 90 % drug was released on the first day, while using PLGA 502H as a milling stabilizer can sustain the drug release for more than 20 days (Figure 3.19). Around 1.5 % poloxamer in the matrix is the critical factor to increase the drug release. Poloxamer 188 is a water-soluble polymer. After being dissolved and leaving the particles quickly upon contact with release medium, water-soluble poloxamer 188 can result in a porous structure, which can not sustain drug release anymore. Water-insoluble PLGA hindered the quick drug release until its swelling and erosion.

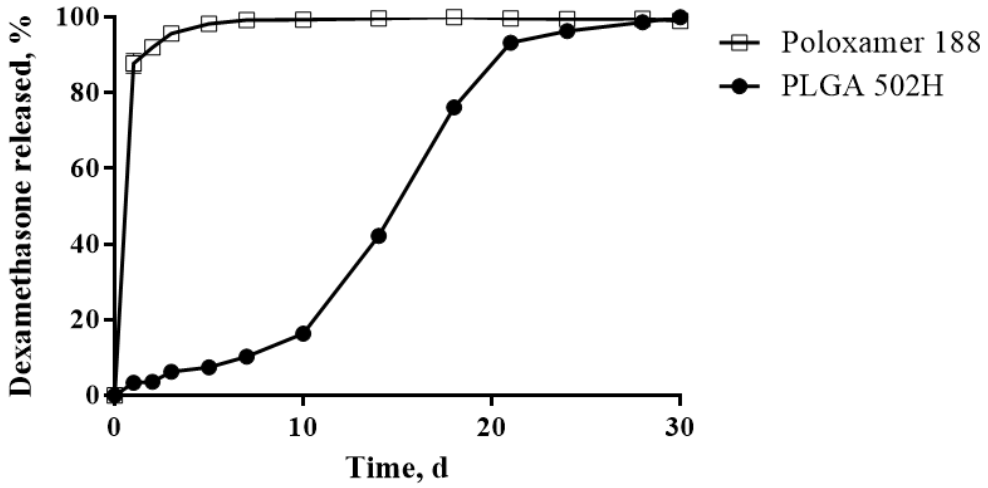


Figure 3.19. *In vitro* release of 20 - 50 μm 10 % nanosized dexamethasone PLGA 503H microparticles with different milling stabilizers.

3.2.4.2. The effect of PLGA types

Drug release profiles of PLGA microparticles can be tailored by varying the basic characteristics of PLGA polymers, such as polymer molecular weight (Mw), capped end-groups, and copolymer ratio [19, 132, 137, 138]. Decreasing the polymer Mw, non-capped end-groups, and increasing the molar ratio of glycolic acid increases the hydrophilicity of the formulation leading to faster polymer degradation rates. In addition, it is known that PLGA degradation occurs mainly through random hydrolysis of ester bonds and is autocatalyzed under acidic conditions. Therefore, the low Mw PLGA 502H may facilitate the degradation via increased water absorption and through generation of acidic oligomers that will result in autocatalysis of the polymer matrix. Increasing the polymer Mw (PLGA 503H), capped end-groups (PLGA 502), and increasing the molar ratio of lactic acid (PLGA 752S) contributed to a higher hydrophobic interaction between drug and polymer. Moreover, decreasing the hydrophilicity of PLGA inhibited water uptake from the release medium, which in turn resulted in lower initial burst release (Figure 3.20). The polymer Mw, capped end-groups, and L:G ratio of PLGA also exerted an effect on subsequent release. As shown in Figure 3.20, the lag phase was longer and drug release amount was less for more hydrophobic PLGA. Following the lag phase, drug is mainly released when the polymer matrix degrades and the drug diffuses from the eroded matrix [69]. It is known that the rate of PLGA degradation decreases with the more hydrophobic nature of PLGA. The sustained release time was in the order of 502H (15 days) < 503H (30 days) < 502 (45 days) < 752S (100 days). This result is consistent with the trend observed in other studies [6, 20, 138, 139], where PLGA 502H, 503H, 502 and 752S can sustain drug release for 14 - 28, 21 - 35, 42 - 56 and 90 -120 days, respectively.

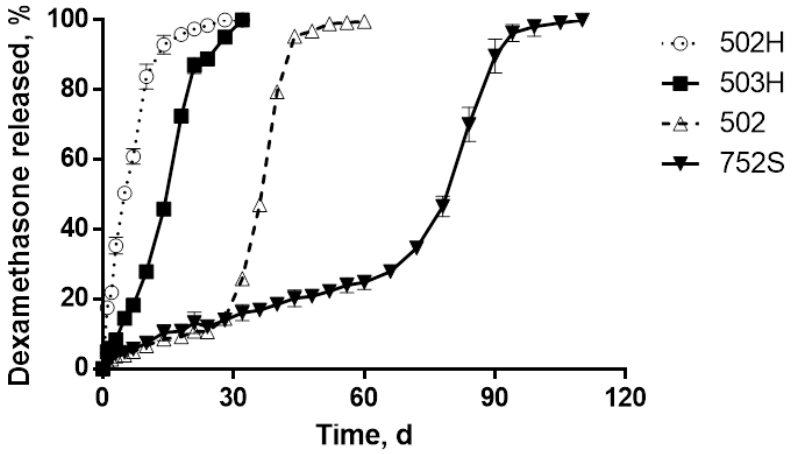
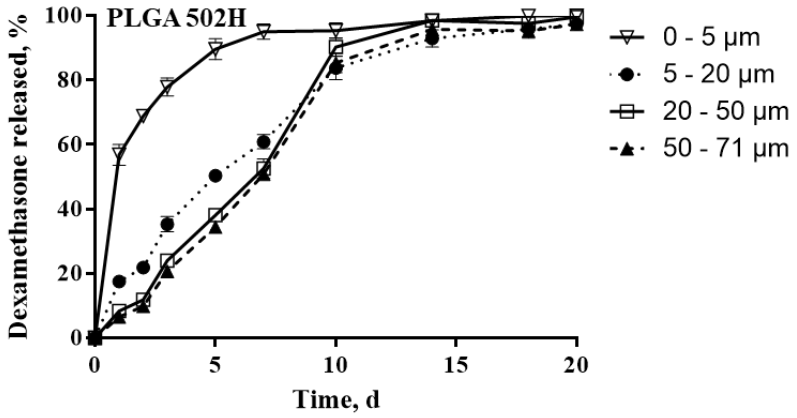


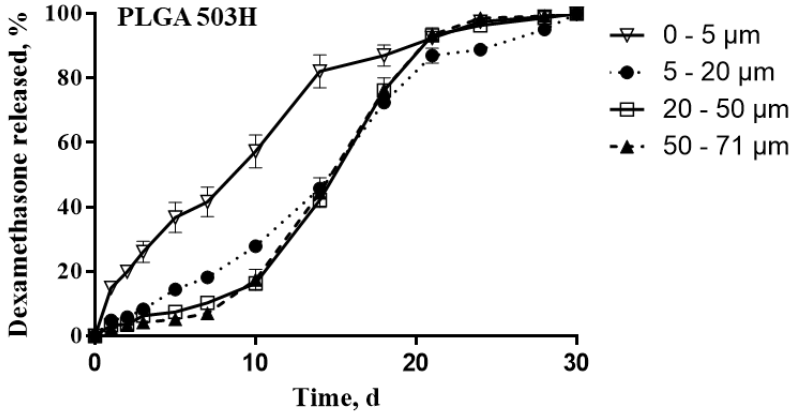
Figure 3.20. *In vitro* release of 20 - 50 μm 10 % nanosized dexamethasone microparticles with different PLGA types.

3.2.4.3. The effect of microparticle size

The effect of microparticle size on the release of drug has also been investigated. Microparticles produced under the same protocol were separated into four groups of sizes: smaller than 5, between 5 and 20, between 20 and 50, and between 50 and 71 μm using different sieves. The result shows a trend of release profiles with the microparticle's sizes (Figure 3.21).



(a)



(b)

Figure 3.21. *In vitro* release of 10 % nanosized dexamethasone from microparticles with different particle sizes and PLGA types: PLGA (a) 502H and (b) 503H.

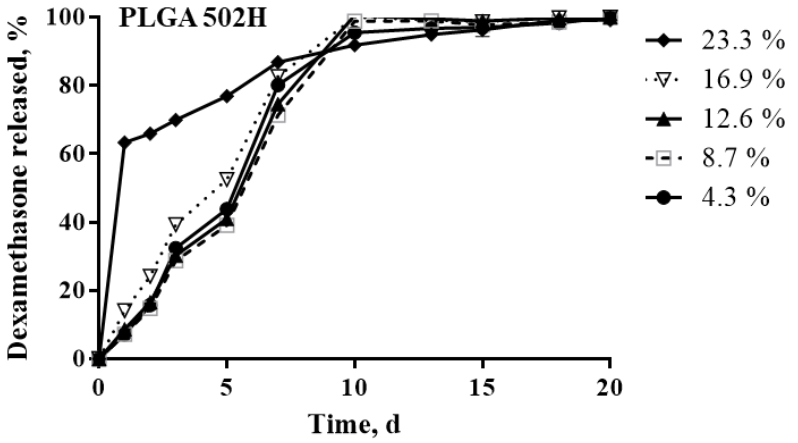
Microparticles with larger sizes have a more sustained release compared to the smaller size microparticles. Besides, a lower initial burst was also observed. This observation was expected as larger microparticles have a smaller surface area to mass ratio and thicker polymer shells, which reduces the diffusion rate. This opens the opportunity to group the microparticles into categories of different release rates via size range discrimination [140]. Therefore, better control of drug release that is required for specific applications can be made [141]. Interestingly, by selecting the proper particle size of PLGA 502H and 503H microparticles, a quasi-linear release was obtained and the lag phase disappeared, e.g. 5 - 20, 20 - 50 and 50 - 71 µm PLGA 502H microparticles and 0 - 5 and 5 - 20 µm PLGA 503H microparticles. Moreover, 0 - 5 µm PLGA 503H microparticles loaded with

nanosized dexamethasone can be successfully prepared and a quasi-linear release was achieved during 20 days, which could be promising for pulmonary drug delivery. For effective pulmonary drug delivery, the optimal particle size is crucial and should fall within the range of 1 - 5 µm [50]. Moreover, PLGA microparticles can overcome the immediate release of a drug to provide sustained effects.

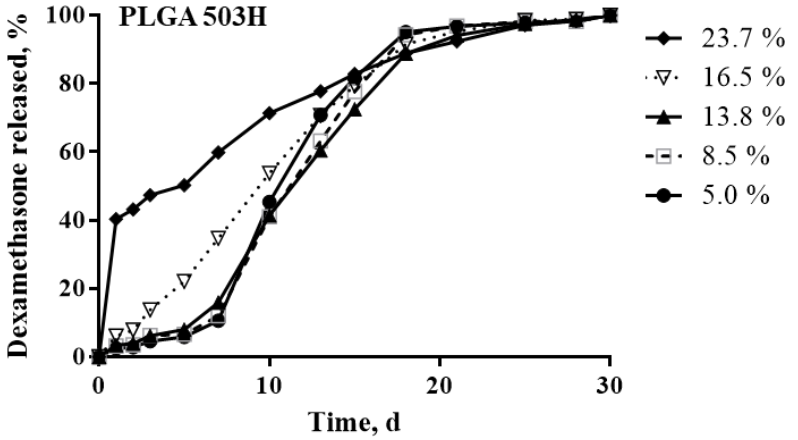
3.2.4.4. The effect of drug loadings

Usually, burst release increases with increasing drug loading due to the elution of surface-associated drugs and a high concentration gradient between microparticle and surrounding release medium [69]. Actual drug loadings ranging from 4.3 % to 23.7 % were chosen for comparison (Figure 3.22). Starting with the lowest loading of around 5 %, the 24 h burst release is just 7.2 % for PLGA 502H microparticles and 2.5 % for PLGA 503H microparticles. As drug loading increases, the burst release also increased as expected. At around 17 % loading, the burst release increased to 14.0 % for PLGA 502H microparticles and 6.1 % for PLGA 503H microparticle. At around 23 % drug loading, the burst release increased significantly to 63.2 % for PLGA 502H and 40.1 % for PLGA 503H. In this case, drug particles that are exposed to the surface should be the first to dissolve, leaving behind pores that may connect to more deeply buried drug particles. Moreover, when increasing drug loading, the probability of drug particle interconnectivity also increases, and more voids and inter-channels left behind by nanosized drug particles are another reason for the high burst. More pores could also be the result of lower PLGA concentration in the oil phase for high drug loading microparticle preparation. Thus, burst release would be expected to increase. After the burst release, the lag phases were eliminated for 4.3 % - 23.3 % drug loading PLGA 502H microparticles and 16.5 % - 23.7 % drug loading PLGA 503H microparticles, which might be caused by adequate hydrophilicity of PLGAs and proper pores and inter-channels formed by nanosized dexamethasone. In sum, selecting suitable drug loading achieves quasi-linear release profiles from

PLGA microparticles loaded with nanosized drug.



(a)



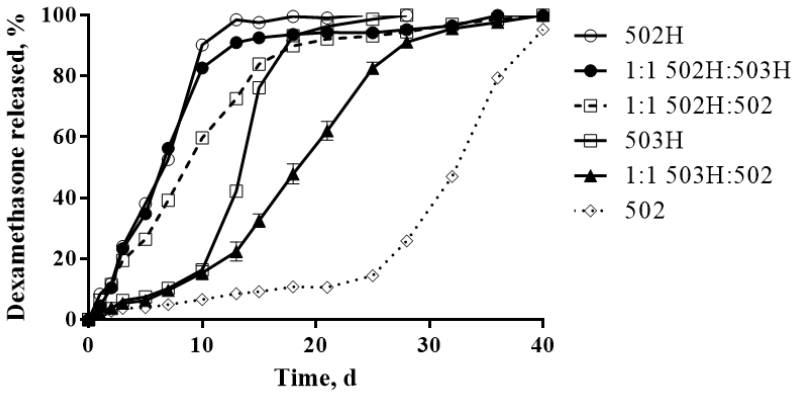
(b)

Figure 3.22. *In vitro* release of nanosized dexamethasone from 20 - 50 µm microparticles with different drug loadings: (a) PLGA 502H and (b) PLGA 503H.

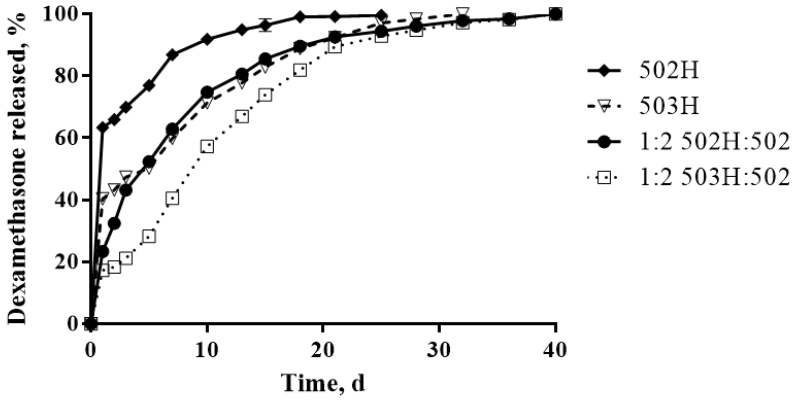
3.2.4.5. The effect of PLGA blends

The effect of blending PLGAs on the *in vitro* drug release of nanosized dexamethasone loaded microparticles was investigated. For 10 % drug loading (Figure 3.23 a), a quasi-linear release is obtained. For 30 % drug loading (Figure 3.23 b), a typical biphasic or triphasic release profile was observed. The burst release and lag time of the microparticles prepared using blends of these two PLGAs were between those two single PLGA microparticles. Using PLGA 502 blended with PLGA 502H or 503H showed an increased initial burst release and

increased drug release at lag phase making the whole release profile more continuous (Figure 3.23 a and b). The increased burst release was speculated to be a result of increased polymer hydrophilicity and consequent increased drug diffusion [122]. Likewise, the drug release at the lag phase or the period of lag phase is considered to be a consequence of the different times required for different polymer degradation. In the case of microparticles prepared using polymers blends, there is a decrease or elimination of the lag phase time and increase in the release rate with increasing the amount of the more hydrophilic PLGAs. Thus using PLGA mixtures is a useful way to achieve linear release of nanosized dexamethasone loaded PLGA microparticles. Moreover, quasi-linear release period from days to months was also obtained by using different PLGA blends.



(a)



(b)

Figure 3.23. *In vitro* release of nanosized dexamethasone from 20 - 50 µm microparticles with PLGA blends: (a) 10 % and (b) 30 % drug loading.

3.2.4.6. The effect of different model drugs

Release profiles of 20 - 50 µm PLGA 503H microparticles loaded with nanosized dexamethasone, hydrocortisone and dexamethasone sodium phosphate were studied. Dexamethasone sodium phosphate released continuously over the whole release period after the burst release (Figure 3.24). Dexamethasone and hydrocortisone show a triphasic release profile. The burst release was in the order of dexamethasone sodium phosphate ＞ dexamethasone ＞ hydrocortisone. Hydrocortisone microparticles have a smaller burst release compared with dexamethasone microparticles since the higher solubility of hydrocortisone in water results in a more thorough washing process and less drug has direct access to the release medium. Dexamethasone sodium phosphate PLGA microparticles prepared by S/O/O coacervation method avoided the water washing process and left more drug to have direct access to the release medium, thus resulting in a high burst release. Dexamethasone sodium phosphate microparticles have the fastest release rate, and hydrocortisone microparticles have a faster release at the lag phase and final release stage compared with dexamethasone microparticles since the higher solubility in water resulting in a higher diffusion capacity. Irrespective of varied *in vitro* release, these results indicated that both, water-soluble and insoluble nanosized model drugs can be effectively encapsulated into PLGA 503H microparticles with sustained *in vitro* release. It should be highlighted that different model drugs need different PLGA types, microparticle sizes, drug loading and PLGA blends as described above to achieve a quasi-linear release.

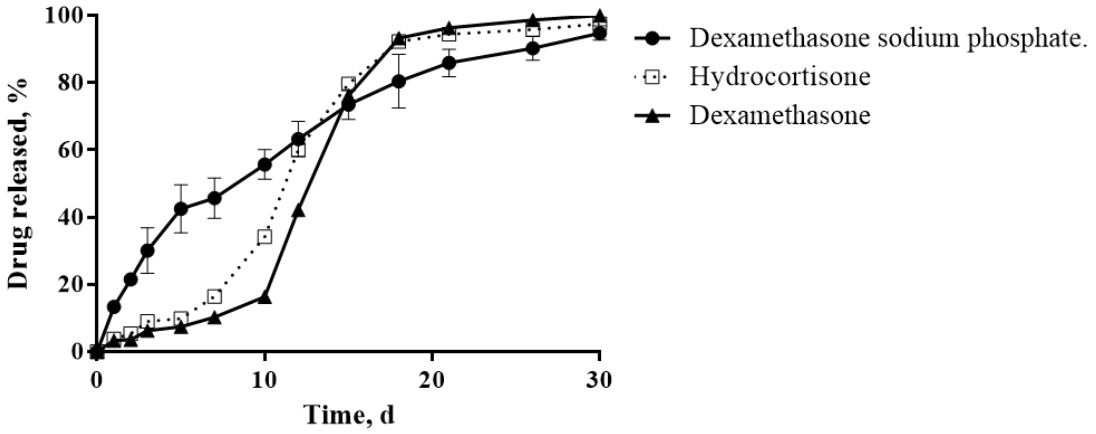


Figure 3.24. *In vitro* release of nanosized dexamethasone, hydrocortisone and dexamethasone sodium phosphate from 20 - 50 µm PLGA 503H microparticles.

3.2.5. Conclusion

Continuous delivery of drugs in microparticles is a widespread problem in the PLGA microparticle drug delivery area and linear release leads to efficient delivery and significantly reduced toxicity hazards. Very few studies have reported encapsulating nanosized drugs into PLGA microparticles to achieve continuous release. No extra stabilizers should be used to manufacture nanosized drugs since a minor amount of stabilizer can impact the release of PLGA microparticles. These goals were met in this study. The wet bead milling in the dichloromethane process yielded very narrow size distribution and included water-soluble and -insoluble nanosized drug particles with PLGA as a milling stabilizer. Furthermore, these nanosized drugs have a stable solid state and low aggregation properties after encapsulation. Various PLGA microparticles loaded with nanosized drug were effectively produced by S/O/W or S/O/O encapsulation method. The uniform distribution of nanosized drug particles in the microparticles reduced the exposure of the drugs to the surface and pores, leading to reduced burst release. By changing PLGA types, selecting microparticle sizes, varying drug loadings, and using PLGA blends, quasi-linear releases without lag phase were obtained. Moreover, the uniform encapsulation of nanosized drug particles

into PLGA microparticles (smaller than 5 μm) with high drug loading, and continuous delivery was achieved, which is promising for specific applications (e.g. pulmonary drug delivery).

3.3. PLGA *in situ* forming implant and microparticle incorporating nanosized drug as long-term drug delivery carriers

3.3.1. Introduction

The PLGA ISI and ISM are being developed as promising delivery systems. ISI, made of biodegradable PLGA dissolved in water-miscible solvents such as N-methyl-2-pyrrolidone, can be injected via a syringe into the body and solidify to form a solid depot after injection. ISM consists of an internal phase containing drug in PLGA-solvent emulsified into an external phase. Upon injection of this emulsion, the internal phase precipitates and forms microparticles. Compared to traditional PLGA implants and microparticles, *in situ* forming systems are easier to use, can be formulated in higher doses and injected via a smaller diameter injection needle. Although these systems are well tolerated and provide sustained release of the incorporated drug over the designated dosing period, their initial drug burst releases were 15 - 80 %, which is high and may cause tissue irritation and sometimes toxicity [142].

To achieve low burst and suitable release profiles, optimization of solvent, PLGA type and concentration, as well as additional ingredients (such as release modifiers and plasticizers) were already considered and oil-in-oil emulsion was investigated to modify PLGA *in situ* forming systems. Triacetin, which is a more hydrophobic solvent, leaves the “depot” very slowly, resulting in slower phase inversion, and a significant reduction in the burst release compared to N-methyl-2-pyrrolidone (NMP) containing systems [143, 144]. Increasing the PLGA concentration and the PLGA molar mass were commonly used to reduce burst release but it was limited because of the viscosity requirements of the polymer solution for injection [145, 146]. To reduce the burst release while maintaining injectability, methods like introducing carriers for the drug to form particulates [147], adding a polymeric controlled-release additive [148], or adjusting the solvent characteristics by mixing a hydrophilic solvent and a hydrophobic solvent

at different ratios [142, 148] were also investigated. When the non-aqueous PLGA solution was mixed with a biocompatible external oil phase, like peanut oil, oil-in-oil emulsion was formed for injection. This oil-in-oil emulsion has a significantly reduced initial burst release since the external oil phase formed a partial barrier between the aqueous medium and the internal polymer solution which slowed down internal solvent diffusion to form a less porous PLGA matrix [61, 64, 149, 150]. However, no study tried to investigate how drug dispersion states and particle sizes would impact the physicochemical property and *in vitro* release of PLGA *in situ* forming systems.

In the previous chapter, nanosized drugs were successfully prepared by non-aqueous wet bead milling in solvents and these nanosized particles were then encapsulated into PLGA microparticles directly by solvent evaporation or coacervation method. These microparticles showed a stable drug solid state, a lower burst release and a more continuous release profile *in vitro*. However, as a limitation, solvent must completely evaporate and microparticles need to dry before use. To further simplify the manufacturing process, PLGA *in situ* forming systems loaded with a nanosized drug are proposed, which can be directly injected into the body without removing the solvent and drying process. With the benefits described above, incorporating nanosized drugs into PLGA *in situ* forming systems can potentially overcome the limitations of microparticle as well as a high initial burst from conventional *in situ* forming systems. The objective of this study is to compare PLGA *in situ* forming systems with different drug dispersion states and particle sizes and systemically investigate PLGA *in situ* forming systems incorporating nanosized drug.

3.3.2. Dexamethasone nanosuspension and PLGA *in situ* forming systems preparation and characterization

Wet bead milling was used as the most suitable technology for nanosizing dexamethasone particles on a small scale [133]. Triacetin was selected as the milling medium based on solubility measurement of dexamethasone and

suitability for *in situ* forming systems manufacturing. The low solubility of dexamethasone (1.82 ± 0.03 mg/g) in triacetin (25 °C) ensured dexamethasone particles were suspended at 5 % (w/w). Wet bead milling of 5.0 % dexamethasone in triacetin for 2 h resulted in 372 nm nanocrystals with a low PDI (0.17). The hydrophobic functional groups of triacetin deposit at the apolar drug particle surface, and then improve the wetting process and stabilize the dexamethasone particles. Moreover, compared with water as the milling medium, increased viscosity of triacetin can avoid the agglomerate of drug particles. After being separated from the milling beads, a milky nanosuspension was obtained (Figure 3.25 b). After 3-month storage, there was no obvious sedimentation of nanosuspension due to smaller drug particle size and increased suspension viscosity, which was caused by high particle number per volume. In contrast, sedimentation of microsuspension was observed within 1-day storage at room temperature (Figure 3.25 c).

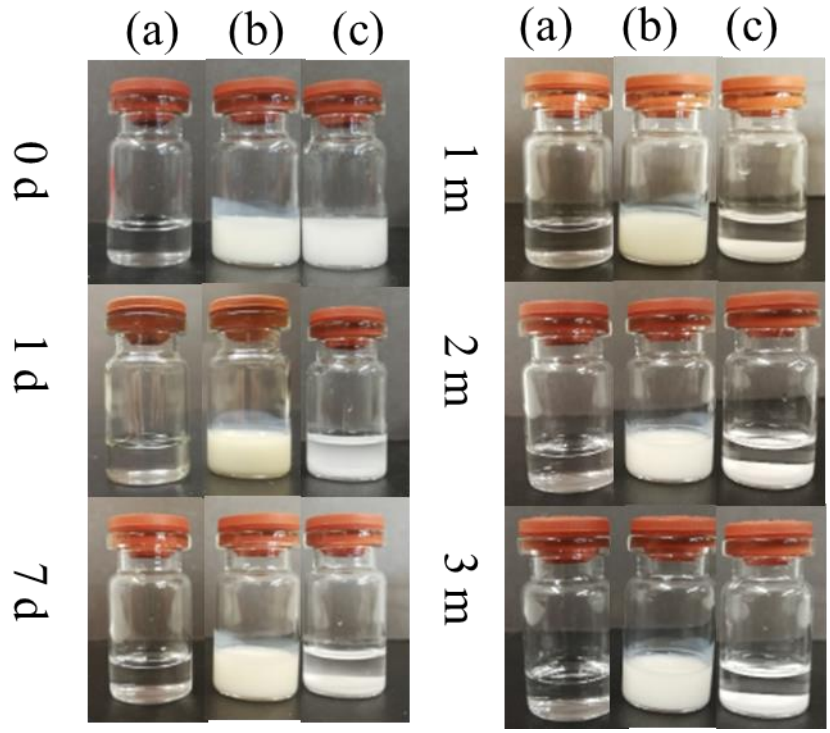


Figure 3.25. Macroscopic pictures of (a) triacetin, (b) 5 % (w/w) dexamethasone triacetin nanosuspension, and (c) 5 % (w/w) dexamethasone triacetin microsuspension stored at room temperature for 3 months.

Although nanosized dexamethasone particles were obtained by milling in triacetin without any stabilizers, there was the possibility of changing polymorphs or forming solvates. DSC indicated no desolvation of dexamethasone (Figure 3.26). The melting peaks of nanosized drugs still existed, but the melting temperatures decreased suggesting a decrease in the degree of crystallinity due to imperfections in the crystal lattice by milling [109, 114]. Moreover, small crystals melt at a lower temperature than large crystals [115]. Comparing original micronized dexamethasone (Figure 3.27 a), the reduced intensity and broadening of the diffraction peaks of nanosized dexamethasone (Figure 3.27 b) may be attributed to the reduced degree of crystallinity, which further supports findings from the DSC. Moreover, the XRPD results indicated nanosized dexamethasone was still in a crystalline state and there was no polymorphism form change after incorporation into ISI (Figure 3.27 d).

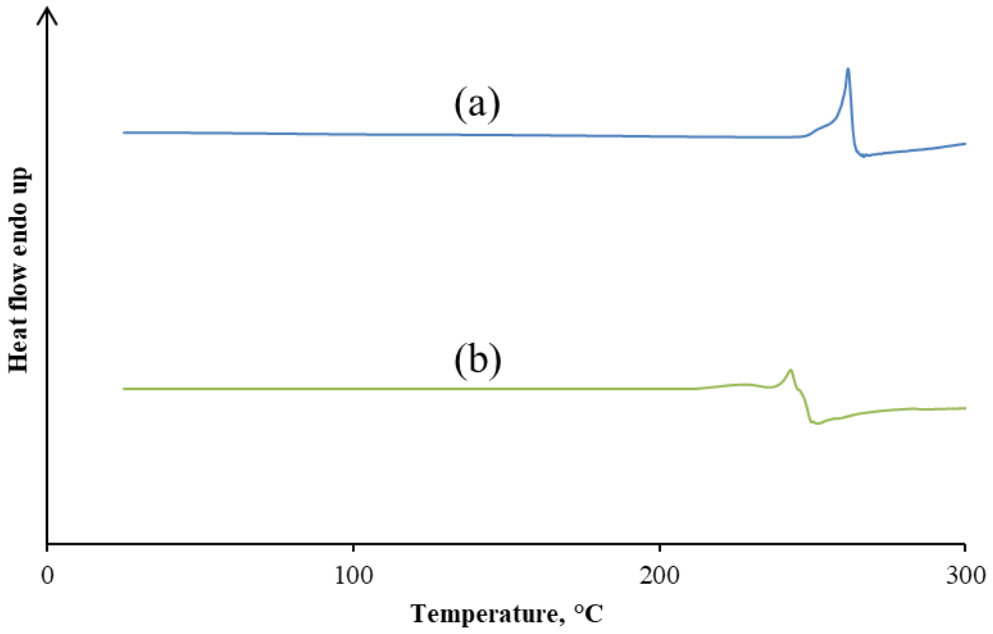


Figure 3.26. DSC thermograms of (a) original micronized dexamethasone and (b) nanosized dexamethasone.

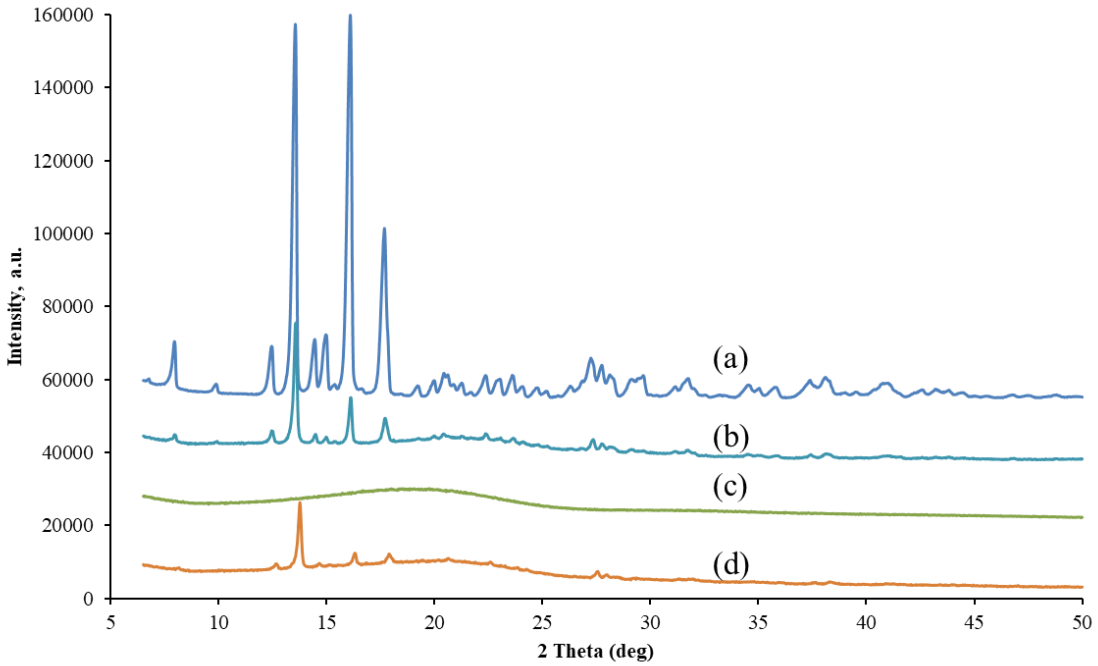


Figure 3.27. XRPD spectra of (a) original micronized dexamethasone, (b) nanosized dexamethasone, (c) PLGA 502H placebo and (d) 10 % nanosized dexamethasone ISI.

To prepare ISIs, PLGA was dissolved in dexamethasone dispersions (triacetin as solvent) and solutions (NMP or a mixture of NMP and triacetin as solvent). A viscous milky polymer suspension or clear polymer solution was obtained (Figure 3.28). Dissolved and nanosized dexamethasone ISIs homogeneities were maintained for at least 3 months, while sedimentation of micronized dexamethasone particles occurred within 3-day storage at room temperature (Figure 3.29). The larger dexamethasone particles easily sedimented and caused a heterogeneous distribution inside the ISI. This was a critical difference as solidification of PLGA dissolved in triacetin may need more than 10 days after incubating in release medium [142]. Moreover, nanosized dexamethasone had no significant change in particle size and PDI during 3-month storage (Table 3.8), confirming ISI incorporating nanosized drug was physically stable. This was due to increased drug particle numbers existing in PLGA triacetin solution resulting in a semi-gel structure with a higher viscosity, avoiding aggregation and sedimentation of nanocrystals. Moreover, the diffusion of dexamethasone molecular was also decreased hindering the recrystallization of dexamethasone

at the surface of nanoparticles.

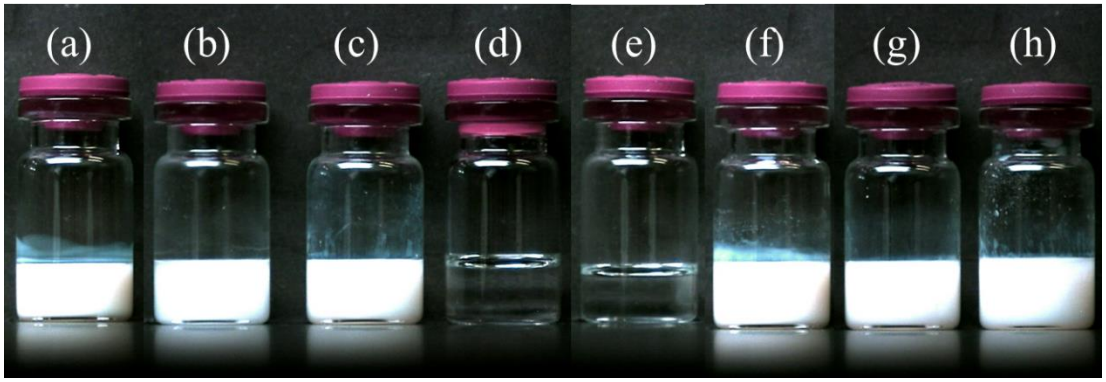


Figure 3.28. Macroscopic pictures of (a) 5 % dexamethasone triacetin nanosuspension, (b) 10 % micronized dexamethasone ISI (100 % triacetin), (c) 10 % nanosized dexamethasone ISI (100 % triacetin), (d) 10 % dissolved dexamethasone ISI (100 % NMP), (e) 10 % dissolved dexamethasone ISI (20 % NMP and 80 % triacetin), (f) 25 % nanosized dexamethasone ISI, (g) 30 % PLGA 502H ISI and (h) 40 % PLGA 502H ISI.

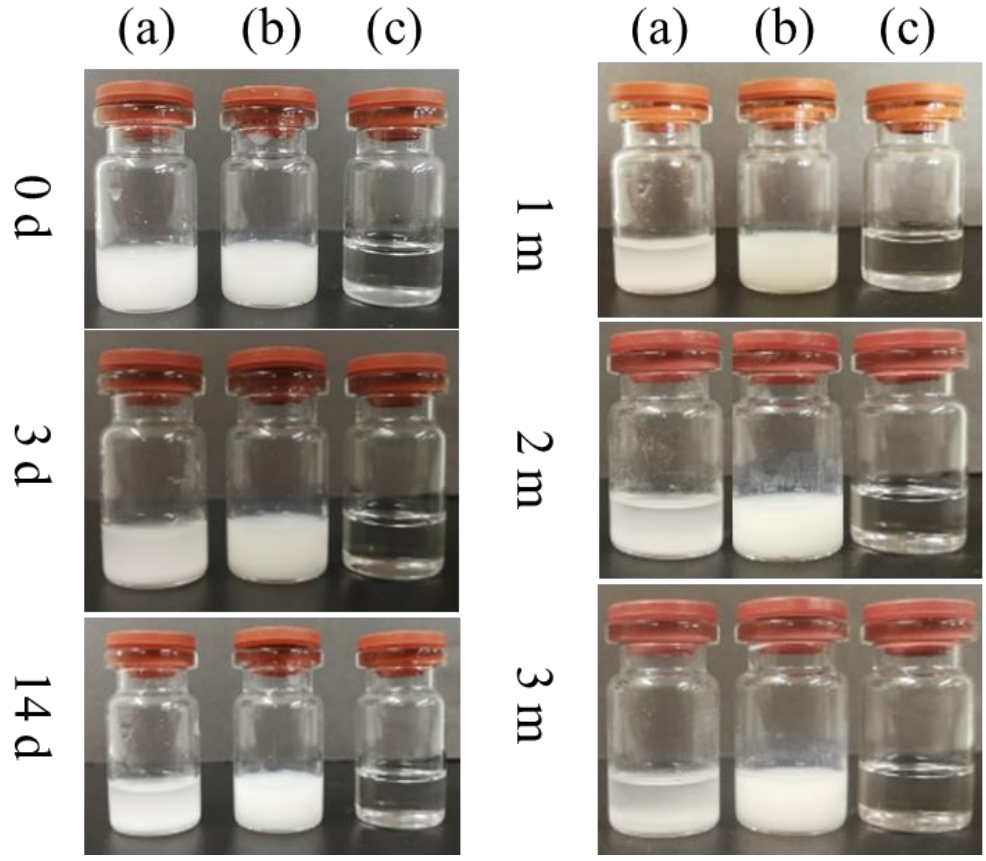


Figure 3.29. Macroscopic pictures of (a) 10 % micronized dexamethasone ISI, (b) 10 % nanosized dexamethasone ISI and (c) 10 % dissolved dexamethasone ISI stored at room temperature for 3 months.

Table 3.8. Particle size of nanosized dexamethasone in PLGA *in situ* forming system (20 % PLGA 502H and 10 % drug loading) stored at room temperature.

Time, month	Z-average, nm	PDI
0	372 ± 14	0.17 ± 0.01
1	368 ± 15	0.17 ± 0.02
3	398 ± 27	0.20 ± 0.01

In polymer solutions, polymer molecules show different chain conformations depending on the quality of the solvent: the viscosity is lower in good solvent than in poor solvent [151]. The viscosity of PLGA in NMP was significantly lower compared to PLGA in triacetin (Table 3.9). This confirmed that NMP is a better solvent for PLGA than triacetin. Decreasing particle size and increasing drug loading can also significantly increase the viscosity of formulations. By increasing nanoparticle number, particle-particle interaction became more significant, thus increasing viscosity from 0.751 to 0.933 Pa·s. Increasing PLGA 502H concentration from 20 % to 40 % can significantly increase the viscosity from 0.933 to 9.119 Pa·s, caused by the stronger intermolecular action of PLGA. Higher viscosities can hinder injection. The injectability of a formulation is of utmost practical importance: if the forces required to inject the drug product are high, the treatment becomes complicated and time-consuming. The injectability was investigated by a texture analyzer. The force required to expulse nanosized dexamethasone formulations was higher compared to the other formulations loaded with dissolved and micronized dexamethasone, which was consistent with the viscosity of these formations. Overall, all formulations had acceptable injectability, except 40 % PLGA ISI. The high viscosity and large maximum injection force of 40 % PLGA ISI may cause an injectability problem and require the use of larger needle sizes (Figure 3.30).

Table 3.9. Composition, viscosity and maximum injection force of ISIs at 20 °C.

ISIs	Triacetin, g	NMP, g	PLGA, g	Dexamethasone, g	Viscosity, Pa·s	Maxmium injection force, N
Micronized (100 % triacetin)	1.00	0.00	0.20	0.02 (micronized)	0.751 ± 0.003	7.60 ± 0.08
Nanosized (100 % triacetin)*	1.00	0.00	0.20	0.02 (nanosized)	0.933 ± 0.011	9.13 ± 0.26
Dissolved (100 % NMP)	0.00	1.00	0.20	0.02 (dissolved)	0.031 ± 0.000	4.19 ± 0.26
Dissolved (20 % NMP and 80 % triacetin)	0.80	0.20	0.20	0.02 (dissolved)	0.336 ± 0.002	5.70 ± 0.19
25 % dexamethasone	1.00	0.00	0.20	0.05 (nanosized)	2.257 ± 0.070	9.81 ± 1.06
30 % PLGA	1.00	0.00	0.30	0.03 (nanosized)	3.225 ± 0.041	24.90 ± 0.31
40 % PLGA	1.00	0.00	0.40	0.04 (nanosized)	9.119 ± 0.080	46.76 ± 1.24

*Nanosized (100 % triacetin) ISI also refers to 10 % dexamethasone ISI and 20 % PLGA ISI.

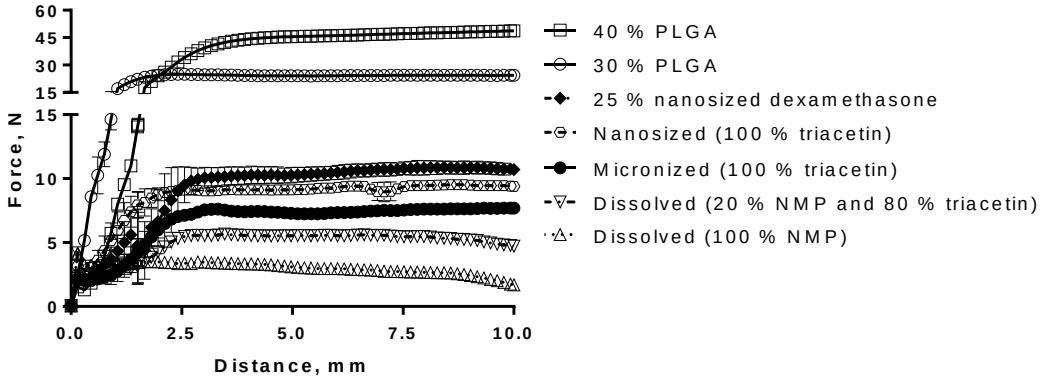


Figure 3.30. Force-distance plot of the investigated ISIs.

After nanosized dexamethasone polymer solution (20 % PLGA 502H and 10 % drug loading) was emulsified into an aqueous external phase of 2.0 % PVA at the ratio of 1:4 using a two-syringe system, ISM was formed. By varying mixing cycles, three groups of microparticles loaded with nanosized dexamethasone with significantly different particle sizes were obtained. Increasing mixing cycles, the particle size of microparticles decreased by more energy input to break emulsion particles.

Table 3.10. Particle size of ISMs by varying mixing cycles.

Mixing cycles	Mean particle size, μm
5	97.4 ± 60.9
10	65.8 ± 42.5
20	29.1 ± 18.6

3.3.3. *In vitro* release

Upon initial contact between the *in situ* forming drug delivery systems and the aqueous medium, water permeated into the implanted delivery system over a period ranging from seconds to weeks, inducing coagulation or solidification of PLGA. Thus, a solid implant was formed. The *in vitro* drug release from the formulations followed a triphasic pattern [152, 153]. A fast initial release phase (burst) was followed by a second slow release phase lasting days or weeks and a third rapid release phase. Because *in situ* forming systems were administered as a liquid, there was a lag time between the injection and the formation of the solid implant. During this period, solvent along with the dissolved or dispersed drug moved out of the formulation quickly causing the initial burst release. After that, a diffusion-controlled slower release phase followed. Finally, when PLGA degraded rapidly an erosion-controlled release phase occurred. The degradation of amorphous PLGA (RG 502H) used in this study was expected within 4 weeks [138, 154]. For the first time, *in situ* forming systems loaded with nanosized dexamethasone were prepared and compared to dissolved and micronized dexamethasone. The effect of PLGA concentration, drug concentration and formulation surface area on *in situ* forming systems loaded with nanosized dexamethasone were also investigated.

3.3.3.1. The effect of drug dispersion states and particle sizes

The drug release from ISIs loaded with micronized, nanosized and dissolved dexamethasone were investigated to understand the effect of drug dispersion

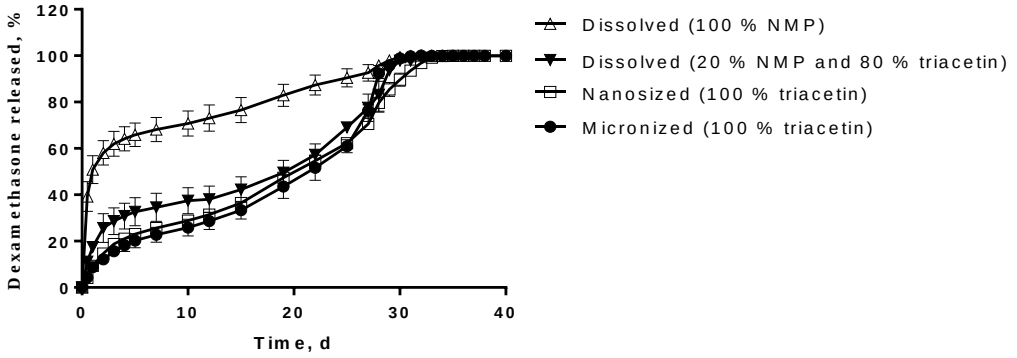
states and particle sizes. Compared to dispersed dexamethasone, dissolved dexamethasone had the largest burst and released the fastest (Figure 3.31 a), which is consistent with their lowest viscosity. However, some care should be taken when comparing these results, since the solvent was different: 100 % NMP or 20 % NMP : 80 % triacetin (dissolved dexamethasone) versus 100 % triacetin (dispersed dexamethasone). The diffusion of NMP into water over a short period caused a larger initial burst release because the NMP carried the dissolved drug into the release medium during the implant formation [155, 156]. When triacetin was used as solvent, which is more hydrophobic and less water-miscible than NMP, its diffusion into the release medium was much slower. Also, dexamethasone in a dispersed state was encapsulated inside the implant resulting in a significantly smaller initial burst release. The quick diffusion of NMP facilitated the hardening of ISIs and resulted in a more porous structure, which would increase the overall drug release rate.

The initial bursts and release rates were supposed to be different for micronized and nanosized dexamethasone. Nanosizing dexamethasone should increase the movement of dexamethasone particles and the apparent solubility of dexamethasone [157, 158], which could increase the burst. However, incorporating nanosized dexamethasone into ISI increased its viscosity, thus decreasing the diffusion rate of triacetin and the movement of dexamethasone particles into the release medium. This gave nanosized dexamethasone ISI a similar initial burst as incorporating micronized dexamethasone (9.3 % versus 8.7 %). The following lag phase of nanosized and micronized dexamethasone ISIs lasted for 10 days, then there were another quick release phases. More drug was released at the lag phase from nanosized dexamethasone ISI when compared to micronized dexamethasone (29.0 % versus 25.8 %). One explanation could be that nanosized particles were uniformly distributed inside the implants easing their release during the lag phase. In contrast, micronized dexamethasone was sedimented before the solidification of the implant. Micronized dexamethasone at the bottom of the implant was less possible to

contact with release medium and diffuse out at lag phase. During the quick release period, release medium penetrated into more viscous PLGA ISI loaded with nanosized dexamethasone slower compared to micronized dexamethasone, resulting in slower PLGA degradation. Nanosized dexamethasone released slower and had a longer overall release period than micronized drug (35 days versus 28 days).

To better understand the effects of drug dispersion state and particle size on the drug release kinetics, the dynamic changes in wet mass and pH were monitored. The wet mass changes reflected the solvent exchange kinetics: solvent leached into the release medium and water penetrated the implants. Dissolved dexamethasone ISI (100 % NMP) exhibited an increase in mass with time (Figure 3.31 b), indicating obvious water uptake. This was consistent with the morphology observation of the implant (Figure 3.32). Very importantly, the addition of 80 % hydrophobic triacetin to the implants lowered increase in mass, which was comparable with pure triacetin ISIs. The implant using triacetin as solvent decreased in mass from day 0 to day 2, which can be attributed to solvent diffusing into the release medium. From day 3 to day 12, the implant mass increased, which was due to more water penetration into the systems. The addition of the more hydrophilic solvent NMP generally increased the rate and extent of this increase in mass. This might eventually be attributable to altered polymer precipitation kinetics: the presence of hydrophilic NMP can be expected to facilitate water penetration into the system, leading to accelerated polymer precipitation and, hence, altered inner implant structures. This correlated with generally faster drug release from these ISIs. During these 12-day study, there was a rough ranking order concerning the effect of drug dispersion state and particle size on the rate and extent in the mass increase of the implants: dissolved > micronized > nanosized. This should be consistent with the observed drug release kinetics, but more drug was released at the lag phase by nanosized dexamethasone. These findings indicated micronized dexamethasone sedimented and heterogeneous distribution resulted in less diffuse out at lag

phase. After day 12, the pH of the release medium and the implant mass decreased significantly, indicating the quick degradation of PLGA (Figure 3.31 c). Furthermore, after day 22, the rate and extent in the mass decrease of the implants: micronized > nanosized, and the pH of release medium: nanosized > micronized. This confirmed the slower degradation of PLGA and explained the longer release at the quick release phase of nanosized dexamethasone ISI. We can conclude that selecting different drug dispersion state and particle size of ISIs can be effectively used to fine-tune their drug releases.



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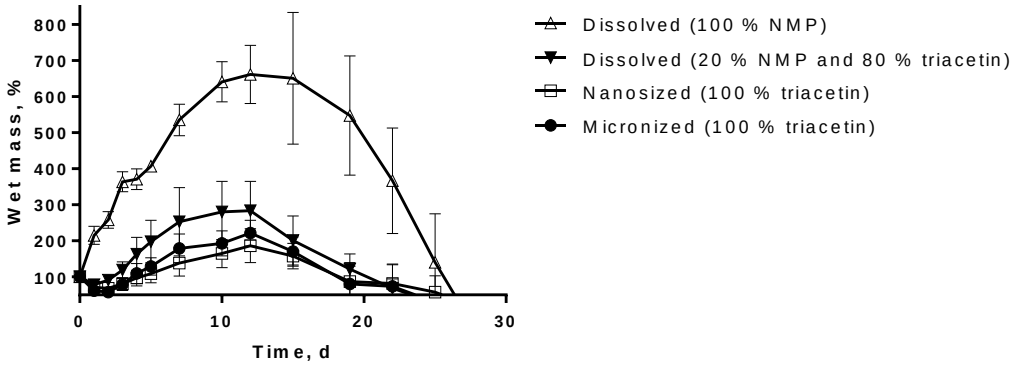

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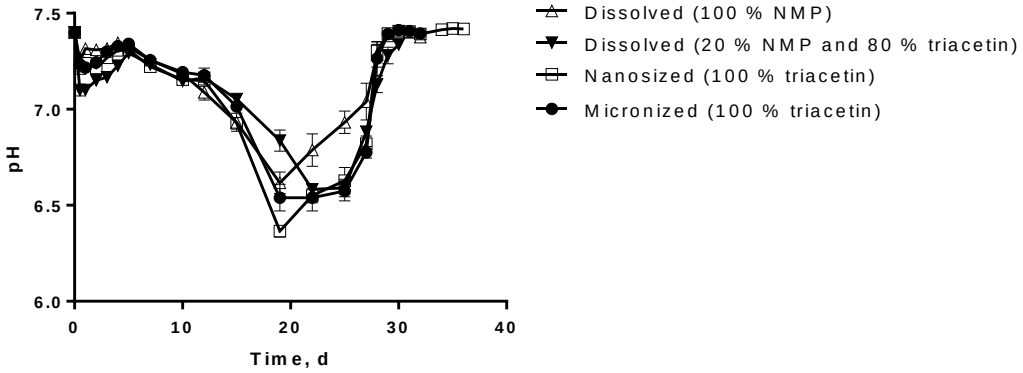


(c)

Figure 3.31. (a) *in vitro* release data, (b) dynamic changes of wet mass and (c) dynamic changes of pH of ISIs loaded with 10 % micronized, nanosized and dissolved dexamethasone.

Twenty-four hours after injection into the buffer medium, PLGA *in situ* forming implants solidified using the NMP as solvent (Figure 3.32). While only the surface of PLGA *in situ* forming implants was hardened by using triacetin as solvent on the first day. It was obvious that PLGA *in situ* forming implants solidification extent by using a mixture of NMP and triacetin (20:80) as solvent was in between pure

NMP as solvent and pure triacetin as solvent. As it can be seen, the investigated PLGA *in situ* forming implants swelled along with time. It has to be pointed out that the “top view” pictures and the “side view” pictures have combined to evaluate the swelling. When looking at the pictures obtained after 3-day incubation, the volume of these PLGA *in situ* forming implants were in the order of dissolved (100 % NMP) > dissolved (20 % NMP and 80 % triacetin) > micronized > nanosized, which was consistent with the solidification rates. It became obvious that NMP as solvent remarkably accelerated implant hardening and water uptake (due to porous structure facilitated water penetrated the implant) and nanosized drug slowed the implant swelling (due to high viscosity hindering water penetrated the implant). After incubation of the PLGA *in situ* forming implants in the buffer medium for 12 days, more transparent matrixes were observed for all samples, indicating the degradation of PLGA and reached the point of quick water uptake, mass erosion and drug release. Moreover, the extent of transparency was in the order of dissolved (100 % NMP) > dissolved (20 % NMP and 80 % triacetin) > micronized > nanosized, which was consistent with the drug release rates. From day 22 to 30, the PLGA *in situ* forming implants of micronized and dissolved dexamethasone disappeared indicating they were quickly eroded and degraded. In contrast, PLGA *in situ* forming implants obtained by using the triacetin as solvent and incorporating nanosized dexamethasone did show a significantly slower mass erosion after 22-day incubation.

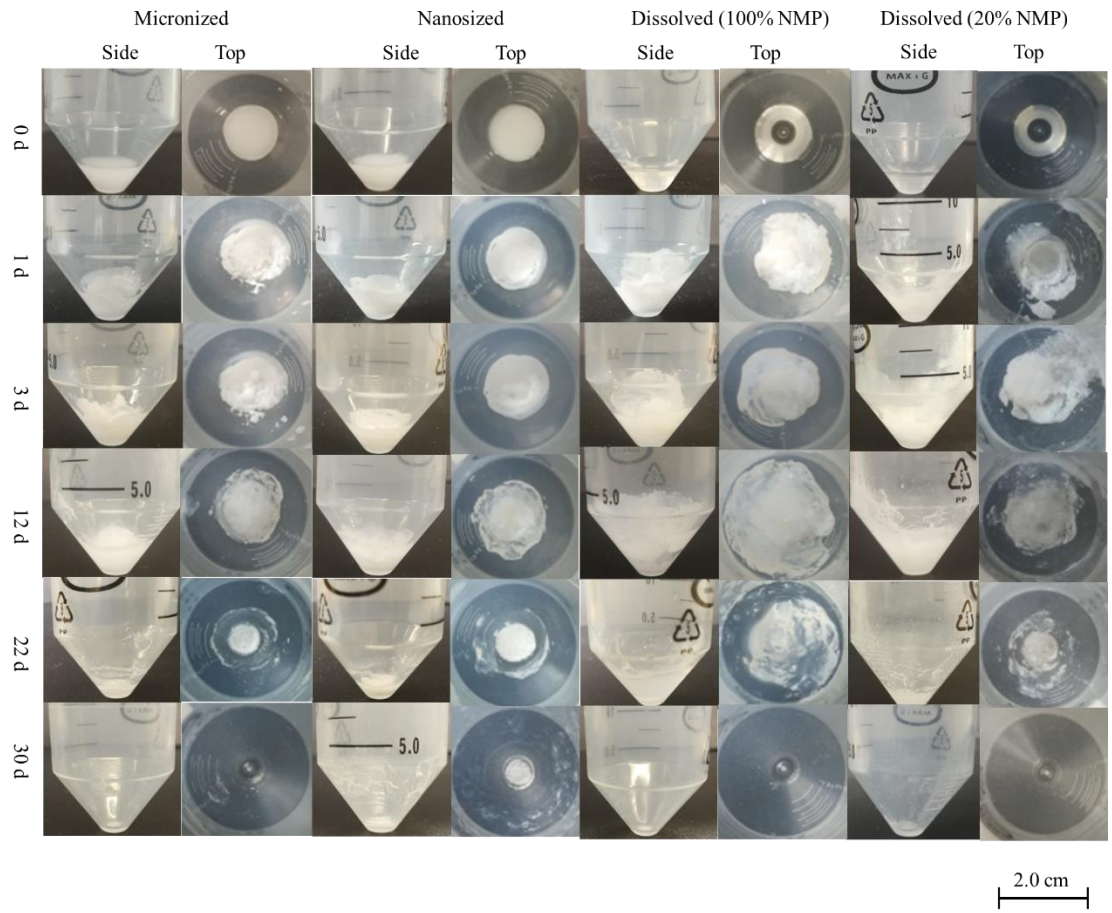
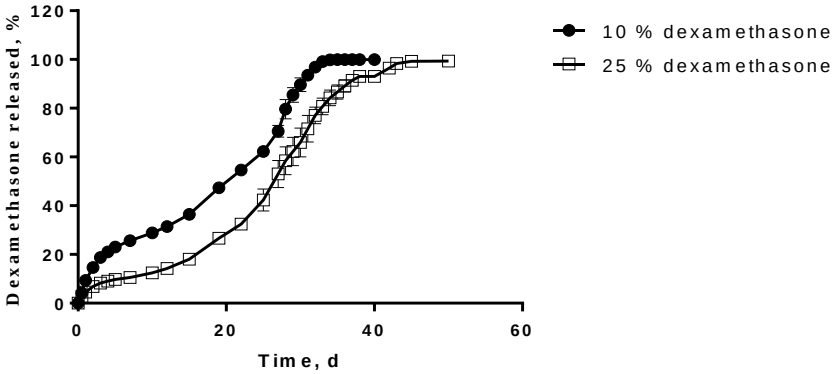


Figure 3.32. Optical macroscope pictures of the top and side views of *in situ* forming implants after different exposure times to the release medium: PLGA 502H *in situ* forming implant loaded with 10 % micronized dexamethasone, nanosized dexamethasone, dissolved dexamethasone (100 % NMP), and dissolved dexamethasone (20 % NMP and 80 % triacetin).

3.3.3.2. The effect of drug loading

The drug loading affected the drug release kinetics: increasing drug loading from 10 % to 25 % decreased the initial burst, extended the lag phase, and increased overall release time (Figure 3.33 a). This was partially attributed to the significant increase of viscosity from 0.933 to 2.257 Pa·s. Figure 3.33 b shows less triacetin diffused out from 25 % drug loading implant on the first day, less water penetrated in 25 % drug loading implant from day 1 to day 12, and slower mass loss in 25 % drug loading implant after day 12. The reason was that high viscosity system avoided the diffusing out of triacetin and penetrating of water, slowing the degradation of PLGA due to lack of water. The postponed PLGA degradation

caused a long release period. As shown by pH change (Figure 3.33 c), there are three phases: (1) no difference from day 0 to day 10, (2) larger pH decrease in 25 % drug loading implants from day 10 to day 18, (3) less pH decrease in 25 % drug loading implants after day 18. In phase 2, less water absorption surpassed the water exchange and then accelerated the degradation of the top layer of 25 % drug loading implant. However, the drug release was similar from day 10 to day 18. Although more PLGA degradation in 25 % drug loading, more water diffused in and water exchanged in 10 % drug loading implants, which also increased drug release. After day 18, water diffused into the implant became difficult in 25 % drug loading, resulting in slower PLGA degradation and a longer release period.



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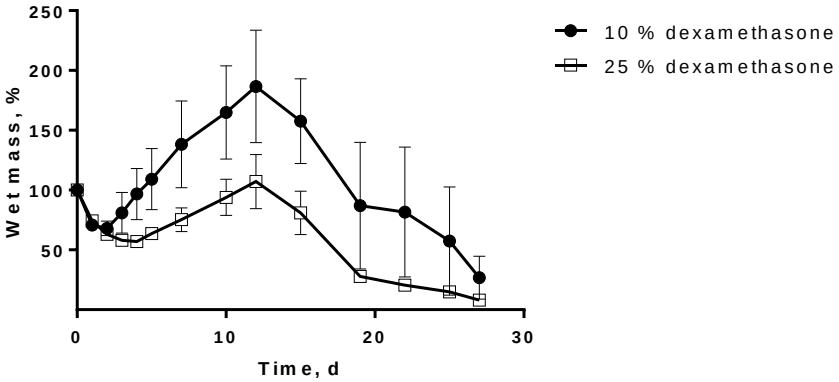

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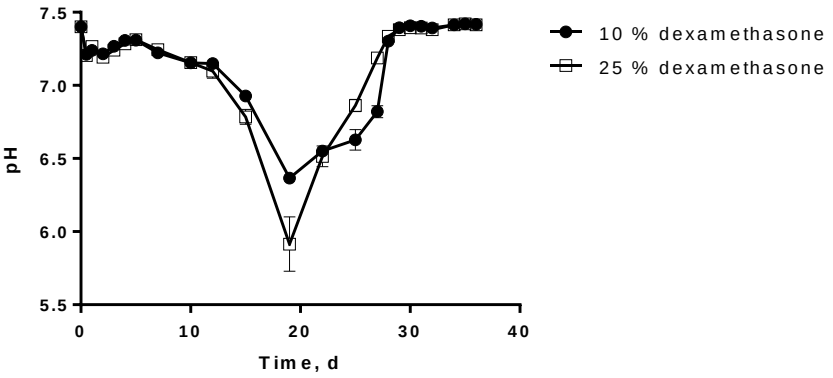


(c)

Figure 3.33. (a) *in vitro* release data, (b) dynamic changes of wet mass and (c) dynamic changes of pH of ISIs loaded with 10 % and 25 % nanosized dexamethasone.

When looking at the morphology pictures obtained after 3-day incubation, the volume of PLGA *in situ* forming implant loaded with 10 % nanosized dexamethasone was greater than 25 % (Figure 3.34). Increased nanosized dexamethasone content slowed the implant swelling (due to high viscosity hindering water penetrated the implant). After incubation for 12 days, implants

loading with 10 % nanosized dexamethasone were more transparent than 25 %, which was consistent with the drug release rates. At day 34, the PLGA *in situ* forming implant loaded with 25 % drug still existed and with 10 % drug disappeared. Increased nanosized dexamethasone content slowed mass erosion.

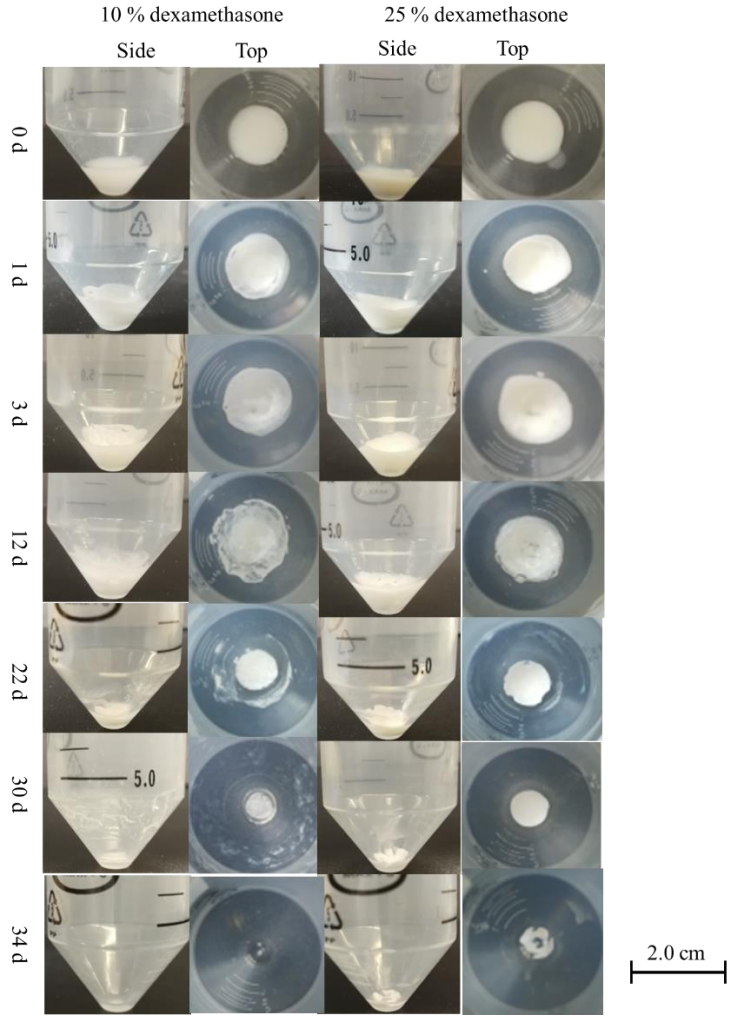


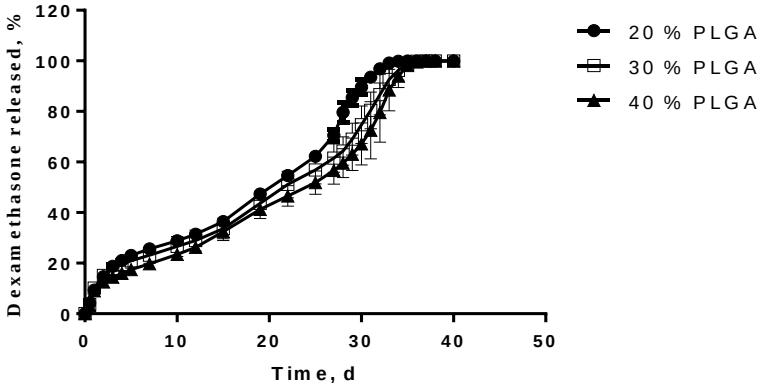
Figure 3.34. Optical macroscope pictures of the top and side views of *in situ* forming implants after different exposure times to the release medium: PLGA 502H *in situ* forming implant loaded with 10 % nanosized dexamethasone and 25 % nanosized dexamethasone.

3.3.3.3. The effect of PLGA concentration

As a potential tool to adjust drug release from PLGA ISIs, the impact of varying PLGA concentrations was studied. Specifically, these implants were based on 20 %, 30 % and 40 % PLGA 502 H at constant PLGA : dexamethasone ratio of 10:1 (w/w). PLGA concentration indeed played a crucial role in drug release

(Figure 3.35 a). Increasing the polymer concentration decreased the initial burst release. Higher concentrations of PLGA in the formulation tended to give higher solution viscosities, lower the implant network porosity and increase the structure density, thus decreasing the diffusion rate of triacetin and movement of drug particles into the release medium [29, 43]. This resulted in a smaller burst. After the burst, dexamethasone release also slightly decreased by adding more PLGA. PLGA precipitation started at the interface of formulation and release medium. Subsequent PLGA precipitation filled the ISIs. In the case of higher polymer concentrations, more polymer was available to fill the interior of the systems, resulting in denser and thicker shells. The denser and thicker the polymer shells caused longer diffusion pathways through the PLGA matrices to be overcome by the trapped drug. Thus, higher polymer concentrations in the formulations led to more barriers and, hence, slower drug release.

Further studies using wet weight and pH change can give a better understanding of mass transport processes. A higher initial PLGA concentration resulted in slower triacetin diffusion out and lower water penetration (Figure 3.35 b). Decreasing pH values of the release medium were indeed observed in all cases (Figure 3.35 c). At higher polymer concentrations, the “pH drop” was more pronounced than in the case of lower PLGA concentrations. This can probably be attributed to the fact that thicker polymer “shells” were created at high PLGA concentrations, resulting in more pronounced autocatalytic effects since the generated short chain acids more slowly diffused out and bases from the release medium more slowly diffused in, due to the longer diffusion pathways to be overcome. Interestingly, this faster PLGA degradation at higher polymer concentrations was not reflected in the drug release kinetics (Figure 3.35 a), demonstrating the dominance of the thickness of the PLGA shells (the lengths of the diffusion pathways through the polymeric matrices) in this case.



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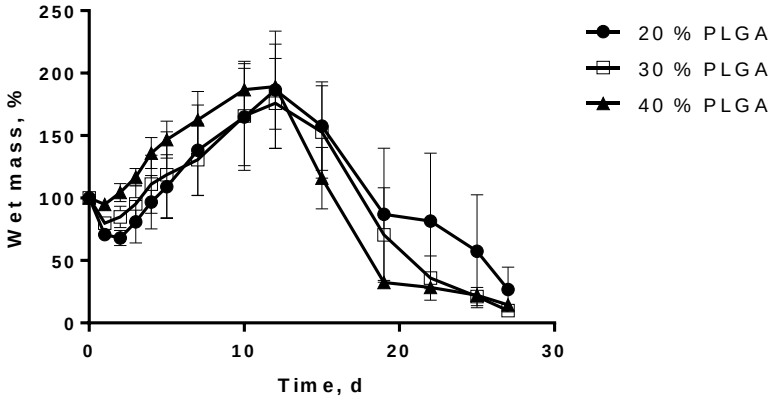

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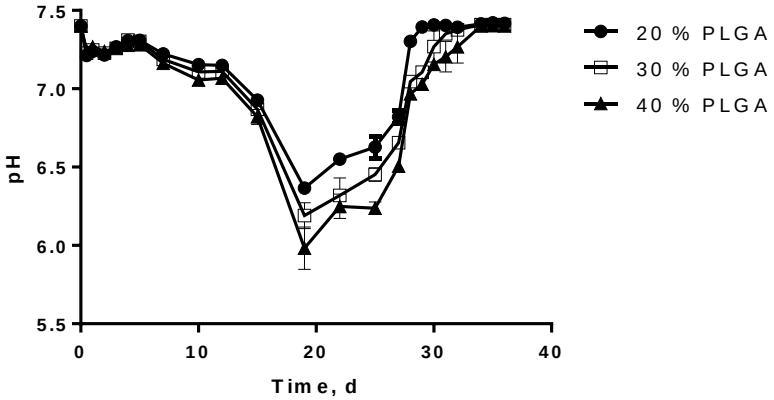


(c)

Figure 3.35. (a) *in vitro* release data, (b) dynamic changes of wet mass and (c) dynamic changes of pH of 20 %, 30 % and 40 % PLGA 502H ISIs loaded with 10 % nanosized dexamethasone.

After 3-day incubation, the volumes of these PLGA *in situ* forming implants were in the order of 20 % PLGA > 30 % PLGA > 40 % PLGA (Figure 3.36). It became obvious that increased PLGA concentration slowed the implant swelling (due to

high viscosity hindering water penetrated the implant). After incubation of the PLGA *in situ* forming implants in the buffer medium for 12 days, the extent of transparency was in the order of 20 % PLGA > 30 % PLGA > 40 % PLGA, which was consistent with the drug release rates. After day 30, the PLGA *in situ* forming implants still existed. Increased PLGA concentration slowed mass erosion. On day 34, 40 % and 30 % PLGA implants still can be observed, but 20 % PLGA implants were no longer visible.

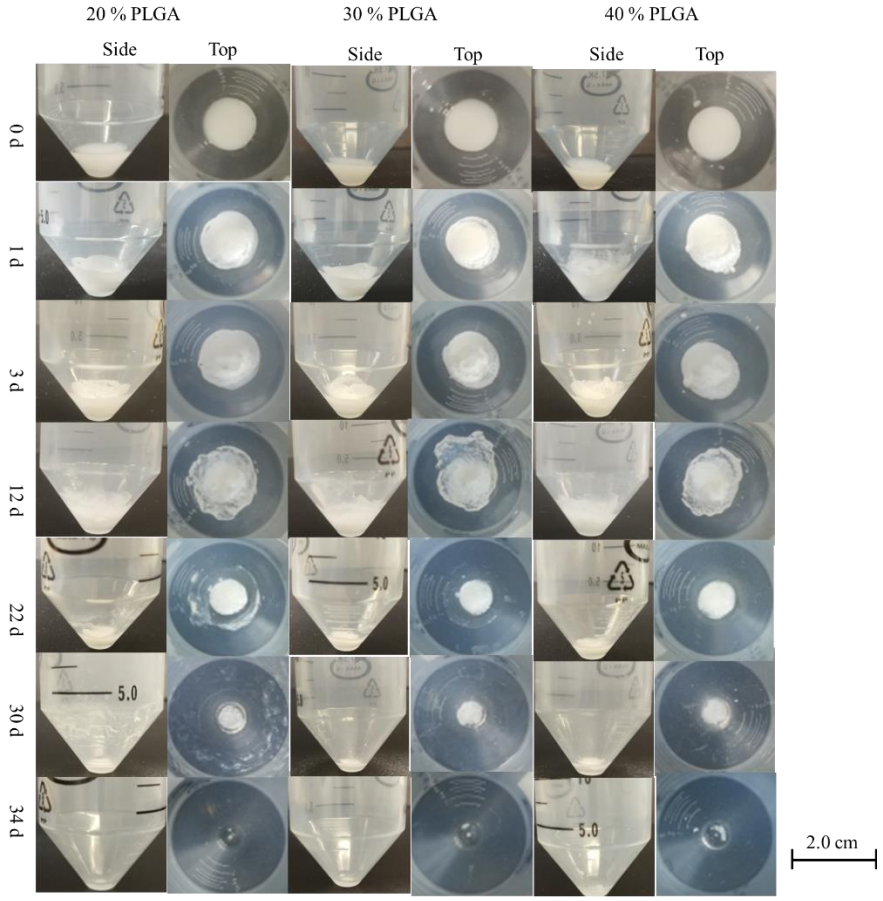


Figure 3.36. Optical macroscope pictures of the top and side views of *in situ* forming implants after different exposure times to the release medium: PLGA 502H *in situ* forming implant of 20 % PLGA, 30 % PLGA and 40 % PLGA.

3.3.3.4. The effect of formulation surface area

Microparticles were produced under the same protocol by varying mixing cycles. Emulsion formation by using two syringes, just before injection, obtained three groups of sizes: 29.1, 65.8 and 97.4 µm. Although ISMs have a similar trend of release profiles to the corresponding ISI (Figure 3.37). ISI released longer than

ISMs and microparticles with larger sizes had a more sustained release compared to the smaller size microparticles. Besides, a lower initial burst was also observed in ISI and 97.4 μm ISM. This observation was expected as these formulations have a smaller surface area to mass ratio and thicker polymer shells, which reduced the diffusion rate. This opens the opportunity to group the microparticles into categories of different release rates via size range discrimination [140]. Therefore, better control of drug release that is required for specific applications can be made [141].

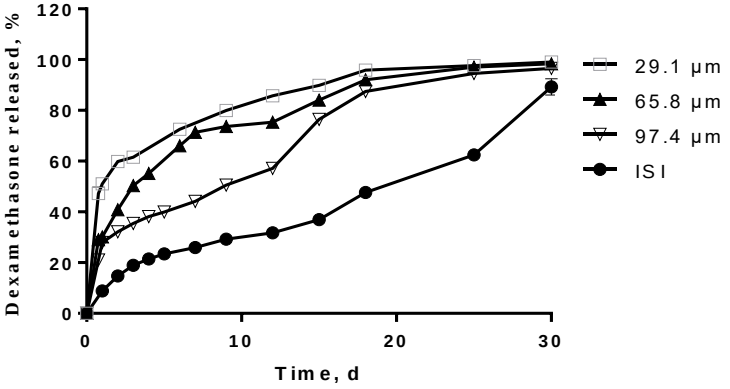


Figure 3.37. *In vitro* release data of dexamethasone from ISMs and ISI loaded with 10 % nanosized dexamethasone.

3.3.4. Conclusion

Compared to dissolved and micronized dexamethasone, the newly developed PLGA *in situ* forming systems incorporating nanosized dexamethasone had superior physical stability, were suitable for injection use, significantly reduced burst effect, released more drug release at lag phase, and extended overall release period. By varying the nanosized dexamethasone loading, PLGA concentration and formulation surface area, a designated release profile can be achieved. Future studies should investigate the *in vivo* performance of PLGA *in situ* forming systems incorporating nanosized drugs.

3.4. Preparation of porous PLGA microparticles containing dexamethasone by using nanosized/micronized sugar particles as porogen

3.4.1. Introduction

Porous microparticles have drawn great attention in the last two decades for their potential applications in many fields, such as drug delivery and tissue regeneration [41, 42, 56]. Low density and large specific surface area are the inherent characteristics of porous microparticles. These unique properties make them suitable as a carrier for pulmonary and gastro-retentive drug delivery. When compared with nonporous microparticles, drug encapsulation efficiency and release kinetics depend on the porosity of the microparticles [159-162]. Porosity generally refers to the presence of cavities on the surface and within the core of microparticles, which are usually interconnected. The number, diameter and structure of pores determine the unique properties of such microparticles.

The emulsion technique is the most popular way to prepare porous microparticles. PLGA microparticles are prepared by O/W emulsions in which droplets of an organic solvent containing PLGA are dispersed in the continuous water phase. Chemical additives can induce pore formation inside and/or at the surface of the microparticles. The use of effervescent agents such as ammonium bicarbonate create porosity through gas evolution within oil droplets [163-165]. Influx water can create porosity especially when double emulsion-solvent evaporation technique is used and the osmotic pressure differs between the internal and the external aqueous phase of the emulsion [5]. Chemical substances, such as sodium chloride, which increase osmotic pressure in the internal water phase have been widely reported to manufacture porous PLGA microparticles [59, 166]. Besides the formation of gas and difference in osmotic pressures in the emulsion method, the generation of porous structure can also be achieved by the encapsulation of a porogen. After solidification of PLGA, the porogen dissolves and leaches into a solvent which is a non-solvent for PLGA, resulting in a porous

structure [167]. The porogen leaching is straightforward in preparation, providing effective control of pore size and porosity by varying the size and the amount of the porogen. However, the required process of porogen extraction is connected with drawbacks and inherent limitations such as leaching with solvents facilitating diffusional mass exchange, which removes not only porogen but also encapsulated drug. Moreover, the porogen usually requires a long time to leach out completely. The lack of interconnectivity may also result in low porosity. Also, organic porogen materials may require the use of an organic solvent for extraction, with residual porogen as well as organic solvent resulting in biocompatibility issues. This is why commonly used porogens are inorganic salts like sodium chloride, sugars like sucrose, linear polymers like poloxamer [57, 168-170]. Salt and sugar particles are the most popular leaching porogens, not only due to their low cost but also the biocompatibility and the convenience to use water as a leaching medium. Although porous scaffolds/films were prepared using leaching of sodium chloride or sugar particles (100 - 250 µm) [169, 171-174], no data is available for preparation of injectable porous microparticles (smaller than 200 µm) using sodium chloride or sugar particles as porogen. This might be due to restrictions in manufacturing, such as small yield and irregular morphology due to procedures using spray drying or supercritical fluids or the slow and possibly incomplete leaching of the porogens, which can be associated with loss of active ingredient.

It has been reported that a solid-in-oil-in-water (S/O/W) emulsion system can also be used to prepare porous PLGA microparticles [175, 176], and the addition of the solid phase in the system was a key factor in forming the porous structure. Takai et. al fabricated porous PLGA microparticles via a S/O/W emulsion technique, where nanosized hydroxyapatite can be an effective emulsifier and stabilize the water drops in the oil phase [175]. They further proved that the addition of the solid phase in the system was a key factor to form the porous structure and concluded that high affinity between solid-polymer and polymer-medium, and low affinity between solid-medium were the best combinations to

obtain porous microparticles with a dense core and a porous layer [176]. However, these solid particles are water-insoluble and stayed inside the microparticles after preparation, which may affect the safety and *in vitro* drug release of microparticles. Many researchers found that porous microparticles could be formed when encapsulating water-soluble drugs by the S/O/W method [116, 125, 177, 178]. During the microparticle preparation, water diffused into the emulsion droplets and dissolved the drug particles. Thus, forming an inner water phase, resulting in the porosity of the microparticles. This process caused the low encapsulation efficiency of water-soluble drugs.

In this study, a simple “one-step” fabrication of porous PLGA microparticles with tunable morphology texture and *in vitro* drug release behaviors using sugar particles as fast-acting water-soluble porogen was investigated. The proposed “one-step” fabrication technique is a S/O/W method using nanosized/micronized sugar particles as porogen that do not require an additional porogen leaching step after solidification. Porous PLGA microparticles were produced using various sugar types, particle size ranges and weight fractions and different PLGA types. Physicochemical properties and drug release were characterized to evaluate the potential of nanosized/micronized sugar particles for manufacturing porous PLGA microparticles. It is hypothesized that nanosized/micronized sugar particles are promising porogen to produce highly tunable porous PLGA microparticles.

3.4.2. Characterization of micronized and nanosized sugars

Sucrose particle diameter was reduced by jet milling from over 20 µm of the raw powder to 2.8 µm (Figure 3.38). Further decrease of particle size via jet milling was limited by development of high electrostatic charges. Wet bead milling was used for nanosizing sugar particles on a small scale. Due to high aqueous solubility of sugars in water, dichloromethane was selected as wet bead milling medium. Sugars, like sucrose, trehalose, and lactose are highly insoluble in

dichloromethane (at 25 °C), which ensures that they are in suspension during milling [179, 180]. Wet bead milling of sugars in pure dichloromethane resulted in 400 nm sucrose crystals as proven using SEM, although strong agglomeration was present after solvent evaporation. PLGA can be used as a steric hindrance to stabilize this dichloromethane system avoiding strong agglomeration of sugar nanoparticles and enabling measurement with PCS. Around 400 nm particles with a low polydispersity index were obtained for sucrose, lactose and trehalose by non-aqueous wet bead milling (Table 3.11).

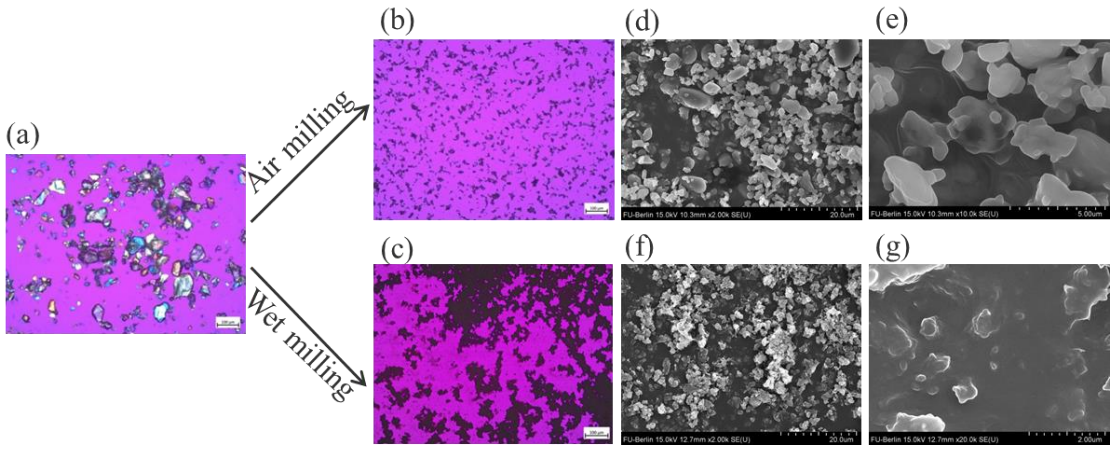


Figure 3.38. Microscopic pictures of (a) as received, (b) micronized and (c) nanosized sucrose; SEM pictures of (d and e) micronized and (f and g) nanosized sucrose.

Table 3.11. Particle size (z-average) and PDI of nanosized sugars.

Sugar	Particle size, nm	PDI
Sucrose	374.0 ± 37.2	0.12 ± 0.01
Lactose	205.5 ± 13.9	0.21 ± 0.04
Trehalose	366.0 ± 29.7	0.29 ± 0.05

3.4.3. Formation of porous PLGA microparticles

Porous PLGA structure can be formed traditionally by a sugar particles leaching process as it is templated from the percolated porogen network. The percolation can only be achieved at high porogen volume fractions and considering time

needed for complete porogen dissolution. So far, no studies reported to produce nanosized sucrose and use these nanosized sugars as porogen to obtain porous PLGA microparticles. In this paper, instead of traditional leaching method, PLGA microparticles were prepared by the S/O/W method using different content of nanosized sugar as porogen. SEM images of particle surface and cross-section indicated that the morphology of these microparticles is completely changed (Figure 3.39). The number of surface pores is increasing with increasing sugar content. The cross-sectional images further indicated that the microparticles consist of two parts which are (i) PLGA walls and (ii) cavities. This morphology is the so-called ‘‘porous’’ structure. The majority of internal cavities are however larger the 400 nm, i.e. larger than the nanosized sucrose particles. This observation suggested that internal cavities were not only formed by porogen leaching. Moreover, the surface of the microparticle contains only a limited number of pores even if the nanosized sucrose content reaches 30 %. This is another indication of the complex pore-forming process by the S/O/W method using nanosized sugar as porogen.

The formation of porous microparticles using S/O/W method can be roughly explained as follows (Figure 3.40). In the first step, a suspension of nanosized sucrose dispersed in PLGA dichloromethane solution was emulsified into water phase, forming a dispersed phase of embryonic oil droplets in an aqueous continuous phase. On contact of the embryonic dichloromethane droplets with the aqueous phase, the influx of water and concomitant outflux of dichloromethane caused phase separation into a PLGA-rich phase and a water-rich phase [18,19]. When more dichloromethane was extracted into the aqueous phase, the boundary of the droplet shrank rapidly at first [29]. As the dichloromethane was extracted, its concentration within the droplet decreased (less than 10 %, w/w) until PLGA precipitated out of solution and a hardened particle formed. During solvent removal, water-soluble sucrose was dissolved partially upon contact with influx water, resulting in increased water fluxed into the oil droplet (Figure 3.41), which was due to dissolved sucrose increasing the

osmotic pressure of the oil phase. Sufficient dichloromethane removal produced a porous skeletal structure of PLGA with porous channels filled with aqueous sucrose solution. In the second stage, the solidified microparticles were dispersed in water. Encapsulated sucrose particle was dissolved and leached out from hardened PLGA microparticles, leaving porous structures.

Indeed, there are some pores or channels in the nanometer range (Figure 3.39) that are similar to the size of nanosized sucrose, indicating some parts of microparticles can harden quickly and fix sucrose particles that were subsequently dissolved, washed out and leave pores or channels correspondingly. With increasing content of sucrose from 1 % to 30 %, the average diameter of large cavities increases from 1.8 to 8.8 μm, while that of surface pores remains constant around 0.4 to 1 μm but number of surface pores increases significantly (Figure 3.39). This was caused by an increase in the fractional volume of the sucrose within the emulsion droplets, which in turn imbibed more water. The number of nanosized pores at the surface was concomitantly increased due to the increased number of nanosized sucrose particles initially fixed at the hardened surface of PLGA microparticles and then washed away leaving more nanosized pores at the surface.

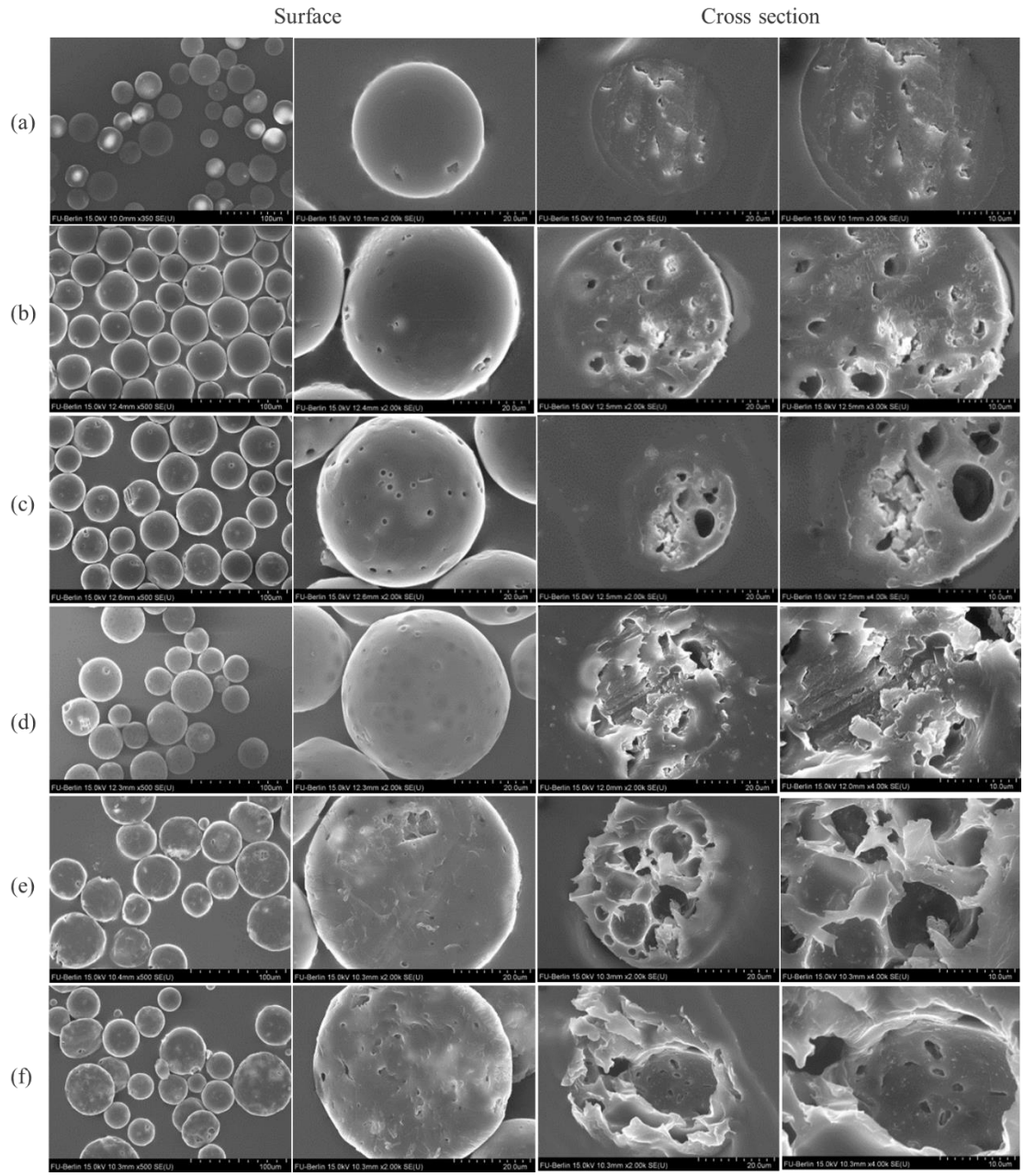


Figure 3.39. SEM pictures of surfaces and cross-sections of porous 10 % dexamethasone PLGA 503H microparticles prepared with (a) 0 %, (b) 1 %, (c) 2.5 %, (d) 5 %, (e) 10 % and (f) 30 % nanosized sucrose as porogen.

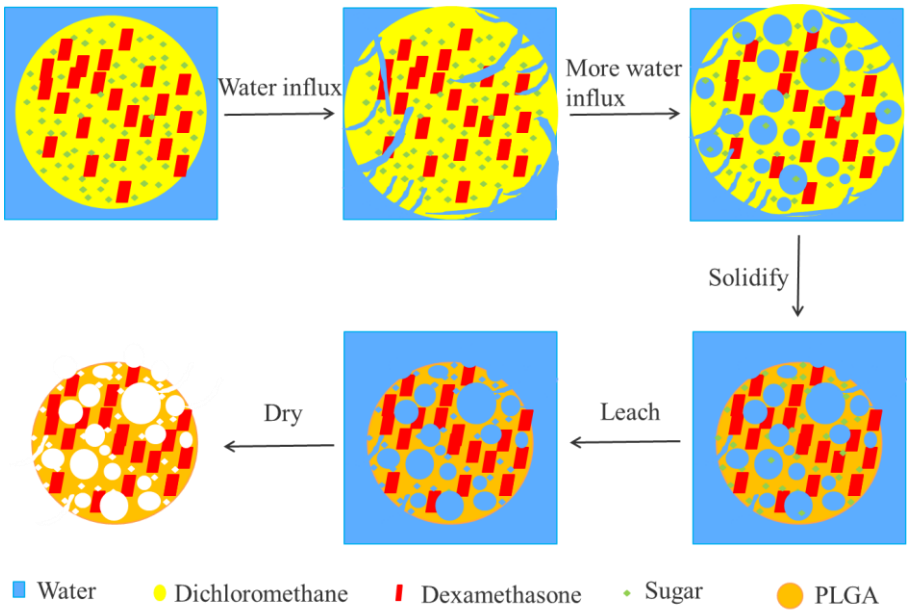


Figure 3.40. Schematic representation of droplet dynamics during the formation process of porous PLGA microparticles using nanosized/micronized sugar particles as porogen.

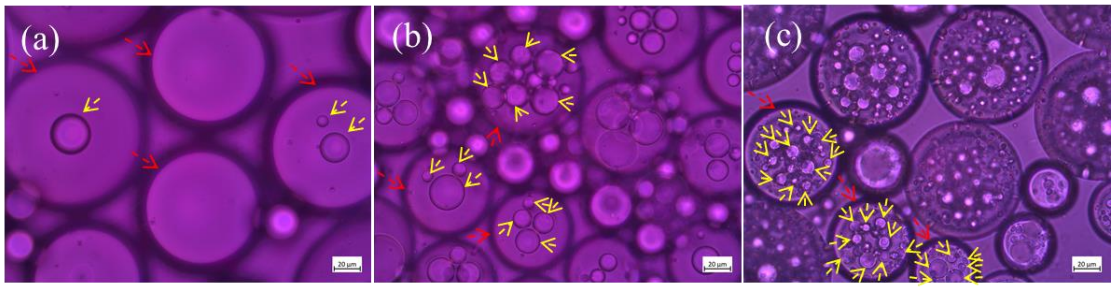


Figure 3.41. Microscopic images of dichloromethane emulions with (a) 0 % sucrose, (b) 2.5 % micronized sucrosse and (c) 2.5 % nanosized sucrose; red arrow: oil droplet; yellow arrow: water droplet.

Although nanosized sucrose particles were expected to wash away during manufacturing due to their high solubility and increased dissolution rate (increased surface area by reduced particle size), there was the possibility of residual sugars inside the PLGA microparticles. DSC of the physical mixture indicated 1 % sucrose still has an obvious melting peak at 185 °C (Figure 3.42 b). However, this melting peak of nanosized sucrose is not observable in porous PLGA microparticles, indicating that the porogen was removed completely or below the limit of detection of DSC. The evaporation process and the following

washing process may provide enough time for all sucrose particles to dissolve and diffuse out the PLGA microparticles. It is also worth noting that dexamethasone melting peak at around 190 – 200 °C is evident within PLGA microparticles using nanosized sucrose as porogen, showing that no impact was made on the incorporated drug (Figure 3.42 d).

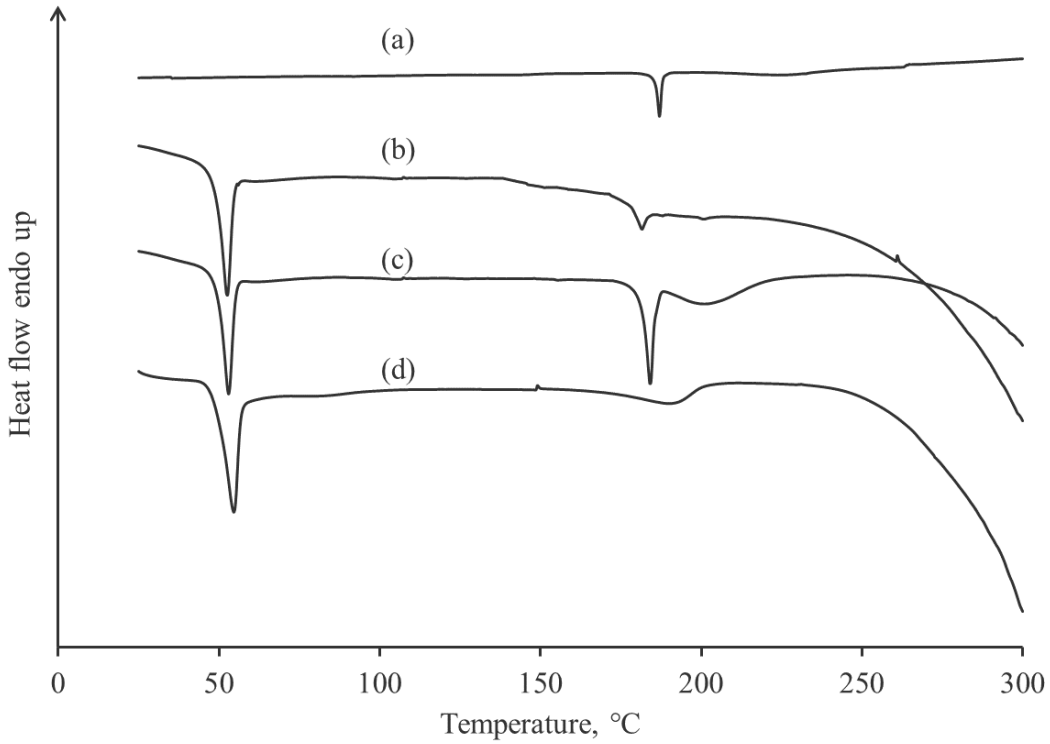


Figure 3.42. DSC thermograms of (a) nanosized sucrose, (b) physical mixture of nanosized sucrose and PLGA 503H (1:99), (c) physical mixture of nanosized sucrose, dexamethasone and PLGA 503H (5:10:85) and (d) 10 % dexamethasone PLGA 503H microparticles using 5 % nanosized sucrose as porogen.

3.4.4. Effect of particle size of sucrose

To know how the particle size of sucrose impacts the PLGA microparticles as porogen, sucrose powder was air milled from more than 20 µm to 2.8 µm (Figure 3.37) and different contents of micronized sucrose were used as porogen to prepare porous PLGA microparticles.

Porous PLGA microparticles were prepared with either micronized 2.8 µm sucrose (Figure 3.43) or nanosized 400 nm sucrose (Figure 3.39) as porogen to

investigate particle size effects. For both, increasing sugar concentration resulted in increased number of pores at the surface as well as inner channels and cavities. When closely comparing the porous structure of microparticles prepared by micronized and nanosized sucrose, there are some clear differences. With similar sucrose content, micronized sucrose resulted first in larger size and decreased number of surface pores and second in a less porous internal structure. At a fixed sucrose content of 10 %, increasing the particle size of sucrose from 400 nm to 2.8 µm, the average diameter of surface pores increases from around 400 nm to 3 µm with the latter one also having significantly fewer surface pores. In contrast, the average internal cavity diameter decreases from 7.1 to 4.3 µm. The diameter of the surface pores is enlarged according to increased sucrose particle size, due to bigger sucrose particles being fixed and larger pores left after leaching [21]. Due to the decreased number of micronized sucrose particles, the surface pore number decreases correspondingly. The decrease in size of cavities from increasing sucrose particle size was probably caused by the reduced dissolution rate of the sucrose, resulting in less influx water (Figure 3.41) and then delayed solidification of the PLGA microparticles. In sum, the cavity and pore diameters can be controlled by adjusting the particle size of sucrose.

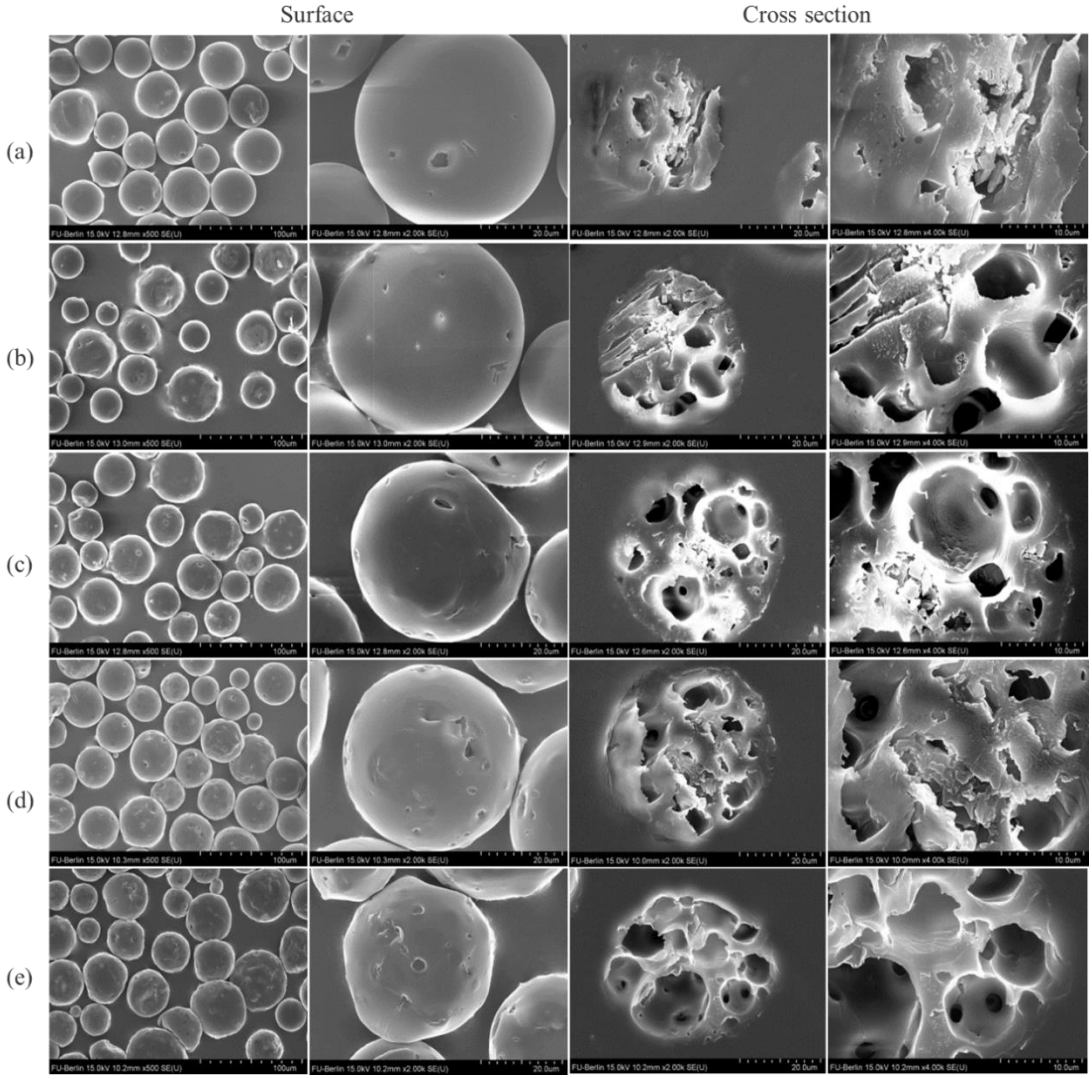


Figure 3.43. SEM pictures of surfaces and cross-sections of porous 10 % dexamethasone PLGA 503H microparticles prepared with (a) 1 %, (b) 2.5 %, (c) 5 %, (d) 10 % and (e) 30 % micronized sucrose as porogen.

The actual drug loading of microparticles decreased with increasing amount of sucrose, independent of sucrose particle size (Table 3.12). Because of the associated increase of porosity, dexamethasone had a higher chance to be in contact with water phase during manufacturing process and was therefore at greater risk of dissolving and leaching.

Table 3.12. Particle size, actual drug loading and surface area of investigated dexamethasone PLGA 503H microparticles.

Sucrose		Mean particle size, µm	Span*	Actual drug loading, %	Surface area, m^2/g
Size	Concentration, %				
Nanosized	0	33.5 ± 2.1	0.43 ± 0.01	9.7 ± 0.1	0.65 ± 0.10
	1	36.4 ± 1.9	0.43 ± 0.02	9.4 ± 0.1	0.84 ± 0.13
	2.5	38.4 ± 3.7	0.44 ± 0.04	8.7 ± 0.2	2.18 ± 0.17
	5	36.5 ± 1.5	0.52 ± 0.07	8.9 ± 0.1	2.90 ± 0.21
	10	30.3 ± 0.4	1.08 ± 0.15	9.0 ± 0.1	4.94 ± 0.39
	30	28.4 ± 0.3	1.27 ± 0.21	7.6 ± 0.2	16.98 ± 0.74
Micronized	1	33.0 ± 0.8	0.55 ± 0.09	9.8 ± 0.1	0.79 ± 0.07
	2.5	31.8 ± 1.2	0.54 ± 0.10	9.8 ± 0.0	1.33 ± 0.12
	5	32.1 ± 1.2	0.51 ± 0.05	9.5 ± 0.2	1.77 ± 0.20
	10	33.3 ± 2.2	0.57 ± 0.03	8.9 ± 0.1	3.15 ± 0.37
	30	33.5 ± 3.1	0.54 ± 0.04	7.4 ± 0.1	11.23 ± 0.69

*Span = (D90 – D10)/D50

Specific surface areas were determined for obtained PLGA microparticles at 25 °C. The specific surface area for the PLGA microparticles without using sucrose as porogen is 0.651 m^2/g. Microparticles with nanosized sucrose as porogen have a specific surface area between 0.84 and 16.98 m^2/g when sucrose content increases from 1 % to 30 %. Microparticles with micronized sucrose as porogen have a specific surface area ranging from 0.79 m^2/g to 11.23 m^2/g when sucrose content increases from 1 % to 30 %. Specific surface area, as an indicator for porosity, is increasing linearly with increasing content of micronized or nanosized sucrose as porogen (Figure 3.44). The measured increase in surface area is consistent with the results seen in the SEM pictures showing a more porous structure at higher sucrose contents. Microparticles produced with micronized sucrose as porogen have a lower specific surface area in accordance with the less porous structure as seen in the SEM pictures.

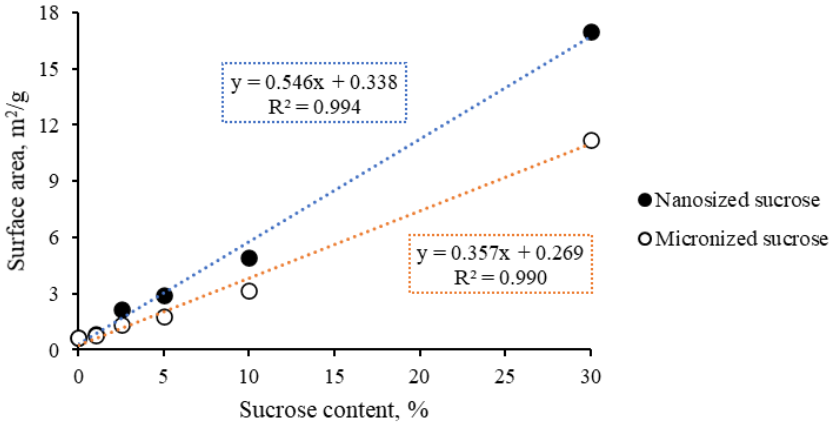
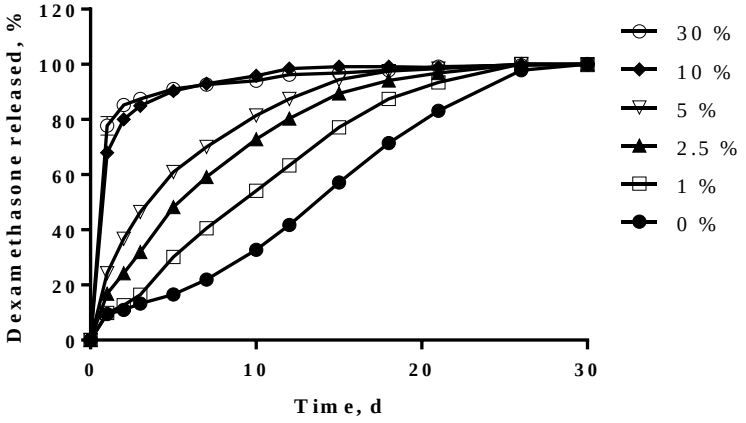


Figure 3.44. Correlation between micronized/nanosized sucrose content as porogen and PLGA microparticle surface area; Linear regression results are from the microparticles either using nanosized sucrose (blue line) or micronized sucrose (orange line) as porogen.

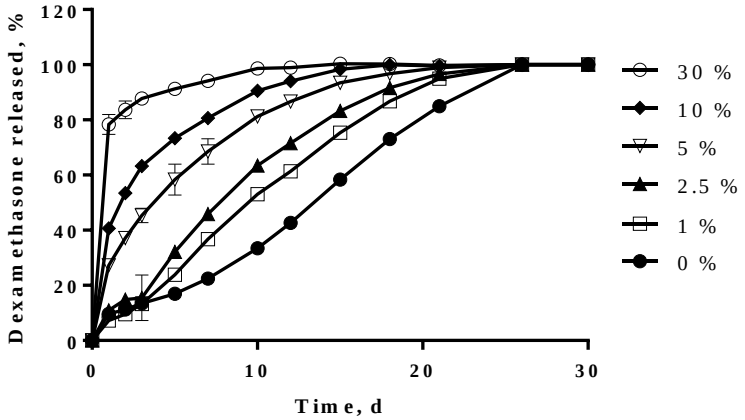
In vitro release studies were conducted to investigate if these porous structures can control the release of encapsulated dexamethasone. The results demonstrated a distinction between the dexamethasone release kinetics from nonporous and porous microparticles (Figure 3.45 a and b). Generally, for similar particle sizes, drug was released faster from porous than from nonporous microparticles, most likely due to the decreased diffusion distance through the polymer. Porosity caused by the introduction of nanosized/micronized sucrose into the systems, modified the release profiles significantly, which include the burst release, lag phase and final quick-release phase.

Above 2.5 % nanosized sucrose, burst release increased with increasing sucrose content, because dexamethasone had more direct access to the release medium due to increased porosity. Lag phase was eliminated or at least characterized by significant drug release at this stage due to the uniform pores created by nanosized sucrose. Less drug was released in those systems during final release due to increased amount of already released drug during previous two phases. Nanosized sucrose can be used to form porous microparticles and to modify the entire release profile even to a linear release. When introduced micronized sucrose instead of nanosized sucrose, the release profiles were also modified

significantly, which was also due to a porous structure formed. However, the release profile adjustment by using micronized sucrose was not as significant as by using nanosized sucrose, because fewer pores and cavities were generated by larger sucrose particles, resulting in smaller specific surface areas. Indeed, there are linear correlations between surface area of porous PLGA microparticles and drug released at burst phase or both burst and lag phase (Figure 3.45 c and d). Below 5 m^2/g the surface area is directly proportional to drug release (either burst phase or both burst and lag phase) with the coefficient of determinations around 0.9. However, when the surface area is larger than 5 m^2/g, the majority of dexamethasone (around 80 %) was released during the burst phase, so that no linear correlation of surface area with drug release is possible above this threshold.



(a)



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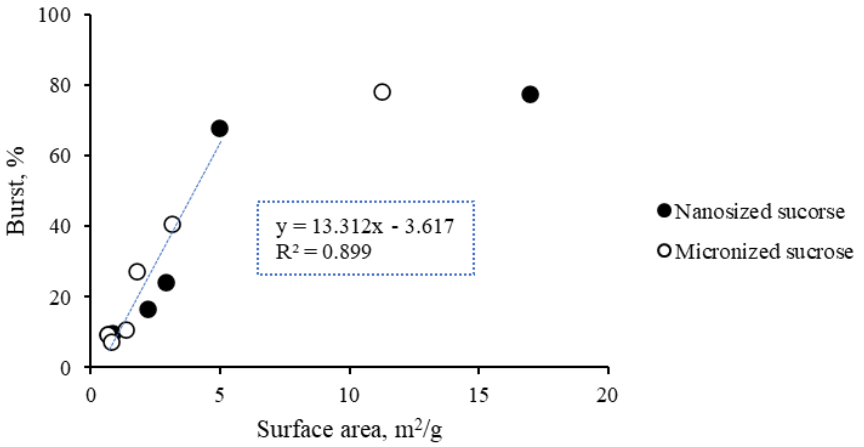

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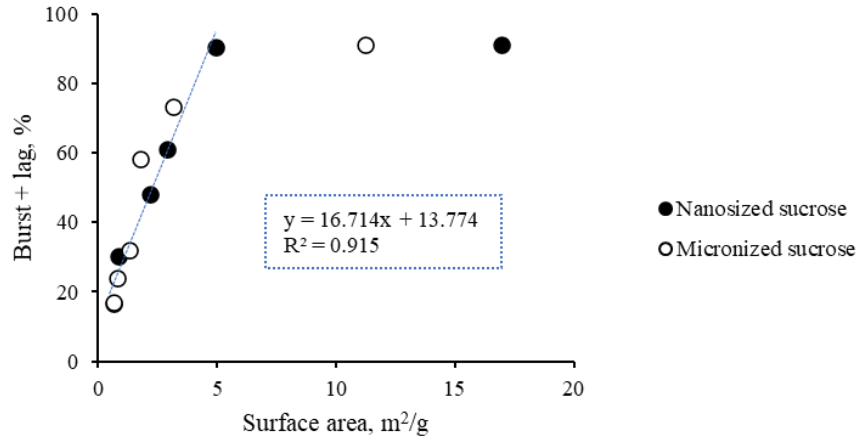
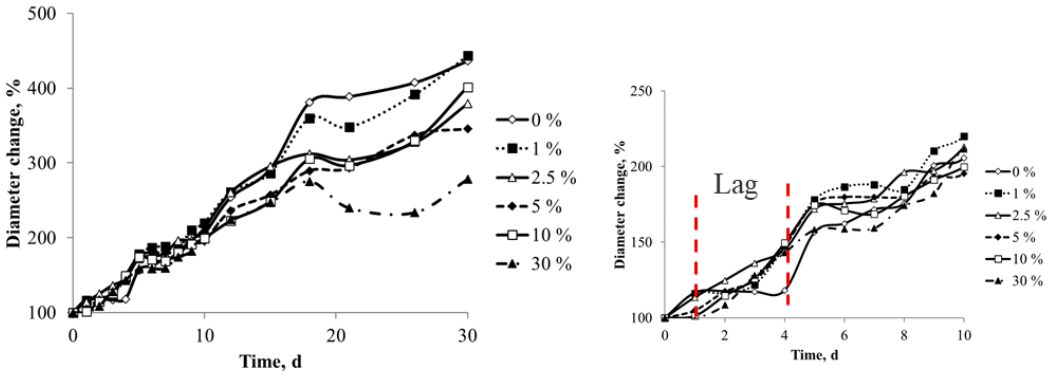


(d)

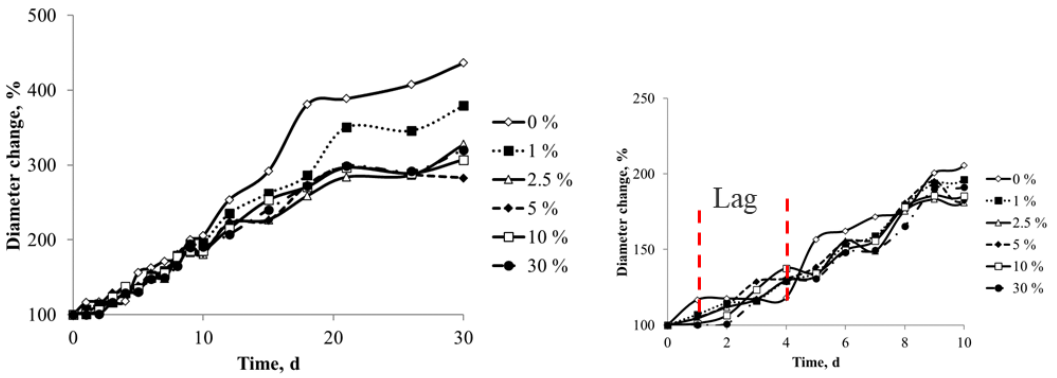
Figure 3.45. *In vitro* release studies of 10 % dexamethasone PLGA 503H microparticles using (a) nanosized and (b) micronized sucrose as porogen; Correlation between PLGA microparticle surface area and percentage of dexamethasone released at (c) burst phase (1 day) or (d) both burst and lag phase (5 days); Linear regression results in (c) and (d) are from the microparticles surface area less than 5 m^2/g no matter of using nanosized sucrose or micronized sucrose as porogen.

To further understand the *in vitro* release of porous PLGA microparticles, dynamic changes in the diameter of single PLGA 503H microparticles were monitored. Interestingly, it was found that the diameter changes correlated well with release profiles (Figure 3.46 and 3.47). During the burst release (the first day), the diameter of microparticles prepared by less sucrose amount increased more, which was due to less sucrose resulted in a denser structure. The burst release was from the diffusion of drug and minor swelling of microparticles. A more porous structure formed by a higher amount of nanosized sucrose resulted in more direct access of dexamethasone to the release medium. During the lag phase (1 - 4

days), the diameter of porous microparticles produced using nanosized sucrose as porogen increased significantly, while the diameter of microparticles produced without porogen remained constant. The swelling was caused by the fragile structure of porous microparticles and resulted in more drug release over the lag phase. During 5 - 12 days, the diameter of all microparticles increased significantly, the quick release happened at this period and release rates were similar. After 12 days, diameter changes were more significant for microparticles without or with less nanosized sucrose, which was due to fewer pores and channels resulting in denser structures. Diameter changes of microparticles prepared with micronized sucrose were not so significant during 1 – 4 days, which was due to fewer channels and thicker PLGA walls. This is consistent with the results of *in vitro* release, where less drug was released during the lag phase of porous PLGA microparticles prepared using micronized sucrose as porogen.



(a)



(b)

Figure 3.46. Dynamic changes in the diameter of single PLGA 503H microparticles loaded with 10 % dexamethasone using (a) nanosized and (b) micronized sucrose as porogen upon exposure to phosphate buffer pH 7.4; Magnifications of first 10 days show on the right.

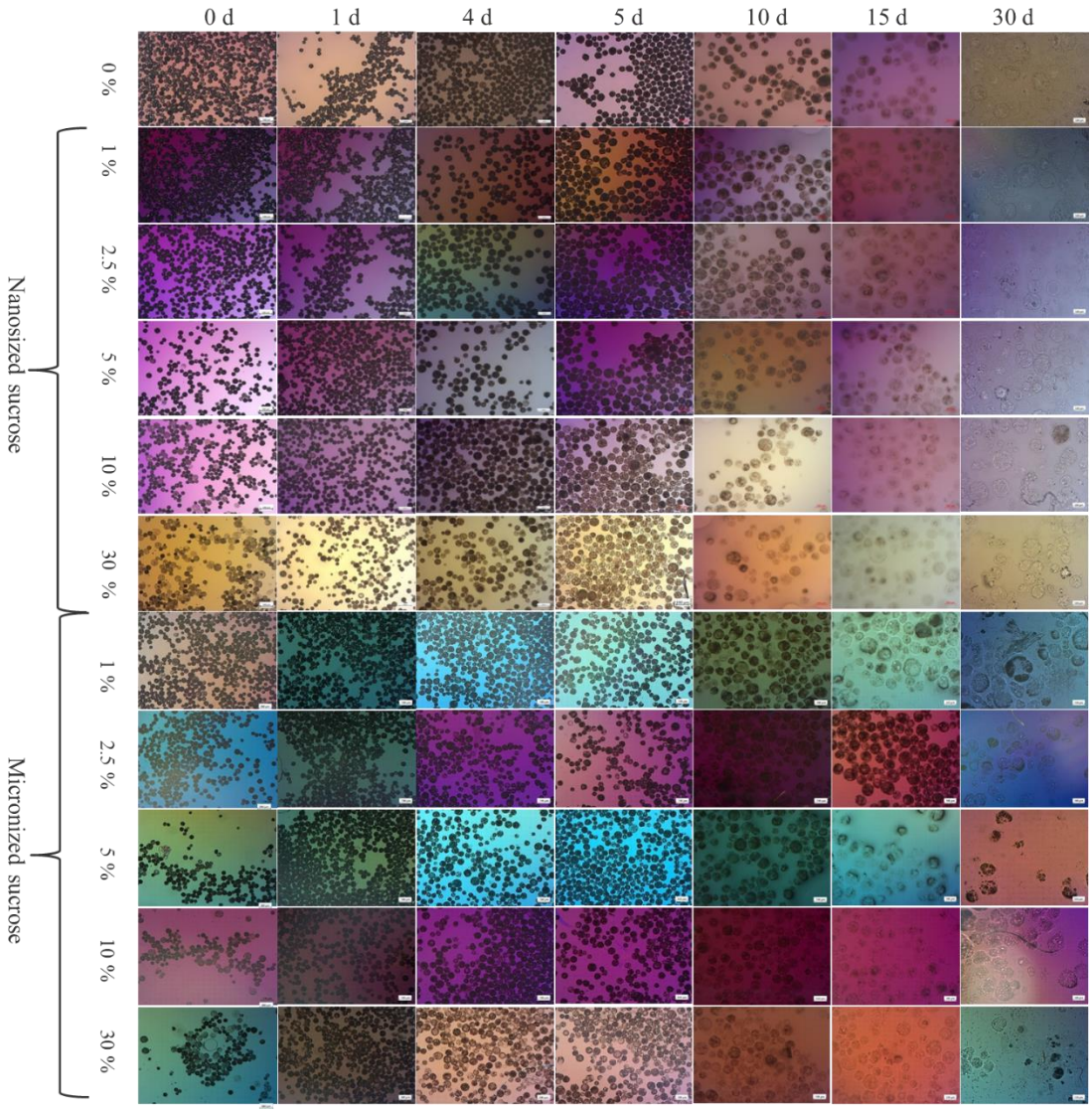


Figure 4.47. Microscopic images of PLGA 503H microparticles loaded with 10 % dexamethasone using nanosized/micronized sucrose as porogen upon exposure to phosphate buffer pH 7.4.

3.4.5. Effect of sugar types and PLGA types

To understand the universal usage of sugar particles, nanosized lactose and trehalose were prepared and used as porogen to manufacture 10 % dexamethasone PLGA 503H microparticles. Furthermore, to extend the release period, equivalent microparticles were produced using more hydrophobic PLGA 502 and PLGA 752S.

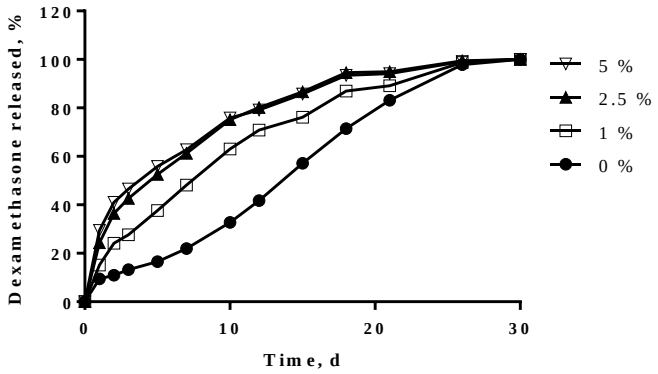
Using nanosized lactose and trehalose to replace nanosized sucrose as porogen

can also modify the *in vitro* release from PLGA microparticles by adjusting their amount (Figure 3.48). Specifically, 1 % nanosized lactose, and 1 % - 2.5 % nanosized trehalose resulted in a linear drug release from 10 % dexamethasone PLGA 503H microparticles. Although nanosized lactose and trehalose were as effective as nanosized sucrose to be fast dissolving porogens and resulted in comparable drug encapsulation efficiencies (Table 3.12 and 3.13), the resulting *in vitro* release profiles were different even with the same sugar content and similar particle size. More investigations are required to identify whether the sugars have different dissolution rates, osmotic pressures and interactions with PLGA, and how these properties could affect the porous structure and drug release.

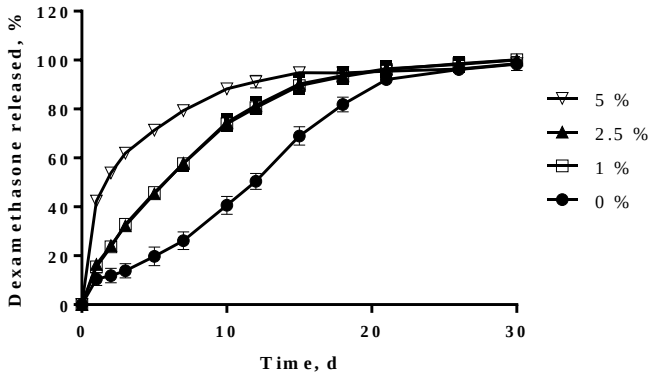
Table 3.13. Particle size and actual drug loading of investigated microparticles.

Formulation		Mean particle size, μm	Span*	Actual drug loading, %
PLGA	Nanosized sugar			
502	0 % sucrose	30.8 ± 1.2	0.31 ± 0.02	7.8 ± 0.2
	0.2 % sucrose	30.9 ± 0.4	0.30 ± 0.01	7.4 ± 0.3
	0.5 % sucrose	32.0 ± 1.5	0.49 ± 0.04	7.0 ± 0.1
	1 % sucrose	32.6 ± 0.7	0.57 ± 0.11	6.7 ± 0.0
	2.5 % sucrose	30.7 ± 0.2	0.46 ± 0.06	6.4 ± 0.0
	5 % sucrose	30.7 ± 0.4	0.61 ± 0.07	5.6 ± 0.2
	10 % sucrose	32.7 ± 0.6	0.58 ± 0.03	3.7 ± 0.1
752S	0 % sucrose	28.8 ± 0.1	0.49 ± 0.02	8.4 ± 0.0
	0.2 % sucrose	26.7 ± 2.4	0.55 ± 0.10	7.1 ± 0.1
	0.5 % sucrose	29.4 ± 0.2	0.42 ± 0.08	6.3 ± 0.1
	1 % sucrose	23.4 ± 1.4	0.63 ± 0.09	5.7 ± 0.2
	2.5 % sucrose	29.8 ± 0.2	0.44 ± 0.02	5.4 ± 0.2
	5 % sucrose	30.3 ± 0.1	0.47 ± 0.02	4.4 ± 0.0
	10 % sucrose	27.6 ± 0.9	0.57 ± 0.05	3.2 ± 0.1
503H	0 % lactose	33.5 ± 0.4	0.43 ± 0.03	9.7 ± 0.1
	1 % lactose	37.7 ± 0.2	0.52 ± 0.02	8.9 ± 0.0
	2.5 % lactose	40.2 ± 1.4	0.51 ± 0.06	8.5 ± 0.2
	5 % lactose	37.0 ± 1.1	0.54 ± 0.06	8.8 ± 0.3
503H	1 % trehalose	31.6 ± 0.7	0.36 ± 0.04	9.4 ± 0.1
	2.5 % trehalose	32.5 ± 0.3	0.54 ± 0.01	9.0 ± 0.2
	5 % trehalose	31.9 ± 1.5	0.62 ± 0.07	8.9 ± 0.1

*Span = (D90 – D10)/D50



(a)



(b)

Figure 3.48. *In vitro* release studies of 10 % dexamethasone PLGA 503H microparticles using nanosized (a) lactose and (b) trehalose as porogen.

To extend the release period, different PLGA types were used to prepare porous PLGA microparticles. However, a pronounced burst was observed especially with increasing porogen amount (Figure 3.49). Moreover, in contrast to PLGA 503H, no linear release was obtained by using PLGA 502 and 752S. However, these results indicated that small amounts of nanosized sucrose can be used to adjust the burst release of PLGA 502 and 752S microparticles. While a theoretical loading of 10 % dexamethasone was targeted, the actual drug loading decreased from 8.4 % to 3.2 %, if the amount of nanosized sucrose content was increased from 0 - 10 % (Table 3.13). The rationale for the high burst and low encapsulation efficiency was based on fundamental mass transport theory as applied to microdroplet dynamics encapsulating dexamethasone. Affinities among drug, polymer, water and dichloromethane in the S/O/W system have been considered using various phase systems. Dexamethasone particles floated or aggregated in

dichloromethane when there was no mixing. Because dichloromethane has a low polarity (relative permittivity of 8.4), it works as a poor dispersing medium for dexamethasone which is slightly hydrophilic. Interestingly, dexamethasone dispersibility has been improved when PLGA 503H was dissolved in dichloromethane. It can be thought that PLGA worked as good dispersant by adsorbing at the dexamethasone surface due to the polar and hydrophobic functional groups PLGA 503H deposited between dichloromethane and apolar drug particle surface [20]. With the carboxyl end group of PLGA 503H, the hydrophilic and hydrophobic differences became more pronounced, which caused enhanced stabilization. However, the carboxyl ends of PLGA 502 and 752S have been esterified, and the stabilization effect of these PLGAs decreased significantly. When water fluxed into the embryonic dichloromethane droplets, more dexamethasone particles deposited at the surface of water and dichloromethane, caused by the poor stabilization effect of PLGA 502 and 752S. These dexamethasone particles were close to the surface of pores and channels, which were easier to wash out during manufacturing resulting in lower encapsulation efficiency and diffuse out during *in vitro* release leading to high burst release. This theory is consistent with the morphology observed by SEM (Figure 3.50). It is obvious that PLGA 502 microparticles have more cavities at the surface, more broken microparticles and irregular channels compared with PLGA 503H microparticles at constant sucrose content. These observations indicated PLGA 503H had a better stabilization effect on systems of drug, polymer, water and dichloromethane than PLGA 502.

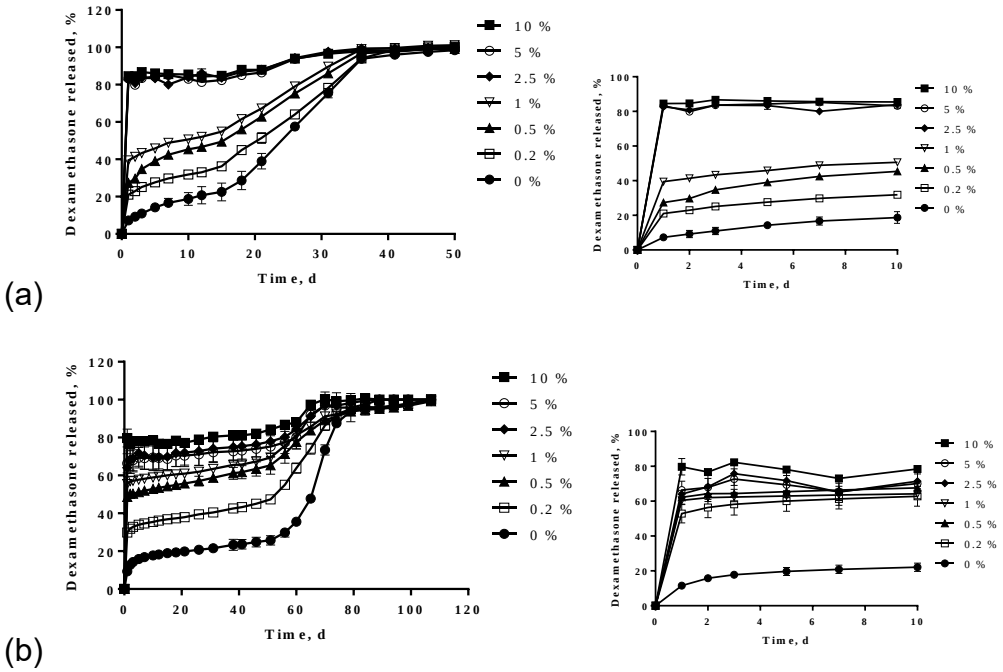


Figure 3.49. *In vitro* release studies of 10 % dexamethasone (a) PLGA 502 and (b) PLGA 752S microparticles using nanosized sucrose as porogen; Magnifications of first 10 days show on the right.

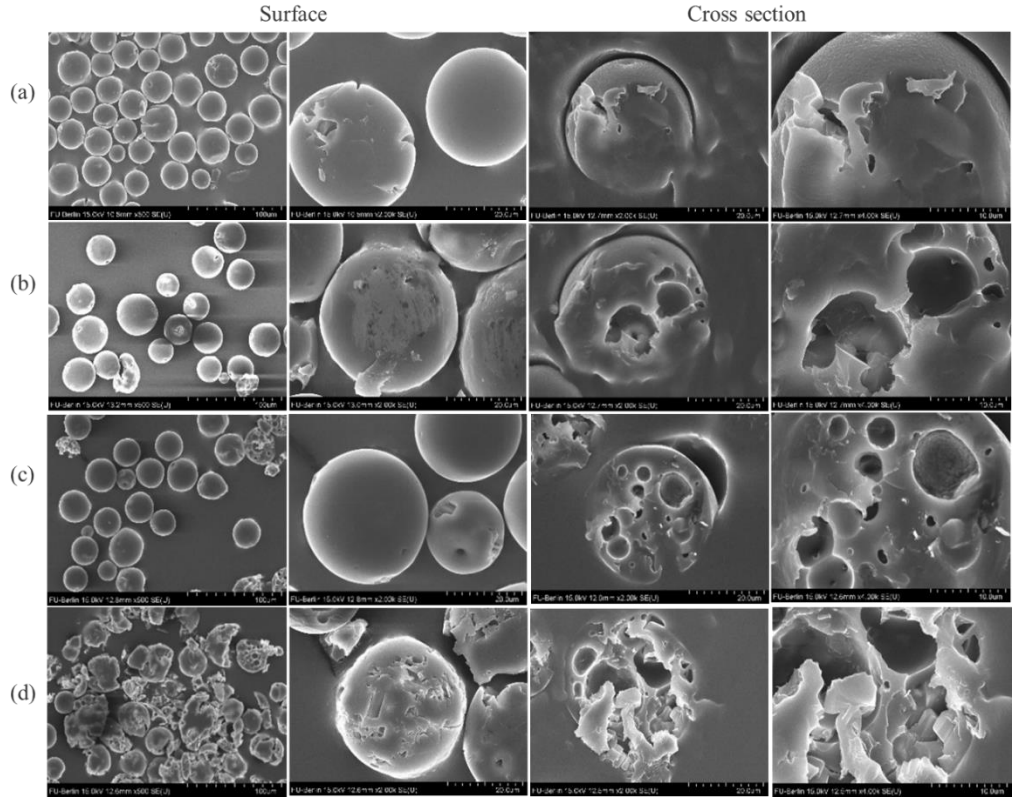


Figure 3.50. SEM pictures of surfaces and cross-sections of porous 10 % dexamethasone PLGA 502 microparticles prepared with (a) 0 %, (b) 0.5 %, (c) 1 % and (d) 5 % nanonized sucrose as porogen.

3.4.6. Conclusion

Micronized and nanosized sugar particles were successfully prepared by jet and wet bead milling respectively. They were effectively used as fast-dissolving porogens to prepare porous PLGA microparticles by the S/O/W method. These porogens were completely leached out during the manufacturing process. By changing particle size, content of micronized/nanosized sugars, different sugars and PLGA types, clinically relevant properties such as morphology, overall porosity, and *in vitro* drug release could be tailored. The latter showed that porous PLGA microparticles can be effectively used as a sustained-release drug delivery system and can obtain a linear release. As a limitation to the proposed method, the resulting pore size can not be controlled only by varying the size of porogen particles. From a future perspective, micronized/nanosized sugar particles might be incorporated into PLGA matrix first, followed by leaching out of the hardened particles, leaving pores expected with the same diameter as encapsulated sugar particles.

4. Summary

PLGA drug delivery systems have been widely investigated as carriers for sustained drug release. But incorporating nanosized drugs or excipients into these systems was not systematically studied until now. This research aimed first to understand the specific properties of PLGA microparticles loaded with nanosized drug when compared to micronized and dissolved drug. Then optimize the manufacturing process and formulation parameters of PLGA drug delivery systems incorporating nanosized drugs. And finally, investigate the possibility of using sugar particles and an optimized manufacturing process to prepare porous PLGA drug delivery systems.

A comparative study of PLGA microparticle properties loaded with micronized, nanosized and dissolved drug

The PLGA microparticles loaded with micronized, nanosized and dissolved dexamethasone (or hydrocortisone) were produced with a solvent extraction/evaporation method and were analyzed in terms of their particle size, encapsulation efficiency, drug solid state, morphology, *in vitro* release and dynamic changes in the diameter. The micronized and nanosized drugs were still in crystalline form after encapsulation into PLGA microparticles with encapsulation efficiencies mostly greater than 85 %, while encapsulating dissolved drug obtained the lowest encapsulation efficiency varying from 32 % to 63 % and dissolved drug recrystallized when increasing drug loading to 30 %. In all varied PLGA types, drug loadings and drug types. PLGA microparticles loaded with dissolved drug released faster than those loaded with nanosized drug, and greatly faster than those loaded with micronized drug during the whole release period. Drug release rates correlated with their diffusion capacities: dissolved $>$ nanosized $>$ micronized. Quasi-linear release was obtained by encapsulating 10 % nanosized dexamethasone into PLGA 502H and 503H microparticles. Quick wetting process and homogenous distribution of nanosized drugs inside microparticles helped to form a uniform inner network and thus eliminated the lag phase. Encapsulating nanosized drug into PLGA microparticles is a promising method to increase encapsulation efficiency, keep a stable solid state and

achieve a continuous release profile.

Milling in PLGA solution facilitates the encapsulation of drug nanocrystals into PLGA microparticles

Dexamethasone, hydrocortisone and dexamethasone sodium phosphate nanosuspensions (200 – 300 nm) were successfully prepared by wet bead milling in dichloromethane using PLGA as a milling stabilizer. PLGA microparticles loaded with nanosized drugs were prepared utilizing a S/O/W solvent evaporation method or S/O/O coacervation method. The nanosized drugs inside the microparticles were homogeneously distributed and in crystalline forms. Most microparticles' encapsulation efficiencies were larger than 80 %. The PLGA microparticles released sustained *in vitro* within some months. By changing PLGA types, selecting microparticle sizes, varying drug loadings, and using PLGA blends, the release profiles could be modified and a quasi-linear release without a lag phase was obtained.

PLGA *in situ* forming implant and microparticle incorporating nanosized drug as long-term drug delivery carriers

Nanosized dexamethasone was prepared by milling directly in triacetin and characterized by particle size and solid state. Dexamethasone was nanosized to 372 nm in triacetin without any stabilizer and had no solid state change. PLGA *in situ* systems was obtained by PLGA addition. Their physical stability, viscosity, injectability, particle size, *in vitro* release, wet mass change, pH change and morphology were investigated. Incorporating nanosized dexamethasone increased systems' viscosities, which resulted in better physical stability for at least 3 months, a smaller burst release, increased drug release during lag phase and a longer release period, compared to dissolved and micronized dexamethasone. Increased nanosized dexamethasone loading and PLGA concentration can increase the viscosity of the formulations and hence tune the release. By decreasing the formulation particle size via emulsification, the release can also be modified significantly. PLGA *in situ* forming systems containing nanosized drug are physically stable, injectable and can obtain a longer sustained

drug release with a low initial burst.

Preparation of porous PLGA microparticles containing dexamethasone by using nanosized/micronized sugar particles as porogen

The usage of nanosized/micronized sugar particles as porogen can introduce porosity within PLGA microparticles containing dexamethasone using S/O/W method. The porosity of the microparticles is caused both by the influx of water into the oil droplets and the encapsulation and subsequent dissolution of sugar particles during the manufacturing process. Porous PLGA microparticles containing dexamethasone were fabricated with different particle sizes, weight fractions and types of sugar particles, and were characterized for morphology, thermal property, particle size, drug content and surface area. Additionally, *in vitro* drug release and swelling of microparticles were conducted on porous PLGA microparticles to estimate the dissolution kinetics of encapsulated dexamethasone. Overall, the introduction of nanosized/micronized sugar particles resulted in porous PLGA microparticles with high encapsulation efficiency. Designed porosity and pore size, as well as modifiable *in vitro* drug release could be achieved via the selection of appropriate particle size and weight fraction of nanosized/micronized sugar particles. The data demonstrate that nanosized/micronized sugar particles are promising porogens for the production of highly tunable porous PLGA microparticles.

The successful completion of this research can instruct the development of PLGA formulations. Firstly, this work will aid future research in PLGA microparticles in terms of choosing drug particle sizes and dispersion states. Secondly, this study will be helpful in the design and development of PLGA formulations loaded with drug nanocrystals in a simplified process by combining non-aqueous wet bead milling and subsequent microencapsulation. Further, this study presents a newly developed PLGA *in situ* forming system incorporating nanosized drug. Finally, this research is also an important step toward the development of porous PLGA microparticles using nanosized sugar particles as porogen which can adjust the drug release.

5. Zusammenfassung

PLGA basierte Systeme wurden umfassend als Depotarzneimittel für eine anhaltende Arzneistofffreisetzung untersucht. Aber die Verkapslung von Arzneistoffen oder Hilfsstoffen in Nanogröße in diese Systeme wurde bisher nicht systematisch untersucht. Diese Forschung dient zunächst dem Verständnis der spezifischen Eigenschaften von PLGA-Mikropartikeln, die mit nanokristallinen Arzneistoffen beladen sind, im Vergleich zu mikronisierten und gelösten Arzneistoffen. Anschließend wurden der Herstellungsprozess und die Formulierungsparameter von PLGA-Formulierungen, die nanokristalline Arzneistoffe enthalten optimiert. Schließlich wurde untersucht, Zuckerpartikel und einen optimierten Prozess zur Herstellung poröser PLGA-Formulierungen zu verwenden.

Eine vergleichende Studie der Eigenschaften von PLGA-Mikropartikeln, welche mit mikronisiertem, nanokristallinem und gelöstem Arzneistoff beladen sind

Die mit mikronisiertem, nanokristallinem und gelöstem Dexamethason (oder Hydrocortison) beladenen PLGA-Mikropartikel wurden mit einem Lösungsmittelextraktions-/Verdampfungsverfahren hergestellt und hinsichtlich Partikelgröße, Verkapselungseffizienz, Arzneistofffestkörperzustand, Morphologie, In-vitro-Freisetzung und dynamischer Veränderungen des Durchmessers analysiert. Die mikronisierten und nanokristallinen Wirkstoffe waren nach der Verkapslung in PLGA-Mikropartikel immer noch in kristalliner Form mit Verkapselungseffizienzen von meist mehr als 85 %, während die Verkapselung gelöster Wirkstoffe die niedrigste Verkapselungseffizienz zwischen 32 % und 63 % ergab und gelöste Wirkstoffe rekristallisierten, wenn die Wirkstoffbeladung auf 30 % erhöht wurde. Bei allen verschiedenen PLGA-Typen, Arzneistoffbeladungen und Arzneistofftypen setzten während der gesamten Freisetzungsdauer PLGA-Mikropartikel beladen mit gelöstem Arzneistoff schneller frei als solche mit nanokristallinem Arzneistoff und viel schneller als solche mit mikronisiertem Arzneistoff. Die Wirkstofffreisetzungsraten korrelierten

mit den Diffusionskapazitäten: gelöst > nanokristallin > mikronisiert. Eine quasi-lineare Freisetzung wurde durch die Verkapseln von 10 % nanokristallinem Dexamethason in PLGA 502H- und 503H-Mikropartikel erhalten. Der schnelle Benetzungsprozess und die homogene Verteilung von nanokristallinem Wirkstoffen innerhalb der Mikropartikel trugen zur Bildung eines einheitlichen inneren Netzwerks bei und eliminierten so die Verzögerungsphase. Die Verkapselung von nanokristallinen Arzneistoffen in PLGA-Mikropartikel ist eine vielversprechende Methode, um die Verkapselungseffizienz zu erhöhen, einen stabilen Festkörperzustand beizubehalten und ein kontinuierliches Freisetzungsprofil zu erreichen.

Das Mahlen in PLGA-Lösung zur Erleichterung der Verkapselung von Arzneistoff-Nanokristallen in PLGA-Mikropartikel

Dexamethason-, Hydrocortison- und Dexamethason-Natriumphosphat-Nanosuspensionen (200 – 300 nm) wurden erfolgreich durch Nassmahlen mit Kugeln in Dichlormethan unter Verwendung von PLGA als Mahlstabilisator hergestellt. Mit nanokristallinen Arzneistoffen beladene PLGA-Mikropartikel wurden unter Verwendung eines S/O/W-Lösungsmittelverdampfungsverfahrens oder eines S/O/O-Koazervationsverfahrens hergestellt. Die nanokristallinen Wirkstoffe waren innerhalb der Mikropartikel homogen verteilt und in kristalliner Form. Die Verkapselungseffizienz der meisten Mikropartikel lag bei über 80 %. Die PLGA-Mikropartikel wurden in vitro innerhalb einiger Monate anhaltend freigesetzt. Durch Änderung des PLGA-Typs, der Mikropartikelgrößen, der Wirkstoffbeladung und Verwenden von PLGA-Mischungen waren die Freisetzungsprofile modifizierbar und es wurde eine quasi-lineare Freisetzung ohne Verzögerungsphase erhalten.

In-situ-bildende PLGA-Implantate und Mikropartikel, die nanokristallinen Arzneistoff enthalten, für die langfristige Arzneistofffreisetzung

Nanokristallines Dexamethason wurde durch direktes Mahlen in Triacetin hergestellt und die Partikelgröße und der Festkörperzustand charakterisiert. Dexamethason hatte in Triacetin ohne jeglichen Stabilisator eine Partikelgröße

von 372 nm und zeigte keine Veränderung des Festkörperzustandes. PLGA-in-situ-Systeme wurden durch PLGA Zugabe erhalten. Ihre physikalische Stabilität, Viskosität, Injizierbarkeit, Partikelgröße, In-vitro-Freisetzung, Nassmassenänderung, pH-Änderung und Morphologie wurden untersucht. Die Einarbeitung von nanokristallinem Dexamethason erhöhte die Viskosität der Systeme, was im Vergleich zu gelöstem und mikronisiertem Dexamethason zu einer besseren physikalischen Stabilität für mindestens 3 Monate, einer geringeren initialen Freisetzung, einer stärkeren Arzneistofffreisetzung in der Verzögerungsphase und einer längeren Freisetzungsdauer führte. Eine erhöhte Beladung mit nanokristallinem Dexamethason und eine erhöhte PLGA-Konzentration können die Viskosität der Formulierungen erhöhen und somit die Freisetzung modifizieren. Durch Verringerung der Partikelgröße der Formulierung kann die Freisetzung ebenfalls signifikant modifiziert werden. In-situ-bildende PLGA-Systeme, die nanokristalline Wirkstoffe beinhalten, sind physikalisch stabil, injizierbar und können eine länger andauernde Wirkstofffreisetzung mit einer geringen Initialfreisetzung erreichen.

Herstellung von porösen Dexamethason-haltigen PLGA-Mikropartikeln, unter Verwendung von nanokristallinen/mikronisierten Zuckerpartikeln als Porogen

Durch die Verwendung von nanokristallinen/mikronisierten Zuckerpartikeln als Porogen kann Porosität innerhalb von Dexamethason-haltigen PLGA-Mikropartikeln, unter Verwendung des S/O/W-Verfahrens erreicht werden. Die Porosität der Mikropartikel wird sowohl durch das Eindringen von Wasser in die Öltröpfchen als auch durch die Verkapselung und anschließende Auflösung von Zuckerpartikeln während des Herstellungsprozesses verursacht. Poröse PLGA-Mikropartikel, die Dexamethason enthielten, wurden mit unterschiedlichen Partikelgrößen, Gewichtsanteilen und Arten von Zuckerpartikeln hergestellt und hinsichtlich Morphologie, thermischer Eigenschaft, Partikelgröße, Wirkstoffgehalt und Oberfläche charakterisiert. Zusätzlich wurden die in-vitro-Arzneistofffreisetzung und das Quellverhalten der porösen PLGA-Mikropartikeln

analysiert, um die Auflösungskinetik von verkapseltem Dexamethason zu untersuchen. Insgesamt führte die Verwendung von nanokristallinen/mikronisierten Zuckerpartikeln zu porösen PLGA-Mikropartikeln mit hoher Verkapselungseffizienz. Eine kontrollierte Porosität und Porengröße sowie eine modifizierbare In-vitro-Wirkstofffreisetzung können durch die Auswahl einer geeigneten Partikelgröße und eines Gewichtsanteils von nanokristallinen/mikronisierten Zuckerpartikeln erreicht werden. Die Daten zeigen, dass nanokristalline/mikronisierte Zuckerpartikel vielversprechende Porogene für die Herstellung von hochgradig kontrollierbaren porösen PLGA-Mikropartikeln sind.

Der erfolgreiche Abschluss dieser Forschung kann die Entwicklung von PLGA-Formulierungen anleiten. Erstens wird diese Arbeit die zukünftige Forschung an PLGA-Mikropartikeln im Hinblick auf die Auswahl von Wirkstoffpartikelgrößen und Dispersionszuständen unterstützen. Zweitens wird diese Studie bei der Gestaltung und Entwicklung von PLGA-Formulierungen hilfreich sein, die mit Arzneistoff-Nanokristallen in einem vereinfachten Verfahren beladen sind, indem nichtwässriges Nassperlenmahlen und anschließende Mikroverkapselung kombiniert werden. Darüber hinaus stellt diese Studie ein neu entwickeltes PLGA In-situ formendes System vor, das nanokristalline Wirkstoffe enthält. Schließlich ist diese Forschung auch ein wichtiger Schritt in Richtung der Entwicklung poröser PLGA-Mikropartikel unter Verwendung von nanokristallinen Zuckerpartikeln als Porogen, welche die Arzneifreisetungfreisetzung regulieren können.

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7. Publications and Presentations

Research publications:

- **C. Zhang**, R. Bodmeier, A comparative study of PLGA microparticles loaded with micronized, nanosized and dissolved drug. (In preparation)
- **C. Zhang**, R. Bodmeier, Milling in PLGA solution facilitates the encapsulation of drug nanocrystals into PLGA microparticles. (In preparation)
- **C. Zhang**, R. Bodmeier, PLGA *in situ* forming implant and microparticle incorporating nanosized drug as long-term drug delivery carriers. (In preparation)
- **C. Zhang**, R. Bodmeier, Preparation of porous PLGA microparticles containing dexamethasone by using nanosized/micronized sugar particles as porogen. (In preparation)

Poster presentations:

- **C. Zhang**, R. Bodmeier, A comparative study of PLGA microparticles loaded with micro-sized and nano-sized dexamethasone, PBP world meeting, May 2021, Vienna, Austria.

8. Curriculum Vitae

For reasons of data protection, the curriculum vitae is not published in the electronic version.

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