DEVELOPMENT OF BIODEGRADABLE IMPLANTS WITH SPECIFIC DRUG RELEASE PROFILES

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry and Pharmacy

of Freie Universität Berlin

by

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2015

1st Reviewer: Prof. Dr. Roland Bodmeier

2nd Reviewer: Prof. Dr. Philippe Maincent

Date of defense: 17.12.2015

To my Family

Acknowledgements

Firs and foremost, I would like to express my sincere gratitude to my supervisor, Prof. Dr. Roland Bodmeier for the opportunity to perform my doctoral project at the Institute of Pharmaceutical Technology, Collage of Pharmacy at the Freie Universität in Berlin. I am very thankful to him for the support and professional guidance contributing to my academic development.

I wish to thank to Prof. Dr. Philippe Maincent for co-evaluating this dissertation.

To all current and former colleagues I am most grateful for the supportive and friendly working environment: to Dr. Andriy Dashevskiy, Dr. Zahra Ghalanbor, Dr. Rebaz H. Ali, Dr. Duangratana Shuwisitkul, May Darwich, Chengcheng Zhao, Marius Heck, Gaith M. Zoubari and Seyedreza Goldoozian; to Kathrin Bürki, Dr. Agnieszka Solik, Luisa Duque, Lisa Beßlich and Rahul Sonawane for proofreading the parts of my dissertation; to Stefan Walter for his generous technical support; to Mrs. Gabriela Karsubke for her assistance with administrative issues.

I would like to thank German Academic Exchange Service (DAAD) for the financial support of my Ph.D. project.

I wish to dedicate my gratefulness to my family, my parents Jovanka and Zivko and to my lovely sister Jovana for the understanding, encouragement and love during all years of my studying.

My warmest thanks go to my beloved husband Nenad for his love, support and endless patience with me all these years.

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CHAPTER

1 Introduction

1.1 Biodegradable Polymers

Over the last few decades, developments in tissue engineering, regenerative medicine and controlled drug delivery attributed to increasing use of biodegradable polymeric excipients. Considerable research and development efforts have focused on application of biodegradable polymers in the medical treatments, mainly in designing of parenteral devices. Taking into account their safety and long-term clinical data as well as predictable degradation profiles, the biodegradable polymers have been utilized in various controlled drug delivery systems, including nanoparticles, microspheres and implants. The biodegradable polymers contain hydrolysable bonds, and thus biodegradation designates the chemical scission of polymer backbone in a physiological environment via hydrolysis or enzyme-catalysed hydrolysis. Degradation leads to polymer erosion. The polymer erosion represents a mass loss of the polymeric matrix via release of the degradation products such as monomers and oligomers (Göpferich 1996). When compared to non-degradable polymers, they have the improved biocompatibility and are degraded in the body, thereby eliminating the need for surgical removal (Siepmann et al. 2012). To be approved for parenteral applications, biodegradable materials must be naturally and completely eliminated from the body by the normal metabolic pathways and their by-products should be biocompatible and toxicologically safe.

Classification of Biodegradable Polymers

According to source, biodegradable polymers are classified as synthetic or natural (biologically derived) (Göpferich 1997; Edlund and Albertsson 2002). Table 1.1 shows the examples of natural and synthetic biodegradable polymers (Mishra et al. 2008).

Table 1.1. List of biodegradable polymers

Natural polymers	Synthetic polymers
Polysaccharides: chitosan, dextran, alginate, hyaluronic acid	Polyesters: Poly(glycolic acid), Poly(lactic acid), Poly(lactic-co-glycolic acid), Poly(caprolactone)
Proteins: collagen, gelatin, albumin, elastin, fibrin	Polyanhydrides
	Polyorthoesters
	Polyurethanes
	Tyrosine-derived polycarbonates
	Polyphosphazenes

Although the natural polymers have specific cell binding affinity, they may show a significant immunogenic response. The control of molecular weight, compositions as well as physicochemical properties of natural polymers is not easy. The main advantage of synthetic biodegradable polymers is a predictable and precise control of degradation kinetics. They allow designing the suitable mechanical, physical, chemical and thermal features (Vert 2005).

In classification of degradable polymers, a distinction is also made between surface (or heterogeneous) and bulk (or homogeneous) eroding materials depending on the erosion mechanism (Figure 1.1) (Alexis 2005). Upon incubation of the polymeric matrix, water penetrates into the amorphous region and disrupts the van der Waals forces and hydrogen bonds causing a decrease in the glass transition temperature (Gentile et al. 2014). Water further induces hydrolytic degradation of backbone i.e. cleavage of covalent bonds, resulting in formation of shorter oligomers and finally monomers and thus a decrease in the polymer molecular weight. The process of hydrolytic degradation is followed by the mass loss of polymer matrix, i.e. the physical disintegration of system as a result of the release of degradation products (Göpferich 1997). If the water penetration proceeds faster than the matrix erodes, degradation will occur throughout the matrix and material will be

lost from the entire polymer volume. This behavior is designed as bulk erosion and it is sometimes referred to homogeneous erosion because mass loss proceeds at a more or less uniform rate throughout the matrix (Edlund and Albertsson 2002). The size and shape of the device remains intact even at later stages of degradation, but the microstructure within the bulk changes considerably. After erosion to a critical degree, the device eventually collapses. However, if water penetration is slow compared to the erosion process, water is consumed mainly on the surface by hydrolysis and mass loss occurs from the surface layers of the device. The size of the device gradually decreases but shape is unchanged and the bulk remains non-degraded (Göpferich 1996). This phenomenon is referred to surface or heterogeneous erosion. Surface eroding devices are hence often preferred over bulk eroding for drug delivery because the erosion is more predictable. However, ideal surface or bulk erosion process is difficult to achieve and most polymers cannot be unequivocally assigned to any category (Göpferich 1996; Edlund and Albertsson 2002).

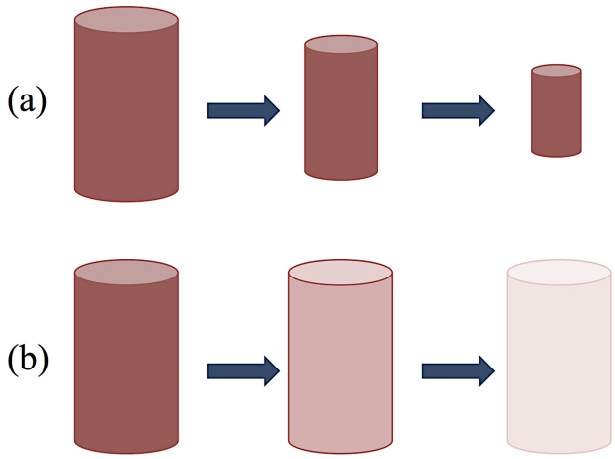


Figure 1.1. Schematic representation of (a) surface and (b) bulk erosion

Poly(lactic-co-glycolic acid) (PLGA)

According to the biodegradable polymer classification, homopolymers poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) as well as their copolymer poly(lactic-co-glycolic acid) (PLGA) are categorized as synthetic, bulk eroding, linear, aliphatic poly(α-esters). Polymers and copolymers of lactic and glycolic acids can be prepared in two ways: by a direct polycondensation reaction of lactic acid and glycolic acid, resulting in polymers of low molecular weight or by a ring opening polymerization of the corresponding cyclic dimers, lactide or glycolide at elevated temperature (Figure 1.2). The ring opening polymerization yields the polymers of high molecular weight and of better mechanical

properties (Gentile et al. 2014; Erbetta et al. 2012; Qian et al. 2011; Wang et al. 2000). L-PLA, D-PLA and D,L-PLA can be produced due to asymmetric carbons in the lactide units. L-PLA and D-PLA forms are semicrystalline, while D,L-PLA is amorphous and thus preferable for drug delivery systems as the drug can be more homogeneously distributed through the polymer matrix (Erbetta 2012).

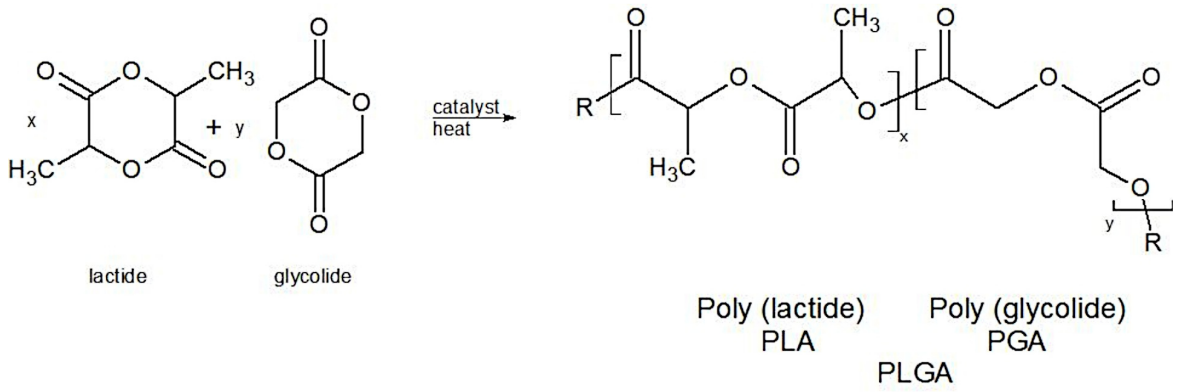


Figure 1.2. PLGA synthesis by ring-opening copolymerization of lactide and glycolide; x and y number of units of lactide and glycolide, respectively (Adapted from Erbetta 2012)

PLGA biodegrades by randomly hydrolysis of its ester bonds by esterases, producing lactic acid and glycolic acid. The lactic acid and glycolic acid are the natural metabolites, which are eliminated from the body via the Krebs cycle in the form of carbon dioxide and water (Figure 1.3) (Merkli et al. 1998). Hence, PLGA is considered as highly biocompatible and toxicologically safe, non-immunogenic, non-carcinogenic polymer.

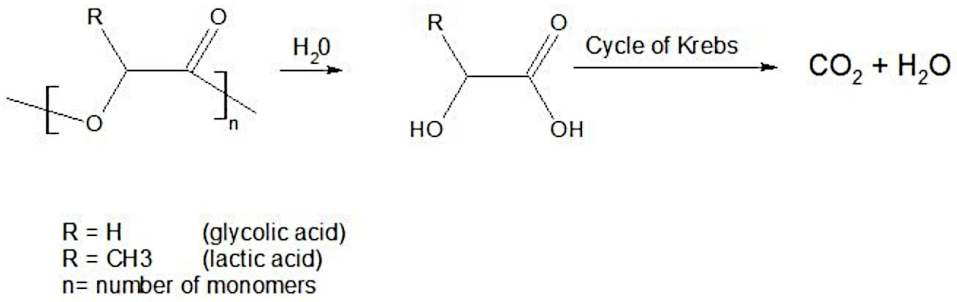


Figure 1.3. Mechanism of PLGA hydrolysis (Adapted from Merkli et al. 1998)

In order to obtain the controlled drug delivery from systems based on PLGA, the parameters affecting polymer degradation have to be considered. Polymer degradation and thus drug release rate are accelerated by higher hydrophilicity in the backbone or end groups, greater reactivity of hydrolytic groups in the backbone, less crystallinity and larger size of the device. Acid end PLGA is slightly more hydrophilic and therefore degrades

faster in water compared to polymers, which are end-capped with esters (Shiah et al. 2011). The ratio of lactic acid and glycolic acid controls the biodegradation rate in the following way; the higher the content of glycolide units, the lower the time required for degradation as compared to predominantly lactide materials (Samadi et al. 2013). Accordingly, the most important factors influencing the degradation rate to be taken into account upon polymer selection are copolymer composition, initial molecular weight and end-group functionalization (Gentile et al. 2014; Tracy et al. 1999). Moreover, other formulation excipients as well as pharmaceutical active ingredients also affect degradation rate.

PLGAs are categorized as bulk eroding polymers as a consequence of heterogeneous hydrolytic degradation of ester bonds resulting in formation of degradation products having carboxylic acid (Ghalanbor et al. 2013). These acid by-products lead to a drastic pH drop inside the PLGA matrix reaching value of pH 2 *in vivo* (Mäder et al. 1996). Fu et al., 2000 visualized and quantitatively determined pH changes within PLGA microspheres. The most acidic part was in the center of the spheres, while higher pH values were detected near the edges. An acidic microenvironment may induce the inflammation at the site of application and can sometimes compromise the stability, solubility, bioavailability, pharmacokinetics and ultimately therapeutic efficiency of the encapsulated drugs (Pitt et al. 1981). Furthermore, an acidic microenvironment causes an autocatalytic acceleration of polymer degradation. As a result of this phenomenon, the slower degradation occurs at the surface of matrix having outer layers of longer mechanical stability. While the polymer degradation starts from the beginning of the incubation, the onset of erosion process occurs after a lag phase. Consequently, the mass loss profile is sigmoidal. Mechanisms that might explain the discontinuous mass loss of bulk-eroding polymers include existing of pH gradient within the matrix, percolation phenomena as well as formation of soluble oligomers (Körber 2010; Göpferich 1997). Degraded polymers within the matrix cannot erode if there is no connection to the outer medium via pores. Hence, when the molecular weight of PLGA oligomers reaches the value of about 1000 g/mol, representing the upper limit for oligomers to be soluble, they start to diffuse out (Park 1994). This suggests that erosion process of PLGA is controlled by the kinetics of the formation of soluble oligomers. This stage denotes the rapid mass loss and system collapses (Edlund and Albertsson 2002).

1.2 PLGA-Based Delivery Systems for Parenteral Application

PLGA is among the few synthetic polymers approved by Food and Drug Administration (FDA) for human clinical use (Makadia 2011). The first suture (Dexon™) prepared by melt extrusion process was based on biodegradable polymer, poly(glycolic acid). Since Dexon™ was introduced to the market in 1969 by Cyanamid, application of PLGAs in medical treatments has been rapidly expanding. Nowadays PLGA is commonly used absorbable material for parenteral controlled drug delivery, tissue engineering (Wang et al. 2010) and vaccination (Feng et al. 2006). In general, PLGAs offer the broad range of release requirements: from a delivery of up to one month (an amorphous polymer with high hydrophilicity), to six months (an amorphous polymer with high molecular weight) and to more than six months (semi-crystalline polymer with a high degree of crystallinity) (Alexis 2005). FDA and European Medicine Agency approved several PLA and PLGA-based drug delivery systems in various therapeutic needs such as cancer, inflammation and other diseases. Numerous parenteral, PLGA controlled delivery technologies were successfully developed and validated. Many products are currently on the market, formulated as extended drug release rod-shaped solid implants, nano- and microspheres, in situ forming implants and microparticles.

Table 1.2 contains the examples of PLGA-based commercial injectable therapeutics. Mainly they are intended for delivery of peptides as well as low molecular weight drugs.

Table 1.2. Examples of marketed drug delivery systems based on PLGA/PLA

Product	Therapeutic	Carrier	Indication	Delivery technology	Duration of action	Route of administration
Zoladex®, Astra Zeneca	Goserelin acetate[2]	PLGA	Breast cancer, endometriosis, prostate cancer	Rod-shaped implant (1.2 mm x 10-12 mm or 1.5 mm x 16-18 mm)	28 days or 12 weeks	Subcutaneous
Ozurdex®, Allergan	Dexamethasone[1]	PLGA	Macular edema, retinal vein occlusion, uveitis	Rod-shaped implant (0.46 mm x 6 mm)	3-6 months	Intravitreal
Profact or Suprefact Depot®, Sanofi-Aventis	Buserelin acetate[2]	PLGA	Prostate cancer, endometriosis	Rod-shaped implant	2 or 3 months (pre-filled syringe contains two or three implants, respectively)	Subcutaneous
Leuprone® HEXAL®, Sandoz/Hexal	Leuprolide acetate[2]	PLGA, PLA	Prostate cancer	Rod-shaped implant (length 1 cm)	1 or 3 months (implants based on PLGA or PLA, respectively)	Subcutaneous
Eligard®, Astellas	Leuprolide acetate[2]	PLGA	Advanced prostate cancer	In situ forming implant	1, 3, 4 or 6 months	Subcutaneous
Atridox®, Tolmar	Doxycycline hyclate[1]	PLA	Chronic adult periodontitis	In situ forming implant	1 week	Subgingival
Bydureon®, Astra Zeneca	Exenatide	PLGA	Type II diabetes mellitus	Microspheres	1 week	Subcutaneous
Enantone®, Takeda	Leuprolide acetate[2]	PLGA, PLA	Prostate cancer, endometriosis, central precocious puberty	Microspheres	4 weeks or 3 months (microspheres based on PLGA or PLA, respectively)	Subcutaneous

Product	Therapeutic	Carrier	Indication	Delivery technology	Duration of action	Route of administration
Lupron Depot® and Lupron Depot-Ped®, Abbvie	Leuprolide acetate[2]	PLGA, PLA	Prostate cancer, endometriosis, fibroids, central precocious puberty (CPP)	Microspheres	1 or 3 months (microspheres based on PLGA; 4 or 6 months (microspheres based on PLA)	Intramuscular
Nutropin Depot®, Alkermes/Genentech (discontinued)	Somatropin (rDNA origin); recombinant human growth hormone (rhGH)[3]	PLGA	Long-term treatment of growth failure	Microspheres	Once- or twice-monthly injections of individual dose	Subcutaneous
Trelstar®, Watson Pharma	Triptorelin pamoate[2]	PLGA	Prostate cancer	Microgranule	1, 3, or 6 months	Intramuscular
Arestin®, OraPharma	Minocycline hydrochloride[1]	PLGA	Periodontitis	Microspheres	3 weeks	Subgingival
Risperdal® Consta, Janssen	Risperidone[1]	PLGA	Schizophrenia	Microspheres	2 weeks	Intramuscular
Somatuline® LA, Ipsen	Lanreotide acetate[2]	PLGA	Acromegaly, thyrotropic adenomas, neuroendocrine tumours	Microspheres	2 weeks	Intramuscular
Sandostatin® LAR®, Novartis	Octreotide acetate[2]	PLGA	Acromegaly, gastro-entero-pancreatic endocrine tumors, neuroendocrine tumors	Microspheres	4 weeks	Intramuscular

[1] Low molecular weight drug
[2] Peptide
[3] Protein

Release Mechanisms in PLGA Drug Delivery Systems

Injectable PLGA drug delivery systems are intended for controlled drug release over a period of several weeks to several months. Although zero-order drug release is the most preferable, these systems often show bi- or tri-phasic drug release profile due to diffusion/heterogeneous degradation of biodegradable polymer (Fredenberg et al. 2011). With increasing device size, release profile can change from first order to sigmoidal shape. (Berchane et al. 2007; Berkland et al. 2003). Phase I is usually referred as an initial burst release. It is diffusion-controlled release of drug at the surface of system or poorly encapsulated particles in pores close to the surface, which initially diffuse out after hydration of system. Wang et al. 2002 did subtle characterization of the initial burst release of a model peptide from PLGA microspheres by correlation of the morphological changes and drug release rate profile during the first 24 h of release. They found three phases of the initial drug release in terms of permeability. The first phase of high permeability corresponds to very high initial release rate due to drug leaching out from the surface or due to the rupture of polymer surface structure caused by osmotic pressure. A pore density at the surface increases and shape of pores becomes round. The phase of high permeability is followed by the phase of medium and low permeability, which corresponds to an increase of swelling and a decrease in the release rate. Decrease in permeability is resulted from polymer chain rearrangement and surface pores closure. During this period diffusion barrier develops and the "skin" type structure with a porous core and less porous surface is observed. Moreover, intrinsically amorphous PLGAs undergo degradation in aqueous medium, which occurs heterogeneously i.e. faster inside a sample then at the surface (Vert et al. 1990). After the initial burst release, phase II follows. This phase is also called a lag phase. The phase II is characterized by a slow drug release through relatively dense polymer. Formation of the skin structure, pore closing, polymer-drug interaction or drug-drug interaction can be also an explanation for the lag phase (Kang and Schwendeman 2006; Kang et al. 2008). In the typical tri-phasic profile, the phase III is a period of faster release due to rapid polymer erosion and thus faster drug diffusion.

Manufacturing Techniques of PLGA Drug Delivery Systems

PLGAs are suitable as degradable polymeric carriers for parenteral controlled drug delivery systems due to ability to be fabricated into a variety of morphologies including

nanoparticles, microparticles, solid implants and in situ forming drug depots (microparticles and implants).

PLGA microparticles can be prepared by different microencapsulation techniques including solvent extraction/evaporation processes, phase separation (coacervation) and spray drying (Jain 2000). A choice of the technique depends on the nature of polymer, the drug, the intended use and the duration of therapy. However, emulsion techniques are most often used, at least in the laboratory scale, due to possibility in adaption for drugs of different physicochemical properties (Wischke and Schwendeman 2012). Considerable number of PLGA based controlled release systems are in the form of microparticles mainly owing to an easy adjustment of doses as well as their advantage as the multiple unit dosage form, i.e. there is no dose dumping due to failure in single carrier. Additionally, benefits of microparticles as drug depots compared to solid implants include their injection through smaller needles. Although the invasive procedures are required upon administration of solid implants by a minor surgical incision or by special injector (trocar), thereby causing inconvenience to the patients, they can be easily remove if early therapy termination is required due to adverse effects. Additional disadvantageous properties of microparticles comprise the reconstitution of microparticles in an aqueous media before administration, the use of organic solvents during preparation step as well as residual organic solvent in the final product (Jain 2000). For some therapeutics such as protein drugs, large interfaces during microencapsulation are destabilizing factor due to interfacial adsorption resulting in denaturation and aggregation of proteins (Perez et al. 2002).

PLGA solid implants are typically formed in a rod-like shape and can be prepared by different techniques, whereby some of them are solvent-free processes in contrast to microencapsulation methods (Witt et al. 2000). The most common preparation techniques of implants are summarized in Figure 1.4.

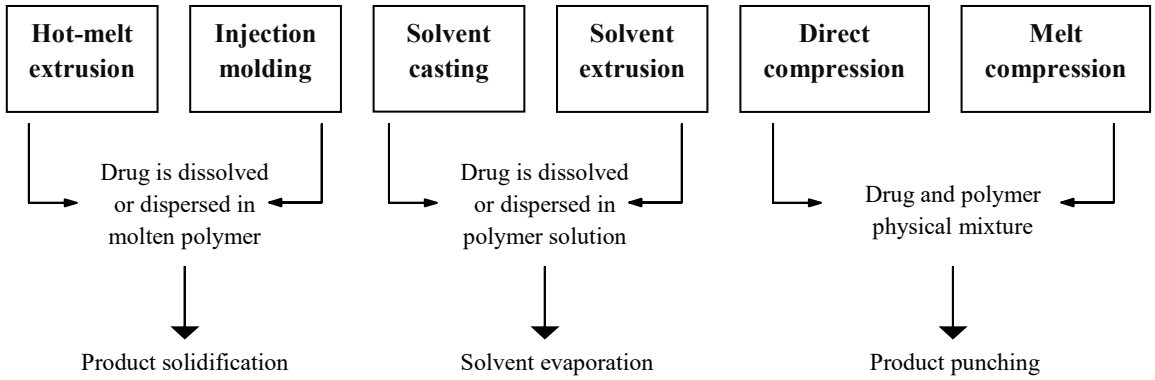


Figure 1.4. Common manufacturing techniques of PLGA implants (Modified and adapted from Wischke and Schwendeman 2012)

Generally, formation of implants starts from a drug-polymer solution, dispersion, molten, or powdered blend. Each manufacturing technique is associated with certain disadvantages. The properties of final product (e.g. porosity) strongly depend on the selected preparation method.

PLGA is a thermoplastic polymer, which can be easily shaped when it is molten. Therefore PLGA is often used as a thermal binder in production of biodegradable implants by process of injection molding or hot-melt extrusion. These techniques can be applied to produce controlled-release formulations via the homogeneous embedding of drug particles in the biodegradable polymers. Injection molding denotes the process of transferring the molten material under high pressure into a closed and shape-specific mold. When material solidifies, the final product is obtained by opening the mold (Quinten et al. 2009). Whereas injection molding is commonly used to fabricate devices of complex shape, hot-melt extrusion is a suitable and often utilized method for preparation of solid rod-shaped PLGA implants. Implants are basically matrix systems with molecularly dispersed or suspended drug. A laboratory-scale extrusion process can be performed using plastic syringe attached to the die at elevated temperature (Zhou et al. 1998; Ghalanbor et al. 2010). Nevertheless, for industrial fabrication of drug-loaded implants a more sophisticated setup for hot melt extrusion with well-controlled process parameters is preferred. For instance, using twin-screw extruder is a fast, one-step process operating in a continuous manner (Wischke and Schwendeman 2012). However, thermooxidative and thermomechanical degradation of polymers as well as degradation of embedded drugs during melt processing (extrusion, injection molding and melt compression) may occur

(Weiler and Gogolewski 1996). Ghalanbor et al. 2010 reported decreasing of the activity of released lysozyme from PLGA implants from 92.5% to 62.5% with increasing extrusion temperature from 100 °C to 105 °C. Rothen-Weinhold et al. 1999 investigated effect of extrusion temperature and time on the purity of somatostatin analogue vapreotide embedded in PLA implant. They revealed no significant degradation of the peptide if the extrusion temperature did not exceed 80-90 °C for up to 2 h.

Solvent associated methods for preparation of PLGA implants comprise solvent casting and solvent extrusion processes. Organic solvents are used for shaping implants at low temperatures and therefore these techniques can be an alternative for formulation of temperature sensitive compounds. Drug-polymer solution or suspension can be casted into the mold or extruded into the silicone tubing from pre-filled syringe. Subsequently, material is subjected to drying in order to evaporate the organic solvent (Desai et al. 2008; Zhou et al. 1998). Zhou et al. 1998 employed solvent extrusion processes in fabrication of PLGA multiple-drug cylindrical implant for intraocular management of proliferative vitreoretinopathy. Desai et al. 2008 published two papers reporting the use of solvent extrusion method in preparing injectable cylindrical PLGA implants. Solvent casting method is commonly utilized in preparation of films or laminar implants (Klose et al. 2008). Considering that PLGA polymers form easy-to-handle laminar implants, different types of circular film-implants (discs) composed of one or more PLGA-layers prepared by a solvent-casting technique alone or combined with compression method were reported (Dorta et al. 2002; García et al. 2002). Multilayer discs with a central drug monolithic layer, or multilayer discs with a central drug reservoir can be produced by compression of pre-casted films (Santoveña et al. 2006). Generally, a drawback of the solvent associated methods is a presence of an organic solvent in the formulation. Gradual solvent removal and hardening of implants may cause demixing of suspended drug particles, Ostwald ripening of precipitating drug aggregates and potential inhomogeneity of the final product. Due to the implant size, the residual solvent is also a challenge associated with application of solvent casting and solvent extrusion techniques (Wischke and Schwendeman 2012).

Preparation of PLGA implants by direct compression is a less stressful method in compared to other processes (Negrín et al. 2004). Compression is a solvent- and heat-free process and thus it is suitable for manufacturing of implants containing thermolabile or moisture-sensitive drugs (Jivraj 2000). PLGA implants prepared by this method

sometimes show the high initial burst release and the short duration of drug release. Therefore the additional methods are necessary in order to retard drug release rate. Negrín et al. 2004 developed PLGA or PLA methadone loaded direct compressed implants for 1-week or 1-month release duration, respectively, as a result of coating by PLA. Onishi et al. 2005 introduced PLGA compressed implant of ketoprofen exhibiting week-long sustained release *in vitro*. Bodmeier and Chen 1989 prepared PLA pellets by a simple direct compression technique without the use of heat or organic solvents intended for either parenteral or oral use. The drug release reduction was achieved by dipping the pellets into methylene chloride for a short period of time. PLA solubilization in methylene chloride and reprecipitation upon solvent evaporation caused formation of dense, less porous film on the surface.

Other approach that can result in decreasing drug release from compressed PLGA matrices is melt compression method. Qian et al. 2001 prepared cylindrical millirods by a compression-heat molding procedure. The solid physical mixture of PLGA microspheres and trypan blue powder was processed at temperature higher than the glass transition temperature of PLGA to allow the annealing of the PLGA microspheres under a constant compression pressure.

1.3 Hot-Melt Extrusion

Hot-melt extrusion (HME) is defined as the process of pumping raw materials with a rotating screw under controlled conditions and at elevated temperature through a die into a product of uniform shape and density. Basically, hot-melt extrusion process comprises three steps within a single device: melting, mixing and shaping. HME was firstly applied within the plastic and food industry. In the 1987, BASF introduced the melt extrusion process based on polymers with a high glass transition temperature (such as polyvinylpyrrolidones) to pharmaceuticals (Görtz et al. 1992). Interest for utilisation of HME in the pharmaceutical industry is steadily growing. This confirms the increased number of research groups that have been demonstrating applicability of HME in the formulation of various dosage forms. Nowadays HME is an attractive alternative to traditional processing methods. It offers many advantages to other techniques. There are

shorter and more efficient processing times to a final product due to combining a few manufacturing operations into a single unit operation. Molten polymers have a role of thermal binder. In addition, it is a solvent and water-free process offering environmental advantages due to elimination of solvents in processing. Accordingly, HME is an alternative to wet granulation process for drug candidates with poor stability during processing caused by hydrolysis. Furthermore, HME provides the intense mixing and agitation caused by the rotating screw and thus contributes to more uniform dispersion. HME is a continuous process and efficient for manufacturing in a big scale and at relatively high throughput rates (Crowley et al. 2007; Breitenbach 2002). Continuous processing allows for good process control and scalability.

HME provides immediate, sustained, modified and targeted drug delivery systems in the form of variety dosages forms such as granules (Liu et al. 2001), pellets (Young et al. 2002), tablets (Zhang and McGinity 2000), implants (Ghalanbor et al. 2010; Ghalanbor et al. 2012), transmucosal, transdermal (Repka et al. 2002) and transungual systems (Mididoddi et al. 2006; Mididoddi and Repka 2007). Increasing number of new chemical entities that face poor bioavailability due to solubility issues significantly increase HME use in the pharmaceutical and OTC industry. HME proved to be a powerful tool for preparing solid molecular dispersion resulting in improved bioavailability.

Although the interest for HME in the pharmaceutical industry is increasing, there are some process limitations, which have to be considered. Both active ingredients and pharmaceutical grade polymers must be thermally stabile at processing temperatures.

Hot-Melt Extruders

Unlike other industries, extruders for the pharmaceutical application must fulfill all cleaning and validation standards. The contact surface and all contact parts of equipment should be made of inert materials and meet the regulatory requirements including the lack of any kind of interactions with the product. Depending on the mechanism by which the rubber compound or the plastic formulation is forced through the die, extruders are classified into two main types: ram and screw type extruders. Ram extrusion operates with a positive displacement ram (piston) that generates high pressures and pushes the material through the die transforming it into extrudates (Figure 1.5). The main drawback of ram extruders is lower homogeneity of extrudates due to limited melting capacity and poor temperature uniformity (Crowley et al. 2007).

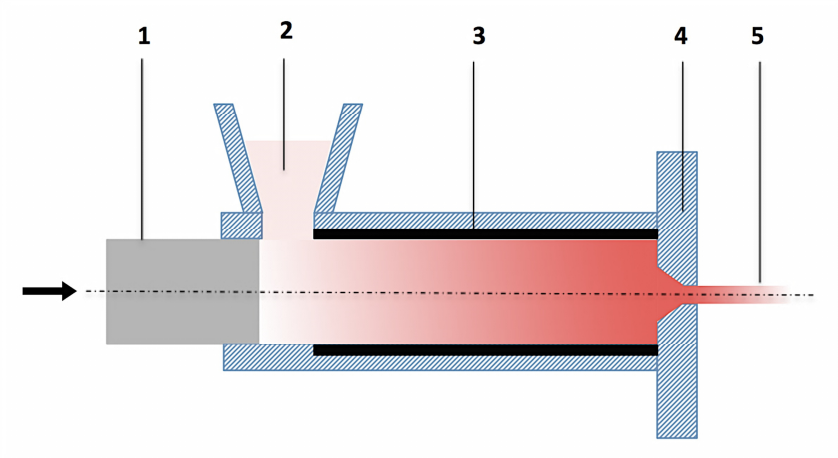


Figure 1.5. Schematic representation of ram extruder; (1) ram, (2) feed hopper (3) temperature controlled barrel, (4) die, (5) extrudate

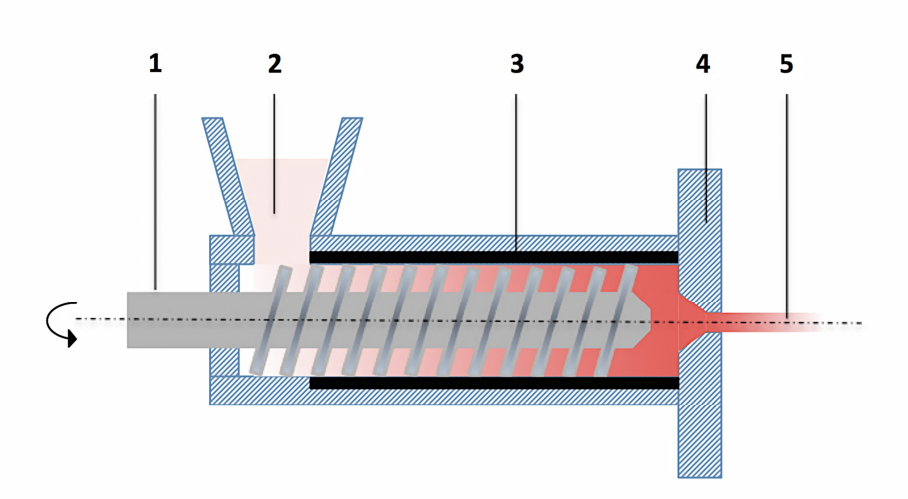


Figure 1.6. Schematic representation of screw extruder; (1) rotating screw, (2) feed hopper (3) temperature controlled barrel, (4) die, (5) extrudate

Contrary to ram extruder, the screw extruder (Figure 1.6) provides more intense mixing and higher shear stress caused by rotating the screw(s). Hence polymer melt viscosity is reduced, mixing of drug-carrier is more efficient and improved homogeneity and uniformity of final dosage form is achieved. In principles, the screw extruder consists of three distinct parts: conveying system for material transport and mixing, die system and downstream auxiliary equipment. Temperature-controlled barrels enclosing screw(s) transport and subsequently force the melt material through a die forming a particular shape. It is possible that barrel consists of different temperature zone. The design of some extruded allowed feeding at different stages (zones) of barrel. Therefore, applicability of HME in formulation of temperature sensitive compounds is allowed by feeding drug at the

last barrel zone into already molten polymer. From the barrel, the material continues to the die. There are different die configurations that determine the shape of extrudates and also affect the physical properties of extrudates. Downstream auxiliary equipment serves for cooling, cutting, collecting or analyzing the extruded dosage form.

Figure 1.7. Thermo Scientific HAAKE MiniLab II, a Micro Compounder with conical twin-screws (Picture taken from www.rheologysolutions.com)

Generally, screw extruders can be designed in two different configurations, as single- and twin-screw extruders. The single screw extruder consists of one screw, which rotates inside the barrel, whereas the twin-screw extruder operates with two screws commonly arranged side by side (Figure 1.7). The screws can either rotate in the same direction (co-rotating extruder, industrially the most important type of extruders) or the opposite direction (counter-rotating extruder). In principles, counter-rotating twin-screw extruder generates high shear forces and high pressure between screws with high possibility of air entrapment in product. The major difference between the single- and twin-screw extruders is material transport mechanism and their mixing capability. The main characteristics of single-screw extruders compared to twin-screw extruders are mechanical simplicity, significantly less cost of investment, poor mixing process, longer residence time of material due to longer length of screw and bigger device size required to achieve an equivalent output. Most of commercial extruders offer a possibility of changing the different screws configurations (Mollan 2003; Crowley et al. 2007). There are two types

of screw elements: conveying and kneading elements (Figure 1.8). The number and arrangement of those elements can be varied depending on requirements such as a high or low shear. Generally, kneading elements are used to introduce shear energy to the extruded materials. In addition, the elements can be arranged in different offset angles (30°, 60° and 90°) and thus screw design encompasses several sections supporting several functions (feeding, mixing, melting or compression and metering) (Kolter et al. 2011).

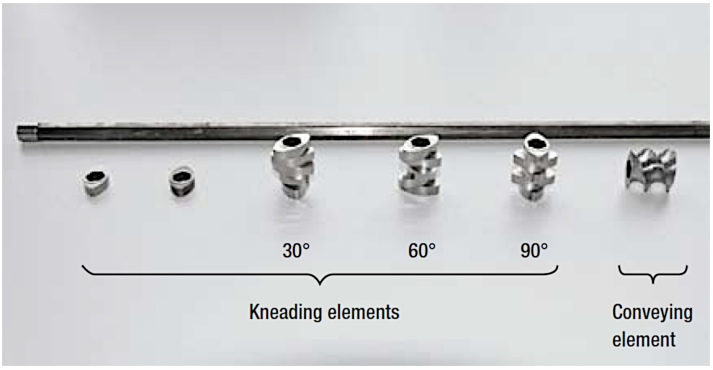


Figure 1.8. Different screw elements: kneading and conveying

The size of extruders is commonly based on screw diameter.

Processing Conditions

In-process control parameters involve barrel and die temperatures, screw speed (rpm), torque or power consumption, pressure and feed rate (Steiner 2003). Processing conditions depend on the chemical stability and physical properties of the thermal polymer such as melt viscosity, molecular weight, glass transition temperature and melting point (in the case of a semi-crystalline polymer). Basically, high melt viscosity is generated upon extrusion of materials with high molecular weight, although the viscosity of most polymers decreases by increasing shear stress. Materials with high glass transition or melting temperature require higher processing temperatures, which is an issue for thermo-sensitive drugs. Therefore, feature making polymer a good candidate for hot-melt extrusion is a suitable thermoplastic behavior i.e. it must be able to deform easily inside the extruder at relatively low processing temperature insuring thermal stability of the all individual compounds and it must be able to solidify upon its exit.

Commonly temperature of the melting zone is set from 15 °C to 60 °C above the melting point of semi-crystalline polymers or the glass transition temperature of amorphous polymers (Crowley et al. 2007). In twin-screw extrusion some process variables can be

modified during the extrusion such as screw speed, barrel temperature and feed rate. On the other hand, changes such as screw and die design and barrel layout are set before process run.

Residence times in the extruder vary depending on the size of the extruder, screw speed, screw configuration and feed rate. Typically residence time is in the range from 0.5 to 10 minutes. With increasing feed rate and screw speed, the residence time of the material decreases and the torque of the machine increases. At higher temperatures, the torque decreases due to a lowering of the melt viscosity of the polymer (Kolter et al. 2011). Torque is directly proportional to melt viscosity. An extruder is design to support the rotating screws at the selected speed while compensating for the generating torque from the material being extruded. Minimum temperatures are required for extrusion. Otherwise, the torque required to rotate the screw overloads the drive unit (Crowley et al. 2007). The torque and speed requirements of a specific formulation and the process condition determine the power requirements of the extruder (Mollan 2003).

Extrusion condition can affect significantly product quality. Influencing parameters are more complex in case of screw extruder than ram extruder. Physical properties of extrudates can vary considerably depending on the extruder type, temperature profile in the barrel, screw configuration, screw speed, feed rate and die profile. Increased screw speed and barrel temperature cause a slight decrease in melt viscosity and pressure inside the barrel and consequently the density of extrudate (Ding et al. 2006).

Chokshi et al. 2007 proposed the preformulation study based on the physic-mechanical characterization of drug and polymer binary mixtures as a predictive tool in establishing the HME process as well as drug/carrier miscibility and compatibility. The most relevant parameters to be investigated are solubility (determined by group contribution method), thermal analysis (differential scanning calorimetry, hot stage microscopy), and rheological analysis (torque rheometer). Thus the selection of appropriate excipients prior the extrusion process is improved depending on requirement for either one phase system (solid solution) or two-phase system (solid dispersion). Basically, drug can be either dispersed (in an amorphous or crystalline state) or dissolved in carrier. If drug and polymer are miscible then mixture shows a single glass transition temperature (T_g) at an intermediate temperature between the T_g of both drug and polymer and depends on the relative proportion of each component according to the Gordon–Taylor equation (Chokshi

et al. 2007; Forster et al. 2001a; Forster et al. 2001b).

$$T_g = \frac{w_1 T_{g,1} + k w_2 T_{g,2}}{w_1 + k w_2}$$

w_1 and w_2 are weight fractions of components 1 and 2, respectively, whereas k has to be evaluated from experimental data and represents unequal contributions of components to the blend. The index 2 in equation refers to the higher T_g component.

Another equation in use is the Fox equation, which is commonly applied to predict the glass transition temperature in miscible polymer blends.

$$\frac{1}{T_g} = \frac{w_1}{T_{g,1}} + \frac{w_2}{T_{g,2}}$$

In compatible systems two T_g values exist, which depend on composition of binary blends. If polymer blends are immiscible, in not an infrequent case, T_g values for pure components do not change with composition (Brostow et al. 2008).

Analysis of the T_g is also useful for prediction as to whether extrudate is in the glassy or rubbery state at the normal storage temperature.

Die Swell Phenomenon

Upon exiting the die, the extrudate diameter increases in relative to diameter of the die from which it is extruded (Crowley et al. 2007). This phenomenon is known as a die swell or the Barus effect (Figure 1.9). To what extent the cross-sectional expansion of extrudate is occurred, depends on the nonlinear viscoelastic properties of the polymer melt such as the shear and the extensional viscosities and the first normal stress difference as well as extrusion conditions (Ganvir et al. 2011). Generally, the viscous behavior of polymer melt determines the throughput, whereas the elastic properties are important for dimensional stability.

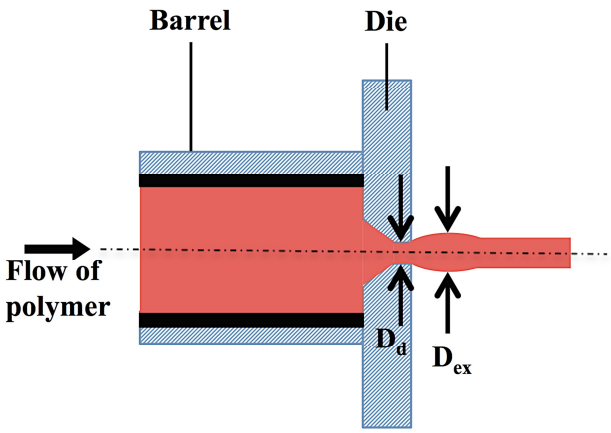


Figure 1.9. Schematic representation of die swell phenomenon; an increase in diameter of the extrudate, D_{ex} upon exiting the die of diameter D_d

The degree of extrudate swell is expressed by the die-swell ratio of extrudate diameter versus die diameter. Although, in the literature the extrudate swell (S) is also described as a function of extruded strand diameter (D_{ex}), the length of the die (L) and the diameter of the die (D_d) (Münstedt 2014).

$$S = \frac{(D_{ex} - D_d)}{D_d}$$

In the polymer rheology die swell is explained as a consequence of various effects such as a normal stress effect, an elastic energy effect, an entropy enlargement effect, an orientation effect and a memory effect. Actually, all of these effects are in correlation to each other and contribute to the die swell phenomenon. Prior entering in the die some polymers have got a roughly spherical confirmation. In the narrow die under the action of shear and compression, the flow rate speeds up and conformation of some polymer chains becomes more elongated. Physical entanglements of some molecular chains begin to disentangle and become uncoiled proportional to time that polymer spends in this condition. During die flow, the resultant stress and strain cannot be relaxed completely because polymers display time dependent stress relaxation. Relaxation time is time for which the polymer has a memory. If the relaxation time is higher than the process time, the process is predominantly characterized as elastic. If the relaxation time is shorter, then the process is viscous. After exiting the die to a strain-free state, the flow rate slows down and individual polymer chains tend to reorient and recover from deformation imposed by the rotating screw. The molecules increase their radius of gyration and they are relaxed in elastic deformation by reentanglement and recoiling. The extruded material contracts in

the flow direction and grows in the normal direction producing the die swell effect. Extent of die swell is inversely related to the number of disentanglements within the die (Wang 2012).

Mendelson et al. 1971 considered the swelling of an extrudate after flow through a die as the unretarded recovery of elastic strain imparted to the polymer by 1) elastic deformation at the entrance and 2) elastic deformation during shearing flow through the die due to the steady shear normal stresses. In longer dies with higher the die length to diameter ratio, the entrance strain declines along the length of the die and the die swell is a consequence of the deformation due to normal stress generated in the shearing field. On the other hand, in shorter dies the entrance deformation predominates.

Liang 2008 investigated an effect of extrusion conditions on die swell behavior of polypropylene/diatomite composite melts. The die swell ratio increases nonlinearly with increasing shear stress and shear rate when temperature is constant, while it decreases with a rise of temperature when the load is fixed. With increasing the die diameter a non-linear increase in die-swell ratio is observed when load and temperature are constant, whereas it reduces non-linearly with increasing the die length to diameter ratio.

In summary, high shear rates and thus compression at the die followed by sudden release of pressure is the main factor responsible for die swell. Shear rate and pressure can be increased by: increasing the output rate (polymer flow stream), decreasing die orifice dimension, reducing the melt temperature, increasing molar mass (molecular weight) and chain branching and decreasing the die length to diameter ratio (Koopmans 1992).

Koopmans 1992 pointed the significance of the temperature surrounding the extrudate on the extrudate swell. If the medium surrounding the extrudate has a lower temperature than the melt, the cooling of the emerging strand results in a lower ultimate extrudate swell.

Extrudate swell can be used to assess the polymer viscoelasticity upon melt extrusion. Through rheological characterization, extrudate swell can also be correlated with the molecular structure of the polymer (molecular weight and extents of crosslinking and long chain branching). A better understanding of such melt behavior will be beneficial for the optimization of processing parameters and the design of extrusion equipment, both of which affect product quality and production cost. Importance of die swell should be taken into consideration in controlling the size and shape as well as the mechanical properties of

the extruded products. All of these parameters are significant in investigation of extruded pharmaceutical dosage forms and may affect drug delivery (Wang 2012).

Materials Used in Hot-Melt Extrusion

In formulation and design of pharmaceutical hot-melt extruded dosage forms the selection of matrix carrier for incorporation of active compound is important step. The physical and chemical properties of the carrier control the drug release from the final dosage form and dictate the processing conditions. Beside thermoplastic carriers an extrusion composition can contain an additional compounds to improve the process and the final product quallity such as plasticizers and other processing aids (release modifying agents, antioxidants, acid receptors, thermal lubricants, light absorbens). Due to mechanical shear stress imposed by the rotating screw and thermal stress caused by reltively high precessing temperature, the formulation stability is an issue. Appart drug stability concern in the pharmaceutical formulations, polymers also may undergo chain scission, depolymerization or thermal degradation. These instability events can be identified by thermoanalytical technics such as differential scanning calorimetry and thermogravimetric analysis or by gel permeation chromatography. Hence additives are often included in the formulation to ensure stability of both drug and carrier.

Plasticizers are typically low molecular weight compounds i.e. diluents in the hot-melt extrusion formulations. Plasticizers are commonly used in small amount to improve the physical and mechanical properties of the final products. They decrease the glass transition temperature and the melt viscosity of polymers by increasing the free volume between polymer chains (Aharoni 1998). The inter-molecular secondary valence forces between plasticizer and polymer make polymer softer and more flexible. Incorporation of plasticizer enables extrusion at relatively lower temperature thus ensuring the stability of drug as well as polymer. Interestingly, in some pharmaceutical dosage forms drugs may also have a plasticizing effect (Crowley et al. 2007, Crowley et al. 2004).

1.4 PLGA Implants Prepared by Hot-Melt Extrusion

Hot melt extrusion (HME) is widely used in formulation of variety pharmaceutical dosage forms. In oral drug formulations it is applied for enhancement of the dissolution rate and bioavailability of a drug, controlling or modifying drug release, taste masking and stabilizing the pharmaceutical active ingredient. HME serves as a suitable tool for production of topical delivery systems such as dermal or transdermal patches as well as parenteral depots like injectable implants (Figure 1.10) and stents.

(a) (b)

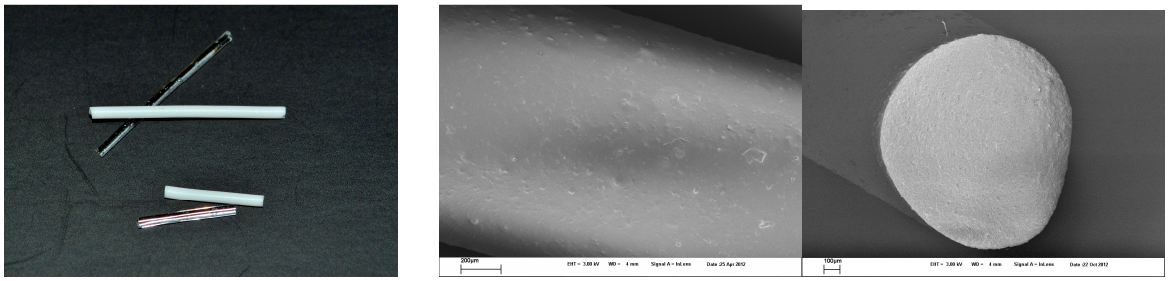


Figure 1.10. Image of PLGA implants prepared by hot-melt extrusion; (a) macroscopically and (b) observed by scanning electron microscope (SEM), the surface of implant (left) and the cross-section (right)

Several biodegradable implants prepared by hot-melt extrusion have been patented. The invention of Shiah et al. 2011 provides implants based on a mixture of hydrophilic end and hydrophobic end PLGA, which deliver active agents (dexamethasone) without a high burst release. The implants are manufactured by double extrusion process and have a size suitable for implantation in an ocular region. A hand held applicator is used to insert one or more implants into the eye. Processes used for the manufacture of the implants by double extrusion process comprise: milling of PLGAs (Resomers RG 502 and RG 502 H), blending of micronized PLGAs and dexamethasone, first extrusion at 105 °C into filament, pelletizing of filament, second extrusion at 107 °C into filament, cutting rods to size (diameter of about 0.1 mm to about 1 mm), packing of rods and sterilization by gamma radiation. A double extrusion process provides the desired filament implant with a uniform distribution of dexamethasone as well as better control of in-batch variability. The factors influencing the release kinetics comprise the particle size and solubility of the active agent, the ratio of active agent to polymer(s), the method of manufacture, the exposed surface area and the erosion rate of the polymer(s). The release of an active agent

from a biodegradable polymer matrix may also be modulated by varying the ratio of hydrophilic end PLGA to hydrophobic end PLGA in the matrix.

Siegel and Winey 2012 invented implants comprising a therapeutic drug in an amount of 10%-60% and polymer containing polylactic acid (PLA) and optionally polyglycolic acid (PGA) in a PLA to PGA molar ratio between 50:50 and 100:0. The risperidone-loaded implants for treating schizophrenia release drug at linear rate. A therapeutic level of the drug in a subject is maintained for a period from 1 to 18 months by administration of the set of biodegradable implants consisting of one or more individual implants. The production of implants comprises the processes such as compression molding, solvent casting and extrusion molding thus providing the implants of different shapes (the rods, disks, cylinders, sheets). The implants are suitable for retention in a body (e.g. subcutaneous tissue).

Currently, there are two PLGA-based implants prepared by the hot-melt extrusion on the market. Astra Zeneca provides a product of the trade name Zoladex®. It is a subcutaneous depot formulation containing goserelin acetate (LHRH agonist) for treatment of breast cancer, endometriosis and prostate cancer. The Zoladex® (3.6 mg and 10.8 mg) product is rod-shaped implant (1.2 mm x 10-12 mm or 1.5 mm x 16-18 mm) formulated for hormone delivery for a period of 28 days and 12 weeks, respectively. Implants are packaged in a sterile ready-to-use disposable applicator with an integrated needle for subcutaneous injection into the anterior abdominal wall under the supervision of a physician (Figure 1.11). Potential benefits of this kind of application compared with microspheres products are that the implants are supplied ready-to-use, with no reconstitution required, making it safe and easy for nurses and physicians to administer. They are also convenient to store at room temperature (not above 25 °C) and no refrigeration is needed.

Figure 1.11. Zoladex® implant in pre-filled syringe

Ozurdex®, Allergan is dexamethasone intravitreal implant approved by FDA in 2009 for the therapy of macular edema associated with retinal vein occlusion as well as noninfectious uveitis. The rod-shaped hot-melt extruded implant (0.46 mm x 6 mm)

consists of the drug embedded in PLGA matrix. Ozurdex® shows the duration of action from three to six months.

Leuprone® developed by Sandoz/Hexal is the only solid biodegradable implant in the leuprorelin molecule class, which is the most used synthetic nonapeptide analogue (agonist) of LHRH. The product has been used in clinical practice for more than seven years and currently it is approved in 36 countries worldwide. The rod-shaped implants in which the active substance is dispersed homogeneously in PLGA (1-month implant) or in PLA (3-month implant) are inserted by subcutaneous injection into the anterior abdominal wall. 1-month implant contains 3.6 mg of therapeutic substance, whereas only 5 mg of drug is dispersed in 3-month implant, which is half amount compared to the first product Trenantone® containing 10.72 mg leuprorelin embedded in microsphers. Leuprorelin is also formulated as 3-month in situ implant Eligard® with drug content of 22.5 mg (Geiges et al. 2013).

Profact or Suprefact Depot® is an implant for subcutaneous application which consists of LHRH analogue buserelin incorporated in PLGA with 75:25 molar ratio. Each applicator contains two identical cream-coloured, biodegradable and biocompatible rods with the total dose of 6.6 mg buserelin acetate formulated as 2-month depot. Product is available also as 3-month depot consisting of three identical rod-shaped implants with the total dose of 9.9 mg.

1.5 PLGA Reservoir Systems

According to physical structure drug delivery systems can be classified as monolithic or reservoir systems. If drug is more or less homogeneously distributed in a continuous matrix formed by the release rate controlling material, the system is monolithic. If drug is located in the center and the release rate controlling material forms a membrane surrounding drug depot, it is reservoir system. A more complex reservoir design consists of a monolithic system containing dissolved and/or dispersed drug, which is surrounded by a release-rate controlling membrane (Siepmann et al. 2012).

Considering the fact that drug release from PLGA monolithic systems is often bi- or tri-

phasic, an application of additional polymeric drug-free layer onto drug containing PLGA matrix could be an effective tool for reducing the common initial burst release and achieving the zero-order drug release rate (Fredenberg et al. 2011). PLGA reservoir systems can be prepared by dip coating process or by brush using a solution of PLGA or PLA in an organic solvent such as methylene chloride or acetone (Negrín et al. 2004; Zhou et al. 1998). However, a more sophisticated method with well-controlled process parameters is preferred, e.g. spray-coating using specialized coating devices (Figure 1.12).

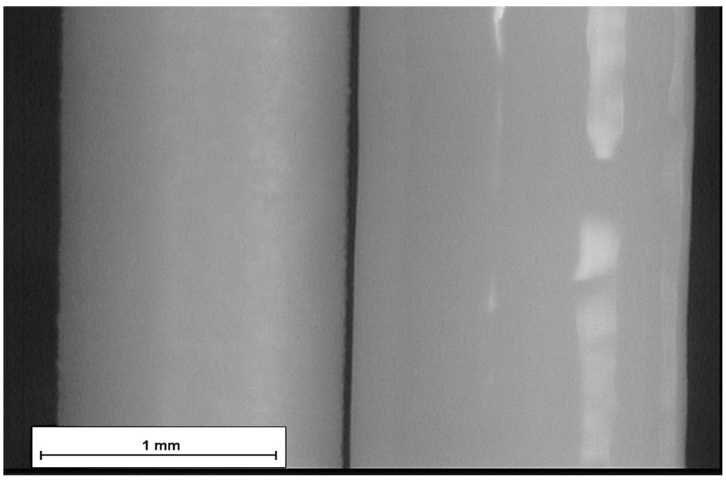


Figure 1.12. Uncoated (left) and spray-coated (right) PLGA hot-melt extruded implants using PLGA organic solution

Alternatively, for preparing PLGA reservoir systems DURIN™ Technology utilizes co-extrusion technique with two extruders connected to a coaxial die (Figure 1.13) (Gibson et al. 2002). Basically, co-extrusion is an improvement to the to the stage of melt extrusion with several advantages over the single hot-melt extrusion. Co-extrusion denotes the simultaneous extrusion of two or more materials creating the co-extrudates. The co-extrudate consists of two or more layers thus forming a lamella or concentric cylinders. The concentric cylindrical co-extrudates have several advantages: simultaneous administration of non-compatible drugs, each presented in a different layer; the external layer may function as a coat to the inner layer, either protecting the native ingredient or tailoring its release; modulation of the release of the drug either by loading the different layers with different amount of drug or by incorporating the drug in different matrices (Quintavalle et al. 2008).

The Memryte™ Implant, Durect is developed for the treatment of Alzheimer's disease using the DURIN™ technology. Co-axial implants are based on biodegradable polyesters

providing sustained release of leuprolide acetate with little to no burst release. The implant is approximately 1.5 mm x 3 cm and allows drug loadings of 60–80% (Stevenson et al. 2012).

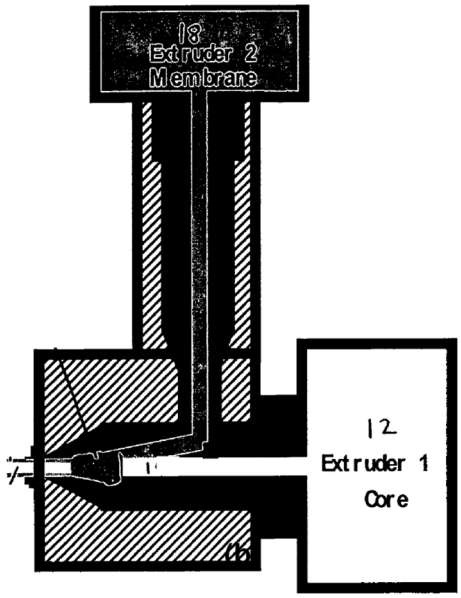


Figure 1.13. Schematic representation of co-extruder that consists of two extruders and co-axial die (Gibson et al. 2002)

1.6 *In Vitro* Drug Release Testing of Parenteral Dosage Forms

Although various *in vitro* release testing are currently applied to investigate drug release from the parenteral long acting systems, there is no pharmacopoeial/regulatory method allowing the standardized and uniform measurements (Delplace et al. 2012). Upon designing of controlled release formulations it is necessary to understand *in vivo* drug release kinetics in order to ensure effective product performance. Testing of each formulation at early stage of development *in vivo* is not practical. Accordingly, there is a need for *in vitro* test being easy to perform and predictive of *in vivo* release. Whereas *in vitro* release studies are mostly conducted in stirred media, controlled release parenteral dosage forms are often administered directly into tissue (e.g. subcutaneous, intramuscular). Parameters that may influence *in vivo* drug release and transport are highly complex. Thus, for *in vitro* release testing only certain critical parameters can be chosen and simulated under standardized and reproducible conditions. Several parameters have to

be considered upon developing *in vitro* release tests including sink conditions, product and drug stability in the media during the study, extent of *in vitro* release, *in vivo* relevance (temperature, agitation, sampling methods, pH and buffer capacity of the medium), accelerated release (due to long-term release) and dissolution vessel selection (Kastellorizios and Burgess 2012).

Common dissolution methods for controlled release parenteral products are mostly non-compendial, although they sometimes include USP apparatus designed for other routes of administration. They are categorized into three general groups: sample and separate-methods, dialysis methods and flow-through methods (Figure 1.14) (Kastellorizios and Burgess 2012; Seidlitz and Weitschies 2012).

The impact of the experimental conditions used for drug release measurements from PLGA parenteral depot systems have been reported in the literature, but not yet fully understood.

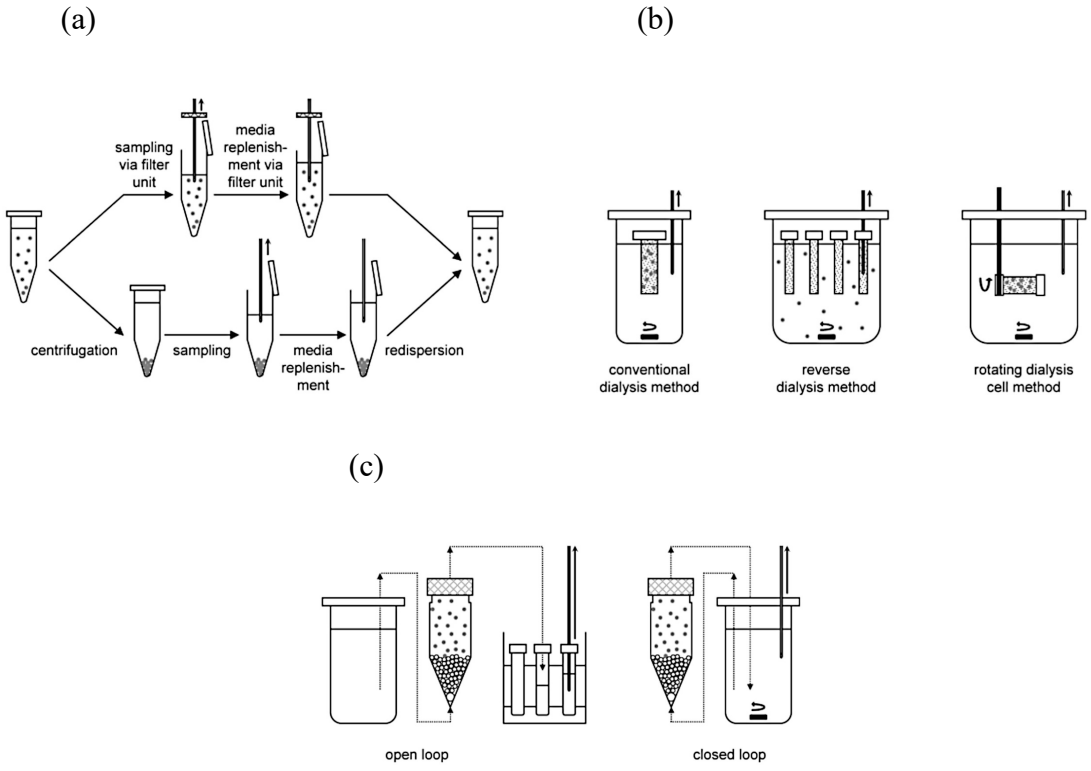


Figure 1.14. Dissolution testing of parenteral dosages forms; (a) sample and separate methods, (b) dialysis methods and (c) flow-through methods (Seidlitz and Weitschies 2012)

Zolnik et al. 2005 and 2006 have developed a modified USP apparatus 4 (flow-through cell) method for *in vitro* release testing of microspheres under real time and accelerated

testing conditions. They have demonstrated the advantages of the USP apparatus 4 method compared to conventional sample and separate method. Later Rawat et al. 2011 investigated the suitability of the modified USP apparatus 4 for possible compendial adaptation for drug release testing of microspheres. The robustness and reproducibility of method was tested using commercially available risperidone PLGA-based microparticles. Risperidone release was not affected by flow rate as well as by minor variations in the method such as amount of microspheres, flow-through cell size, size of glass beads. However, the significant difference in release was observed by slight variation in temperature. Delplace et al. 2012 tested PLGA-based microparticles with agitated and "non-agitated" flasks and tubes, flow-though cells as well as agarose gels showing more or less sensitive/robust drug release profiles.

Although, the flow-through apparatus was recommended in the FIP/AAPS guidelines on dissolution/*in vitro* release testing of novel/special dosage forms including also subcutaneous implants, a major limitation of the apparatus is that the implant is directly placed in the flow of the medium. This is not a fully representation of the *in vivo* environment. Beside modification in size of the cell in order to accommodate implants, further adjustments are necessary (Iyer et al. 2006). Implants are dosage forms that are subcutaneously placed with the aid of surgery or a hypodermic needle. Thus, *in vivo* conditions denote an implant being surrounded by the adipose tissue. Toward more realistic *in vitro* release test for implants, the utilization of hydrogel materials as the tissue-mimicking compartments is a promising option (Klose et al. 2008; Delplace et al. 2012; Semmling et al. 2013).

1.7 Objectives

The purpose of this study was to develop biodegradable implants with specific drug release patterns, from zero-order to pulsatile drug delivery rate. Particular goals were:

a. To reduce the initial drug release from the PLGA hot-melt extruded implants by controlling the process and formulation parameters
b. To investigate the effect of post-preparation thermal treatment, i.e. curing on the reduction of initial burst release from the PLGA hot-melt extruded implants
c. To evaluate the drug release mechanisms from the PLGA hot-melt extruded implants as a function of drug solubility and loading as well as PLGA type
d. To analyse how drugs of different water solubility affect the properties of PLGA matrix regarding the mechanism and extent of polymer degradation as well as ability to swell and uptake water
e. To modulate drug release profiles from the PLGA hot-melt extruded implants by applying a drug-free PLGA layer based on different polymer type and coating level
f. To asses the swelling ability and the drug release from the PLGA implants *in vitro* and *ex vivo* with focus on evaluation of biorelevant dissolution test for subcutaneous implants
g. To develop a high drug-loaded reservoir systems based on polyethylene glycol or calcium hydrogen phosphate compressed cores and drug-free PLGA or PLA shell

CHAPTER

2 Materials and Methods

2.1 Materials

Model Drugs

Dexamethasone sodium phosphate (CHEMOS GmbH, Regenstaut, Germany); Prednisone (micronized) (FAGRON GmbH & Co. KG, Barsbüttel, Germany); Theophylline (BASF AG, Ludwigshafen, Germany)

Table 2.1. Properties of model drug

Drug	Drug solubility[1] (mg/ml)	Melting point (°C)	Molecular weight (g/mol)
Dexamethasone sodium phosphate	466.7	242.6	516.4
Theophylline	10.4	271.3	180.1
Prednisone	0.2	243.52	358.4

[1] at 37 °C in phosphate buffer pH 7.4 (USP) (50 mM)

Polymers

Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 502 H (PLGA 502 H); Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 504 (PLGA 504); Poly(D,L-lactide) Resomer® R 203 S (PLA 203 S) (Evonik Industries AG Pharma Polymers, Darmstadt, Germany)

Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 502 (PLGA 502); Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 503 H (PLGA 503 H); Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 503 (PLGA 502); Poly(D,L-lactide-co-glycolide) 65:35, Resomer® RG 653 H (PLGA 653 H); Poly(D,L-lactide-co-glycolide) 75:25, Resomer® RG 752 H (PLGA 752 H); Poly(D,L-lactide-co-glycolide) 75:25, Resomer® RG 756 S (PLGA 756 S) (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany)

Table 2.2. Properties of biodegradable polymers

Polymer name	Inherent viscosity[1] (dL/g)	Molecular weight (g/mol)	Glass transition temperature (°C)	End group
PLGA 502 H	0.16-0.24	7,000-17,000	42-46	Acid
PLGA 502	0.16-0.24	7,000-17,000	42-46	Ester
PLGA 503 H	0.32-0.44	24,000-38,000	44-48	Acid
PLGA 503	0.32-0.44	24,000-38,000	44-48	Ester
PLGA 504	0.45-0.60	38,000-54,000	46-50	Ester
PLGA 653 H	0.32-0.44	24,000-38,000	46-50	Acid
PLGA 752 H	0.14-0.22	4,000-15,000	42-46	Acid
PLGA 756 S	0.71-1.00	76,000-115,000	49-55	Ester
PLA 203 S	0.25-0.35	18,000-28,000	46-50	Ester

[1] Inherent viscosity is measured at 0.1% w/v in chloroform at 25 °C

Polyethylene glycol (PEG) 1500, 4000 and 6000 (Lutrol® E, BASF AG, Ludwigshafen, Germany)

Other Excipients

Stearic acid; acetyltributyl citrate (ATBC); potassium dihydrogen phosphate (Carl Roth GmbH+Co.KG, Karlsruhe, Germany); calcium hydrogen phosphate anhydrous (Merck KGaA, Darmstadt, Germany); sodium hydroxide (Carl Roth GmbH+Co.KG, Karlsruhe, Germany)

Solvents

Ethyl acetate (Carl Roth GmbH & Co. KG, Karlsruhe, Germany); acetone; dichloromethane (Merck KGaA, Darmstadt, Germany); tetrahydrofuran (Carl Roth GmbH & Co. KG, Karlsruhe, Germany)

Tissues and Other Materials

Porcine adipose tissue and piece of chicken tissue with skin were obtained from a local slaughterhouse. The tissue was cut into pieces having size of 2 cm x 2 cm and of 1 cm thickness.

Catheters: 0.20 ml and 0.05 ml CAP.PLASMA Tyvek width: 20 cm Lot.No.: T110220 and 1.60 ml CAP. PLASMA HMTS LOT/Batch 0410001 75 mm

Hydrogel: Agarose, Carl Roth GmbH+Co.KG, Karlsruhe, Germany

Fat emulsion: Lipofundin® MCT 20%, B. Braun, Melsungen AG, Melsungen, Germany

2.2 Methods

Preparation of Implants

Hot-Melt Extrusion with a Twin-Screw Extruder (HME)

The extrusion process was performed using a co-rotating twin-screw hot-melt extruder (HAAKE MiniLab Rheomex CTW5, Thermo Scientific, Karlsruhe, Germany) at different process parameters: screw speed of 5 and 20 rpm; die diameter of 0.75 and 1 mm, extrusion temperature 65 °C, 90 °C and 100 °C. Polymer and drug were manually premixed using a mortar and pestle. Powder blends of 5 g were fed into the preheated barrel by utilizing force feeder. At the end of barrel the cylindrical die was attached resulting in strand of higher diameter compared to diameter of the die from which it was extruded. The first strand came out about 13-25 minutes after the feeding of the powder blend. Extruded material was collected and weighed in order to determine the yield of the

process. The cylindrical strands were cut into rods of 5 mm length.

During process the torque values were recorded continuously and the maximum value was considered to reflect the melt viscosity of extrusion material.

Milling of Extrudates

One fraction of extrudates based on prednisone and PEG were milled prior compression by ball mill (10 min, 40 rpm), Retsch MM2000, Retsch GmbH Haan, Germany.

Compression

Drug and carrier powder were manually premixed in different ratios on a weight base using a mortar and pestle. Besides the physical mixture, compressed implants were also prepared using the milled extrudates. Stearic acid (2% w/w) as lubricant was added into powder mixture or to the milled extrudates and shortly mixed. Materials were compressed using punch and die set of 2 mm in diameter with a concave surface. 108 mg of material was weighed and subsequently filled manually into the die consisting of 18 matrices. The direct compression process was performed with an equipped single punch tableting press (EK0, Korsch AG, Berlin, Germany)

Hardness testing of the compressed implants was performed using a texture analyzer (TA.XT plus, Stable Micro Systems, Winopal Forschunungsbedarf GmbH, Ahnsbeck, Germany).

Spray Coating

Spray coating process of the PLGA extruded rod-shaped implants was performed in a self-built pan device. A rotating glass vessel with attached deflectors at equal distance was used as the pan. Coating material consisting of 2% w/w polymer solution in ethyl acetate and acetone was sprayed by a spray gun (Walther, Wuppertal, Germany). Batch size was 20 g using as placebo colored hot-melt extruded rods based on ethyl cellulose having the same diameter and length as PLGA implants. Process was performed at room temperature. Processing parameters were as follows: nozzle diameter of 1 mm, atomizing air pressure of 0.5 bar and spray rate of 0.15 g/min. Weight gain was calculated by the following equation.

$$\text{Weight gain (\%)} = \frac{\text{Weight of coated implants} - \text{Weight of uncoated implants}}{\text{Weight of uncoated implants}} \times 100$$

Spray coating process of the compressed implants was performed in a rotor mini-coater using 2% w/w polymer solution in ethyl acetate and acetone as a coating material. Batch size was 50 g. The colored spheres of the same diameter as the diameter of compressed uncoated implants were used as placebo. Process was performed at room temperature. Processing parameters were as follows: atomizing air pressure of 1.4 bar, air flow of 10 bar and spray rate of 0.3 g/min.

Curing Process

Laboratory-scale curing process was performed by placing the hot-melt extruded rod-shaped implants of 5 mm length in an aluminium pan and subsequently in a pre-heated oven at certain temperature (50 °C, 60 °C and 70 °C) for a predetermined time (15 min, 5 h and 15 h). Some fractions of implants were cured at 40 °C and 75% RH. In order to assess the process feasibility at a bigger scale, a large number of implants was placed in the narrow vessel and curing at different conditions was performed.

Drug Extraction from Implants

To quantitatively detect the actual drug loading of implants, 40 ml or 20 ml of 2M NaOH was added to a vial containing the accurately weighed prednisone or dexamethasone sodium phosphate implant, respectively (one implant per vial, n=3). The vials were place into sonication bath (Bandelin Sonorex RK 512H, Bandelin Sonorex Super RK 255H, Retsch) until the implants were completely dissolved. The solution was analyzed by UV/VIS spectrophotometer with a Peltier thermostatted cell holder (Agilent 8453, Agilent Technologies Inc., Palo Alto, USA) equipped with UV-Chemstation biochemical analysis software at 243 nm or 242 nm, for prednisone or dexamethasone sodium phosphate implants, respectively and with single wavelength background correction at 400 nm.

Additionally, extraction of prednisone from PLGA-based hot-melt extruded implants was performed using an organic solvent. Weighed amounts of implants and corresponding physical mixtures were dissolved in 10 ml of dichloromethane (one implant per vial, n=3). Samples were diluted with solvent in ratio of 1:10 and anlyzed using UV/VIS spectrophotometer at 241 nm. Drug-free implants were prepared and subjected to the same procedure. The obtained solution was used as a blank. PLGA did not show any significant UV absorbance in the selected wavelength range.

In Vitro Drug Release Study

Implants were placed in glass vials filled with 50 mM phosphate buffer pH 7.4 (USP), which was used as release medium (one implant per vial, n=3). For dexamethasone sodium phosphate and theophylline implants 20 ml of medium was used, whereas for prednisone implants 40 ml of medium was required in order to maintain the sink condition during the drug release study. The vials with implants were incubated in a horizontal shaker (37 °C, 80 rpm) (GFL 3033, Gesellschaft für Labortechnik GmbH & Co. KG, Burgwedel, Germany). At predetermined time points, 2 ml of sample was withdrawn and replaced with fresh medium. Drug concentrations in the samples were quantified spectrophotometrically at 242 nm, 272 nm and 243 nm for dexamethasone sodium phosphate, theophylline and prednisone formulations, respectively and with single wavelength background correction at 400 nm.

Remaining drug content was quantitatively detected when the drug release studies were terminated. The implants were withdrawn from the release medium and dried in the vacuum oven (Heraeus oven VT 5042 EKP, Hanau, Germany coupled with a chemistry hybrid pump, Vacuubrand GmbH, Wertheim, Germany). 10 ml of 2M NaOH was added to a vial containing the dried implant. When the implant was completely dissolved, the final solution was analyzed by UV/VIS spectrophotometry at the wavelength mentioned above.

The drug release profiles of uncured and cured implants extruded at 100 °C and screw speed of 5 rpm were compared using the similarity factor f_2 (Moore and Flanner 1996; Stevens et al. 2015)

$$f_2 = 50\log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n} W_t(R_t - T_t)^2\right]^{-0.5} 100\right\}$$

where R_t and T_t are the percentage drug dissolved at a time point t from the reference (uncured implants) and the test profile (cured implants); n is the total number of sampling time points; W_t is an optional weight factor, which was set to 1 in the present study. Release profiles with f_2 value above 50 are considered similar to the reference profile (<10% difference), whereby a value of 100 represents identical profiles.

Water Uptake and Mass Loss

Water uptake and mass loss were determined gravimetrically using Mettler M3 microbalance (Mettler Toledo, Gießen, Germany) at the same incubation conditions as described above for the drug release study. The implants were weighed accurately (Initial weight) and immersed in phosphate buffer pH 7.4 (USP) (one implant per vial, n=3). At predetermined time points, the implants were removed from the medium, blotted with tissue paper to remove surface medium and then weighed (Wet weight). The implants were dried in the vacuum oven overnight until constant weight (Dry weight) (Heraeus oven VT 5042 EKP, Hanau, Germany coupled with a chemistry hybrid pump, Vacuubrand GmbH, Wertheim, Germany). Water uptake and mass loss were calculated by the following equations:

$$\text{Water uptake (\%)} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Dry weight}} \times 100$$

$$\text{Mass loss (\%)} = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Initial weight}} \times 100$$

Ex Vivo Drug Release Study, Water Uptake and Swelling Extent

Implants based on PLGA 502 H and loaded with 10% and 30% of dexamethasone sodium phosphate were accurately weighed (Initial weight) using Mettler M3 microbalance (Mettler Toledo, Gießen, Germany). Diameter and length of the rod-shaped implants were also measured and volumes were calculated accordingly (Initial volume). The implants were then inserted into the pieces of the adipose tissue with the aid of needle having the same diameter as the implants. The samples were subsequently immersed in 20 ml phosphate buffer pH 7.4 (USP) in petri dishes (one sample per petri dish, n=3). Simultaneously, the same implant formulations were placed in glass vials filled with 20 ml of release medium (one implant per vial, n=3). All samples were incubated in a horizontal incubation shaker (37 °C, 80 rpm) (GFL 3033, Gesellschaft für Labortechnik GmbH & Co. KG, Burgwedel, Germany) during 24 h. At predetermined time points the implants were withdrawn from the adipose tissue and release medium, blotted with paper to remove surface medium and then weighed (Wet weight). The diameters and lengths of the wet implants were measured and volumes were calculated (Wet volume). Subsequently, the implants were dried in the vacuum oven (Heraeus oven VT 5042 EKP, Hanau, Germany coupled with a chemistry hybrid pump, Vacuubrand GmbH, Wertheim, Germany)

overnight until constant weight (Dry weight). The dried implants were dissolved in 10 ml 2M NaOH and the residual drug content was determined by UV/VIS spectrophotometry. Water uptake of implants was calculated by the equation introduced above, whereas swelling extent was calculated by the following equation:

$$\text{Swelling extent (\%)} = \frac{\text{Wet volume}}{\text{Initial volume}} \times 100$$

Simulation of Counter-Pressure of Adipose Tissue on Implants

Catheters of different balloon volume (0.05 ml, 0.20 ml and 1.60 ml) placed in the adipose tissue were inflated with water, and texture analyzer (TA.XT plus, Winopal Forschunungsbedarf GmbH, Ahnsbeck, Germany) was used for detection of resistance i.e. counter-pressure of the tissue to the extension of balloon (Figure 2.1). The required forces applied by the texture analyzer probe to squeeze out a certain volume of water in the balloon, when the balloon was free in the air or inserted into the tissue, were detected.

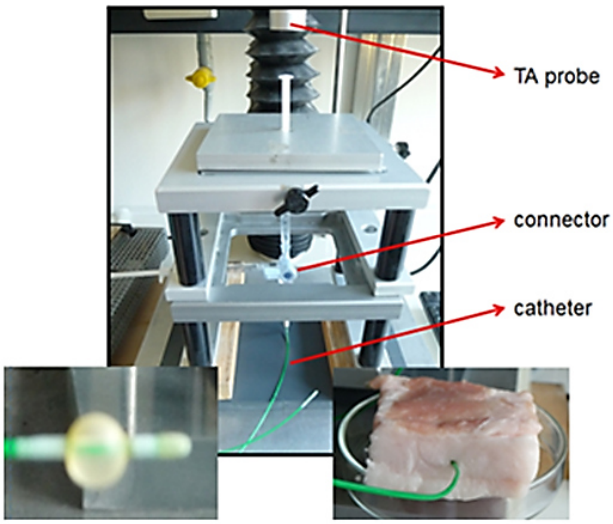


Figure 2.1. Experimental set up for simulation of counter-pressure of adipose tissue on implants

Thus, force versus distance diagram was obtained (Figure 2.2). The profile could be divided into three phases. An initial increase of force occurred quicly resulting in a peak value. The first peak represented the force required to initiate the syringe plunger. Afterwards, a filling of the catheter tube with water continued and the force value was slightly increased until reached the second peak value. The peak represented the onset of expansion of balloon. When balloon was free in the air, the second peak was followed by the last phase of the constant force that corresponded to the extension of balloon freely to the maximal volume. However, when the balloon was inserted into the tissue, the second

peak was followed by a phase of an increase in the force. This phase demonstrated the resistance of the tissue to the extension of balloon. The recorded force values (n=5) in the last phases of the diagram for balloon in the air and balloon in the tissue were averaged and their difference considered as the counter-pressure of the tissue.

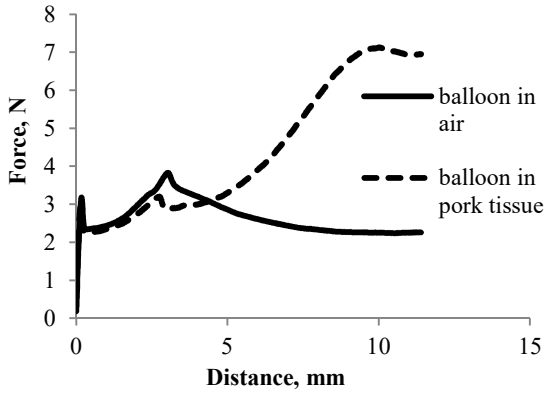


Figure 2.2. Force versus distance diagram

Preparation of Agarose Gel

Agarose powder was weighed and dissolved in the certain amount of the hot, purified water under stirring at 90 ˚C. Evaporated water was added during cooling at a temperature of approximately 50 ˚C. Prior to gelation the polymer solution was casted into a mold and was allowed to solidify at room temperature.

Differential Scanning Calorimetry (DSC)

DSC-studies of drug and polymer powders, physical mixtures or implants were performed with a DSC821e (Mettler Toledo AG, Giessen, Germany) coupled with a Mettler TC15 TA-controller. Samples were accurately weighed in 40 µl aluminum crucibles, closed and a pinhole was introduced into the lid. DSC scans were recorded from 0 °C to 300 °C using a heating rate of 10 °C/min under nitrogen atmosphere. To determine glass transition temperature (T_g), samples were subjected to heat–cool–heat cycle to remove thermal history of the sample. Thermographs were normalized by drug weight.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was used for determination of the water content in drug powder, dexamethasone sodium phosphate. Sample was accurately weighed in 40 µl aluminum crucibles, closed and a pinhole was introduced into the lid. The weight change

of a solid sample was detected upon exposure to an elevated temperature. The sample was heated from 25 °C to 120 °C with a heating rate of 20 °C/min and then kept at 120 °C for one hour. The mass loss of sample (m~5 mg) was detected by a Mettler TC15 TA-controller coupled with a Mettler TG 50 thermobalance (Mettler Toledo AG, Giessen, Germany).

Determination of Water by the Karl Fischer Method

To determine the water content of dexamethasone sodium phosphate, C20 Coulometric Karl Fisher titrator was used (Mettler-Toledo AG, Analytical, Schwerzenbach, Switzerland). The accurately weighed drug (21.92 mg) was dissolved in 5 ml of dehydrated methanol. Firstly, various aliquots of dehydrated methanol (n=4) were added to the titration vessel and titrate to the end-point with Karl Fischer reagent (Hydranal®-Coulomat AD, Sigma-Aldrich CHEMIE, GmbH, Steinheim). Various aliquots of drug solution in methanol (n=4) were then injected into titration vessel and titrate to the end-point with Karl Fischer reagent. Subsequently, the water content was calculated.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR-spectra were generated with an Excalibur 3100 FTIR spectrophotometer (Varian Inc., Palo Alto, USA). The spectra from drug and polymer powder, physical mixture and ground implants (with a mortar and pestle) were collected using a horizontal ATR accessory with a single reflection diamond crystal (Pike Miracle, Pike Technologies, Madison, USA). Sixty four scans at 4 cm^{-1} resolution were averaged and spectral contributions coming from water vapor in the light pass were subtracted using Varian software (Resolution Pro 4.0). Finally, all spectra were treated with a 13-point smoothing function.

PLGA Degradation Study and Molecular Weight Determination

Hot-melt extruded implants (10-15 mm long; 20 mg weight) were placed in glass vials filled with 50 mM phosphate buffer pH 7.4 (USP) (one implant per vial, n=3). For the prednisone implants 40 ml of medium was used, whereas for dexamethasone sodium phosphate implants and pure PLGA 502 H implants vials were filled with 20 ml of medium. The vials were incubated in a horizontal shaker (80 rpm, 37 °C) (GFL 3033, Gesellschaft für Labortechnik GmbH & Co. KG, Burgwedel, Germany). At predetermined time points degraded implants were withdrawn, vacuum dried for 24 h and dissolved in 1

ml of tetrahydrofuran. The turbid samples were centrifuged at 17000 rpm for 15 minutes (HeraeusTM BiofugeTM, Thermo Fisher Scientific Inc., Waltham, MA, USA) to remove the particles. The samples were analyzed for quantifying the molecular weight distribution of the remaining polymer by gel permeation chromatography. Gel permeation chromatography (GPC) analysis was carried out using Shimadzu (Shimadzu, Tokyo, Japan) LD-10 liquid chromatograph equipped with degasser, pump, auto-injector and column oven in combination with Viscotek triple detector (TDA-300, Viscotek, Malvern Instruments Ltd., Malvern, UK) operated in double mode (differential refractive index, viscosimetry). A column with a linear range from 500 g/mol to 18,000 g/mol (Mesopore 7.5 µm x 300 mm; Varian Inc., Darmstadt, Germany) was used as stationary and tetrahydrofuran as mobile phases. The sample concentration was 20 mg/ml with the corresponding injection volumes of 25 µl. Column and detector were operated at 30 °C and the flow rate was 1 ml/min. A universal calibration method (third-order polynomial fit, R^2: 0.99996) was applied to determine the molecular weights of PLGA, which was obtained from polystyrene standards with peak molecular weights of 1,260 g/mol, 2,360 g/mol, 4,920 g/mol, 9,000 g/mol, 19,880 g/mol (Varian Inc., Darmstadt, Germany). Data acquisition was performed using Omnisec software (Viscotek, Malvern Instruments Ltd., Malvern, UK).

The pH and drug content in the release medium was monitored at each time point with a pH-meter (Sartorius, Sartorius AG, Göttingen, Germany) and UV/VIS spectrophotometer with a Peltier thermostatted cell holder (Agilent 8453, Agilent Technologies Inc., Palo Alto, USA) equipped with UV-Chemstation biochemical analysis software.

Determination of Implant Morphology by Optical Light Macroscope

Implant morphology, rupturing behavior of coated implants as well as determination of implant size was studied using a macroscope (Inteq Informationstechnik GmbH, Berlin, Germany). The implants before incubation and during the drug release study were observed at the predetermined time point. The magnification of macroscope was adjusted to obtain a clear observation. The images were recorded by image analysis software (EasyMeasure, Inteq Informationstechnik GmbH, Berlin, Germany).

Scanning Electron Microscopy (SEM)

Scanning electron microscopy, SEM (Zeiss Gemini LEO 1550) was employed to analyze the structure of implants (surface porosity and drug distribution). All samples were sputtered with a gold/palladium mixture to avoid electron charging of the samples during SEM analysis. The samples were studied using an operating voltage of 3 kV and different magnifications.

Drug Solubility Study

Excess amount of the model drug (prednisone, theophylline, dexamethasone sodium phosphate) was placed into 3 ml of phosphate buffer pH 7.4 (USP). The samples were incubated in a horizontal incubation shaker (GFL 3033, Gesellschaft für Labortechnik GmbH & Co. KG, Burgwedel, Germany) at 37 °C and 80 rpm for 48 h. The saturated drug solution was filtered and diluted to achieve an appropriate concentration for analysis using spectrophotometry. The pH of final solution was confirmed to be 7.4. The drug content corresponding to drug solubility at certain conditions was quantified by UV/VIS spectrophotometer with a Peltier thermostatted cell holder (Agilent 8453, Agilent Technologies Inc., Palo Alto, USA) equipped with a UV-Chemstation biochemical analysis software at 243 nm, 272 nm and 242 nm for prednisone, theophylline and dexamethasone sodium phosphate, respectively and with single wavelength background correction at 400 nm. The experiment was run in duplicate for each analyzed drug.

CHAPTER

3 Results and Discussion

3.1 Reduction of Initial Burst Release from Hot-Melt Extruded Poly(lactide-co-glycolide) Implants by Controlling Process and Formulation Parameters

The purpose of this study was to optimise the extrusion process and to investigate the effect of curing on the initial drug release from hot-melt extruded implants as a function of curing temperature/time, molecular weight of biodegradable polymer, drug loading and solubility.

3.1.1 Background

PLGA-based drug delivery systems commonly show bi- or tri-phasic release profiles due to diffusion/heterogeneous degradation of biodegradable polymers (Berchane et al. 2007; Berkland et al. 2003; Fredenberg et al. 2011). Tri-phasic release profile is particularly observed in the case of larger particles or dosage forms. Phase I is usually referred as initial burst. The initial burst denotes diffusion-controlled release of drug at the surface of the system or poorly encapsulated particles in the pores close to the surface, which diffuse out immediately after hydration of system (Wang et al. 2002). Phase II, also known as a lag phase, is commonly characterized by a slow drug release through relatively dense polymer. Formation of the skin structure, pore closing, polymer-drug interaction or drug-drug interaction are the explanations for the lag phase (Kang and Schwendeman 2006; Kang et al. 2008). Phase III is often described as the second burst indicating the period of faster drug release due to polymer erosion.

Although official definition of burst release has not been established, some researches consider the burst as the amount of drug released from parenteral delivery system up to 24 h after administration. This phenomenon is also described as a change in the drug release kinetics independent of specific time points (Wright and Burgess 2012). The burst effect is observed in systems with low molecular weight active ingredients as well as with peptides and proteins. Controlled release formulations containing hydrophilic drugs are more likely to show the initial burst.

Despite the fact that in certain drug therapies the initial burst release is a part of a therapeutic regimen, mostly it is considered as undesirable. The amount of drug released in the initial stage is often unpredictable and cannot be precisely and reproducibly controlled. In addition, the high initial release may reach the toxic limit and thus can be pharmacologically dangerous (Huang and Brazel 2001). Even if the initial burst release does not cause any adverse effect, it is economically and therapeutically inefficient because this amount of drug is excreted before its pharmacological effects are manifested. Therefore, understanding of physical mechanisms of the burst effect and the approaches to prevent burst have been occupying an attention of many researchers.

Several formulation parameters can be varied in order to reduce the initial burst release

from PLGA-based drug delivery systems: polymer type, molecular weight or modification of polymer (Jaraswekin et al. 2007; Luan and Bodmeier 2006; Ravivarapu et al. 2000a; Bodmeier et al. 1992); drug loading, particle size of drug or modification of drug (Ghalanbor et al. 2012; Desai et al. 2008; Ravivarapu et al. 2000b; Bodmeier et al. 1992); system porosity (Ahmed and Bodmeier 2009; Jaraswekin et al. 2007); preparation method (Jaraswekin et al. 2007) and additives (Ghalanbor et al. 2010). Additionally, Ahmed et al. 2012 achieved the reduction in burst release by coating PLGA microparticles with a drug-free PLGA layer.

Drug-loaded PLGA implants for subcutaneous injection are typically prepared in a rod-like shape by process of hot-melt extrusion (HME) at elevated temperatures. However, extrusion conditions affect significantly the product quality. Physical properties of extrudates can vary considerably depending on the extruder type, temperature profile in the barrel, screw configuration, screw speed, feed rate and die profile. Furthermore, the die swell phenomenon plays an important role in controlling the size, shape as well as mechanical properties of the extruded products (Wang 2012). Considering the fact that altering of implant density has a large influence on drug release pattern due to effect on drug diffusion and PLGA degradation, adjusting the processing parameters could be an efficient tool in obtaining the desired drug release profile.

Therefore, the purpose of this study was to reduce the initial burst and to achieve linear drug release kinetics by manipulating processing and formulation parameters. In addition, the effect of curing i.e. post-preparation thermal treatment as an approach to decrease surface porosity of extruded PLGA implants was investigated.

3.1.2 Effect of Processing Conditions

Hot-melt extrusion is increasingly used as a drug delivery technology in the pharmaceutical industry. It is a solvent-free, one-step process and can be operated in a continuous manner (Wischke and Schwendeman 2012). In addition, hot-melt extrusion represents an efficient method for producing uniform solid dispersions in the form of injectable implants based on PLGA due to the intense mixing and agitation caused by the rotating screws (Breitenbach 2002; Ghalanbor et al. 2010; Gosau, and Müller 2010).

However, the most common issue regarding drug release pattern from hot-melt extruded PLGA implants is the initial burst release, particularly for systems loaded with highly water-soluble actives. Besides taking into an account the formulation parameters, the possibility to modify the initial burst release as well as a shape of drug release curve by controlling the process parameters was investigated.

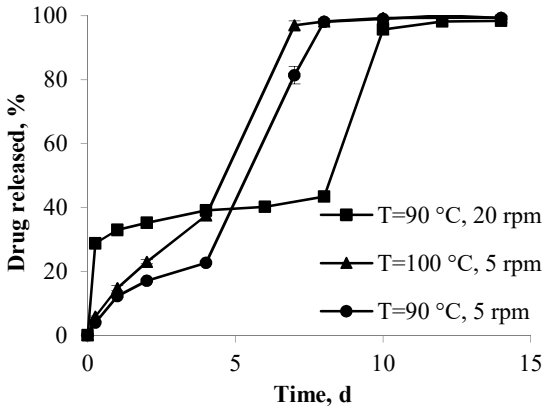


Figure 3.1.1. Effect of processing parameters (barrel temperature and screw speed) on drug release from PLGA 502 H implants containing 10% w/w dexamethasone sodium phosphate; die diameter was 1 mm

For this purpose, PLGA-based implants loaded with 10% dexamethasone sodium phosphate were prepared under different extrusion conditions; barrel temperature and screw speed (Figure 3.1.1). The matrices extruded at 90 °C and a screw speed of 20 rpm showed the typical tri-phasic drug release pattern with initial burst release of about 30%, followed by a lag phase and phase of fast drug release. Moreover, the increased extrudate diameter relative to the diameter of the die was observed. This was attributed to the die swell phenomenon occurred to extrudate upon exiting the die. Taking into account this phenomenon, it was hypothesized that die swell might contribute to higher implant porosity, which in turn could cause faster medium uptake and drug diffusion in the initial phase (Crowley et al. 2007). In addition, as a significant length of extrudate was formed, the influence of gravity caused sagging, i.e. the elongation of extrudate. The elongation of extrudate could result in the formation of cracks on the emerging strand along with the reduction of the die swell effect (Koopmans 1992). Accordingly, slower screw speed during extrusion could allow faster cooling of emerging strand before reaching a significant length to be elongated by gravity. Thus, the cracks formation on the completely non-hardened implants could be prevented contributing to higher implant porosity. Other benefit of the slower screw speed during extrusion was the reduction of the die swell ratio.

Moreover, Liang 2008 showed that an increase of operating barrel temperature also led to the reduced die swell ratio.

Therefore, in order to increase polymer matrix density, the extrusion parameters were varied. With decreasing screw speed from 20 rpm to 5 rpm and maintaining barrel temperature at 90 °C, the drug release in the initial phase as well as the lag phase were decreased. However, the entire drug release profile was still tri-phasic. In addition to the decreased screw speed to 5 rpm, an increase of the production temperature from 90 °C to 100 °C resulted to almost a linear drug release pattern. Higher polymer matrix density caused the accelerated PLGA degradation and thus elimination of the lag phase (Klose et al. 2006; Siepmann et al. 2005).

3.1.3 Curing Effect

By controlling process parameters, the desired drug release profile from hot-melt extruded PLGA implants was successfully achieved. However, the extrusion process was slowed down and performed at higher temperature. High-energy input mainly related to the elevated temperature is unsuitable operating condition causing problems in manufacturing of temperature sensitive compounds, in particular peptide and protein drugs (Crowley et al. 2007; Mauriac and Marion 2006). Therefore, alternative approach to reducing the porosity of the implants and consequently the initial burst release was necessary.

Ahmed and Bodmeier in 2009 proposed a non-aqueous curing technique at a temperature near or above the glass transition temperature of the polymer as a tool to reduce the initial burst release from porous microparticles.

The main goal of the further study was to investigate how post-preparation thermal treatment, i.e. curing of the PLGA extruded matrices at relatively low temperatures when compared to the process temperature affected the drug release.

Effect of Curing Temperature on Dexamethasone Sodium Phosphate Release from Implants of Different Diameters

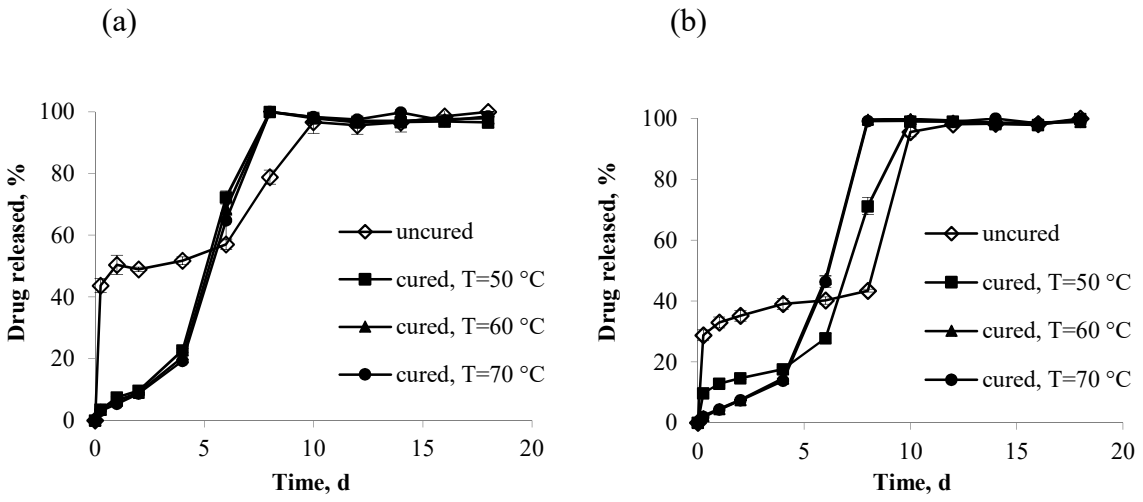


Figure 3.1.2. Effect of short-term curing (15 min) at different temperatures on dexamethasone sodium phosphate release from PLGA 502 H implants of (a) ~0.8 mm and (b) ~1.1 mm diameter with 10% w/w drug loading

With an increase in implant diameter, a slower initial drug release rate and a longer lag phase were observed from hot-melt extruded PLGA implants (Figure 3.1.2). Subsequently, the implants were exposed to post-preparation thermal treatment for 15 minutes at temperatures from 50 °C to 70 °C. The initial burst release of highly soluble dexamethasone sodium phosphate was gradually reduced with increasing curing temperature. Increase of the curing temperature above 50 °C for implants with a diameter of ~0.8 mm or 60 °C for implants with a diameter of ~1.1 mm showed no further effect on the initial burst release. The influence of curing was more pronounced in the case of smaller diameter implants. Curing at 50 °C led to a decrease the drug release in the initial phase from 50% to 7% and from 30% to 12% within one hour for implants with a diameter of ~0.8 mm and ~1.1 mm, respectively. This was presumably attributed to higher die swell ratio of polymeric matrices extruded at the same extrusion temperature and screw speed, but utilizing die of smaller diameter, thus resulting in the formation of more porous implants. In addition, a more effective matrix densification was achieved with decreasing implant size due to the higher heat transfer.

It was anticipated that the curing associated changes to the surface morphology of implant might be observed. The strong attenuation of the burst release was very likely related to the surface pore modification. In order to prove that curing at temperature above the glass

transition of the polymer led to the decrease in porosity, surfaces of the implants were analysed by scanning electron microscope (Figure 3.1.3).

(a) (b)

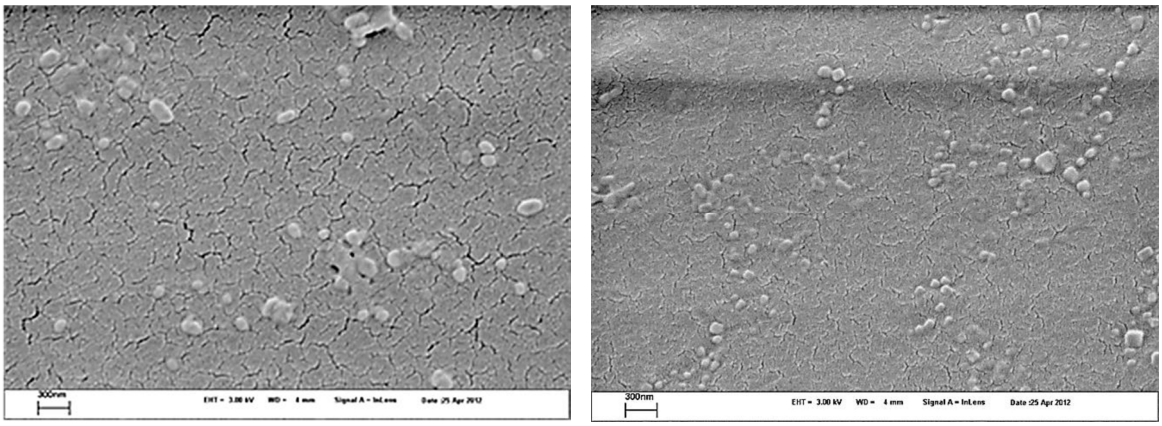


Figure 3.1.3. Surface of implant of ~1.1 mm diameter containing 10% w/w dexamethasone sodium phosphate (a) before and (b) after curing at 50 °C analysed by scanning electron microscope (SEM)

Cracks and channels in the uncured implant were created due to elongation of the completely non-hardened extruded strand upon exiting the die as well as owing to the die swell phenomenon (Figure 3.1.3.a). This facilitated the burst release by increasing the effective surface area and decreasing the resistance to water uptake and drug diffusion (Allison 2008; Koopmans 1992). SEM analysis of the implants before and after exposure to curing revealed a general smoothing of the implant's surface with markedly reduced cracks and channels (Figure 3.1.3.b). This surface modification was attributed to the increased chain mobility of the polymeric matrix at 50 °C, a temperature above glass transition of PLGA 502 H. The polymer particles coalescence observed in the cured implant proved that this phenomenon was a main factor for the decreased diffusion of highly soluble drug in the initial phase.

Although the second burst release occurred at equal rate in both the cured and uncured implants, a decrease in duration of lag phase was more pronounced with the intensification of curing process (e.g. at higher curing temperature and longer curing time). This suggested that the changes in the internal porous structure also could have taken place.

The significance of the external pores in mechanism of drug release was reported by Ehtezazi et al. 2000 indicating that small number of the exit holes on the PLA microsphere's surface were the rate-determining factor for the first order drug release rate.

Pore closure is known approach resulting in a decrease of the burst release (Wang et al. 2002; Ahmed and Bodmeier 2009). Hence, the ability to reduce surface porosity by curing is of high interest to control drug release from PLGA implants.

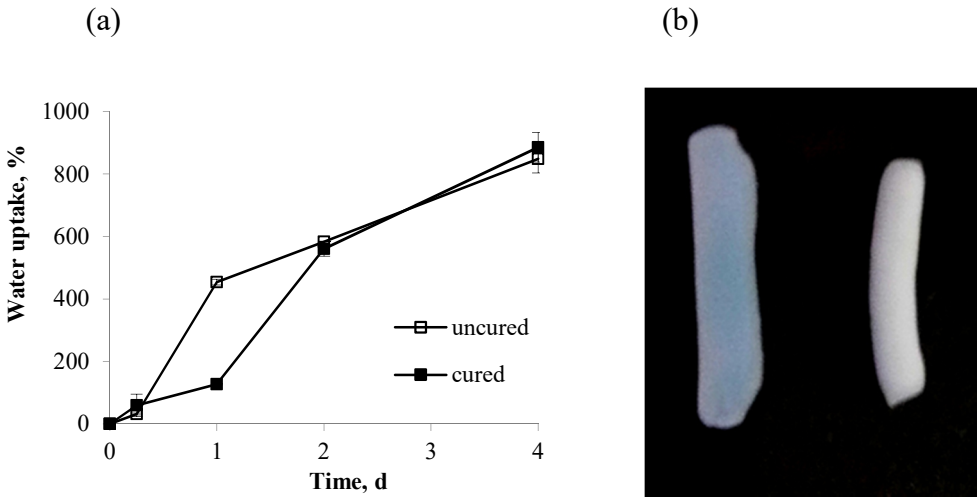


Figure 3.1.4. (a) Water uptake and (b) image of uncured (left) and cured (T=50 °C, 15 min) (right) PLGA 502 H implants of ~0.8 mm diameter containing 10% w/w dexamethasone sodium phosphate after one day of incubation

Considering the results of SEM analyses and drug release study, less water uptake of the cured implants in comparison to uncured ones was expected. Figure 3.1.4.a illustrates a difference of 300% in water uptake value within the first day of incubation i.e. initial phase of drug release. Remodelling of implant's surface at temperatures above glass transition of PLGA resulted in the reduced surface porosity and consequently slowed down water penetration in the matrix. Due to a decrease in medium absorption after curing, considerable difference in morphology of the uncured and cured matrices were noticed after the first day of incubation (Figure 3.1.4.b). Cured implants appeared to be less transparent and smaller in size because of limited swelling ability.

Curing of Implants Extruded at Higher Process Temperature and Reduced Screw Speed

By increasing extrusion temperature and decreasing screw speed, it was supposed that the cracks of extrudates were minimized and the die swell ratio was reduced. Therefore curing had no significant effect in further reduction of the drug release (Figure 3.1.5). According to the value of similarity factor (f_2=72.25), the drug release profiles from uncured and cured implants extruded at 100 °C and screw speed of 5 rpm could be considered similar.

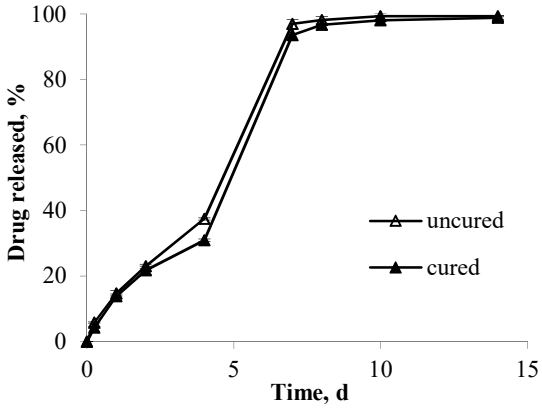


Figure 3.1.5. Effect of curing at 50 °C for 15 min on dexamethasone sodium phosphate release from PLGA 502 H implants extruded at 100 °C and screw speed of 5 rpm

Effect of Curing Time on Dexamethasone Sodium Phosphate Release from Implants with Different Drug Loading

Furthermore, the exposure time of implants to elevated temperature was varied and the influence on dexamethasone sodium phosphate release was investigated.

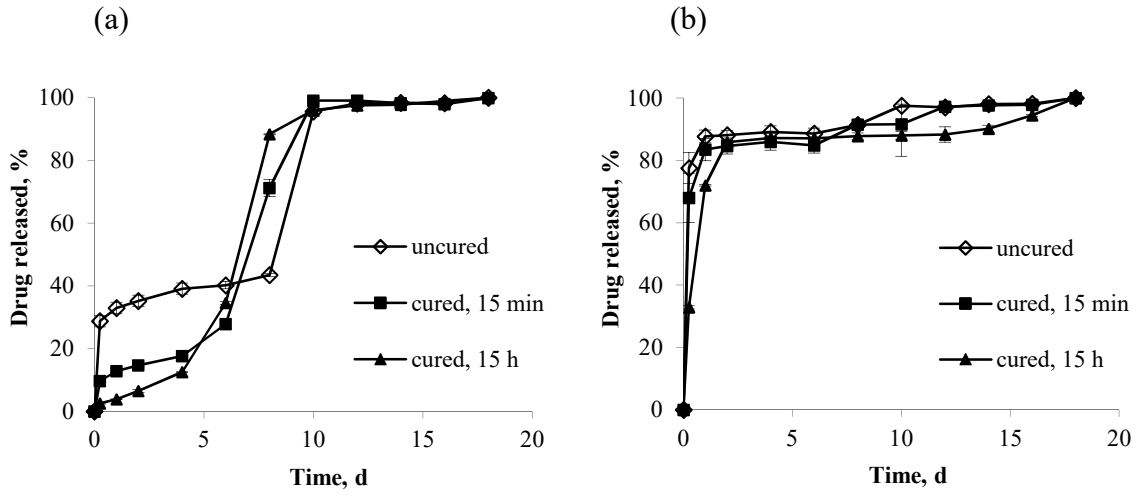


Figure 3.1.6. Effect of curing time at 50 °C on drug release from PLGA 502 H implants containing (a) 10% and (b) 30% w/w dexamethasone sodium phosphate

The initial burst release of highly soluble drug was gradually reduced upon longer curing (Figure 3.1.6). However, short-term curing was more effective in the formulation with lower drug content (Figure 3.1.6.a). PLGA implants containing 10% dexamethasone sodium phosphate showed a decrease in the initial burst release from 30% to 10% within the first day of incubation as a consequence of curing at 50 °C for 15 minutes, while the reduction of burst was only 10% for the higher drug-loaded implants. This was due to a

higher exposure of polymer to drug crystals on the implant's surface in the formulation with lower drug content.

As a result of curing, formulations with 10% drug loading showed the considerable reduction of the initial burst release, but the drug release patterns were still tri-phasic i.e. non-linear. On the other hand, formulations with 30% drug loading showed the fast drug release despite the curing.

Curing at 40 °C and 75% RH

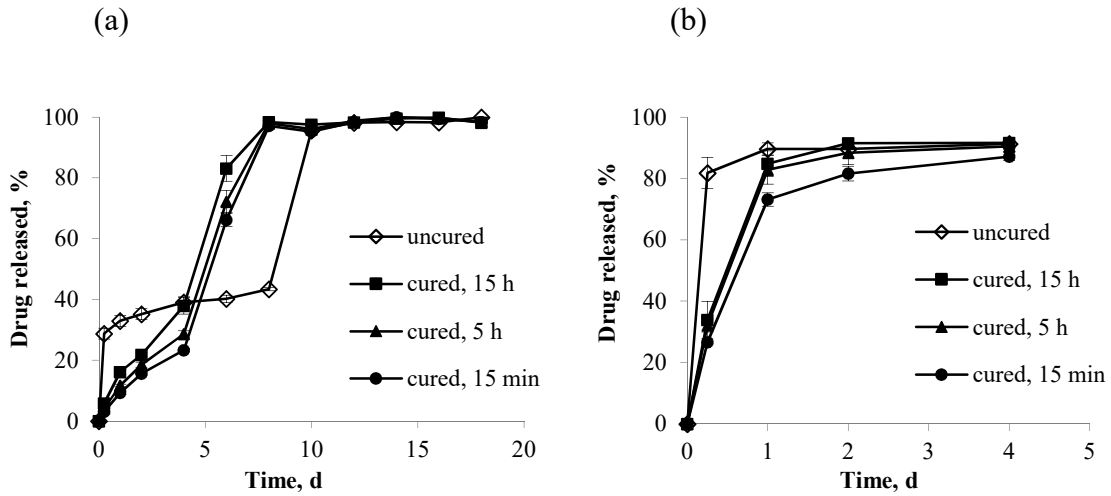


Figure 3.1.7. Effect of curing at 40 °C and 75% RH on dexamethasone sodium phosphate release from PLGA 502 H implants containing (a) 10% and (b) 30% w/w drug

In order to eliminate a lag phase and achieve linear drug release profile, the curing conditions were varied. Implants were cured in the chamber with increased humidity and a relatively low temperature of 40 °C (Figure 3.1.7). Water acts as a plasticizer for the PLGA systems increasing polymer chain mobility and rearrangement and thus facilitates polymer particle coalescence (Wang et al. 2002; Shah et al. 1992; Steendam et al. 2001). The increased humidity contributed in the densification of polymeric matrices at lower temperature compared to the above discussed curing process performed at temperatures higher than 40 °C and at 12% RH. Curing in low humidity environment (12% RH) at 40 °C was not able to induce polymer chain mobility resulting in an absence of any effect on the drug release profile. Similarly, the burst release from PLGA microspheres was reduced at high humidity treatment, but only in formulation containing surfactant with a plasticizing effect upon PLGA, whereas there was no effect on the release in formulations with surfactant acting as an antiplasticizer (Bouissou et al. 2006, Ye et al. 2010).

A slightly increased drug release was observed from implants loaded with both 10% and 30% dexamethasone sodium phosphate with the longer curing process (Figure 3.1.7). This could be explained by migration of highly water-soluble drug, dexamethasone sodium phosphate, which might be dissolved in the absorbed moisture. Drug migration was facilitated by the plasticizing effect of water vapor, which increased the free volume and molecular mobility of PLGA.

Curing at 40 °C and 75% RH proved to be a successful post-preparation treatment in modulation of drug release kinetics from PLGA hot-melt extruded matrices. Reduced initial burst as well as the linear drug release profile was achieved from formulation containing 10% dexamethasone sodium phosphate (Figure 3.1.7.a). On the other hand, the drug release rate for formulation with 30% drug loading was still fast with 70% drug released in the initial phase (Figure 3.1.7.b).

Table 3.1.1. Moisture uptake of implants containing 10% w/w dexamethasone sodium phosphate during curing at 40 °C and 75% RH

	15 min	**5 h**	**15 h**
Moisture uptake, %	0.41 ± 0.03	1.27 ± 0.06	1.80 ± 0.03

Presence of moisture might be an issue in terms of potential instability of pharmaceutical active compounds and dosage forms, thus altering the storage lifetime. Considering this fact, moisture uptake of implants was monitored at predetermined time points during curing at 40 °C and 75% RH. After 15 hours the absorbed humidity was 1.80 ± 0.03% (Table 3.1.1). Subsequently, implants were dried in the vacuum oven until constant mass and subjected to dissolution study. Drug release profiles before and after drying step were identical. The obtained value of moisture uptake was consistent with previous reported data for poly(D,L-lactic acid) showing percent weight changes between 1.1% and 1.3% for poly(D,L-lactic acid) with molecular weights ranged of 12,500-136,500 g/mol, respectively (Steendam et al. 2001). PLGA-based microsphere manifested the similar moisture uptake (up to 2%) over increasing relative humidity up to 95% (Bouissou et al. 2006).

Effects of Curing under Different Conditions on Dexamethasone Sodium Phosphate Release from Implants with Higher Drug Loading

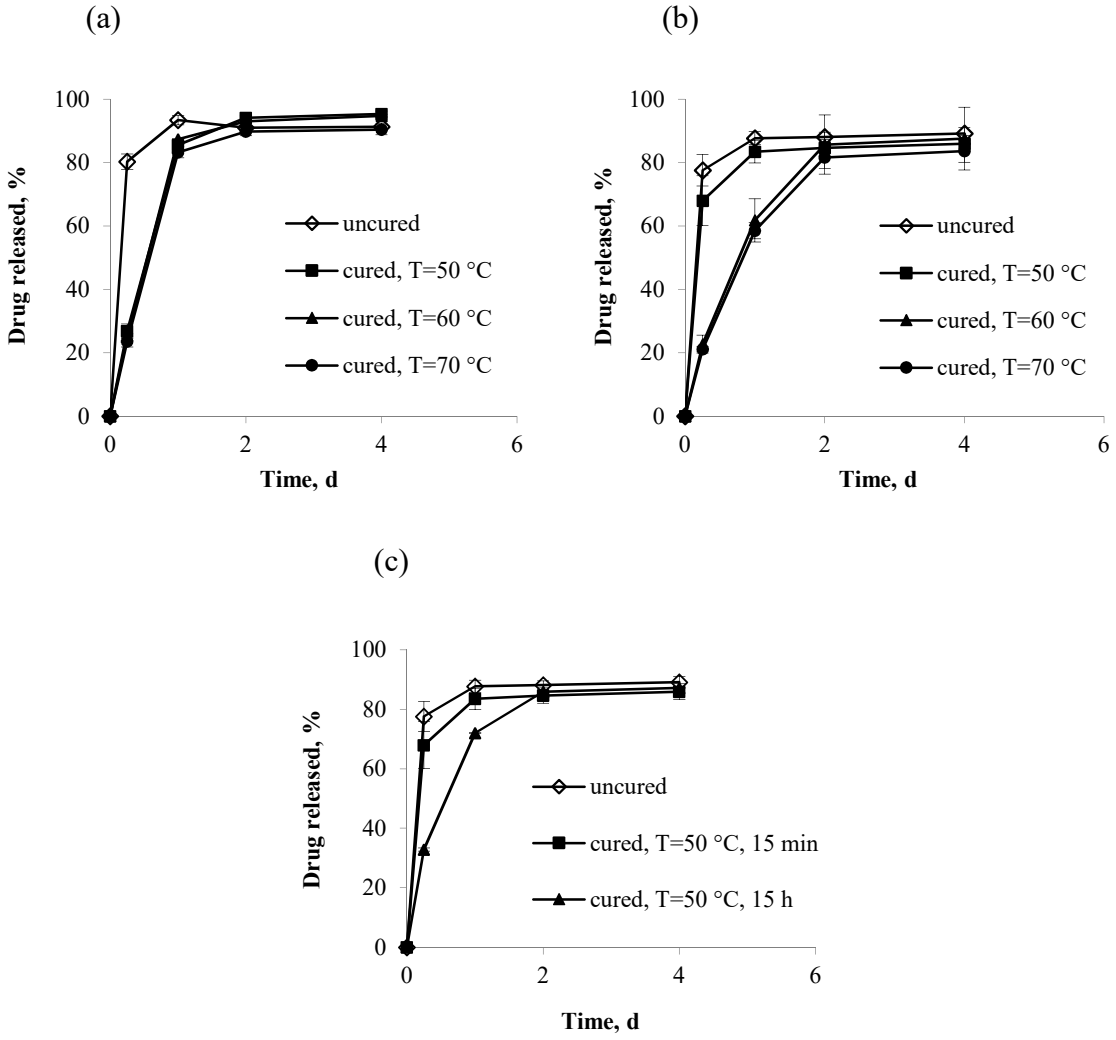


Figure 3.1.8. Effects of curing at different temperatures/time on drug release from PLGA 502 H implants of (a) ~0.8 mm and (b), (c) ~1 mm diameter containing 30% w/w dexamethasone sodium phosphate; curing time for (a) and (b) was 15 min

The initial burst release of dexamethasone sodium phosphate from implants containing 30% drug was gradually reduced upon more intense curing i.e. at higher curing temperatures and longer curing time (Figure 3.1.8). Although the initial burst from implants of ~0.8 mm diameter was decreased remarkably, from 80% to 20% after 6 hours of incubation, complete drug release was detected on the second day (Figure 3.1.8.a). In summary, irrespective of implant's diameter, curing time or temperature, the curing process was unsuccessful in controlling dexamethasone sodium phosphate release from formulations with 30% drug loading. Implants showed fast drug release profiles. This was

attributed to exceeding of drug percolation threshold i.e. drug crystals being in contact and forming network within matrix with easy access to the implant surface, thus resulting in the fast drug release by diffusion.

Therefore, spray coating with drug-free polymer layer was proposed as a necessary procedure in order to retard dexamethasone sodium phosphate release from formulations with drug content above percolation threshold. Thus, reservoir system consisting of a drug-loaded core and polymeric shell was developed. This will be discussed in the following chapters.

Effect of Curing on Drug Release from Implants Loaded with Drugs of Different Solubility

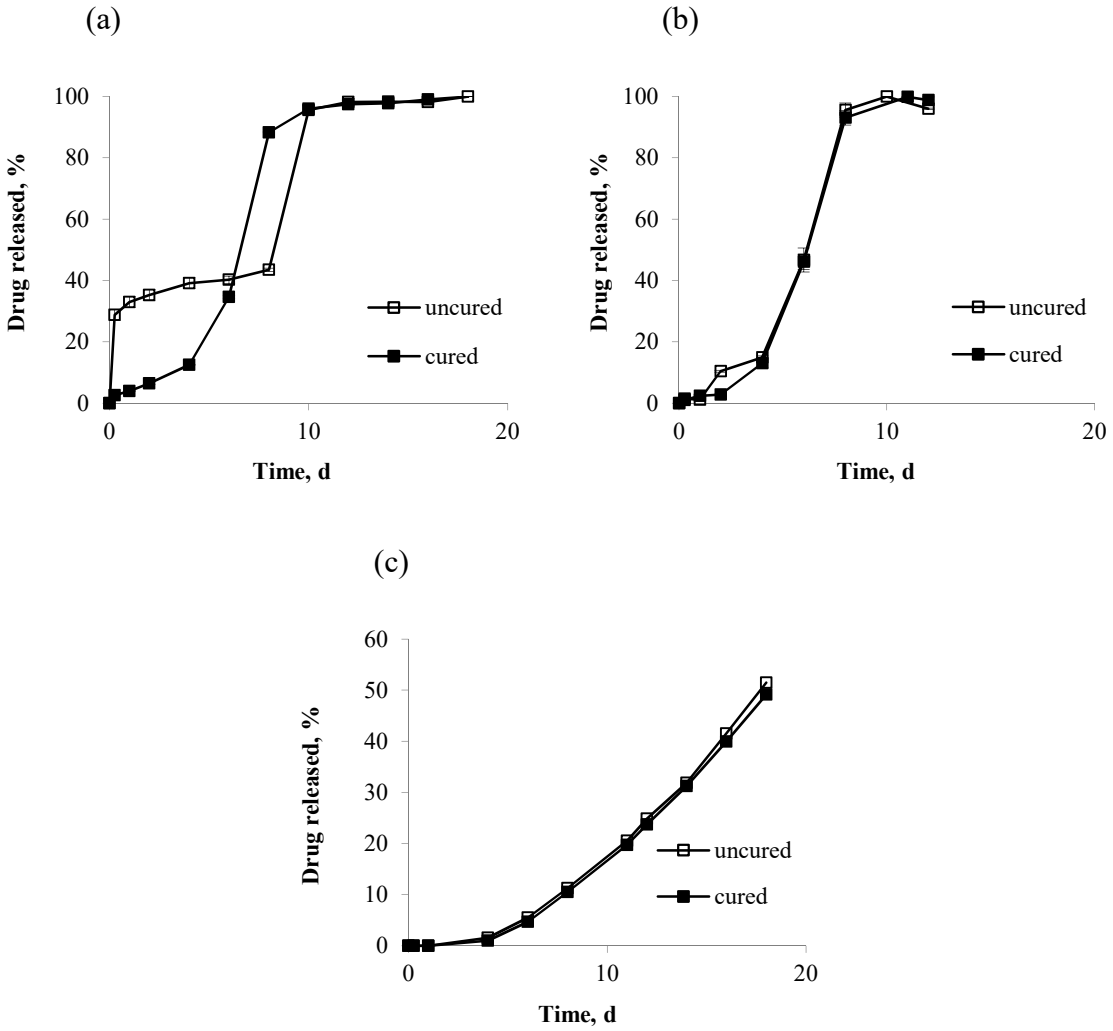


Figure 3.1.9. Effect of curing at 50 °C for 15 h on drug release from 10% w/w drug-loaded PLGA 502 H implants with drugs of different solubility: (a) dexamethasone sodium phosphate (466.7 mg/ml), (b) theophylline (10.4 mg/ml) and (c) prednisone (0.2 mg/ml)

The influence of curing on drug release kinetics from PLGA 502 H extruded implants containing 10% drug of different solubility was further investigated. The curing effect at temperature of 50 °C for 15 h gradually decreased with decreasing drug solubility, and therefore having no effect on drug release from implants loaded with prednisone, slightly soluble drug with a solubility of 0.2 mg/ml. As discussed above, by decreasing surface porosity and water uptake, curing reduced the rate of drug diffusion initially. The higher reduction of the initial burst i.e. diffusion-controlled phase was observed as a consequence of curing at higher drug solubility.

Effect of Curing on Drug Release from Implants Based on PLGAs of Different Molecular Weights

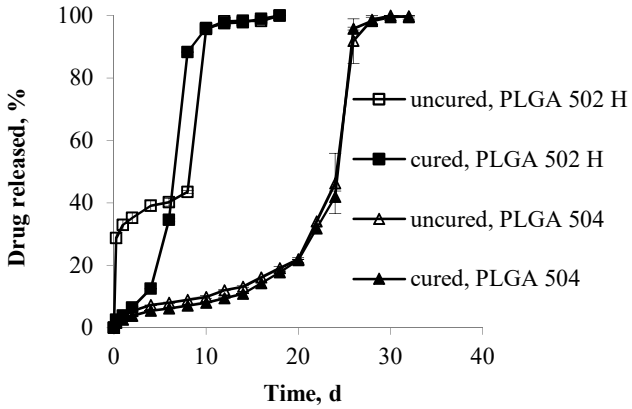


Figure 3.1.10. Effect of curing at 50 °C for 15 h on dexamethasone sodium phosphate release from 10% w/w drug-loaded implants based on PLGAs of different molecular weights

As it was proven above, the surface remodeling process required a degree of plasticity in the PLGA matrix, which caused the associated structural changes as observed by SEM. Thermal treatment of implants at temperature above the glass transition of the polymer led to an increase of polymer chain mobility and their rearrangement. Considering that glass transition is related to chain length, it was assumed that smoothing of the implant's surface with markedly reduced cracks and channels would not occur upon curing of the implant based on higher molecular weight polymer. Comparison of drug amount released in the initial phase from the cured PLGA implants based on different molecular weight PLGAs indicated that curing effect was more pronounced for implants prepared with PLGA of lower molecular weight and glass transition (Figure 3.1.10). Taking into account the glass transition temperatures of the PLGAs being in the range of 42-46 °C and 46-50 °C for PLGA 502 H and PLGA 504, respectively, it could be concluded that mobility of

PLGA 504 chains was slower at the certain and predetermined curing temperature. Therefore, the initial burst from cured PLGA 504 implants was not considerably reduced in contrast to PLGA 502 H implants. In addition, end-capped PLGA 504 contributed to a lower initial drug release from both uncured and cured implants.

Feasibility of Curing Process

Characterization of mechanical stability and assessment of implant shape upon curing is also important with respect to packing into the specialized applicator that allows easy administration of this dosage form.

Laboratory-scale curing process was performed by placing the implants in the aluminium pan and subsequently in the pre-heated oven at the certain temperature for predetermined time. Short-term heating for 15 minutes was feasible at temperatures ranging from 50 °C to 70 °C. The cured implants did not adhere to the bottom of the pan and did not change shape. However, long-term heating for 15 h at temperature of 50 °C caused the implants to adhere to the bottom. Moreover, after long-term heating at 70 °C, the implants were molten, deformed in shape and attached to the bottom.

In order to assess the process feasibility at a bigger scale, a large number of implants was placed in the narrow vessel and curing at different conditions was performed. Short-term heating at temperature of 50 °C was feasible, but at higher temperatures the implants stuck to each other.

Accordingly, the recommendation for the successful large-scale curing at higher temperatures would be either operating in fluidized bed coater or slowly shaking during the process in order to prevent sticking of implants.

3.1.4 Conclusion

The initial burst release of highly soluble dexamethasone sodium phosphate from PLGA-based implants could be successfully reduced by controlling the process parameters during hot-melt extrusion. The operating factors considerably affected the physical properties of the extruded polymeric matrices and thus influenced the drug release behavior. By

increasing extrusion temperature and decreasing screw speed, the extrudates of higher density were obtained due to the reduction of die swell and the prevention of cracks formation. The typical tri-phasic drug release profile from PLGA 502 H implants was modified and a linear dexamethasone sodium phosphate release was achieved. However, upon extrusion under these conditions, the material was exposed to a high temperature for a longer time, which potentially might cause instability of heat-labile actives.

Alternatively, the ability to controllably reduce the implant's surface porosity by a short-term curing at relatively low temperatures when compared to the process temperatures is of great interest with respect to the reduction of the initial burst release and is preferable for thermolabile hydrophilic therapeutic compounds. The effect of curing temperature, time and relative humidity on the polymer chain mobility was evaluated. The desired linear drug release profile was successfully achieved, whereas the structural integrity and regular shape of implants could be preserved. Therefore, a brief exposure of PLGA implants to curing process proved to be an effective method to reduce the initial burst release of highly water-soluble drug.

3.2 Poly(lactide-co-glycolide) Implants Prepared by Hot-Melt Extrusion and Spray Coating Process; *In Vitro/Ex Vivo* Drug Release Study

The purpose of this study was to investigate the drug release properties of the uncoated and coated PLGA-based hot melt extruded matrices as a function of drug solubility and loading as well as to evaluate a biorelevant dissolution test for subcutaneous implants.

3.2.1 Background

Biodegradable polymers used as drug release controlling matrices in commercialized parenteral products mainly include polyesters such as poly(lactide) (PLA) and poly(lactide-co-glycolide) (PLGA) (Körber 2007). Generally, PLGAs are categorized as bulk eroding polymers as a consequence of heterogeneous hydrolytic degradation of ester bonds resulting in formation of degradation products having carboxylic acid (Ghalanbor et al. 2013). The acidic degradation products cause an autocatalytic acceleration of polymer degradation. The process of hydrolytic degradation is followed by the mass loss of polymer matrix (Göpferich 1997). In contrast to surface eroding polymers, PLGA matrix erodes slower than the water penetration proceeds, thus degradation occurs throughout the matrix and material is lost from the entire matrix volume. This behavior is sometimes referred to homogeneous erosion because mass loss proceeds at a more or less uniform rate throughout the matrix (Edlund and Albertsson 2002). The size and shape of the device remains intact even at later stages of degradation, but the microstructure within the bulk changes considerably. After erosion to a critical degree, the device eventually collapses.

In the formulation of parenteral PLGA-based delivery systems the modulation of the degradation of a polymer matrix represents a strategy to control drug release. Many factors are known to influence the degradation rate of PLGA: the ratio of the monomers used during synthesis, molecular weight, selection between ester and acid terminated polymers, properties of incorporated drug, additives, size, geometry and porosity of the device as well as the surrounding conditions (Tracy et al. 1999; Alexis 2005; Gentile et al. 2014). In addition, several approaches have been used to modulate release of drugs from PLGA systems including blending with polymers of different hydrophobicity/hydrophylicity or blending with different molecular weights of the same polymer (Bodmeier et al. 1989) as well as copolymerizing with polyethylene glycol (Milacic and Schwendeman 2014; Witt et al. 2000). Beside polymer selection, the rate of drug delivery is influenced by the design of dosage form as well as the manufacturing technique. Controlled release delivery devices can be divided into reservoir and matrix systems. However, biodegradable systems based on PLGA are mainly matrix systems, which can be in the form of solid implants (Ghalanbor et al. 2013), nanoparticles (Danhier 2012), microparticles (Ye et al. 2010) and delivery systems that form in situ (Dong et al.

2006; Patel et al. 2010). PLGA implants for controlled release of therapeutics are usually cylindrical matrices, which can be in the millimeter to centimeter scale, facilitating the high drug loadings.

The objective of this study was to investigate the drug release rate from the PLGA implants as a function of polymer type, drug solubility and loading as well as the influence of incorporated drug on the matrix degradation and erosion. Hot-melt extrusion as a single-step process was applied to prepare solid dispersions via the homogeneous embedding of dug particles in PLGA as a release-controlling polymer. In order to modulate the release of drugs from PLGA-based hot-melt extruded implants, spray coating process with PLGA organic solution was further applied. Thus, reservoir system with a drug-loaded core and drug-free shell was obtained. Effect of PLGA type in the coating as well as effect of varied coating levels on release of different soluble model drugs were investigated. This approach may serve as a promising tool for modulation of drug release especially from systems for parenteral application. Thereby, the potential toxicity problems of other release-modifying additives could be eliminated.

3.2.2 Drug Release from Uncoated Implants

Effect of Core Type

Rod-shaped PLGA-based implants of ~5 mm length and ~1 mm diameter were prepared by hot-melt extrusion. Dexamethasone sodium phosphate and prednisone as the model drugs of different water solubility were homogeneously embedded into the polymeric matrix based on different types of PLGA.

The initial release phases of dexamethasone sodium phosphate from PLGA implants containing 30% of the highly water-soluble drug were high, from 50% to 90% within the first day of incubation (Figure 3.2.1.a). The high initial burst was followed by a phase of no release until onset of polymer erosion, which contributed to liberation of the entrapped active ingredient. This trend was observed regardless of the polymer molecular weight as well as monomer ratio of PLGA. Matrix systems based on more hydrophobic PLGAs could only slightly reduce burst release of dexamethasone sodium phosphate, but the

entire drug release pattern was still fast. This was due to a high drug loading, which was above percolation threshold, as shown in a further study.

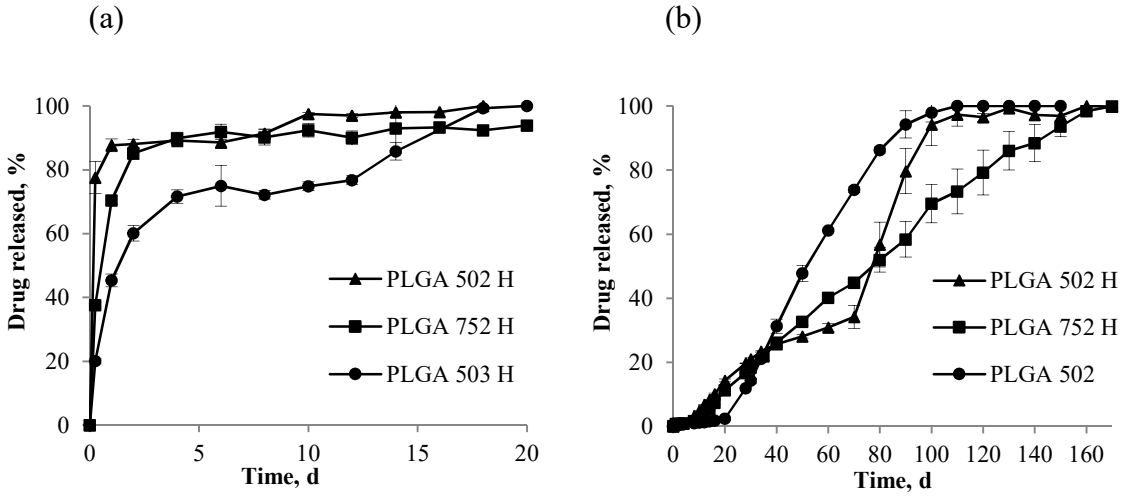


Figure 3.2.1. Effect of polymer type on drug release from implants loaded with 30% w/w drugs of different water solubility: (a) dexamethasone sodium phosphate (466.7 mg/ml) and (b) prednisone (0.2 mg/ml)

Interestingly, incorporation of the slightly soluble model drug prednisone into the PLGA hot-melt extruded matrices led to either bi- or multi-phasic drug release pattern with a complete absence of the initial burst release (Figure 3.2.1.b). Instead, all these implants showed lag phases before onset of prednisone release with duration dependent on the polymer type. Owing to a high prednisone content embedded in the polymeric carrier, hydrophilicity of the entire system might be altered. Poor drug solubility was a limiting factor for the matrix wetting resulting in the delayed prednisone dissolution. Hence, PLGA 502 H and PLGA 752 H implants demonstrated the lag phase of 8 to 10 days. The longest non-release period of almost 20 days for the implants based on end-capped PLGA 502 was probably caused by the slowest water uptake and drug dissolution due to even higher hydrophobicity of the matrix with ester terminated polymer chains. After the lag phase, a phase of sustained and linear drug release followed for implants based on PLGA 752 H and PLGA 502. Any change in drug release kinetics such as fast release due to delayed onset of polymer erosion was not observed as it has been reported for bulk eroding PLGA (Göpferich 1997). When comparing the slope of the release phases from implants based on PLGA 752 H and PLGA 502, higher slope, i.e. the higher prednisone release rate was observed with PLGA 502 matrices than PLGA 752 H. This was attributed to slower degradation rate of PLGA 752 H due to a higher content of lactide units.

Surprisingly, PLGA 502 H prednisone matrices showed a multi-phasic drug release pattern. After a lag phase and a phase of drug liberation, the rate of prednisone release slowed down between the 30th and 70th day of incubation, continuing with faster drug release until completeness of the release. Additional studies were performed to evaluate this phenomenon.

Incorporation of a high content of the slightly soluble drug in the PLGA matrix resulted in atypical drug release behaviour. It was very likely that matrix properties were changed in terms of water absorption, polymer degradation and erosion depending on the solubility of embedded drugs.

Effect of Drug Loading

In order to explain the mechanism of drug release as well as effect of the drug solubility on the PLGA 502 H matrix properties, implants loaded with different drug content were investigated.

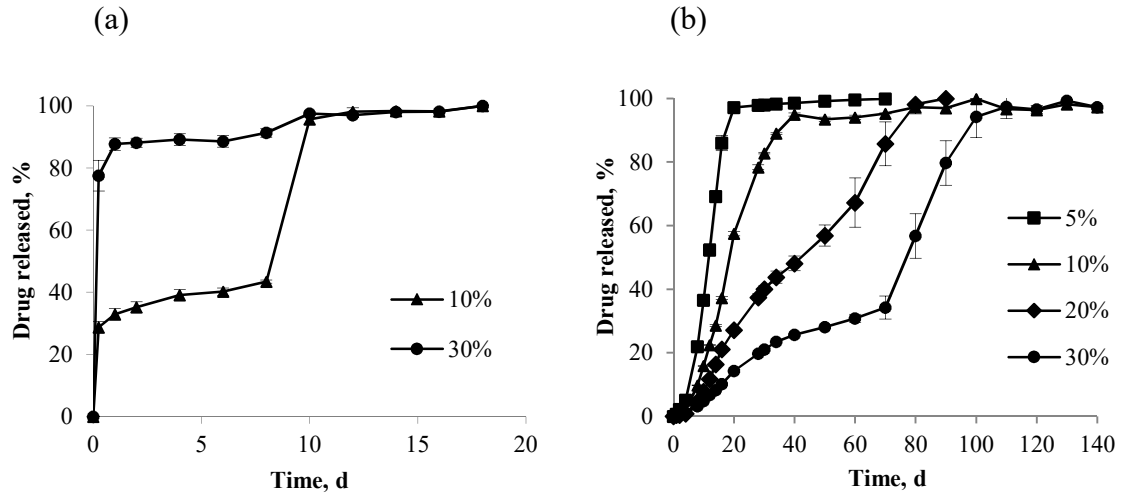
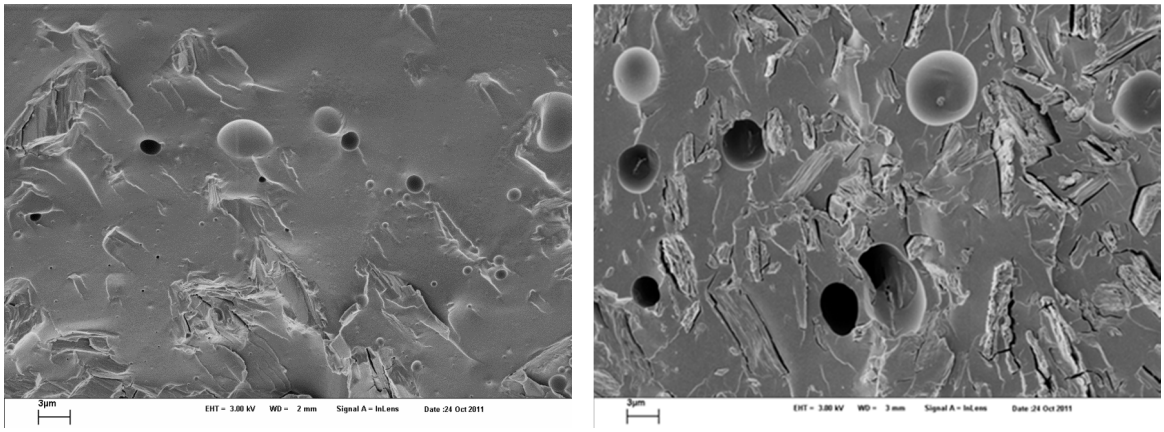


Figure 3.2.2. Effect of drug loading on release from PLGA 502 H implants containing (a) dexamethasone sodium phosphate and (b) prednisone

Drug release curves of PLGA 502 H implants containing both 10% and 30% dexamethasone sodium phosphate followed the typical tri-phasic profile (Figure 3.2.2.a). The magnitude of the initial burst release was in correlation with drug loading. With an increase of dexamethasone sodium phosphate loading up to 30%, the initial burst release was increased because drug crystals were connected in a network structure within the matrix (Figure 3.2.3.c and d). 30% dexamethasone sodium phosphate loading was above the percolation threshold, and thus the highly soluble drug was released quickly by

diffusion forming a porous system. 90% of embedded drug was liberated within one day of incubation, whereas the rest of 10% entrapped drug was released after one week as a consequence of matrix erosion. However, in the implants with lower dexamethasone sodium phosphate content, there were less drug particles situated on or near the surface of the implant. Drug crystals were separated and surrounded by the dense polymer (Figure 3.2.3.a and b), which slowed down the rate of drug diffusion in the initial phase.

(a) (c)



(b) (d)

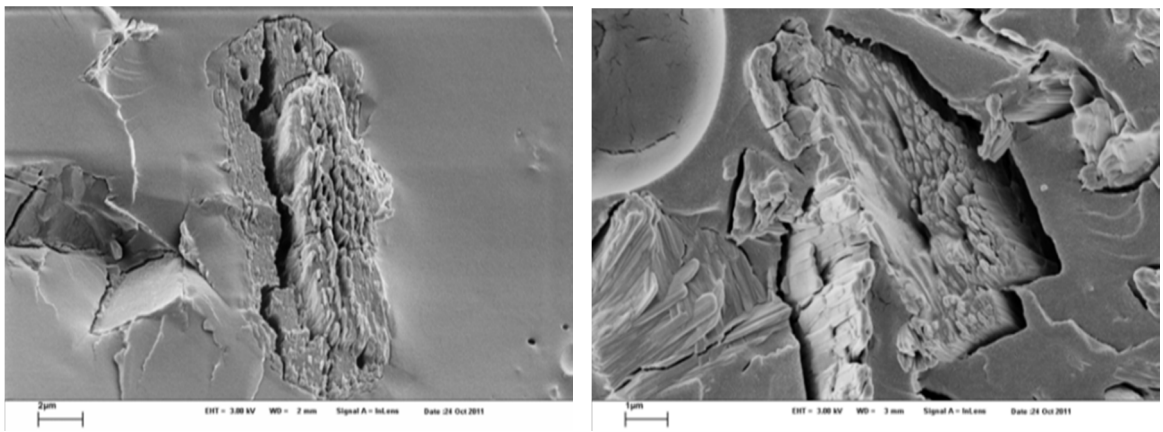


Figure 3.2.3. Scanning electron microscope (SEM) images of cross section of dexamethasone sodium phosphate implants based on PLGA 502 H before incubation; (a) and (b) 10% w/w drug loading; magnification 3 µm and 2 µm, respectively; (c) and (d) 30% w/w drug loading; magnification 3 µm and 1 µm, respectively

On the other hand, the higher the prednisone loading, the slower slightly soluble drug release was observed, reaching the release completeness after four months for implants with 30% drug content (Figure 3.2.2.b). All formulations containing different amount of

active substance manifested a lag phase from two to eight days depending on the drug loading. Afterwards, the implants loaded with 5% and 10% of the drug demonstrated a phase of constant sustained release over twenty and forty days, respectively, and thus resulting in the bi-phasic drug release pattern. However, the higher loaded formulations showed a change in drug release kinetics in terms of decreasing drug release rate after one month of incubation leading to the multi-phasic profiles. This trend was observed only in the formulations containing higher prednisone amount and based on PLGA 502 H.

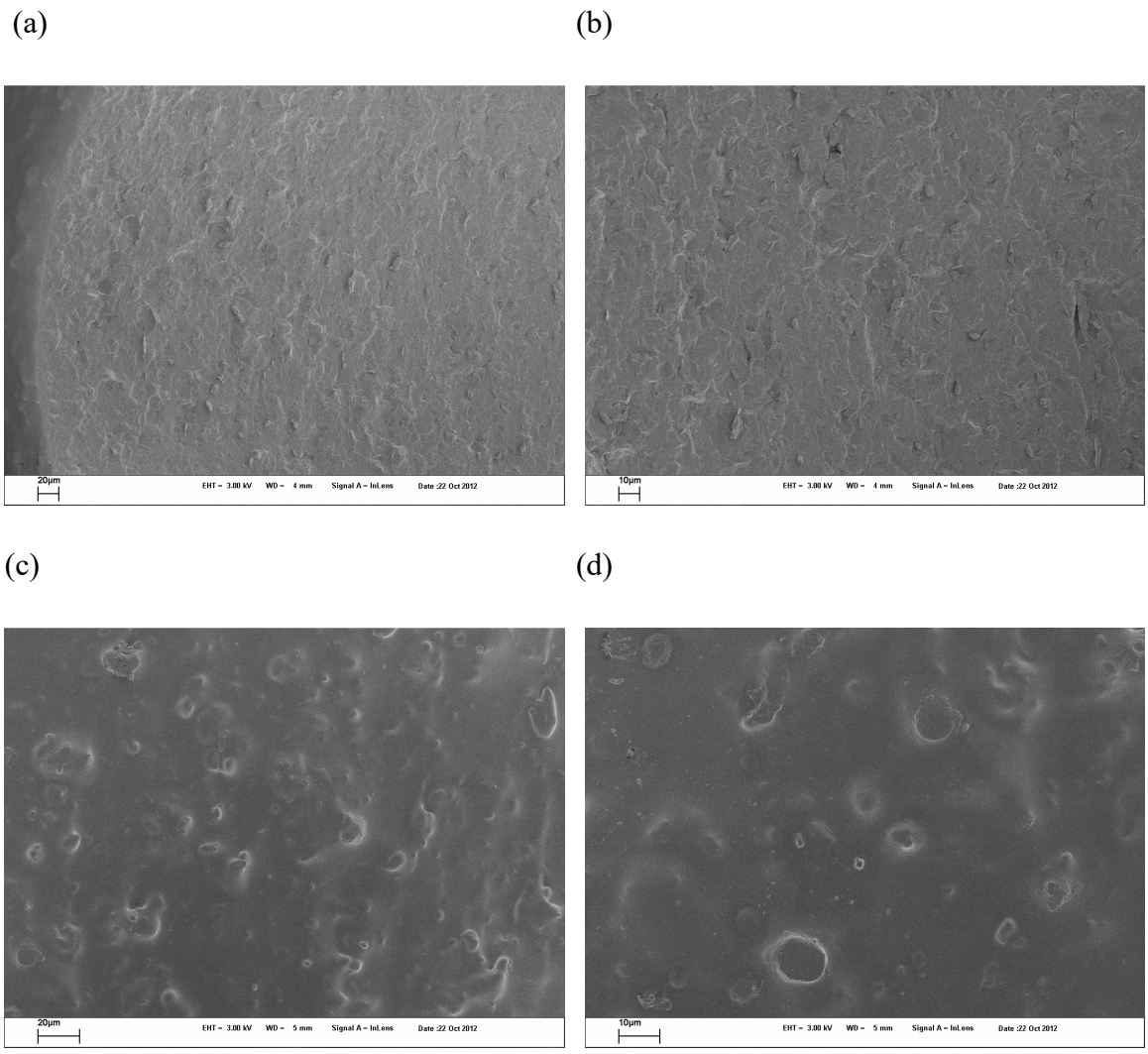


Figure 3.2.4. Scanning electron microscope (SEM) images of prednisone implants based on PLGA 502 H containing 30% w/w drug; (a) and (b) the cross section of implant; magnification 20 µm and 10 µm, respectively and (c) and (d) the surface of implant; magnification 20 µm and 10 µm, respectively

SEM analyses were performed in order to have a better insight about microstructure of the hot-melt extruded matrices loaded with the drugs of different solubility. Both prednisone and dexamethasone sodium phosphate implants had a compact and homogenous matrix

immediately after preparation (Figure 3.2.3 and 3.2.4). Images of the prednisone implants revealed a smooth surface and the uniform drug distribution within the matrix without regions with large drug crystals due to micronized prednisone particles. Any kind of drug layering or accumulation as a potential reason for the multi-phasic prednisone release was not observed (Figure 3.2.4).

In addition, when comparing the cross-section of prednisone and dexamethasone sodium phosphate implants, the big pores and voids around dexamethasone sodium phosphate crystals were detected, whereas for the prednisone containing implants it was not observed (Figure 3.2.3 vs. Figure 3.2.4). Diameters of pores were proportionally higher with increasing of drug content, from around 1 µm to 3 µm in implants with 10% and 30% drug loading, respectively. This was attributed to exceedingly hygroscopic character of dexamethasone sodium phosphate. During hot-melt extrusion process at high temperature, the formation of pores occurred due to rapid evaporation of the bound and adsorbed water. This phenomenon may additionally contribute to an increase of system porosity and the initial burst release in matrixes containing highly soluble drugs.

According to the United States Pharmacopeia (USP 38) the sum of the percentage of water content and alcohol content in dexamethasone sodium phosphate does not exceed 16%. Thermogravimetric analysis (TGA) of the pure drug revealed 15% mass loss upon heating. However, according to the fifth edition of the International Pharmacopoeia published in 2015, the water content in dexamethasone sodium phosphate is not more than 10%, whereas the ethanol content is not more than 3%. Beside TGA, Karl Fischer method is the most commonly used techniques for determination of the total water content of pharmaceutical solids (Khankari et al. 1992). The result for the water content of dexamethasone sodium phosphate determined by Karl Fischer method was 12.76%.

Drug Stability during Hot-Melt Extrusion Process

Although literature reports an absence of interaction between the model drugs and the PLGA terminal carboxylic groups during the microencapsulation process, there is no conformation about drug stability at elevated temperature during hot-melt extrusion. Jaraswekin et al. 2007 demonstrated via NMR spectroscopy that ionic interaction with carboxylic groups of PLGA and the negatively charged dexamethasone sodium phosphate could not take place. In the case of prednisone, Giovagnoli et al. 2008 showed a slight

initial adsorption of prednisone onto the surface of PLGA 502 H microspheres during the release study over 160 days at 37 °C in PBS (pH 7.4). However, they also reported that this process was reversible and was followed by drug re-precipitation.

In order to explain the mechanism responsible for the multi-phasic prednisone release from the high drug-loaded PLGA 502 H implants, physical state of drug in the extrudates was analysed by DSC.

(a) (b)

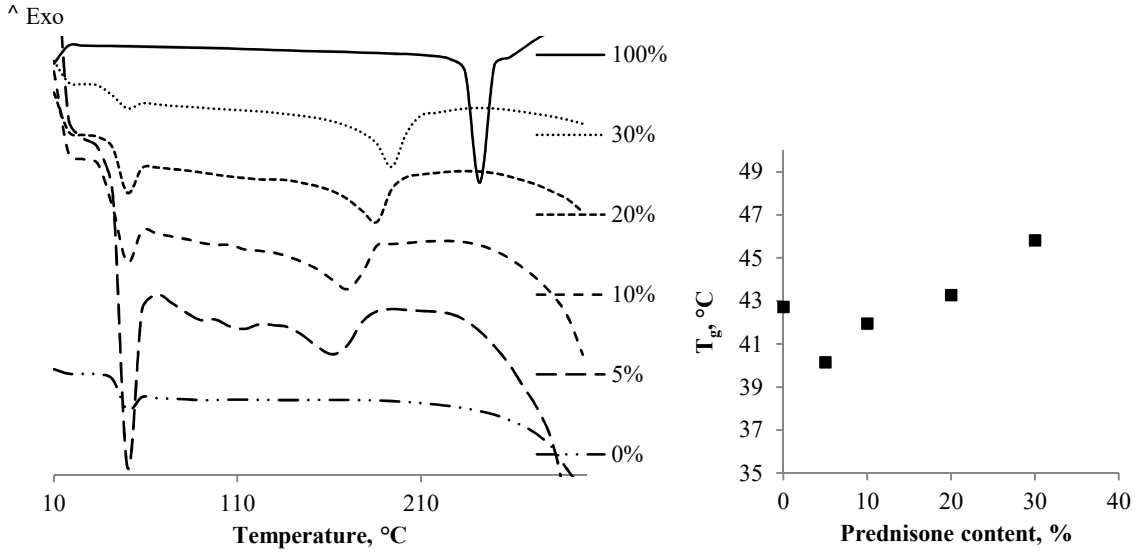


Figure 3.2.5. (a) DSC-thermograms of pure prednisone and PLGA 502 H extruded implants with different drug content (values are normalized by prednisone weight); (b) effect of prednisone content on the glass transition temperature (T_g) of PLGA 502 H extruded implants

The thermal behavior of implants indicated the presence of intact prednisone crystals within the polymeric matrix independent of drug loading ranged from 5% to 30% (Figure 3.2.5.a). This confirmed that prednisone was not dissolved i.e. molecularly dispersed in the molten polymer during processing at elevated temperature. With decreasing prednisone content the drug melting point values as well as the polymer glass transition values were gradually reduced (Figure 3.2.5). This behavior suggested that at higher prednisone content, mobility of the polymer chains was hindered and the polymeric matrix was more rigid. Similarly, it could be concluded that upon incubation in the release medium at 37 °C, the plasticizing of polymer in the implants containing higher prednisone loading might be restricted and slower in comparison to the implants with lower prednisone content. This also might contribute in retardation of the slightly soluble drug

release from the higher drug-loaded implants.

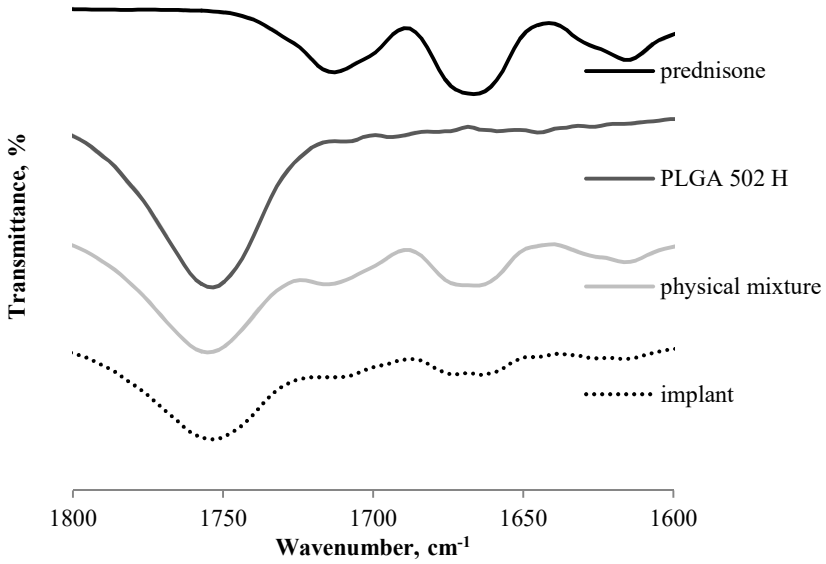


Figure 3.2.6. FTIR spectra of pure prednisone, PLGA 502 H, physical mixture and extruded implant containing 30% w/w drug; Peaks represent carbonyl band of PLGA at 1750 cm^{-1} and stretching vibration of carbonyl band and cyclohexadiene of prednisone at 1700 $cm^{-1}$and 1660 cm^{-1}, respectively

Extruded implant and physical mixture composed of PLGA 502 H and 30% prednisone were analyzed by FTIR. The characteristic carbonyl band of PLGA at 1750 cm^{-1} and both characteristic bands of prednisone such as stretching vibration of carbonyl at 1700 cm^{-1} and cyclohexadiene at 1660 cm^{-1} appeared in FTIR spectra from both implant and physical mixture. Hence, it could be concluded that the preparation process had no effect on the prednisone physical state and any kind of strong covalent drug-polymer interaction did not occur (Figure 3.2.6). In addition, the embedded prednisone in PLGA matrices was completely extracted using both organic solvent and sodium hydroxide solution. This result as well as complete prednisone release at the end of dissolution test also contributed in the conclusion of an absence of drug-polymer covalent interaction during hot-melt extrusion.

Although DSC, FTIR and extraction results showed prednisone stability after processing at elevated temperature, the reason for the multi-phasic prednisone release from the PLGA 502 H-based implants containing higher drug content was not still elucidated.

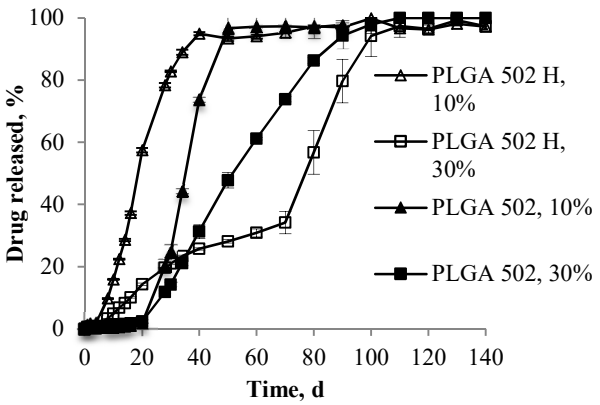


Figure 3.2.7. Prednisone release from 10% and 30% w/w drug-loaded implants based on PLGA 502 H and end-capped PLGA 502

The same trend was observed when comparing the release profiles from implants based on the end-capped i.e. ester terminated PLGA 502 and acid terminated PLGA 502 H with 10% and 30% drug loading (Figure 3.2.7). Due to higher hydrophobicity of PLGA 502 compared to its non end-capped analogue, longer lag phase and slower the entire drug release pattern was expected. However, unlike PLGA 502 H implants, PLGA 502-based formulations demonstrated the bi-phasic drug release kinetics.

(a) (b)

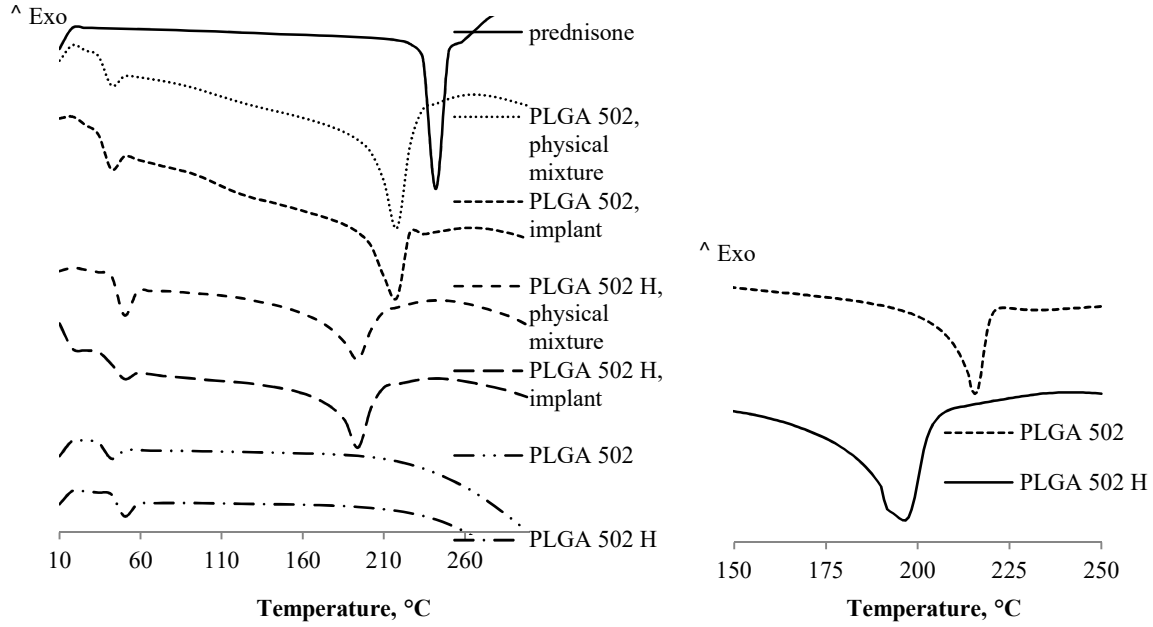


Figure 3.2.8. (a) DSC-thermograms of pure prednisone, PLGA 502, PLGA 502 H, drug-polymer physical mixtures and extruded implants containing 30% w/w drug (values are normalized by prednisone weight); (b) magnified scans of prednisone melting peaks obtained from implants based on PLGA 502 and PLGA 502 H

The prednisone melting peaks of drug-polymer physical mixtures and implants obtained from end-capped and non end-capped PLGA were observed at different temperatures (Figure 3.2.8.a). Moreover, the appearance of drug melting peak originated from PLGA 502 H and PLGA 502 implants was not the same (Figure 3.2.8.b). The melting peak of prednisone in PLGA 502 H implants was broader and partially separated when compared to the sharp peak observed for PLGA 502 implants.

This observation in addition to the increased glass transition temperature in formulation composed of PLGA 502 H and 30% prednisone was correlated to eventual a negligible weak interaction of the drug with the polymeric matrix during processing (Figure 3.2.5.b). Weak and reversible non-covalent bonds between the drug and the polymer, which could occur both during the preparation process and the incubation period, might serve as an explanation for the multi-phasic prednisone release pattern.

Effect of Drug Solubility on Release Mechanism from PLGA Uncoated Implants

Figure 3.2.9 demonstrates the effect of drug loading on release rate from implants composed of PLGA 502 H and different water-soluble drugs. With increasing of the highly water-soluble drug content, an increase in the initial burst release was observed due to fast dexamethasone sodium phosphate diffusion and the formation of a more porous system. On the contrary, with the higher drug loading of slightly soluble drug, the slower prednisone release was obtained.

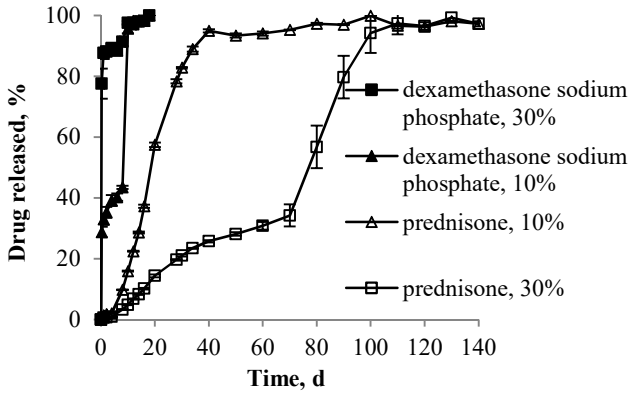


Figure 3.2.9. Effect of drug loading on release from PLGA 502 H hot-melt extruded implants containing model drugs of different solubility: dexamethasone sodium phosphate (466.7 mg/ml) and prednisone (0.2 mg/ml)

Most probably, the physicochemical properties of the incorporated drugs considerable affect the resulting release patterns, especially at high initial drug loadings. The drug release was in correlation with medium uptake, mass loss and degradation rate of PLGA implants during incubation (Figure 3.2.10, 3.2.11 and 3.2.12). The same trend was observed by analyzing morphology and swelling of implants loaded with drugs of different solubility (Figure 3.2.13). Dexamethasone sodium phosphate is freely soluble in an aqueous medium and at pH 7.4 is present mainly in its di-anionic form, whereas the slightly soluble prednisone is a neutral molecule. Hence, the solubility of incorporated drugs was a key factor influencing the mechanism and rate of release as well as PLGA matrix properties in terms of ability to absorb water and to degrade.

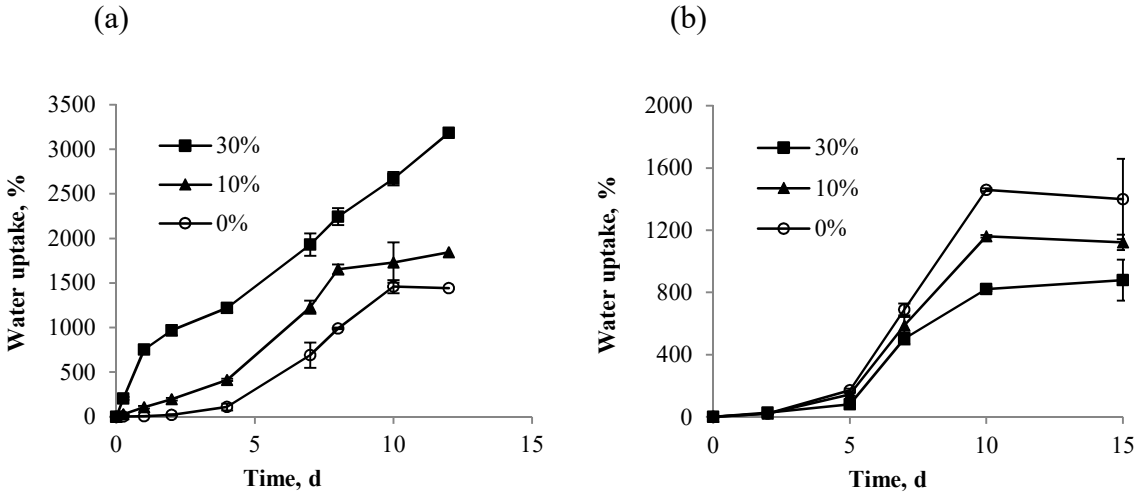


Figure 3.2.10. Water uptake of PLGA 502 H implants containing (a) dexamethasone sodium phosphate and (b) prednisone during incubation in phosphate buffer pH 7.4 at 37 °C

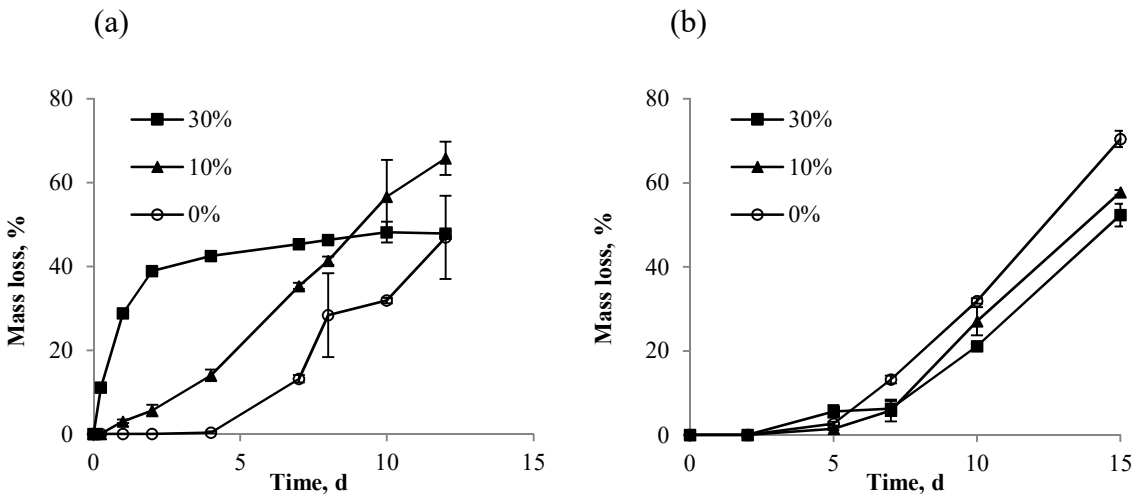


Figure 3.2.11. Mass loss of PLGA 502 H implants containing (a) dexamethasone sodium phosphate and (b) prednisone during incubation in phosphate buffer pH 7.4 at 37 °C

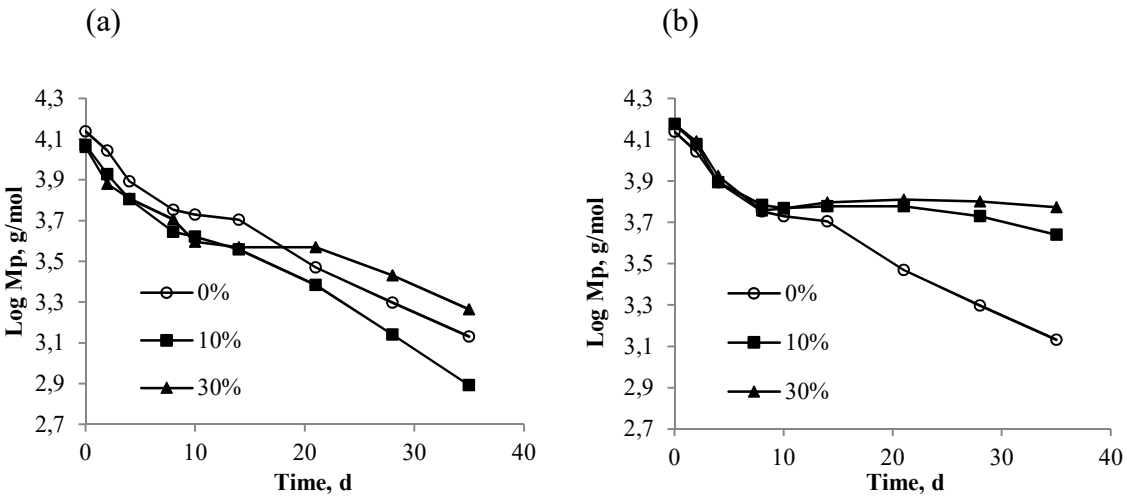


Figure 3.2.12. Semi-log plot of the peak molecular weight of PLGA 502 H implants containing (a) dexamethasone sodium phosphate and (b) prednisone during incubation in phosphate buffer pH 7.4 at 37 °C

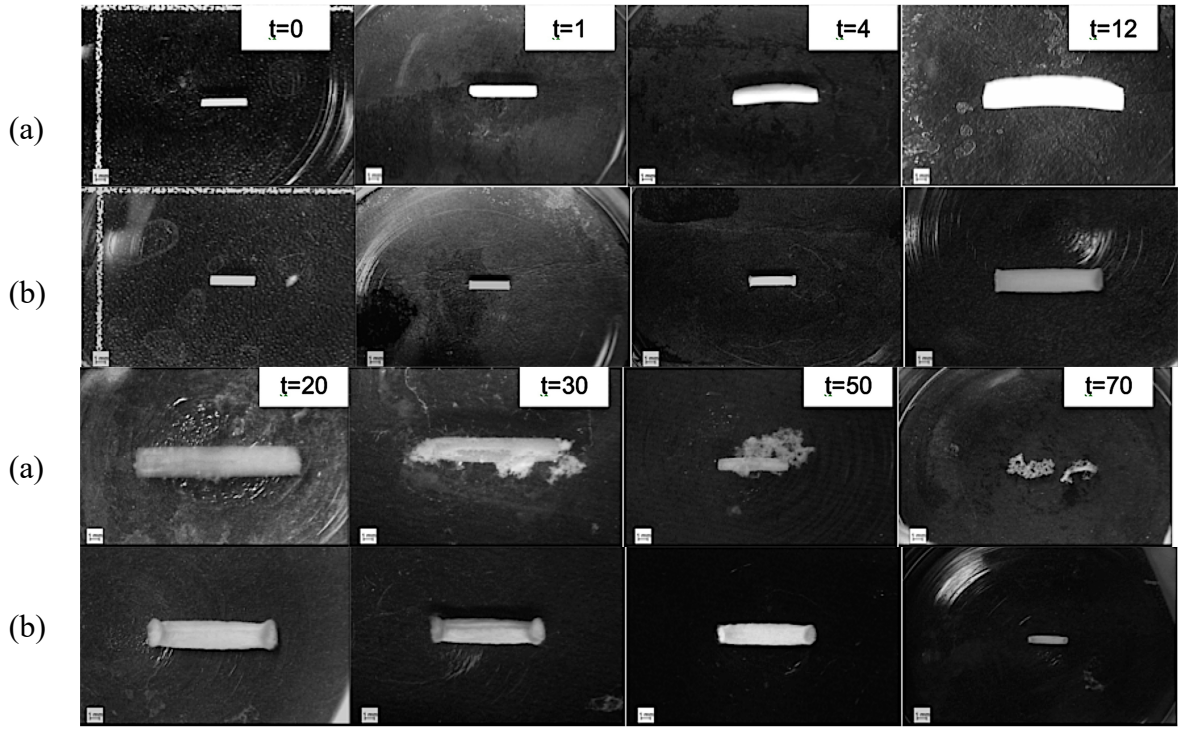


Figure 3.2.13. Morphology of PLGA 502 H implants containing 30% w/w (a) dexamethasone sodium phosphate and (b) prednisone during incubation in phosphate buffer pH 7.4 at 37 °C

Hydrolytic degradation of blank PLGA 502 H implants was initiated from the beginning of incubation due to slight water penetration into the polymeric matrix within the first week (Figure 3.2.10). Initially the free carboxylic end groups facilitated the polymer degradation (Figure 3.2.12). The delayed onset of mass loss was caused by formation of water-soluble PLGA oligomers (1000 g/mol) capable to diffuse out of the system (Körber

2010) (Figure 3.2.11). After one week the accelerated phase of water uptake was in correlation with decreasing the peak of the lognormal molecular weight distribution of PLGA (log Mp) as well as with a rapid mass loss i.e. erosion of the system (Figure 3.2.10, 3.2.11 and 3.2.12).

The change in water uptake is a possible way of investigating the hydrophilic nature of materials. Higher content of freely water-soluble drug could facilitate water penetration and thus contributed to the creation of a highly porous polymer network upon drug leaching (Figure 3.2.10.a). Alteration in the polymer hydrophilicity caused a change in the degradation process. Faster degradation rate compared to blank PLGA 502 H implants was observed for 10% drug loaded implants. Incorporation of 30% drug resulted in the same behavior during the first two weeks of incubation. However, these implants demonstrated a decrease of degradation rate from the second week onwards (Figure 3.2.12.a). This could be attributed to the reduced autocatalytic effect due to earlier outflux of acidic degradation products from the highly porous matrix created by the complete drug release during the first week of incubation. Moreover, increase of dexamethasone sodium phosphate loading led to faster mass loss compared to blank PLGA 502 H implants (Figure 3.2.11.a). Morphological study of the high drug loaded implant revealed the fast and extensive swelling within the first 12 days of incubation, which was in correlation with the water uptake observed for this formulation (Figure 3.2.10.a). Implants retained their shape and integrity until the 20^{th} day despite the accelerated polymer degradation whereby the peak molecular weight approached the molecular weight of soluble PLGA (log Mp~3). This could be explained by the presence of slow degrading region at the surface, which suddenly disintegrated completely due to a decrease of mechanical stability, indicating a bulk erosion process (Figure 3.2.13.a).

On the other hand, presence of lipophilic drug increased hydrophobicity of the PLGA implants and hindered water diffusion into the system, slowing down polymer degradation in comparison to blank PLGA extrudates. Initially, no remarkable changes were observable in drug release, water uptake, mass loss or morphology of prednisone implants (Figure 3.2.9, 3.2.11.b and 3.2.13.b). This indicated that only small amount of absorbed water was tightly bound by polar groups of the polymer, thus initiating onset of hydrolytic degradation. Most probably, prednisone molecules remained immobilized due to the absence of free water domains, which were able to dissolve the slightly soluble drug. Over the time, more water molecules penetrated into the matrix facilitating prednisone release

and causing slow implant swelling. After 10 days of incubation water uptake and the peak molecular weight reached a plateau level (Figure 3.2.10.b and 3.2.12.b). Implants appeared to have more swollen edges and a furrowed surface, indicating that the mass loss was confined to the surface of the system (Figure 3.2.13.b). From the 20th day onwards implant decreased in size gradually, but the shape remained unchanged until the 160th day implying surface erosion. Significant reduction in the implant's size occurred from the 50th to 70th day corresponding to the accelerated prednisone release phase starting from the 70th day (Figure 3.2.9). FTIR spectra of prednisone implants after 80 days of incubation revealed the presence of both PLGA and prednisone confirming that PLGA 502 H was not degraded to water-soluble oligomers within this time interval (Figure 3.2.14). In addition, this was evidence that any covalent interaction between prednisone and PLGA or its degradation products during incubation did not occur and thus, release completeness was achieved.

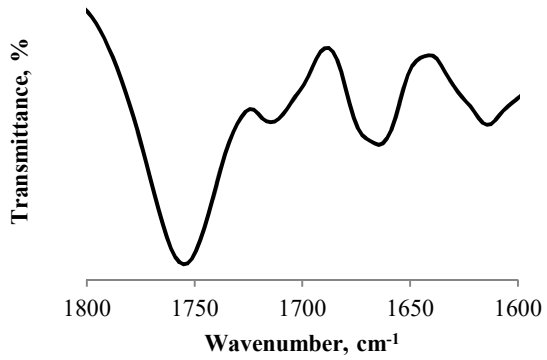


Figure 3.2.14. FTIR spectra of 30% w/w prednisone loaded PLGA 502 H implants after 80 days of incubation; Peaks represent carbonyl band of PLGA at 1750 cm^{-1} and stretching vibration of carbonyl band and cyclohexadiene of prednisone at 1700 cm^{-1}and 1660 cm^{-1}, respectively

3.2.3 Drug Release from Coated Implants

Effect of PLGA Coating on Drug Release from PLGA 502 H Extruded Implants Loaded with Drugs of Different Solubility

Spray coating with PLGA organic solution was applied as a tool in an attempt to modulate drug release from the extruded PLGA matrices loaded with a high content of different

soluble drugs. The special emphasis was on the reduction of the extremely high initial burst release of dexamethasone sodium phosphate and elimination of the multi-phasic prednisone release profile from PLGA 502 H implants.

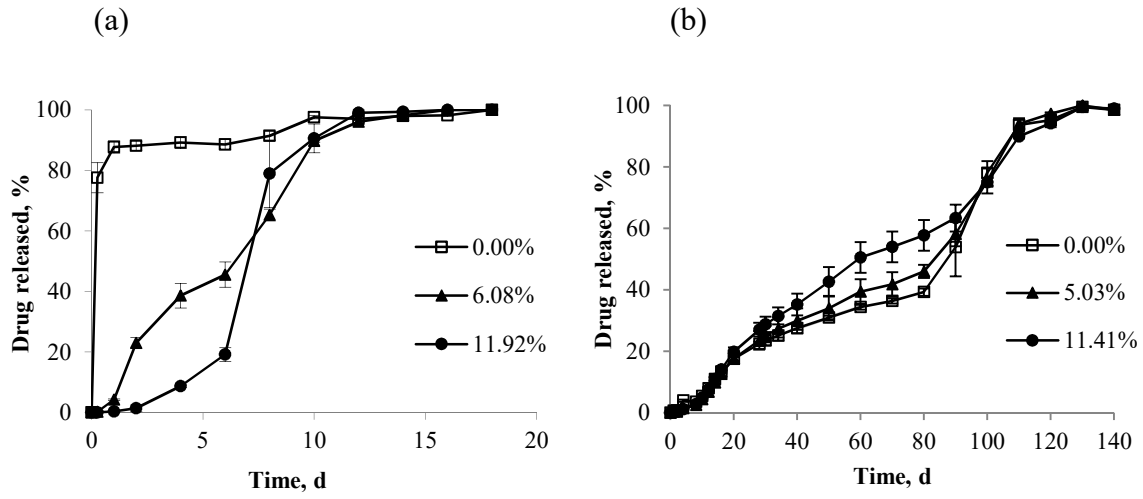


Figure 3.2.15. Effect of different PLGA 502 H coating level on drug release from PLGA 502 H implants containing 30% w/w (a) dexamethasone sodium phosphate and (b) prednisone

Applying drug-free PLGA 502 H layers of different thickness onto the drug-containing PLGA 502 H core had an inverse effect on the drug release depending on the solubility of active ingredient (Figure 3.2.15). As discussed above, the initial rapid medium uptake and the drug diffusion caused the high burst from uncoated implants loaded with 30% of highly soluble drug. With increasing coating level of polymer, dexamethasone sodium phosphate release decreased (Figure 3.2.15.a). The polymeric shell acted as a barrier for water penetration, thus slowing down drug dissolution. On the macroscopic image the difference in morphology of uncoated versus coated matrices after one day of release was noticed (Figure 3.2.16). Due to higher water uptake and extensive swelling the uncoated implant was larger.

On the other hand, prednisone release was not affected by the coating within the first 20 days of incubation (Figure 3.2.15.b). However, from the 20th to 100th day, implants coated with higher coating level had a faster prednisone release. This was attributed to the PLGA shell acting as a barrier for diffusion of PLGA degradation products. Accumulation of acidic by-products led to enhancement of pH gradient within the system. This acidic microenvironment caused an autocatalytic acceleration of polymer degradation inside the core. Thus, the faster core degradation and prednisone release were obtained at higher coating levels, which provided the stronger barriers.

(a) (b)

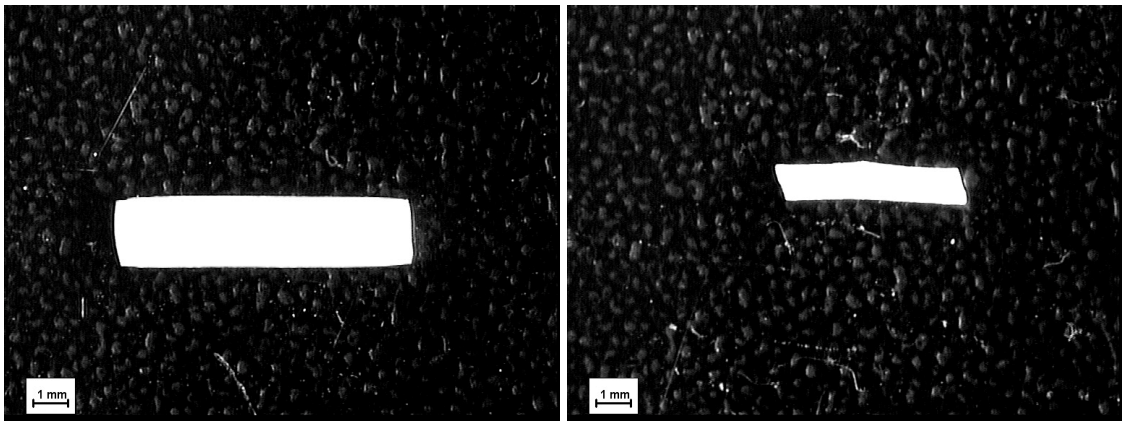


Figure 3.2.16. Morphology of PLGA 502 H implants containing 30% w/w dexamethasone sodium phosphate after one day of incubation; (a) uncoated and (b) coated with PLGA 502 H (11.92% coating level)

Higher content of lactide units in the structure of PLGA provides slower hydrolytic degradation. Therefore, it was assumed that using PLGA 752 H instead of PLGA 502 H as a coating material could prevent diffusion of acidic degradation products from the core for a longer period of time. Thus the phase of slow prednisone release would be even more accelerated.

(a) (b)

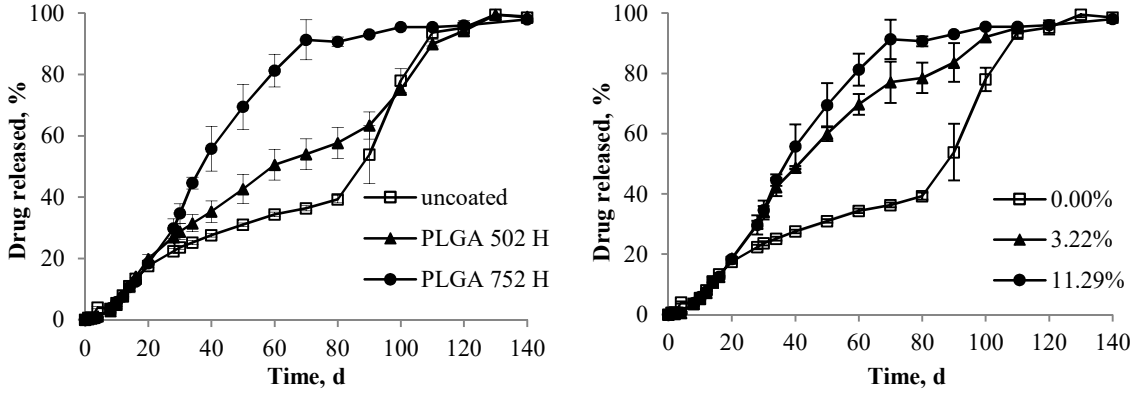


Figure 3.2.17. Effect of (a) different type of polymer in the coating (11% coating level) and (b) different coating level of PLGA 752 H on drug release from implants based on PLGA 502 H with 30% w/w prednisone loading

Indeed, PLGA 752 H was more efficient in elimination of the slow prednisone release phase when compared to PLGA 502 H (Figure 3.2.17.a). Coated PLGA 502 H implants

with higher coating level of PLGA 752 H approximated zero-order drug release kinetics (Figure 3.2.17.b).

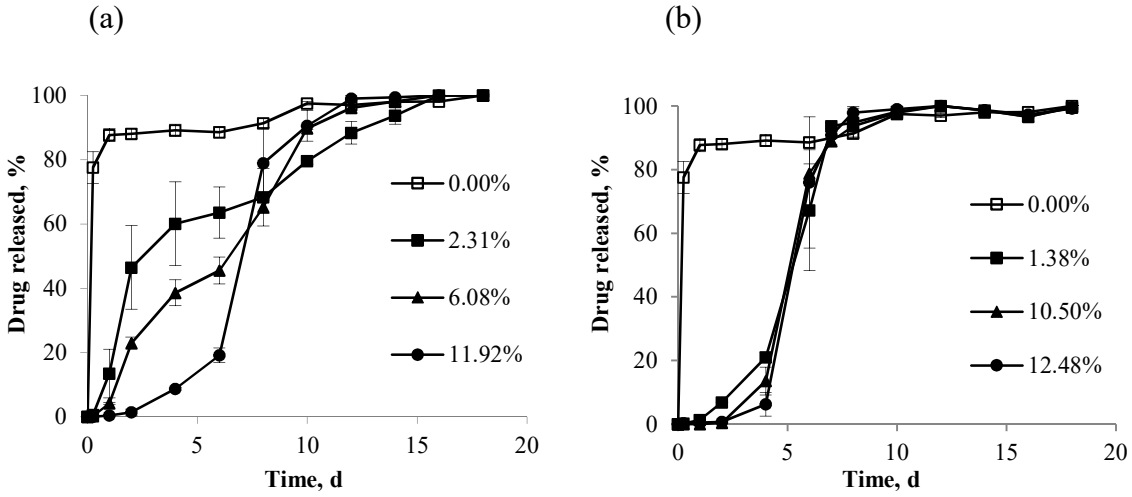


Figure 3.2.18. Effect of different coating level of (a) PLGA 502 H and (b) PLGA 752 H on release from implants based on PLGA 502 H containing 30% w/w dexamethasone sodium phosphate

Dexamethasone sodium phosphate release profile was modified from fast to a linear or to a pulsatile depending on the polymer type in the coatings. PLGA 502 H coatings provided gradual reduction of dexamethasone sodium phosphate release with increasing coating level (Figure 3.2.18.a). Thus, implants coated with 6.08% coating level of PLGA 502 H showed a linear drug release profile. Due to higher content of lactide units in the structure, PLGA 752 H shell was more hydrophobic and more efficient in reduction of medium uptake initially when compared to PLGA 502 H. Therefore, at all coating levels the implants coated with PLGA 752 H showed a period of no release i.e. lag phase up to 2 days which was accompanied by a rapid and complete drug release (Figure 3.2.18.b).

Effect of Curing on Drug Release from Coated Implants

Previously, it was shown that curing process at elevated temperature could reduce dexamethasone sodium phosphate liberation from uncoated PLGA 502 H extruded implants due to remodelling of implants surface, i.e. decreasing of surface porosity (Chapter 3.1). However, curing of the coated implants had a negligible effect on the drug release (Figure 3.2.19). Probably the sprayed polymer layer filled the cracks on the surface of implant and in addition, the curing did not change the mechanical properties of the film prepared by spraying of organic polymer solution.

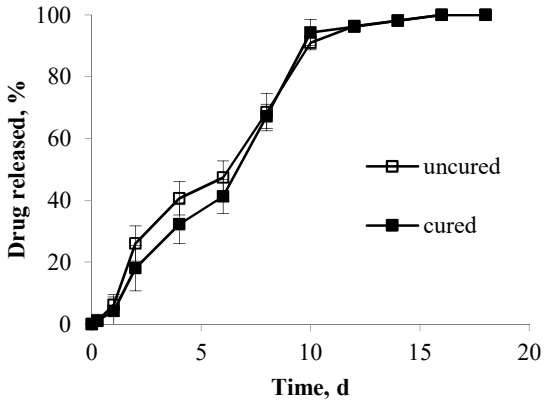


Figure 3.2.19. Effect of curing at 50 °C for 15 min on drug release from PLGA 502 H implants containing 30% w/w dexamethasone sodium phosphate and coated with 6.08% PLGA 502 H coating level

Effect of Variation of Ratio Coated/Uncoated Surface of Implant on Drug Release

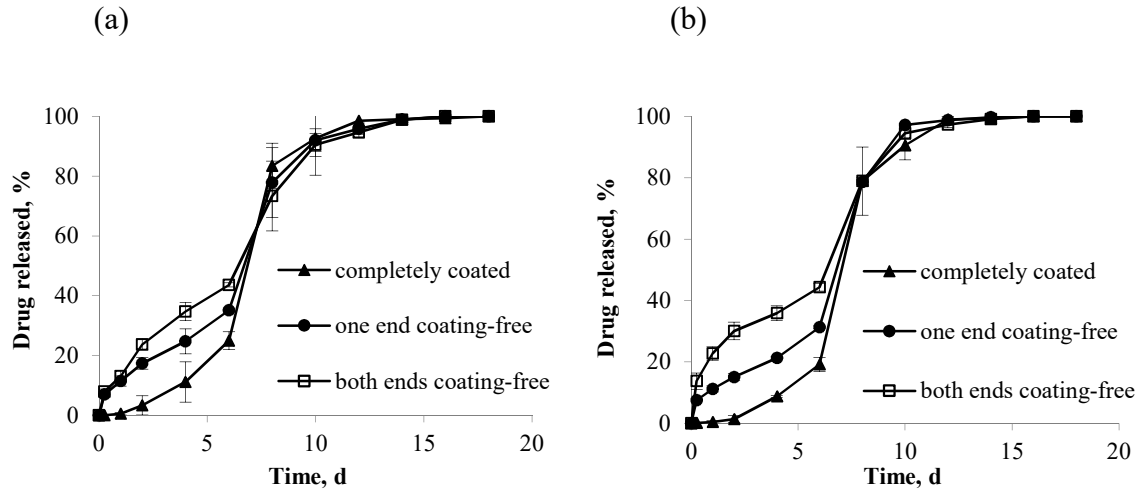


Figure 3.2.20. Effect of variation of ratio coated/uncoated surface of implant on drug release from PLGA 502 H implants containing 30% w/w dexamethasone sodium phosphate and coated with (a) 9.30% and (b) 11.92% PLGA 502 H coating level

Considering that the implants coated with PLGA 502 H at higher coating levels showed the sigmoidal drug release profiles, removing the polymer layer from one or both ends of coated cylindrical implants was applied as a tool for drug release modulation (Figure 3.2.20). Nearly linear dexamethasone sodium phosphate release was achieved from the coated implants with 9.30% coating level and the coating-free both ends (Figure 3.2.20.a).

Modulation of Prednisone Release by Changing Core Material; PEG as Drug Carrier in Core

Instead of PLGAs as matrix formers for the drug-containing cores, a possibility of using polyethylene glycol (PEG) as an alternative drug carrier was investigated. PEG is biocompatible and approved for parenteral use, but less expensive excipient in compared to PLGA. Owing to its solubilisation ability, the higher drug content was incorporated in the matrices. Using PEGs of different molecular weight in the formulation allowed preparation of implants by hot-melt extrusion. Subsequently, implants were coated by spray-coating process with organic solution of PLGA.

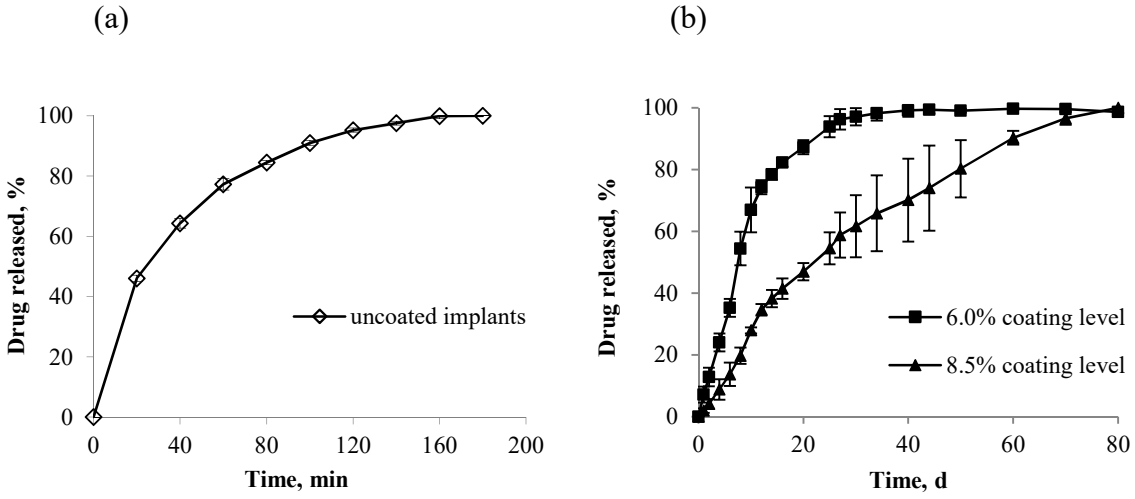


Figure 3.2.21. Prednisone release from implants based on PEG 1500:PEG 4000 (1:1) containing 50% w/w drug; (a) uncoated and (b) coated implants with different coating level of PLGA 502 H

Uncoated PEG-based implants exhibited a rapid prednisone release with 100% drug released within 3 hours (Figure 3.2.21.a). However, applying PLGA 502 H-based layer prolonged drug release up to 80 days (Figure 3.2.21.b). Implants coated with higher coating level demonstrated slower and bi-phasic drug release profile whereby a phase of constant release until the 14th day was accompanied by the phase of slight decreased drug release rate.

Upon incubation in the release medium, water penetrated through the PLGA 502 H shell and an osmotic pressure was built up in the reservoir as PEG dissolved. In addition, acting as a solubilizer, PEG facilitated prednisone dissolution and osmotic pressure pumped the drug out through the coating layer. Within four days of incubation the content inside PLGA shell seemed to be partially dissolved (Figure 3.2.22). Probably erosion of polymer

in shell caused leaking of dissolved PEG thus reducing prednisone dissolution rate from the 14th day of incubation.

(a) (b)

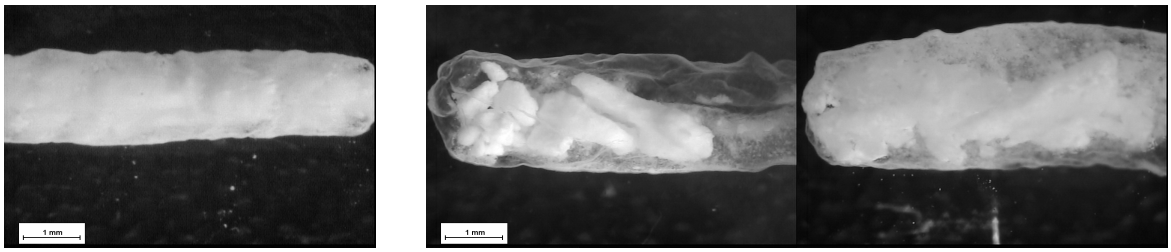


Figure 3.2.22. Morphology of implants based on PEG 1500:PEG 4000 (1:1) containing 50% w/w prednisone; (a) PLGA 502 H coated implant with 6.0% coating level after one day of incubation; (b) PLGA 502 H coated implant with 6.0% (left) and 8.5% (right) coating level after four days of incubation

Therefore, PLGA of later onset of degradation and erosion was selected as a coating material in the next formulation.

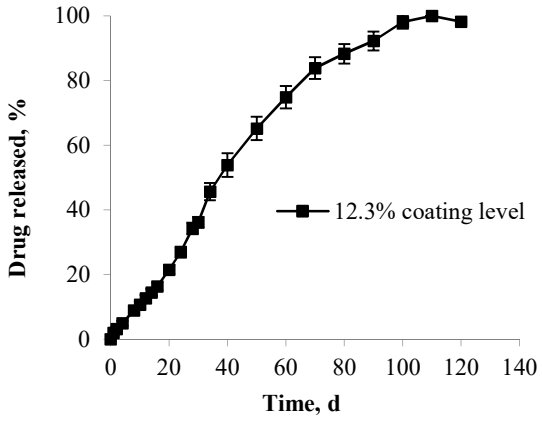


Figure 3.2.23. Prednisone release from implants based on PEG 1500:PEG 4000 (1:1) containing 50% w/w drug and coated with PLGA 653 H

Applying more hydrophobic polymer having higher ratio of lactide to glycolide units, such as PLGA 653 H at 12.3% coating level resulted in extended zero-order drug release up to three months (Figure 3.2.23).

3.2.4 Conclusion

Solubility of drug embedded in the PLGA hot-melt extruded matrices played an important role in drug release mechanism. Particularly at higher loadings, drugs of different solubility proved to be able to alter the PLGA matrix properties regarding hydrophilicity and mechanism and extent of polymer degradation and erosion.

An increase of dexamethasone sodium phosphate loading increased the initial burst release, swelling ability, water uptake, mass loss and polymer degradation when compared to blank PLGA implants. In contrast, with increasing prednisone loading, slower drug release, water uptake, mass loss and polymer degradation were observed. Moreover, dexamethasone sodium phosphate implants retained their shape and integrity until they disintegrated completely indicating bulk erosion. On the other hand, after a slight initial swelling prednisone implants decreased their size gradually implying surface erosion.

Spray coating with a drug-free PLGA polymer layer was proposed as an effective tool for modulation of drug release from the PLGA 502 H extruded implants. With increasing coating level of biodegradable polymer, implants containing the highly soluble drug showed slower release because the shell acted as a barrier for water penetration thus decreasing drug dissolution. Nearly linear or pulsatile drug release profile was achieved depending on the type of PLGA in the shell. The drug release from the coated prednisone implants was independent of the coating within first two weeks of incubation. The PLGA shell acted as a barrier for diffusion of acidic PLGA degradation products thus accelerated the core degradation and prednisone release. PLGA of slower hydrolytic degradation in the shell was able to modify prednisone release profile from the multi-phasic to nearly linear.

An alternative to PLGA, PEG as carrier in core for the slightly soluble drug in combination with PLGA 653 H-based shell at 12.3% coating level had a potential for achieving the extended zero-order drug release up to three months.

3.2.5 *In Vitro/Ex Vivo* Drug Release Study: Assessment of Swelling and Drug Release of PLGA-Based Implants at Site of Application

In study on *in vitro* drug release from uncoated PLGA-based hot-melt extruded matrices as a function of drug solubility and loading (see 3.2.2) it was observed that PLGA implants loaded with the highly soluble drug abundantly swell in an aqueous medium increasing their volume several times (Figure 3.2.13.a). Therefore, the issue was what would be the swelling ability of implants and drug release at the site of administration, i.e. in the subcutaneous adipose tissue.

In Vitro/Ex Vivo Drug Release, Swelling and Water Uptake of PLGA-Based Implants

The subcutaneous adipose tissue represents a loose association of lipid-filled cells, which are held in a framework of collagen fibers. Human abdominal adipose tissue is viscoelastic in nature (Patel et al. 2004). Size of human adipocytes varies from 30 to 70 µm, whereas the adipocytes of the fattened pig have a diameter of 70 µm or grater. Hence, the porcine middle layer is considered as a comparable to the deep subcutaneous layer in the abdominal region of humans (Geerligs et al. 2008).

In order to investigate the drug release and swelling properties of PLGA implants in the subcutaneous tissue *ex vivo*, the study was performed using tissue originated from porcine. Results were compared with commonly used *in vitro* dissolution test for implants, performed in a vial containing phosphate buffer pH 7.4 in an incubation shaker at 37 °C.

In vitro study revealed an increase in the drug release, water uptake and swelling extent of the implants with increasing loading of the highly soluble drug (Figure 3.2.24 and 3.2.25). This behavior was discussed above (see 3.2.2). However, *ex vivo* study was not discriminative for implants of different drug loading. PLGA matrices containing 10% drug showed nearly the same drug release as well as ability to absorb water and to swell in both conditions, *in vitro* and *ex vivo* (Figure 3.2.24 and 3.2.25.a). Contrary, the drug release, water uptake and swelling extent of 30% drug loaded implants were considerably reduced upon *ex vivo* testing (Figure 3.2.24 and 3.2.25.b). This phenomenon was attributed to restricted space for implants swelling in *ex vivo* experiment. In addition, the adipose tissue might apply pressure on implants, thus constraining swelling and hindering water absorption.

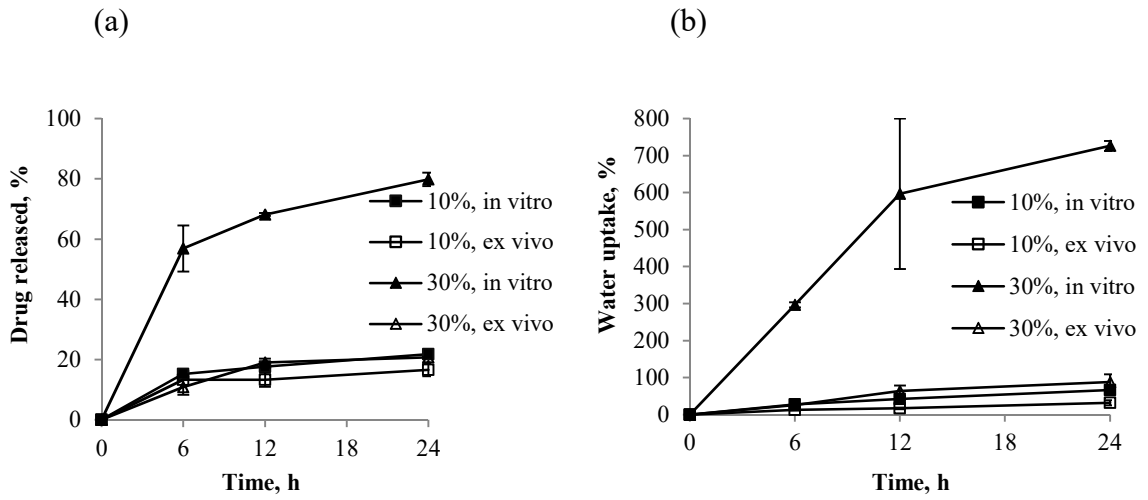


Figure 3.2.24. (a) Drug release and (b) water uptake of PLGA 502 H implants containing 10% and 30% w/w dexamethasone sodium phosphate, *in vitro* and *ex vivo*

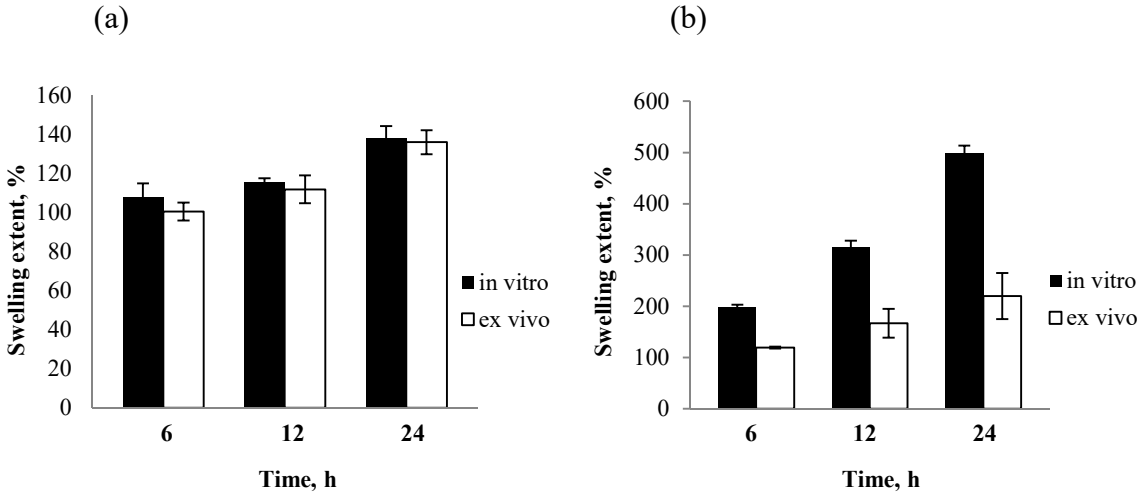


Figure 3.2.25. Swelling behavior of PLGA 502 H implants containing (a) 10% and (b) 30% w/w dexamethasone sodium phosphate, *in vitro* and *ex vivo*

Simulation of Counter-Pressure of Adipose Tissue on Implants

In order to determine the pressure induced by the tissue during implant swelling, the experimental setup was created to simulate this process. Catheters of different balloon volume placed in the tissue were inflated with water, whereas texture analyzer was used for detection of resistance i.e. counter-pressure of the tissue to the extension of balloon.

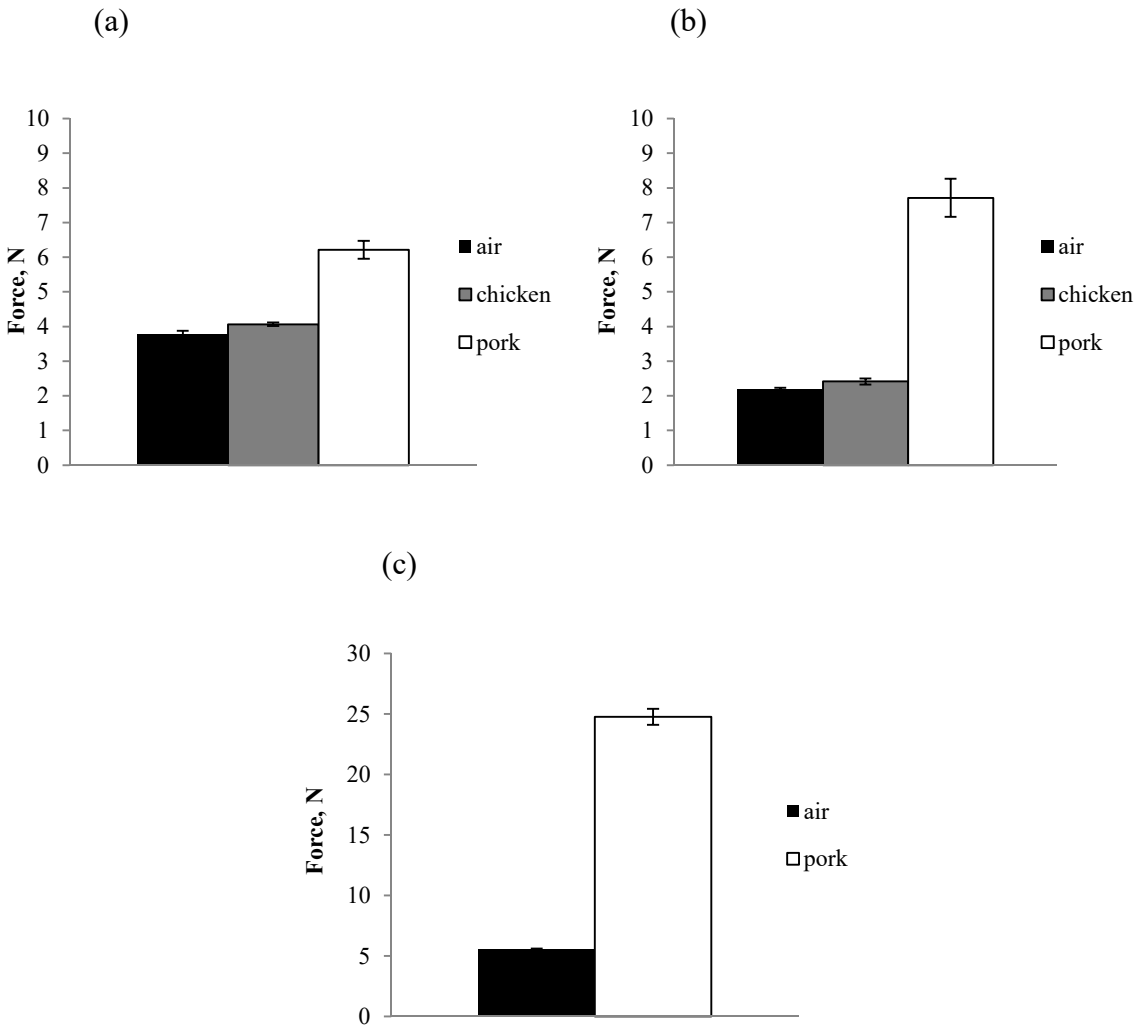


Figure 3.2.26. The required force to squeeze out a certain volume of water in the balloon, wherein the balloon is in the air, in chicken tissue or in pork tissue; (a) catheter 0.05 ml, (b) catheter 0.20 ml, (c) catheter 1.60 ml

The force required to inflate the balloon in the chicken tissue was the same in comparison to the balloon being freely in the air due to loose and thin layer of the chicken subcutaneous tissue (Figure 3.2.26.a and 3.2.26.b). The expanded balloon was intimately underneath the skin. However, the required force to squeeze out the certain volume of water in the balloon, wherein the balloon was in the porcine tissue was notably higher compared to balloon in the air (Figure 3.2.26). Considering the similarity of porcine and human tissue, these values were more relevant. In addition, with increasing the balloon volume, the force was also increased. This indicated that implants with higher ability to swell would be more restricted by surrounding tissue at the side of administration. Moreover, the result was in correlation with the *in vitro/ex vivo* testing of implants (Figure 3.2.24 and 3.2.25).

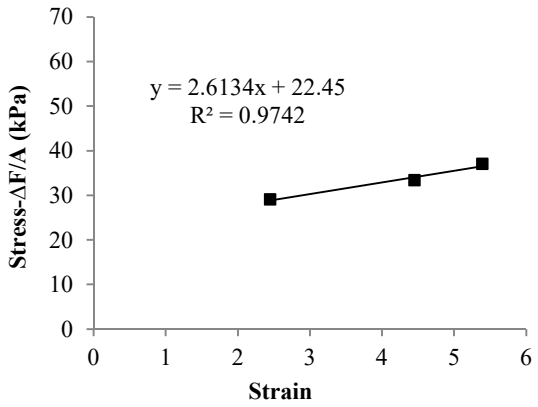


Figure 3.2.27. Stress-ΔF/A (kPa) vs. strain curve for determination of elastic modulus of adipose tissue

In order to characterize a mechanical property of adipose tissue expressed as elastic modulus, the stress versus strain was plotted (Figure 3.2.27). The stress represented the measured force per balloon surface, whereas the strain was amount by which the length of the balloon changed per original length of the catheter. Linear dependence between stress and strain was observed for the balloons of different volume in the range of 0.05 ml to 1.60 ml. Slope of the obtained curve corresponded to the elastic modulus of adipose tissue. Value of 2.61 kPa was consistent with previously reported data confirming the validity of the method. *Ex vivo* abdominal subcutaneous tissue of rat showed a linear and viscoelastic behavior up to 50% strain and modulus of 2.75 kPa (Iatridis et al. 2003). A Samani et al. 2007 investigated *ex vivo* breast fat tissue resulting in Young's modulus of 3.2 kPa. Geerligs et al. 2008 reported somewhat higher shear modulus of 5.6 kPa resulting from porcine subcutaneous fat tissue, whereas the linear region considered to be only up to 0.1% strain.

Due to the long-term drug release study of parenteral implants, the next step was selection of hydrogel as a tissue mimicking material having the similar mechanical properties as the adipose tissue. Thus, the gel could be used for prediction of the drug release and the implant's swelling for a long period of time. Agarose was a good candidate for this purpose based on the following properties: diffusion as dominant transport mechanism, stability over the long period of time, possibility to adequately insert the dosage form, ease of handling and transparency to visualize dosage form (Semmling et al. 2013). Other researchers used the agarose gels of different concentrations for drug release testing. Thus, 2% agarose gels was utilized for dissolution testing of drug-eluting stents, whereas 0.6% agarose gel was used for in vitro release study of PLGA microparticles (Semmling et al.

2013; Klose et al. 2009; Delplace et al. 2012). However, the correlation between the gel mechanical properties and subcutaneous tissue has not been evaluated yet.

Therefore, the gels of different agarose concentration (2.0%, 3.0%, 3.5% and 4.0%) were prepared based on water or fat emulsion (Lipofundin® MCT 20%) and subsequently tested with the catheter of 0.20 ml by texture analyzer (Figure 3.2.28). The required force to squeeze out a certain volume of water in the balloon, wherein the balloon was in the porcine adipose tissue was 7.71± 0.55 N (Figure 3.2.26.b). 4.0% agarose gel showed the mechanical behavior similar to porcine adipose tissue and could be potentially used as a tissue mimicking material.

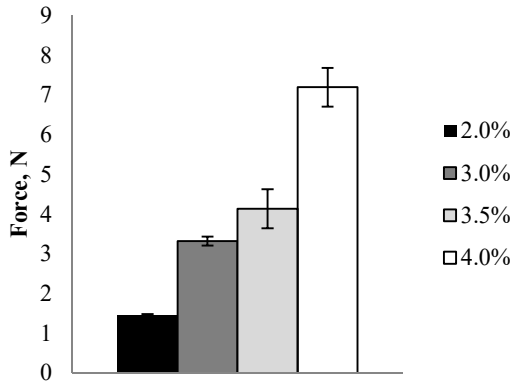


Figure 3.2.28. The required force to squeeze out a certain volume of water in the balloon (catheter 0.20 ml), wherein the balloon is in the gels of different concentrations

3.3 Biodegradable High Drug-Loaded Reservoir Systems: from Zero-order to Pulsatile Drug Delivery

The purpose of this study was to develop a high drug-loaded system with controlled drug release by applying a thin layer of biodegradable drug-free polymeric coating onto drug-loaded compressed cores. The special emphasis was on system flexibility and applicability in terms of drug solubility and loading.

3.3.1 Background

The main goal in the field of controlled drug delivery is to obtain zero-order release kinetics in order to achieve constant plasma or tissue levels. For applications such as the treatment of some systemic or chronic diseases, this approach, which is similar to continuous intravenous administration, may be desirable. However, releasing pharmaceutical substances at a constant rate may not be the optimal manner of delivery for all therapeutics (Liu et al. 2007). In the therapy of bacterial infection or cancer, cells become tolerant and develop resistance if the same active compound is administered continuously (Göpferich 2000; Göpferich 1997). Also, in certain therapies a specific, non-zero order drug release, such as pulsatile release, may be necessary in order to adapt with the circadian rhythms or need of the body (Bussemer et al. 2003).

Pulsatile drug delivery systems are characterized by the rapid and complete release within a short time-period immediately after a predetermined lag-phase with no or only little drug being released (Krögel and Bodmeier 1999; Kikuchi and Okano 2002). This may be preferred in many cases, e.g. in delivery of some hormones and antigens (Iskakov et al. 2002). In a treatment of hormone diseases especially for those that in nature are released in a pulsatile fashion, delivery systems may be designed to mimic this pattern (Jiang et al. 2000). Clinically this is demonstrated for calcitonin, human corticotropin-releasing hormone, gonadotropin releasing hormone, luteinizing hormone-releasing hormone and growth hormone (Wuthrich et al. 1992; Jimoh et al. 1995; Jiang et al. 2000). Pulsed delivery systems offer the possibility of single-shot vaccine if initial and booster release of the antigen can be achieved from one system in which timing of booster release is controlled (Medlicott and Tucker 1999).

Pulsatile dosage forms are classified as single- or multiple-pulse systems and can be designed as pre-programmed systems that deliver drugs depending on the device's structure or triggered systems that release drug in response to some environmental or physiologic variables (Vogelhuber et al. 2001; Kikuchi and Okano 2002; Sershen and West et al. 2002; Guse et al. 2006). The most pulsatile delivery systems are reservoir devices based on polymeric materials releasing a drug rather instantly after a certain period of time, although systems showing sustained release after the lag phase have also been reported in the literature (Vogelhuber et al. 2001).

Controlled drug delivery implants formulated with poly(lactide-co-glycolide) (PLGA) often shows typical tri-phasic drug release profiles; an initial burst release and a rapid second burst release separated by a time interval of no drug release, i.e. lag phase (Shuwisitkul 2011). Although the burst release in some cases could be favourable in terms of designing pulsatile drug delivery devices, the quantity of the burst cannot be precisely controlled (Huang and Brazel 2001). Therefore, it is preferable to achieve a pulsatile release without the initial burst and with predictable lag time lag time, which can be varied in order to cover a broad range of therapeutic requirements.

The main goal of this study was to formulate biodegradable, programmable delivery systems for parenteral application with specific drug delivery rates over a long period of time. The dosage form should provide the therapeutic agent either in a constant manner or after a predetermined lag phase. Considering that programmed release profiles of a therapeutic agent rely on the initial design of the drug delivery system, preparing implants with the reservoir structure could be an option to achieve a well-controlled zero order or a pulsatile release. Therefore, a high drug-loaded system was developed by applying drug-free polymeric coatings onto drug-loaded compressed cores. The system is cost-effective due to a thin layer of PLGA or PLA coating necessary to retard the drug release, whereas the core is based on calcium hydrogen phosphate or polyethylene glycol, the biocompatible and less expensive excipients. The potential drug instability caused by acidic microclimate upon PLGA degradation is omitted. In addition, using compression process for the core preparation offers further advantages: it is simpler and more suitable for thermo sensitive drugs because neither solvents nor heat are applied contrary to commonly used processes such as hot-melt extrusion or microencapsulation techniques. Moreover, using compression punch and die set of 2 mm in diameter makes the system appropriate in size for a subcutaneous application. The study comprises the formulation of PLGA-coated reservoir implants containing low molecular weight model drugs. System applicability was investigated regarding different drug solubility and loading.

3.3.2 Formulation of Reservoir Systems Containing a Slightly Soluble Drug

Prednisone Release from Reservoirs Consisting of Hot-Melt Extruded/Compressed Cores and PLGA 502 H Coatings

It has been reported that compression properties of drugs with an excipient were successfully improved by co-fusion method. Melting before compression was demonstrated to be a method for enhancing tableting properties as well as an increased dissolution rate and improved bioavailability of poorly water-soluble drugs. (Ndindayino et al. 2002a; Ndindayino et al. 2002b). Furthermore, studies on the melt-extrusion of polyols indicated that highly compressible powders might be produced by extrusion of polyols or polyols/drug mixtures.

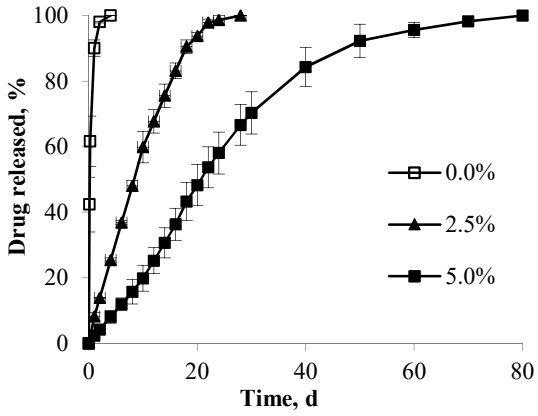


Figure 3.3.1. Effect of PLGA 502 H coating level on prednisone release from reservoirs consisting of hot-melt extruded/compressed cores based on prednisone:PEG 6000 (1:1)

A blend of prednisone:PEG 6000 (1:1) was hot-melt extruded, milled and compressed. Subsequently, the cores were coated with PLGA 502 H and the effect of the coating level of PLGA 502 H was investigated.

Uncoated implants showed 90% drug release within 24 h of incubation (Figure 3.3.1). In order to extend prednisone release and to develop a system capable of providing sustained drug release profiles over longer periods of time, the cores were coated with biodegradable polymer. The rate of drug release from coated implants was directly dependent on the coating level. Application of 2.5% and 5% of PLGA 502 H coatings led to extended release until one and two months, respectively. Thus, desired linear

prednisone release was achieved from biodegradable reservoir systems consisting of hot-melt extruded/compressed cores loaded with 50% prednisone and PLGA 502 H shells.

Effect of Core Preparation Process on Prednisone Release from Uncoated and Coated Implants

However, the hot-melt extrusion process is performed at elevated temperatures and therefore it could be unsuitable for formulation of the heat labile actives. Hence, a simple direct compression process could be an alternative in the core preparation.

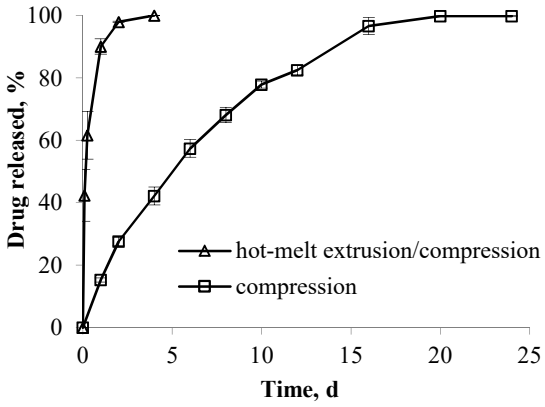
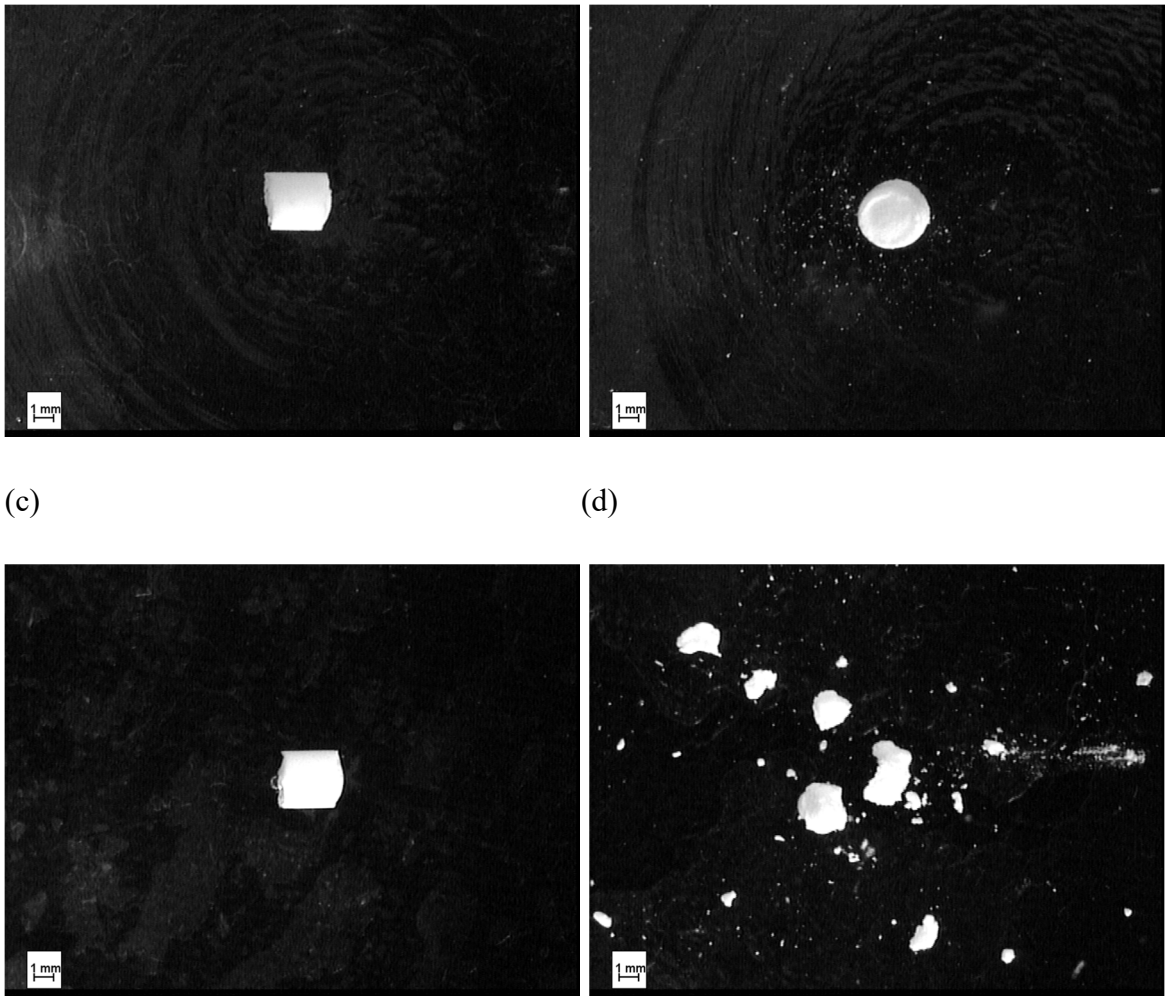


Figure 3.3.2. Drug release from uncoated hot-melt extruded/compressed and directly compressed implants based on prednisone:PEG 6000 (1:1)

Figure 3.3.2 illustrates the prednisone release from the uncoated implants based on prednisone:PEG 6000 (1:1) prepared by utilisation of different process technologies. Drug release was complete within 2 days and 20 days of incubation from hot-melt extruded/compressed and directly compressed cores, respectively. The significantly slower drug release from directly compressed matrices indicated that the hot-melt extrusion process plays an important role in increasing prednisone dissolution rate. Additionally, faster disintegration of the hot-melt extruded/compressed cores was observed (Figure 3.3.3).

(a) (b)



(c) (d)

Figure 3.3.3. Morphology of uncoated implants immersed in phosphate buffer and incubated in a shaker at 37 °C, 80 rpm; implants prepared by direct compression before incubation (a) and after 6 h of incubation (c), implants prepared by hot-melt extrusion/compression process before incubation (b) and after 6 h of incubation (d)

In order to investigate whether during the hot-melt extrusion process the drug was molecularly dispersed i.e. dissolved in the molten carrier (PEG 6000), a solubility study with physical mixture and hot-melt extruded powder was performed.

PEG appeared to be a solubilizer for the slightly soluble prednisone gradually increasing dug solubility up to six times within one month compared to saturation solubility of pure prednisone having value of 0.2 mg/ml (Figure 3.3.4). However, after 20 days the prednisone solubility from the physical mixture and the hot-melt extruded powder reached the same value.

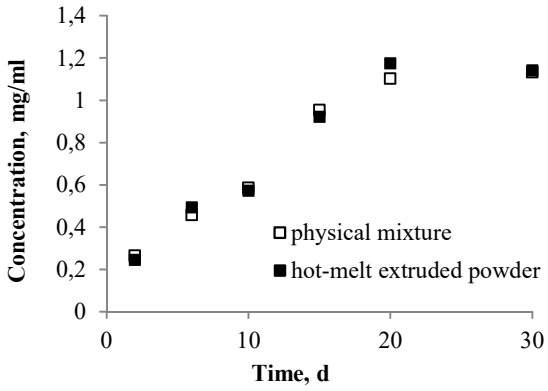


Figure 3.3.4. Prednisone solubility study of physical mixture and hot-melt extruded prednisone:PEG 6000 (1:1) powder

An explanation for the faster dissolution rate of prednisone and faster disintegration of uncoated implants prepared by hot-melt extrusion/compression process compared to implants prepared by direct compression is probably attributed to the co-fusion method. During the hot-melt extrusion process at temperatures sufficient to melt the polymer, but not high enough to melt the drug, the molten polymer covers the drug crystals efficiently due to the intense mixing in the extruder. Additionally, the DSC test showed a slight decrease of PEG melting peak and fusion enthalpy, which could indicate a partial solubility of the drug in the molten carrier (Table 3.3.1).

Table 3.3.1. Carrier melting behaviour analysed by DSC

Prednisone-PEG 6000	**T_{peak} (°C)**	**ΔH_f (J/g)**
Physical mixture	65.13	226.01
Hot-melt extrudate	62.22	192.13

Unlike the hot-melt extruded/compressed prednisone:PEG 6000 (1:1) cores coated with PLGA 502 H, which showed linear drug release, the coated direct compressed matrices released drug in a multiphasic manner (Figure 3.3.5): a non-linear drug release profile comprised of two phases of faster drug release separated by a lag phase. Accordingly, the method of core preparation affected not only the rate, but also the shape of the drug release curve.

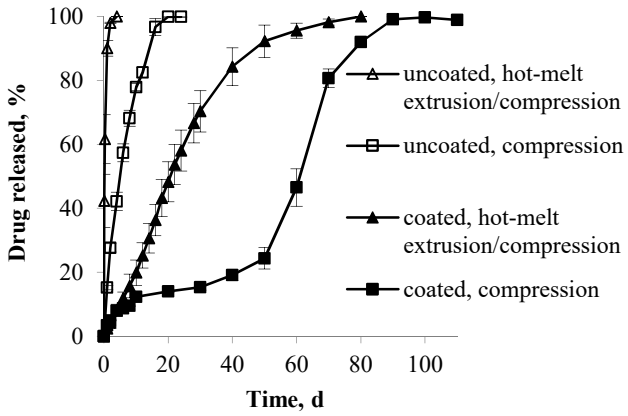


Figure 3.3.5. Prednisone release from reservoir systems based on prednisone:PEG 6000 (1:1) cores prepared by hot-melt extrusion/compression process or direct compression and coated with 5% coating level of PLGA 502 H

The morphology of coated compressed and hot-melt extruded/compressed matrices after 20 days of release was considerably different (Figure 3.3.6). A non-disintegrated core of intact shape and the swollen polymer firmly attached to the core were observed with the coated compressed implants (Figure 3.3.6.a). On the other hand, a loose and transparent coating having a shell-like shape was observed around the partially disintegrated hot-melt extruded/compressed core (Figure 3.3.6.b). Faster medium uptake and core disintegration probably caused an expansion of the PLGA layer accelerating its degradation and erosion. Thus, the friable PLGA shell acted as a porous membrane for prednisone release. Osmotic pressure inside the core created due to dissolved PEG pumped the drug out resulting in constant drug release rate from coated hot-melt extruded/compressed implants.

(a) (b)

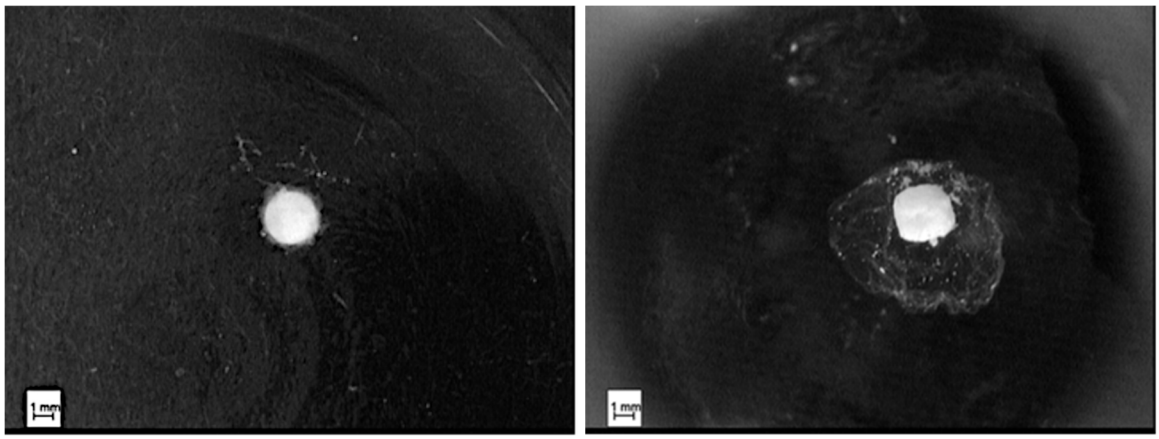


Figure 3.3.6. Morphology of coated implants with 5% PLGA 502 H coating level after 20 days of release study; cores prepared by a) direct compression process and b) hot-melt extrusion/compression process

Effect of PLGA502 H Coating Level on Prednisone Release from Reservoirs Consisting of Directly Compressed Cores

In order to thoroughly investigate the effect of variable PLGA 502 H coating level on prednisone release from compressed cores and identify the reason for changing the drug release kinetics from linear to non-linear upon coating, implants were subjected to drug release study and analysed macroscopically.

(a) (b)

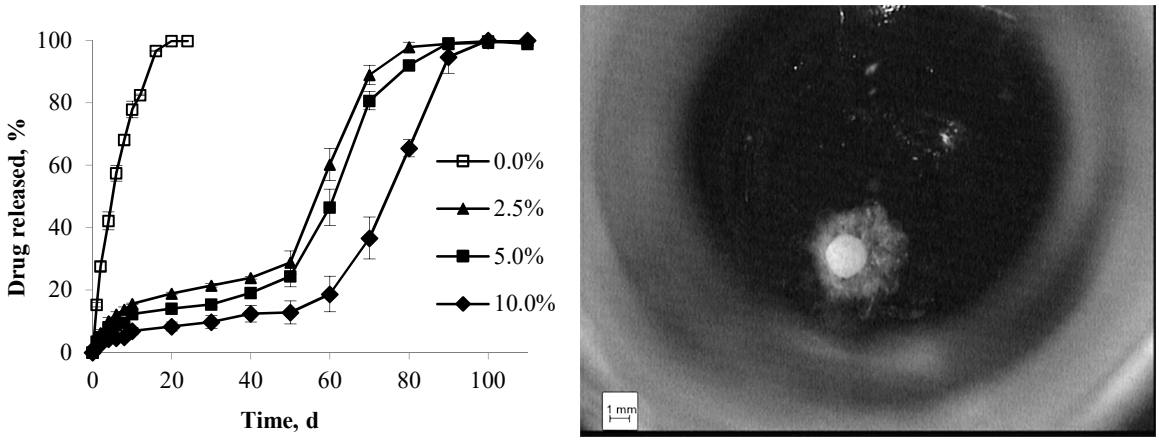


Figure 3.3.7. (a) Effect of PLGA 502 H coating level on drug release from reservoirs consisting of compressed cores based on prednisone:PEG 6000 (1:1); (b) morphology of coated implant with 10% PLGA 502 H coating level after 16 days of release study

With increasing coating level of PLGA 502 H onto the compressed cores, lower initial release and longer lag phases were observed, whereas the second phases of fast release occurred at the same rate (Figure 3.3.7.a). Upon immersion in an aqueous medium, the PLGA coating absorbed water and swelled abound (Figure 3.3.7.b) thus there was a longer drug diffusion pathway which might cause the longer lag phase in coted implants with higher coating level. In addition, water acted as plasticizer and the polymer chain mobility could be increased causing pore closure and decreasing the drug release during the lag phase (Kang et al. 2006). The lag phase was followed by rapid release phase due to PLGA erosion i.e. sudden mass loss.

Therefore, in order to achieve zero-order drug release profile from coated implants consisting of directly compressed cores and to eliminate the lag phase caused by abound swelling of the PLGA 502 H shell, a less swellable polymeric coating could be a potential solution.

Effect of PLA 203 S Coating on Prednisone Release from Reservoirs Consisting of Directly Compressed Cores

Generally, zero-order release kinetics is desirable for long-term releasing formulations, thus providing a constant plasma drug level. In order to avoid the multiphase drug release from coated implants containing directly compressed core, a less swellable polymer such as PLA 203 S was applied as a coating material. On contrary to formulations with PLGA 502 H coating, the PLA 203 S coated implants showed nearly zero-order drug release profiles. Duration of the drug release was directly dependent upon the coating level (Figure 3.3.8). Coating levels ranging from 0%, to 2.5%, to 5% and up to 10% showed complete drug release within 20, 40, 80 and 140 days, respectively. The shell based on PLA 203 S in comparison to PLGA 502 H is more hydrophobic owing to the absence of glycolide units in the polymer structure as well as ester terminated group. In addition, PLA 203 S has a higher molecular weight, thus demonstrating a considerably slower degradation rate. Due to degradation time up to six months the shell was able to maintain the integrity of implants during the entire drug delivery period providing a constant prednisone release rate.

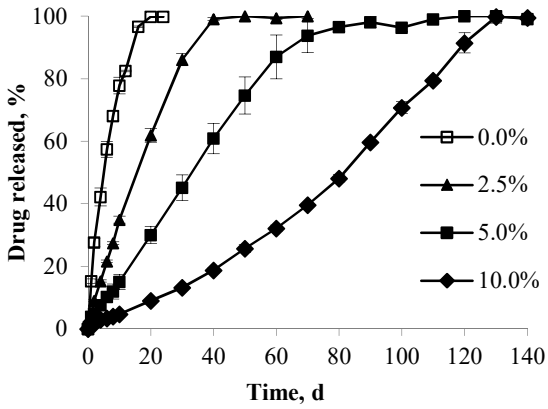


Figure 3.3.8. Effect of PLA 203 S coating level on drug release from reservoirs consisting of directly compressed cores based on prednisone:PEG 6000 (1:1)

Directly Compressed Cores Based on PEG of Different Molecular Weight

Uncoated implants showed extended and linear drug release profiles with a release rate depending on the molecular weight of PEG (Figure 3.3.9). Complete prednisone was released from the implants based on higher molecular weight PEG 6000 within 20 days, whereas the implants prepared with PEG 1500 showed faster drug release with 100% drug released until the 12th day of incubation.

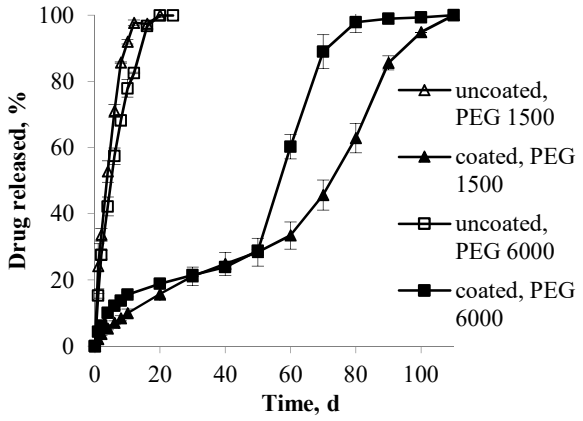


Figure 3.3.9. Prednisone release from implants consisting of directly compressed cores based on PEG of different molecular weight coated with 2.5% coating level of PLGA 502 H

While both uncoated implants provided a constant drug delivery, prednisone release from coated reservoir systems was non-linear independent of the molecular weight of PEG. Surprisingly, formulations based on PEG of lower molecular weight showed a slower release rate. In the initial phase water penetrated through the coating film and dissolved both the polymer and the drug. Dissolved drug can diffuse out through the shell. Water uptake caused swelling of the PLGA coating and thus longer drug diffusion pathways and slower the drug release rate. After the lag phase, which lasted until the 50th day in both formulations followed the phase of faster drug release caused by PLGA erosion. Interestingly, prednisone release from implants based on PEG 1500 was slower in the final stage of drug release. Considering that PEG acted as a solubilizer for the slightly soluble drugs such as prednisone (Figure 3.3.4), an explanation for this phenomenon might be that low molecular weight PEG could diffuse out through the degraded and porous PLGA film after 50 days of incubation. Thus prednisone solubility inside the system was reduced leading to a decreased prednisone dissolution rate.

Effect of PLGA 504 Coating on Prednisone Release from Reservoirs Consisting of Directly Compressed Cores

In order to prove the hypothesis that PEG 1500 diffused out of the system through the porous PLGA 502 H shell after the 50th day of release was a main reason for slowing down prednisone release rate, PLGA of higher molecular weight was selected as coating material. It was expected that erosion onset of shells based on PLGA 504 (Mw 38000-54000 g/mol) occurred later compared to PLGA 502 H (Mw 7000-17000 g/mol) and thus the intact coating would keep PEG 1500 inside the reservoir for a longer period of time.

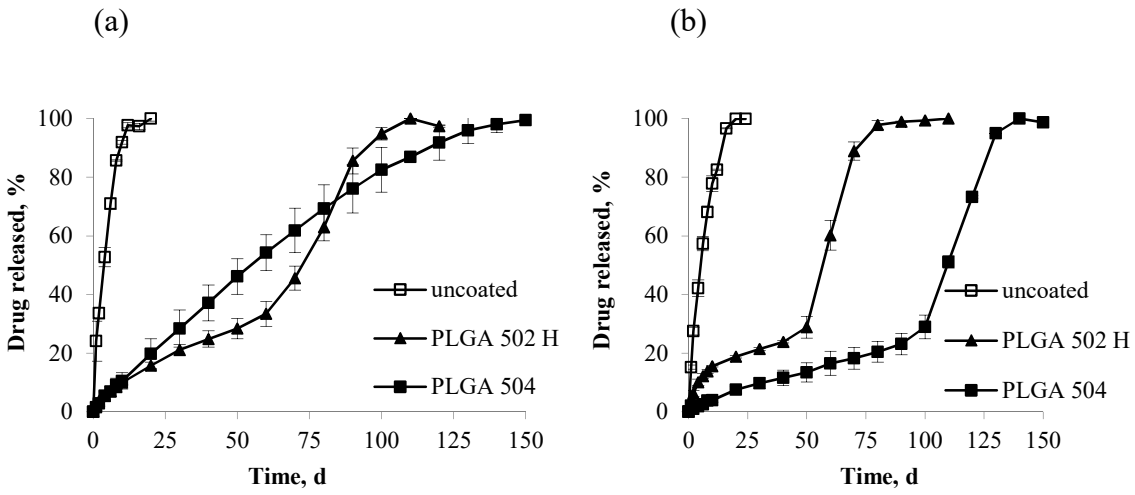


Figure 3.3.10. Drug release from implants consisting of directly compressed cores based on (a) prednisone:PEG 1500 (1:1) and (b) prednisone:PEG 6000 (1:1) and coated with 2.5% coating level of PLGA

Indeed, implants based on PEG 1500 and coated with PLGA 504 showed faster prednisone release with a constant drug release rate compared to implants coated with PLGA 502 H which released drug in the multiphasic manner (Figure 3.3.10.a).

On the other hand, formulations with the higher molecular weight carrier PEG 6000 in the core showed the three-phasic release profile independent of the type of coating polymer (Figure 3.3.10.b). Lower initial release and longer lag phase were observed from reservoirs coated with PLGA 504. The decrease in the initial phase was due to slower drug diffusion through higher molecular weight polymer upon immersion in the release medium, whereas later erosion onset i.e. mass loss of PLGA 504 compared to PLGA 502 H contributed in the prolongation of the lag phase.

3.3.3 Formulation of Reservoir Systems Containing a Highly Soluble Drug

In order to demonstrate the system's flexibility and applicability regarding different solubility of actives, drug delivery reservoir systems were formulated with the highly soluble model drug dexamethasone sodium phosphate with a solubility of 466.7 mg/ml.

Dexamethasone Sodium Phosphate Release from Reservoirs Consisting of Directly Compressed PEG 6000 Cores and PLGA 502 H Coatings

The incorporation of 50% dexamethasone sodium phosphate into cores based on PEG 6000 resulted in fast drug release from both uncoated and coated implants. 100% drug was released from the uncoated formulation within 20 minutes (Figure 3.3.11.a), whereas the PLGA 502 H coating slightly contributed in decreasing the drug release rate (Figure 3.3.11.b). Independent of coating level, all coated systems showed fast release. The maximal retardation of 24 h was obtained from implants coated with 20% of PLGA 502 H.

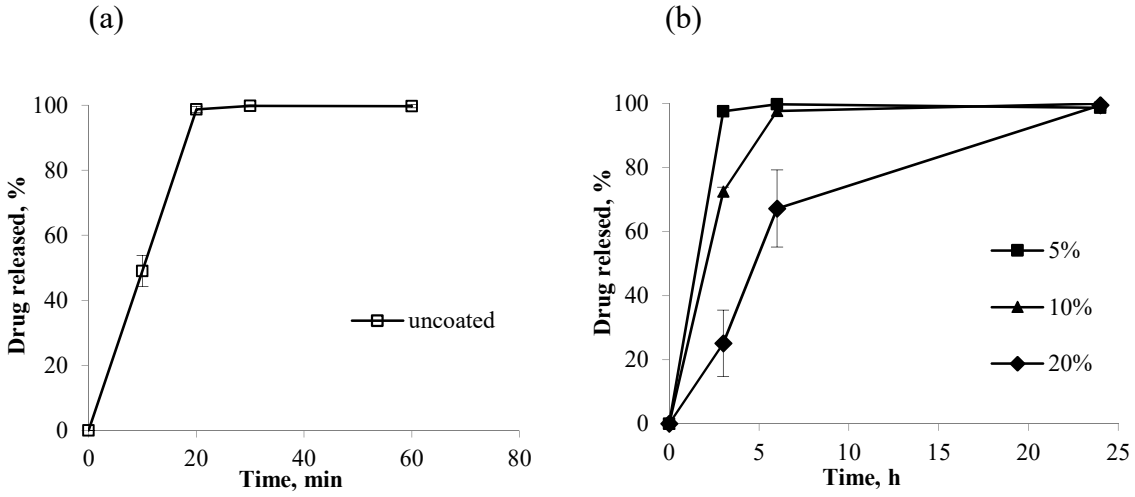


Figure 3.3.11. Drug release from implants consisting of directly compressed cores based on dexamethasone sodium phosphate:PEG 6000 (1:1); (a) uncoated implants and (b) coated implants with different coating level of PLGA 502 H

Morphological analysis of reservoirs during incubation revealed the main mechanism of drug release to be a rupture of PLGA films (Figure 3.3.12). Upon incubation in the release medium, water diffused through the shell and a high osmotic pressure is built up in the reservoir since dexamethasone sodium phosphate and PEG are dissolved. This pressure led to the perforation of the film. On the macroscopic image the holes and ruptures in the PLGA shell were clearly visible.

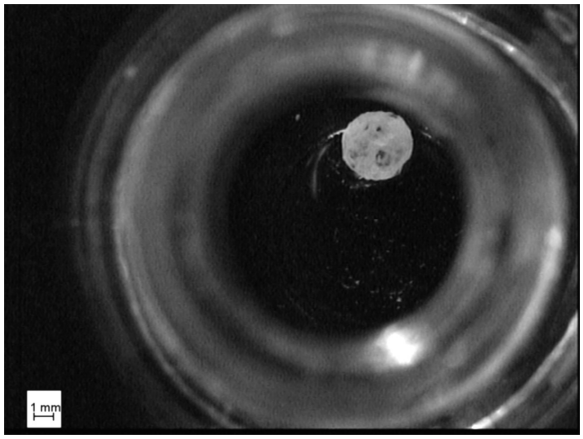


Figure 3.3.12. Morphology of coated implant with 20% PLGA 502 H coating level after 2 days of release study

Dexamethasone Sodium Phosphate Release from Reservoirs Consisting of Compressed PEG 6000 Cores and PLA 203 S Coatings

Considering the mechanism of drug release from PLGA 502 H coated implants, PLA 203 S was next selected as a coating polymer. The shell based on PLA 203 S in comparison to PLGA 502 H is more hydrophobic owing to an absence of glycolide units in the polymer structure as well as ester terminated group. Due to its more hydrophobic nature it was expected to decrease absorption of water.

(a) (b)

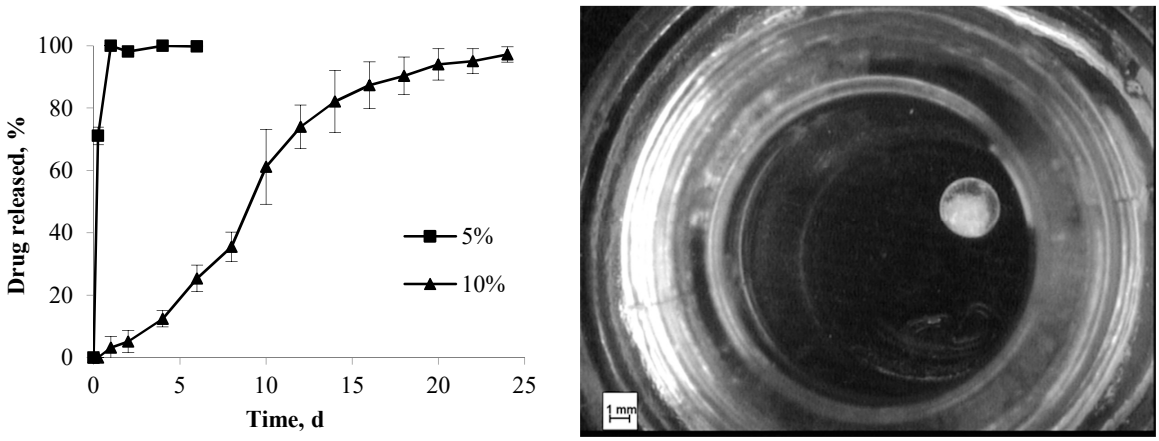


Figure 3.3.13. (a) Effect of PLA 203 S coating level on drug release from reservoirs consisting of compressed cores based on dexamethasone sodium phosphate:PEG 6000 (1:1); (b) Morphology of coated implant with 10% PLA 203 S coating level after 6 days of release

The drug release profile of implants coated with 5% coating level of PLA 203 S still demonstrated fast and complete release within one day. On the other hand, 10% coating

level of PLA film was able to successfully retard dexamethasone sodium phosphate release for a period of three weeks (Figure 3.3.13.a). This was achieved due to the hydrophobic properties of polymer, which led to a slower water uptake and at higher coating level was able to withstand osmotic pressure formed inside the core. The intact PLA 203 shell with no visible perforation was observed on the macroscopic image after one week of drug dissolution test (Figure 3.3.13.b).

In order to make the PLA coating more flexible to withstand the high osmotic pressure created inside the core longer and thus to obtain a formulation with extended release at lower coating level, the water insoluble plasticizer was added to the polymer spray solution. Cores with a lower content of dexamethasone sodium phosphate were also investigated because loading of highly soluble drug strongly affects osmotic pressure.

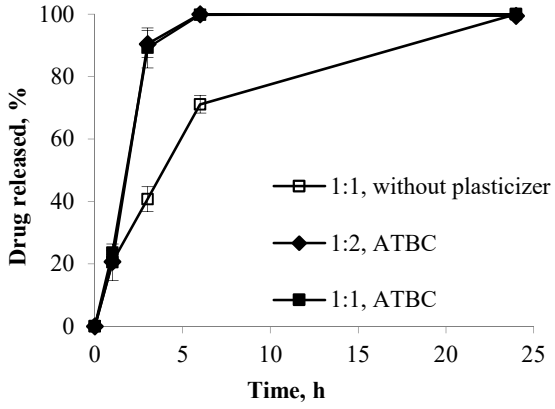


Figure 3.3.14. Effect of ATBC (10% w/w) in the PLA 203 S coating (5% coating level) on drug release from reservoirs consisting of compressed cores based on dexamethasone sodium phosphate:PEG 6000 (1:1, 1:2)

The addition of plasticizer in the polymer coating did not reduce the drug release (Figure 3.3.14). On the contrary, dexamethasone sodium phosphate release was faster in the presence of plasticizer. This can be explained by a lowering of the Tg of polymer in the coating to 30 °C. Therefore, at 37 °C, plasticized PLA films were above the Tg, in the rubbery state, thus having higher elongation but lower puncture strength values which in turn can cause film rupture at an earlier phase of drug release (Kranz et al. 2000). In addition, drug loading did not affect dexamethasone sodium phophate release from formulations containing plasticizer in the coating film.

Dexamethasone Sodium Phosphate Release from Reservoirs Consisting of Directly Compressed Calcium Hydrogen Phosphate Cores and PLGA 503 Coatings

Changing the core base and decreasing the loading of the highly soluble drug were investigated as an approach to decrease an osmotic pressure in the reservoir. In order to achieve extended dexamethasone sodium phosphate release, calcium hydrogen phosphate as insoluble excipient was selected instead of PEG.

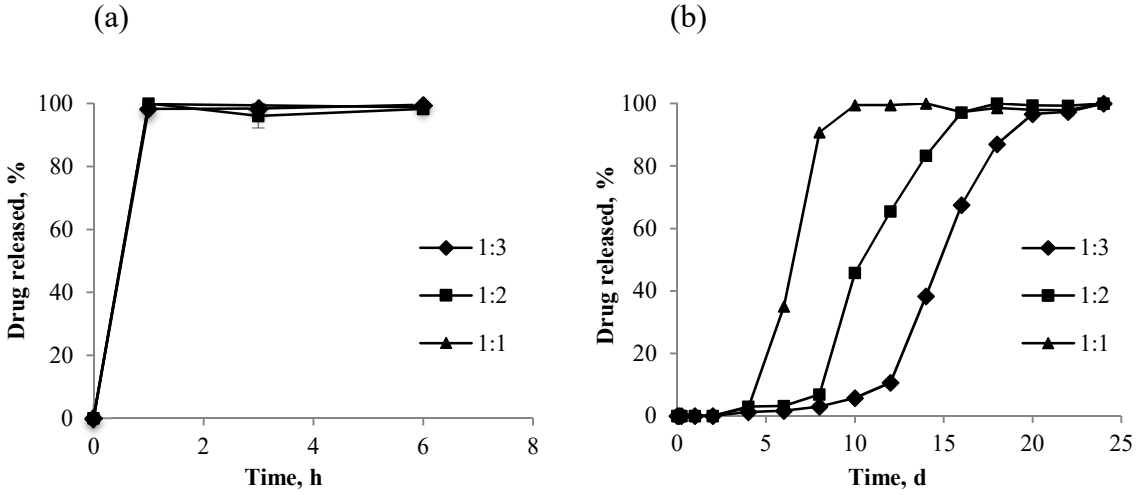


Figure 3.3.15. Drug release from reservoirs consisting of directly compressed cores based on dexamethasone sodium phosphate:calcium hydrogen phosphate (1:1, 1:2, 1:3); (a) uncoated implants and (b) coated implants with 5% coating of PLGA 503

Uncoated implants containing the highly soluble dexamethasone sodium phosphate and calcium hydrogen phosphate showed rapid drug release at all ratios (1:1, 1:2, 1:3) with 100% release within one hour (Figure 3.3.15.a). This was due to fast core disintegration as observed during the macroscopic analysis of incubated implants (Figure 3.3.16). However, coating with PLGA 503 at a level of 5% led to a pulsatile drug release profile that was discriminative for reservoirs with different drug loadings (Figure 3.3.15.b). A lag phase was followed by a phase of rapid drug release due to rupturing of the biodegradable film as it was indicated by macroscopic images of coated implants at the end of the drug release study (Figure 3.3.17). Reservoirs with higher drug content in the core (1:1 ratio) appeared to be partially empty due to drug liberation, while the shell in this implant seemed more porous and perforated than in implants with lower dexamethasone loading (1:3 ratio). The increase of the dexamethasone sodium phosphate:calcium hydrogen phosphate ratio from 1:1, 1:2 to 1:3 led to prolongation of the lag phase from four to six and to eight days, respectively. This can be explained by the increase of osmotic pressure

within the reservoir caused by the increase in loading of highly soluble drug. Thus, the rupture of the polymer shell occurred earlier in implants with higher dexamethasone sodium phosphate content.

(a) (b) (c)

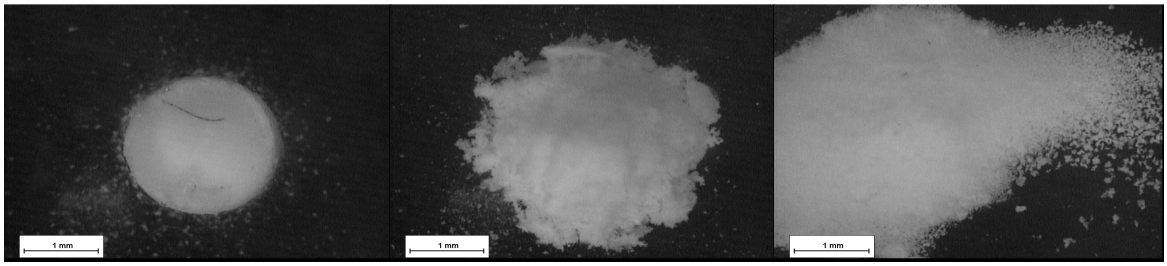


Figure 3.3.16. Morphology of uncoated cores based on dexamethasone sodium phosphate:calcium hydrogen phosphate (1:1); (a) before incubation, (b) after 10 minutes and (c) after 60 minutes of incubation

(a) (b) (c)

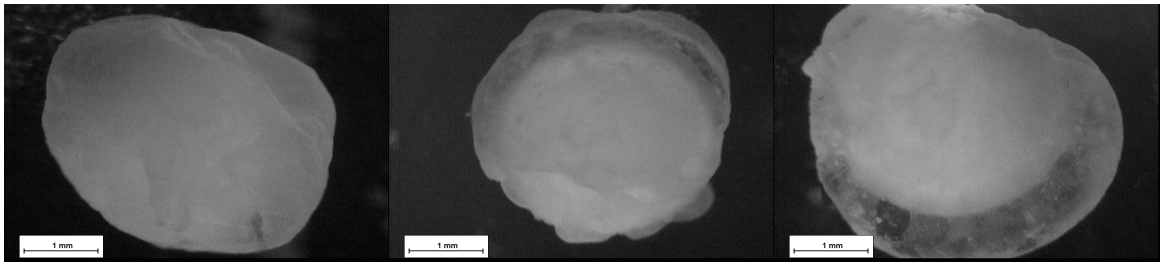


Figure 3.3.17. Morphology of coated implants with 5% PLGA 503 coating level after 26 days of release; cores based on dexamethasone sodium phosphate:calcium hydrogen phosphate (a) (1:3); (b) (1:2) and (c) (1:1)

Modulation of the lag phase was not only possible by changing the content of dexamethasone sodium phosphate, but also by varying the coating level of biodegradable polymer. With increasing the coating level of PLGA 503 from 2.5% to 5.0% onto cores based on dexamethasone sodium phosphate:calcium hydrogen phosphate 1:2, the lag times were extended from one to six days (Figure 3.3.18).

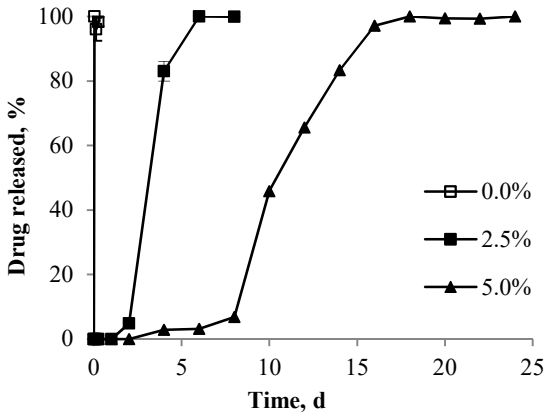


Figure 3.3.18. Effect of PLGA 503 coating level on drug release from reservoirs consisting of compressed cores based on dexamethasone sodium phosphate:calcium hydrogen phosphate (1:2)

Dexamethasone Sodium Phosphate Release from Reservoirs Consisting of Directly Compressed Calcium Hydrogen Phosphate Cores and PLGA 756 S Coating

In designing of programmable delivery systems which release drug in a pulsed manner, the main target is to develop devices capable of providing therapeutic agent after a predetermined off-release period i.e. lag phase depending on the therapeutic needs. A maximum lag time of one week was achieved with formulations of calcium hydrogen phosphate cores coated with PLGA 503 as discussed previously.

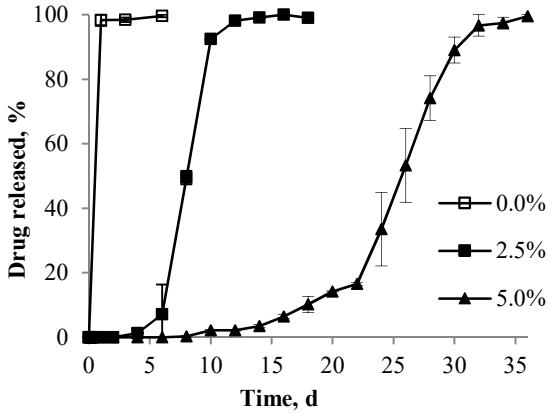


Figure 3.3.19. Effect of PLGA 756 S coating level on drug release from reservoirs consisting of compressed cores based on dexamethasone sodium phosphate:calcium hydrogen phosphate (1:3)

In order to further prolong the lag phase, a more hydrophobic polymer compared to PLGA 503 was utilized for the spray coating process. PLGA 756 S was selected as a polymer with higher monomer ratio of lactide to glycolide and also a higher molecular weight. Accordingly, the film based on PLGA 756 S was more lipophilic; water penetration was slower and pulsatile drug release from coated implants with 5% coating level occurred

after two weeks. Lower dexamethasone sodium phosphate content (1:3 ratio) and thus, a lower osmotic pressure inside the cores also contributed to the prolongation of the lag phase.

3.3.4 Conclusion

The high drug-loaded biodegradable delivery systems for parenteral application capable of releasing the drug either in zero-order or in a pulsed manner were developed.

Incorporating a high dose of the slightly soluble model drug prednisone in the biodegradable reservoir system based on PEG cores and coated with PLGA or PLA rate-controlling shell offered the ability for continuous drug delivery lasting from weeks to several months. Zero-order prednisone release was adjustable by the choice of core preparation method, core base, coating material as well as coating level of biodegradable polymer.

For the highly soluble model drug dexamethasone sodium phosphate, the systems with compressed cores based on calcium hydrogen phosphate and coated with PLGA offered the ability for pulsatile drug delivery. This system enabled varying lag times from days to weeks before drug release was initiated. Drug loading, coating level of biodegradable polymer as well as the type of PLGA played an important role in tailoring the duration of the lag phase.

CHAPTER

4 Summary

In the formulation of controlled drug delivery systems the main goal is to obtain zero-order release kinetics in order to achieve constant plasma or tissue levels. This is, however, one of the challenges in the development of parenteral biodegradable implants based on poly(lactide-co-glycolide) (PLGA). PLGA implants commonly show a tri-phasic drug release profile due to drug diffusion and heterogeneous polymer degradation. Therefore, the purpose of this study was to modulate the drug release pattern from PLGA implants with special emphasis on the reduction of the initial burst release. However, releasing pharmaceutical substances at a constant rate may not be the optimal manner of delivery for all therapeutics. Thus, the aim of work was also to develop biodegradable implants with pulsatile drug release profiles and with the possibility to predict and vary the lag time before the onset of drug release.

In this study, the focus was not only on the variation of formulation parameters but as well on the process parameters to modify the initial burst release as well as the entire drug release profile. For this purpose, 10% dexamethasone sodium phosphate loaded PLGA implants were prepared using a twin-screw extruder. The matrices extruded at 90 °C and a screw speed of 20 rpm showed the typical tri-phasic drug release pattern with initial burst drug release of about 30%, followed by a lag phase and rapid release phase. The process parameters considerably affected the physical properties of the extruded polymeric implants and thus influenced the drug release behavior. By increasing the extrusion temperature from 90 °C to 100 °C and decreasing screw speed from 20 rpm to 5 rpm, the same formulation showed a linear drug release. Extrudates of higher density were obtained due to the reduction of die swell and the prevention of cracks formation. However, under

these extrusion conditions, the material is exposed to a high temperature for a longer time. Alternatively, the feasibility of reducing the implant's surface porosity by a short-term curing at relatively low temperatures when compared to the process temperatures was investigated. This is of great interest with respect to the reduction of the initial burst release of thermolabile hydrophilic drugs. The effect of curing temperature, time and relative humidity was evaluated. Curing at 40 °C and 75% RH proved to be a successful post-preparation treatment to achieve a linear drug release profile from PLGA implants loaded with 10% of highly soluble drug. The drug release profile was modified, whereas the structural integrity and regular shape of implants could be preserved.

The effect of solubility of the embedded drug in PLGA hot-melt extruded matrices was further examined. Highly soluble dexamethasone sodium phosphate and slightly soluble prednisone were used as the model drugs. With increasing dexamethasone sodium phosphate loading from 10% to 30% the initial burst release, swelling ability, water uptake, mass loss and polymer degradation were increased when compared to blank PLGA implants. In contrast, with increasing prednisone loading, slower drug release, water uptake, mass loss and polymer degradation were observed. Moreover, dexamethasone sodium phosphate implants retained their shape and integrity despite accelerated polymer degradation. This could be explained by the presence of slow degrading region at the surface, which suddenly disintegrated completely, indicating a bulk erosion process. Prednisone implants, on the other hand, gradually decreased in size after a slight initial swelling, but the shape remained unchanged. This implied a surface erosion process. The study clearly proved that drugs of different solubility, particularly at higher loadings, were able to alter PLGA matrix properties regarding its hydrophilicity, extent and mechanism of polymer degradation and erosion.

PLGA implants loaded with the highly soluble dexamethasone sodium phosphate showed the typical tri-phasic drug release profiles. From 50% to 90% of the embedded drug was released within one day of incubation from the formulations based on different types of PLGA and with 30% drug loading. The extremely high initial burst release was due to the connection of the drug crystals in a network structure within the matrix. The rest of entrapped active was released after one week as a consequence of matrix erosion. PLGA implants loaded with the slightly soluble prednisone mainly demonstrated bi-phasic drug release kinetics with a lag phase followed by a phase of constant sustained drug liberation. Exceptions to this trend were the prednisone implants based on PLGA 502 H and loaded

with higher drug contents (20% and 30%), which showed a multi-phasic drug release pattern. SEM, DSC, FTIR and extraction results confirmed prednisone stability after processing at elevated temperature. Weak and reversible non-covalent bonds between prednisone and PLGA 502 H, which could form both during the preparation process and incubation period, might serve as an explanation for the multi-phasic prednisone release pattern.

Spray coating with a drug-free PLGA polymer layer was proposed as an effective tool for modulation of drug release from PLGA 502 H extruded implants loaded with a high content of drugs of different solubility. The special emphasis was on the reduction of the extremely high initial burst release of dexamethasone sodium phosphate and the elimination of the multi-phasic prednisone release profile from PLGA 502 H implants. Depending on the type of PLGA in the shell nearly linear (PLGA 502 H) or pulsatile (PLGA 752 H) drug release profiles were achieved. With increasing coating level of biodegradable polymer, implants containing the highly soluble drug showed slower release because the shell acted as a barrier for water penetration and thus decreased the drug dissolution. In contrast, the drug release from prednisone implants was unchanged by PLGA coating within the first two weeks. After two weeks, the prednisone release from coated implants was increased, because the PLGA shell acted as a barrier for diffusion of the acidic PLGA degradation products and thus accelerated the autocatalytic core degradation. By the use of PLGA type of slower hydrolytic degradation as coating material, the prednisone release profile was successfully modified from multi-phasic to nearly linear.

Additionally, alternatives to PLGA as the core material were investigated. By using PEG as a carrier in hot-melt extruded cores containing slightly soluble drug in combination with a PLGA 653 H-based shell at 12.3% coating level, extended zero-order drug release up to three months was achieved.

The study further comprises the formulation of PLGA coated reservoir implants consisting of high drug-loaded compressed cores and drug-free polymeric shells. The incorporation of 50% of the slightly soluble model drug prednisone in the biodegradable reservoir system based on PEG cores and coated with PLGA or PLA as rate-controlling layer provided a continuous drug delivery lasting from weeks up to several months. Zero-order prednisone release was adjustable by the choice of core preparation method, core and

coating material as well as coating level. For the highly soluble model drug dexamethasone sodium phosphate, systems with compressed cores based on calcium hydrogen phosphate and coated with PLGA offered the ability for pulsatile drug delivery. These implants provided adjustable lag times from days up to weeks before drug release was initiated. Drug loading, coating level of biodegradable polymer as well as the type of PLGA played an important role in tailoring the duration of the lag phase. In addition to controlled drug release the system offers several other advantages. Direct compression is a simpler core preparation process in compared to hot melt extrusion and more suitable for thermosensitive drugs. Profitability is increased due to only a thin layer of PLGA or PLA coating necessary to retard the drug release, whereas the core is based on calcium hydrogen phosphate or polyethylene glycol, the biocompatible and less expensive excipients. Furthermore, potential drug instabilities caused by the acidic microclimate upon PLGA degradation are omitted.

Additionally, in order to evaluate the swelling ability and drug release of PLGA-based implants at the site of application, implants were investigated *in vitro* in a vial containing buffer solution and *ex vivo* in porcine adipose tissue. Upon *ex vivo* testing, implants with higher ability to swell were more restricted by the surrounding tissue and demonstrated considerably reduced drug release, water uptake and swelling extent. Therefore, the pressure induced by the adipose tissue on implants upon swelling was simulated and hydrogel material was selected to predict the swelling of implants and drug release for a long period of time. From a series of different concentrations, 4% agarose gel exhibited a mechanical behavior similar to porcine adipose tissue and could be potentially used as a tissue mimicking material for more relevant *in vitro* drug release testing.

In conclusion, the study provided a solution for the reduction of the initial burst release of highly soluble drugs from PLGA hot-melt extruded implants by manipulation of process and formulation parameters. This study also demonstrated the potential of a thin sprayed PLGA layer in controlling the drug release from the biodegradable reservoir systems loaded with a high content of different water soluble drugs. Finally, zero-order and pulsatile drug release kinetics were successfully achieved.

CHAPTER

5 Zusammenfassung

Das Hauptziel bei der Formulierung von Arzneiformen mit kontrollierter Wirkstofffreisetzung ist eine Freisetzungskinetik nullter Ordnung, um dadurch konstante Wirkstoffspiegel in Plasma und Gewebe zu erzielen. Dies ist eine der größten Herausforderungen bei der Entwicklung von parenteralen, bioabbaubaren Implantaten, welche auf Polylactid-co-Glycolid (PLGA) basieren. PLGA-Implantate weisen aufgrund der Wirkstoffdiffusion und der heterogenen Polymerdegradation normalerweise ein dreiphasiges Freisetzungsprofil auf. Das Ziel dieser Arbeit war es daher, die Wirkstofffreisetzung aus PLGA-Implantaten zu modulieren. Der Schwerpunkt lag dabei speziell auf der Reduktion des initialen Bursts. Eine konstante Freisetzungsrate ist aber nicht für alle Arzneistoffe optimal. Daher war eine weitere Zielsetzung bei dieser Arbeit, die Entwicklung von bioabbaubaren Implantaten mit pulsatilen Freisetzungsprofilen, bei welchen die Lag-Zeit vor der Wirkstofffreisetzung vorhergesagt und variiert werden kann.

Der Fokus dieser Arbeit lag nicht nur auf den Formulierungsparametern, sondern auch auf den Prozessparametern, um durch deren Veränderung sowohl den initialen Burst wie auch das ganze Freisetzungsprofil zu beeinflussen. Zu diesem Zweck wurden PLGA-Implantate mit 10% Dexamethason-Natriumphosphat mit einem Zweischneckenextruder hergestellt. Die Matrizen, welche bei 90 °C und einer Drehzahl von 20 Umdrehungen pro Minute extrudiert wurden, wiesen das typische dreiphasige Freisetzungsprofil auf. Beim initialen Burst wurden ca. 30% des Wirkstoffs freigesetzt. Danach folgten eine Lag-Phase und anschließend eine schnelle Freisetzung. Die Prozessparameter hatten einen wesentlichen Einfluss auf die Beschaffenheit der extrudierten Polymerimplantate und beeinflussten so die Wirkstofffreisetzung. Wenn die Prozesstemperatur von 90 °C auf 100 °C erhöht und

die Drehzahl von 20 auf 5 Umdrehungen pro Minute reduziert wurde, wies die gleiche Formulierung eine lineare Wirkstofffreisetzung auf. Extrudate mit höherer Dichte wurden erzielt durch das Reduzieren der Extrudatausdehnung und das Verhindern der Rissbildung. Jedoch ist das Material bei der Extrusion unter diesen Bedingungen über einen längeren Zeitraum einer hohen Temperatur ausgesetzt. Alternativ dazu wurde daher untersucht, ob die Oberflächenporosität der Implantate auch durch ein kurzes Tempern reduziert werden kann. Beim Tempern sind die Temperaturen im Vergleich zu den Prozesstemperaturen bei der Extrusion relativ niedrig. Dies ist von großem Interesse für das Reduzieren des initialen Bursts von thermolabilen, hydrophilen Wirkstoffen. Es wurde untersucht, welchen Einfluss Temperatur, Dauer und relative Luftfeuchtigkeit bei der Temperung haben. Tempern nach der Herstellung bei 40 °C und 75% relativer Luftfeuchtigkeit erwies sich als erfolgreiches Mittel, um für PLGA-Implantate mit 10% leicht löslichem Wirkstoff ein lineares Freisetzungsprofil zu erhalten. Das Freisetzungsprofil wurde verändert, die Integrität und ebenmäßige Form der Implantate hingegen blieben erhalten.

Der Effekt der Löslichkeit des Wirkstoffs, welcher durch Schmelzextrusion in eine PLGA-Matrix eingebettet ist, wurde weiter untersucht. Das leicht lösliche Dexamethason-Natriumphosphat und das schwer lösliche Prednison wurden dabei als Modellarzneistoffe verwendet. Eine Erhöhung des Dexamethason-Natriumphosphat-Gehaltes von 10% auf 30% erhöhte den initialen Burst, die Quellfähigkeit, die Wasseraufnahme, den Massenverlust und den Polymerabbau im Vergleich zu wirkstofffreien PLGA-Implantaten. Im Gegensatz dazu war bei einer Erhöhung der Prednison-Beladung eine Verlangsamung der Freisetzung, der Wasseraufnahme, des Massenverlustes und des Polymerabbaus zu beobachten. Außerdem behielten die Implantate mit Dexamethason-Natriumphosphat ihre Form und Integrität trotz des beschleunigten Polymerabbaus. Dies kann damit erklärt werden, dass es an der Oberfläche einen Bereich mit langsamem Abbau gibt, welcher dann auf einmal komplett zerfällt und auf einen Bulkerosionsprozess schließen lässt. Im Unterschied dazu nahm die Größe der Prednison-Implantate nach einer leichten, anfänglichen Quellung kontinuierlich ab, während sich ihre Form nicht veränderte. Dies deutet auf eine Oberflächenerosion hin. Die Untersuchung bestätigte eindeutig, dass Wirkstoffe unterschiedlicher Löslichkeit – insbesondere bei höheren Beladungen – die Eigenschaften der PLGA-Matrix hinsichtlich Hydrophilie sowie

Mechanismus und Ausmaß des Polymerabbaus und der Polymererosion beeinflussen können.

Mit Dexamethason-Natriumphosphat beladene PLGA-Implantate zeigten das typische dreiphasige Freisetzungsprofil. 50% bis 90% des Wirkstoffs wurde innerhalb einer Inkubationszeit von einem Tag freigesetzt aus Formulierungen mit unterschiedlichen PLGA-Typen bei einer Arzneistoffbeladung von 30%. Der extrem hohe initiale Burst ergibt sich dadurch, dass die Wirkstoffkristalle in der Matrix in einem Netzwerk miteinander verbunden sind. Der verbleibende Anteil des Wirkstoffs wurde nach einer Woche als Folge der Matrixerosion freigesetzt. Mit dem schwer löslichen Prednison beladene PLGA-Implantate wiesen hauptsächlich zweiphasige Freisetzungskinetiken auf. Diese waren gekennzeichnet durch eine anfängliche Lag-Phase, gefolgt von einer Phase, in der der Wirkstoff über einen längeren Zeitraum konstant freigesetzt wurde. Ausnahmen stellten Prednison-Implantate auf Basis von PLGA 502 H dar, welche mit höheren Arzneistoffmengen (20% und 30%) beladen waren. Diese wiesen multi-phasische Freisetzungskinetiken auf. SEM, DSC, FTIR und Extraktionsergebnisse bestätigten die Prednison-Stabilität nach der Verarbeitung bei erhöhten Temperaturen. Schwache, reversible, nicht-kovalente Bindungen zwischen Prednison und PLGA 502 H, die sich während der Herstellung und der Inkubation ausbilden können, sind eine der möglichen Erklärungen für das multi-phasische Freisetzungsprofil von Prednison.

Eine Sprühbeschichtung mit einer wirkstofffreien PLGA-Polymerschicht wurde vorgeschlagen als effektives Mittel für die Modulierung der Wirkstofffreisetzung aus PLGA 502 H-Extrudatimplantaten mit einer hohen Beladung mit Wirkstoffen unterschiedlicher Löslichkeit. Der Fokus lag dabei auf der Reduzierung des extrem hohen initialen Bursts von Dexamethason-Natriumphosphat und der Vermeidung von multi-phasischen Freisetzungsprofilen von Prednison in PLGA 502 H-Implantaten. Je nach PLGA-Typ in der Hülle konnten annähernd lineare (PLGA 502 H) oder pulsatile (PLGA 752 H) Freisetzungsprofile erreicht werden. Mit steigender Schichtdicke des bioabbaubaren Polymers verlangsamte sich die Freisetzung des leicht löslichen Arzneistoffs, da die Hülle als eine Penetrationsbarriere für Wasser diente und somit die Auflösung des Wirkstoffs verzögerte. Im Gegensatz dazu wurde die Wirkstofffreisetzung aus Prednison-Implantaten in den ersten beiden Wochen durch den Überzug nicht verändert. Nach den zwei Wochen war die Prednison-Freisetzung erhöht, da die PLGA-Hülle als Barriere für saure PLGA-Abbauprodukte diente und somit den Abbau des Kerns

beschleunigte. Durch das Verwenden eines PLGA-Typs mit langsamerem, hydrolytischem Abbau als Überzugsmaterial konnte für die Prednison-Freisetzung statt eines multiphasischen Profils ein annähernd lineares Profil erzielt werden.

Zusätzlich wurden Alternativen zu PLGA als Kernmaterial untersucht. Mithilfe von PEG als Trägermaterial für die Schmelzextrusion in Kombination mit einer PLGA 653 H-Hülle (12.3% Überzugsmenge) konnte für den schwer löslichen Wirkstoff eine Freisetzung nullter Ordnung über einen Zeitraum von drei Monaten erreicht werden.

Weiterhin beinhaltete die Studie die Formulierung von PLGA-beschichteten Reservoir-Implantaten, die aus verpressten Kernen mit hoher Wirkstoffbeladung und einer wirkstofffreien Polymerschicht bestanden. Das Einarbeiten von 50% schwer löslichem Prednison in bioabbaubare Reservoirsysteme bestehend aus einem PEG-Kern mit einer PLGA- oder PLA-Ummantelung zur Regulierung der Freisetzungsrate resultierte in einer kontinuierlichen Wirkstofffreisetzung über Wochen bis hin zu mehreren Monaten. Die Freisetzung nullter Ordnung von Prednison konnte durch die Auswahl der Herstellungsmethode für den Kern, des Kern- und Beschichtungsmaterials und des Beschichtungsgrades eingestellt werden. Für die leicht lösliche Modellsubstanz Dexamethason-Natriumphosphat stellten Systeme mit einem verpressten Kern aus Calciumhydrogenphosphat, die mit PLGA beschichtet waren, eine Möglichkeit dar, um eine pulsatile Wirkstofffreisetzung zu erzielen. Diese Implantate boten einstellbare Lag-Zeiten von Tagen bis Wochen, bevor die Wirkstofffreisetzung einsetzte. Die Wirkstoffbeladung, der Beschichtungsgrad mit bioabbaubarem Polymer sowie der PLGA-Typ spielten bei der Modulierung der Dauer der Lag-Phase eine wichtige Rolle. Zusätzlich zur kontrollierten Wirkstofffreisetzung bietet das System einige weitere Vorteile. Im Vergleich zur Schmelzextrusion stellt die Direktverpressung einen einfachen Herstellungsprozess dar, welcher zudem besser für hitzeempfindliche Wirkstoffe geeignet ist. Außerdem wird die Wirtschaftlichkeit optimiert, da lediglich eine dünne PLGA- oder PLA-Ummantelung zur Freisetzungsverzögerung benötigt wird, wohingegen der Kern auf den Materialien Calciumhydrogenphosphat oder Polyethylenglykol basiert. Diese sind ebenfalls biokompatibel, gleichzeitig aber deutlich günstiger. Hinzu kommt, dass potentielle Wirkstoffinstabilitäten, die durch das saure Mikroklima aufgrund des PLGA-Abbaus hervorgerufen werden können, wegfallen.

Um die Quellfähigkeit und die Wirkstofffreisetzung am Wirkort abschätzen zu können, wurden Implantate *in vitro* in einem Reagenzglas in Pufferlösung und *ex vivo* in Fettgewebe vom Schwein untersucht. Bei *ex vivo* Untersuchungen wurden Implantate mit einer höheren Quellfähigkeit durch das umgebende Gewebe stärker im Quellvorgang beschränkt, sodass sie eine beträchtlich verringerte Wirkstofffreisetzung, Wasseraufnahme und Quellung aufwiesen. Aus diesem Grund wurde der Druck, der durch das Fettgewebe auf die Implantate bei deren Quellung ausgeübt wird, simuliert. Ein Hydrogel-Material wurde ausgewählt, um die Quellung der Implantate und die Wirkstofffreisetzung über einen langen Zeitraum vorherzusagen. Aus einer Versuchsreihe mit verschiedenen Konzentrationen wies vier-prozentiges Agarosegel ein vergleichbares mechanisches Verhalten wie Fettgewebe vom Schwein auf. Es könnte daher als Gewebeimitationsmaterial für besser korrelierende *in vitro* Freisetzungstests verwendet werden.

Insgesamt lieferte die Studie einen Lösungsansatz für die Reduzierung des initialen Bursts von leicht löslichen Wirkstoffen aus PLGA-Schmelzextrudaten durch die Variation der Herstellungs- und Formulierungsparameter. Die Studie zeigte auch das Potenzial einer dünnen, aufgesprühten PLGA-Ummantelung, um die Freisetzung aus bioabbaubaren Reservoirsystemen mit hoher Wirkstoffbeladung zu kontrollieren für Wirkstoffe mit unterschiedlicher Löslichkeit. Freisetzungskinetiken nullter Ordnung und Freisetzungsprofile pulsatiler Art konnten erfolgreich entwickelt werden.

CHAPTER

6 References

A

Aharoni, S.M., 1998. Increased Glass Transition Temperature in Motionally Constrained Semicrystalline Polymers; Polymers for Advanced Technologies 9, 169-201 (1998)

Ahmet, A.R. and Bodmeier, R., 2009. Preparation of preformed porous PLGA microparticles and antisense oligonucleotides loading; European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 264-270

Ahmet, A.R., Elkharraz, K., Irfan, M. and Bodmeier, R., 2012. Reduction in burst release after coating poly(D,L-lactide-coglycolide) (PLGA) microparticles with a drug-free PLGA layer; Pharmaceutical Development and Technology, 2012; 17(1):66–72

Alexis, F., 2005. Factors affecting the degradation and drug-release mechanism of poly(lacticacid) and poly[(lactic acid)-co-(glycolic acid)]; Polym Int 54:36-46 (2005) DOI: 10.1002/pi.1697

Allison, S.D., 2008. Analysis of initial burst in PLGA microparticles; Expert Opinion on Drug Delivery (2008) 5(6):615-628

B

Berchane, N.S., Carson, K.H., Rice-Ficht, A.C. and Andrews, M.J., 2007. Effect of mean diameter and polydispersity of PLG microspheres on drug release:

Experiment and theory; International Journal of Pharmaceutics 337 (2007) 118-126

Berkland, C., Kim, K.K. and Pack, D.W., 2003. PLG microsphere size controls drug release rate through several competing factors; Pharmaceutical Research, Vol. 20, No. 7, July 2003

Bodmeier, R. and Chen, H., 1989. Evaluation of Biodegradable Poly(lactide) Pellets Prepared by Direct Compression; Journal of Pharmaceutical Sciences, Vol. 78, No. 10, October 1989

Bodmeier, R., Oh, K. and Chen, H., 1989. The effect of the addition of low molecular weight poly(dl-lactide) on drug release from biodegradable poly(dl-lactide) drug delivery systems; International Journal of Pharmaceutics, 51 (1989) 1-8

Bodmeier, R., Kissel, T. and Traechslin, E., 1992. Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems; Journal of Controlled Release, 21 (1992) 129-138

Bouissou, C., Rouse, J.J., Price, R. and van der Walle, C.F. 2006. The Influence of Surfactant on PLGA Microsphere Glass Transition and Water Sorption: Remodeling the Surface Morphology to Attenuate the Burst Release; Pharmaceutical Research, Vol. 23, No. 6, June 2006

Bussemer, T., Dashevsky, A. and Bodmeier, R., 2003. A pulsatile drug delivery system based on rupturable coated hard gelatin capsules, Journal of Controlled Release, 93 (2003) 331-339

Breitenbach, J., 2002. Melt extrusion: from process to drug delivery technology; European Journal of Pharmaceutics and Biopharmaceutics, 54 (2002) 107-117

Brostow, W., Chiu, R., Kalogeras, I.M. and Vassilikou-Dova, A., 2008. Prediction of glass transition temperatures: Binary blends and copolymers; Materials Letters 62 (2008) 3152-3155

C

Chokshi, R.J., Sandhu, H.K., Iyer, R.M., Shah, N.H., Malick, A.W. and Zia, H., 2005.

Characterization of Physico-Mechanical Properties of Indomethacin and Polymers to Assess their Suitability for Hot-Melt Extrusion Process as a Means to Manufacture Solid Dispersion/Solution; Journal of Pharmaceutical Sciences, Vol. 94, No. 11, November 2005

Crowley, M.M., Fredersdorf, A., Schroeder, B., Kucera, S., Prodduturi, S., Repka, M.A. and McGinity, J.W., 2004. The influence of guaifenesin and ketoprofen on the properties of hot-melt extruded polyethylene oxide films; European Journal of Pharmaceutical Sciences 22 (2004) 409-418

Crowley, M.M., Zhang, F., Repka, M.A., Thumma, S., Upadhye, S.B., Battu, S.K., McGinity, J.W. and Martin, C., 2007. Pharmaceutical Applications of Hot-Melt Extrusion: Part I; Drug Development and Industrial Pharmacy, 33:909-926, 2007

D

Danhier, F., Ansorena, E., Silva, J.M., Coco, R., Breton, A.L. and Préat, V., 2012. PLGA-based nanoparticles: An overview of biomedical applications; Journal of Controlled Release 161 (2012) 505-522

Delplace, C., Kreye, F. Klose, D. Danède, F., Descamps, M., Siepmann, J. and Siepmann, F., 2012. Impact of the experimental conditions on drug release from parenteral depot systems: From negligible to significant; International Journal of Pharmaceutics 432 (2012) 11-22

Desai, K.G.H., Mallery, S.R. and Schwendeman, S.P., 2008a. Formulation and Characterization of Injectable Poly(DL-lactide-co-glycolide) Implants Loaded with N-Acetylcysteine, a MMP Inhibitor; Pharmaceutical Research, Vol. 25, No. 3, March 2008

Desai, K.G.H., Mallery, S.R. and Schwendeman, S.P., 2008b. Effect of formulation parameters on 2-methoxyestradiol release from injectable cylindrical poly(DL-lactide-co-glycolide) implants; European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 187-198

Ding, Q., Ainsworth, P., Plunkett, A., Tucker, G. and Marson, H., 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based

expanded snacks; Journal of Food Engineering 73 (2006) 142–148

Dong, W.Y., Körber, M., López Esguerra, V. and Bodmeier, R., 2006. Stability of poly(D,L-lactide-co-glycolide) and leuprolide acetate in in-situ forming drug delivery systems; Journal of Controlled Release 115 (2006) 158-167

Dorta, M.J., Santoveña, A., Llabré M. and Fariña, J.B., 2002. Potential applications of PLGA film-implants in modulating in vitro drug release; International Journal of Pharmaceutics 248 (2002) 149-156

E

Edlund, U. and Albertsson, A.C., 2002. Degradable polymer microspheres for controlled drug delivery, Advances in Polymer Science, Vol. 157 (2002) 67-112

Erbetta, C.D.C., Alves, R.J., Resende, J.M., Freitas, R.F. and Sousa, R.G., 2012. Synthesis and Characterization of Poly(D,L-Lactide-co-Glycolide) Copolymer; Journal of Biomaterials and Nanobiotechnology, 2012, 3, 208-225

Ehtezazi, T., Washington, C. and Melia, C.D., 2000. First order release rate from porous PLA microspheres with limited exit holes on the exterior surface; Journal of Controlled Release 66 (2000) 27-38

F

Feng, L., Qi, X.R., Zhou, X.J., Maitani, Y., Wang, S.C., Jiang, Y. and Nagai, T., 2006. Pharmaceutical and immunological evaluation of a single-dose hepatitis B vaccine using PLGA microspheres; Journal of Controlled Release 112 (2006) 35–42

Forster, A., Hempenstall, J., Tucker, I. and Rades, T., 2001a. Selection of excipients for melt extrusion with two poorly water-soluble drugs by solubility parameter calculation and thermal analysis; International Journal of Pharmaceutics 226 (2001) 147-161

Forster, A., Hempenstall, J., Tucker, I. and Rades, T., 2001b. The Potential of Small-Scale Fusion Experiments and the Gordon-Taylor Equation to Predict the Suitability of Drug/Polymer Blends for Melt Extrusion; Drug Development and Industrial Pharmacy, 27(6), 549-560 (2001)

Fredenberg, S., Wahlgren, M., Reslow, M. and Axelsson, A., 2011. The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems-A review; International Journal of Pharmaceutics 415 (2011) 34-52

Fu, K., Pack, D.W., Klibanov, A.M. and Langer, R., 2000. Visual evidence of acidic environment within degrading Poly(lactic-co-glycolic acid) (PLGA) microspheres; Pharmaceutical Research, Vol. 17, No. 1, 2000

G

Ganvir, V., Gautham, B.P., Polc, H., Bhamlad, M.S., Sclesi, L., Thaokar, R., Lele, A. and Mackley, M., 2011. Extrudate swell of linear and branched polyethylenes: ALE simulations and comparison with experiments; J. Non-Newtonian Fluid Mech. 166 (2011) 12-24

García J.T., Dorta, M.J., Munguía, O., Llabrés M. and Fariña, J.B., 2002. Biodegradable laminar implants for sustained release of recombinant human growth hormone; Biomaterials 23 (2002) 4759-4764

Geerligs, M., Peters, G.W.M., Ackermans, P.A.J., Oomens, C.W.J. and Baaijens, F.P.T., 2008. Linear viscoelastic behavior of subcutaneous adipose tissue; Biorheology 45 (2008) 677-688

Geiges, G., Schapperer, E., Thyroff-Friesinger, U., Engert, Z.V. and Gravel P., 2013. Clinical development of two innovative pharmaceutical forms of leuprorelin acetate; Therapeutic Advances in Urology (2013) 5(1) 3-10

Gentile, P., Chiono, V., Carmagnola, I. and Hatton, P.V., 2014. An Overview of Poly(lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering; International Journal of Molecular Sciences 2014, 15, 3640-3659; doi:10.3390/ijms15033640

Ghalanbor, Z., Körber, M. and Bodmeier, R., 2010. Improved Lysozyme Stability and Release Properties of Poly(lactide-co-glycolide) Implants Prepared by Hot-Melt Extrusion; Pharmaceutical Research, Vol. 27, No. 2, February 2010

Ghalanbor, Z., Körber, M. and Bodmeier, R., 2012. Protein release from poly(lactide-co-glycolide) implants prepared by hot-melt extrusion: Thioester formation as a reason for incomplete release; International Journal of Pharmaceutics 438 (2012) 302–306

Ghalanbor, Z., Körber, M. and Bodmeier, R., 2013. Interdependency of protein-release completeness and polymer degradation in PLGA-based implants; European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 624-630

Giovagnoli, S., et al., 2008. Physicochemical Characterization and Release Mechanism of a Novel Prednisone Biodegradable Microsphere Formulation; Journal of Pharmaceutical Sciences, Vol. 97, No. 1, January 2008

Gibson, J.W, Tipton, A.J., Holl, R. and Meador S., 2002. Zero-order prolonged release coaxial implants; Pub. No.: US20030007992 A1, US Patent Appl. No.: US 10/177,997

Gombotz, W.R. and Pettit, D.K., 1995. Biodegradable polymers for protein and peptide drug delivery; Bioconjugate Chemistry 6 (1995) 332-351

Gosau, M. and Müller, B.W., 2010. Release of gentamicin sulphate from biodegradable PLGA-implants produced by hot melt extrusion; Pharmazie 65: 487-492 (2010)

Göpferich, A., 1996. Mechanisms of polymer degradation and erosion; Biomoterials 17 (1996) 103-114

Göpferich, A., 1997. Polymer bulk erosion; Macromolecules 1997, 30, 2598-2604

Göpferich, A., 1997. Bioerodible implants with programmable drug release; Journal of Controlled Release 44 (1997) 271-281

Göpferich, A., 2000. Implants with phased release of medicaments; Pub. No.: US6086908 A, US Patent Appl. No.: US 09/142,227

Görtz, H.H., Klimesch, R., Lämmerhirt, K., Lang, S., Sanner, A. and Spengler, R., 1992. Verfahren zur Herstellung von festen pharmazeutischen Formen, EP 0240904 B1

Guse, C., Koennings, S., Blunk, T., Siepmann, J. and Goepferich, A. 2006. Programmable implants-from pulsatile to controlled release; International Journal of

Pharmaceutics. 314 (2006) 161-169

H

Huang, X., and Brazel, C.S., 2001. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems; Journal of Controlled Release, 73 (2001) 121-136

I

Iatridis, J.C., Wu, J., Yandow, J.A. and Langevin, H.M. 2003. Subcutaneous Tissue Mechanical Behavior is Linear and Viscoelastic Under Uniaxial Tension; Connective Tissue Research, 44: 208-217, 2003

Iskakov, R.M., Kikuchi, A. and Okano T., 2002. Time-programmed pulsatile release of dextran from calciumalginate gel beads coated with carboxy-n-propylacrylamide copolymers; Journal of Controlled Release 80 (2002) 57-68

Iyer, S.S., Barr, W.H. and Karnes, H.T., 2006. Profiling In Vitro Drug Release from Subcutaneous Implants: A Review of Current Status and Potential Implications on Drug Product Development; Biopharmaceutics & Drug Disposition 27: 157-170 (2006)

J

Jain, R.A., 2000. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices; Biomaterials 21 (2000) 2475-2490

Jaraswekin, S., Prakongpan, S. and Bodmeier, R., 2007. Effect of poly(lactide-co-glycolide) molecular weight on the release of dexamethasone sodium phosphate from microparticles; Journal of Microencapsulation, March 2007; 24(2): 117-128

Jiang, H.L. and Zhu, K.J. 2000. Pulsatile protein release from a laminated device comprising polyanhydrides and pH-sensitive complexes; International Journal of Pharmaceutics, 194 (2000) 51-60.

Jimoh, A.G., Wise, D.L., Gresser, J.D. and Trantolo D.J., 1995. Pulsed FSH release from an implantable capsule system; Journal of Controlled Release 34 (1995) 87-95

Jivraj, M., Martini, L.G. and Thomson, C.M., 2000. An overview of the different excipients useful for the direct compression of tablets; Pharmaceutical Science & Technology Today Vol. 3, No. 2 February 2000

K

Kang, J. and Schwendeman, S.P., 2006. Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins; Molecular pharmaceutics, Vol. 4, No. 1, 104-118

Kang, J., Lambert, O., Ausborn, M. and Schwendeman, S.P., 2008. Stability of proteins encapsulated in injectable and biodegradable poly(lactide-co-glycolide)-glucose millicylinders; International Journal of Pharmaceutics 357 (2008) 235-243

Kastellorizios, M. and Burgess, D.J., 2012. In Vitro Drug Release Testing and In Vivo/In Vitro Correlation for Long Acting Implants and Injections in J.C. Wright and D.J. Burgess (eds.), Long Acting Injections and Implants, Advances in Delivery Science and Technology; DOI 10.1007/978-1-4614-0554-2_23, © Controlled Release Society, 2012

Khankari, R.K., Law, D. and Grant, D.J.W., 1992. Determination of water content in pharmaceutical hydrates by differential scanning calorimetry; International Journal of Pharmaceutics, 82 (1992) 117-121

Kikuchi A. and Okano T., 2002. Pulsatile drug release control using hydrogels; Advanced Drug Delivery Reviews 54 (2002) 53-77

Klose, D., Azaroual, N., Siepmann, F Vermeersch, G. and Siepmann, J., 2009. Towards More Realistic In Vitro Release Measurement Techniques for Biodegradable Microparticles; Pharmaceutical Research, Vol. 26, No. 3, March 2009 (©2008)

Klose, D., Siepmann, F., Elkharraz, K., Krenzlin, S. and Siepmann, J., 2006. How porosity and size affect the drug release mechanisms from PLGA-based microparticles; International Journal of Pharmaceutics 314 (2006) 198-206

Klose, D., Siepmann, F., Elkharraz, K. and Siepmann, J., 2008. PLGA-based drug delivery systems: Importance of the type of drug and device geometry; International Journal of Pharmaceutics 354 (2008) 95-103

Kolter, K., Karl, M., Nalawade, S. and Rottmann N., 2011. Hot-Melt Extrusion with BASF Pharma Polymers Extrusion Compendium; 2nd edition June 2011, BASF SE

Koopmans R.J., 1992. Extrudate Swell Time of High Density Polyethylene. Part II: Dependency and Effects of Cooling and Sagging; Polymer Engineering and Science, Mid-December 1992, Vol. 32, No. 23

Körber, M., 2007. In situ forming biodegradable microparticles for sustained parenteral delivery of protein drugs; PhD Thesis, Freie Universität Berlin 2007

Körber, M., 2010. PLGA Erosion: Solubility- or Diffusion-Controlled?; Pharmaceutical Research (2010) 27:2414-2420

Kranz H., Ubrich, N., Maincent, P. and Bodmeier, R., 2000. Physicomechanical Properties of Biodegradable Poly(D,L-lactide) and Poly(D,L-lactide-co-glycolide) Films in the Dry and Wet States; Journal of Pharmaceutical Sciences, Vol. 89, No. 12, December 2000

Krögel I. and Bodmeier, R., 1999. Floating or pulsatile drug delivery systems based on coated effervescent cores; International Journal of Pharmaceutics 187 (1999) 175-184

L

Liang, J.Z., 2008. Effects of extrusion conditions on die-swell behavior of polypropylene/diatomite composite melts; Polymer Testing 27 (2008) 936-940

Liu, J., Zhang, F. and McGinity, J.W., 2000. Properties of lipophilic matrix tablets containing phenylpropanolamine hydrochloride prepared by hot-melt extrusion; European Journal of Pharmaceutics and Biopharmaceutics 52 (2001) 181-190

Liu, X., Pettway, G.J., McCauley, L.K. and Ma, P.X., 2007. Pulsatile release of parathyroid hormone from an implantable delivery system; Biomaterials 28 (2007) 4124-4131

Luan, X. and Bodmeier, R., 2006. Modification of the tri-phasic drug release pattern of leuprolide acetate-loaded poly(lactide-co-glycolide) microparticles; European Journal of Pharmaceutics and Biopharmaceutics 63 (2006) 205-214

M

Makadia, H.K. and Siegel, S.J., 2011. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier; Polymers 2011, 3, 1377-1397; doi:10.3390/polym3031377

Mauriac, P. and Marion, P., 2006. Use of ethanol as plasticizer for preparing subcutaneous implants containing thermolabile active principles dispersed in a PLGA matrix; Pub. No.: US 2006/0171987 A1, US Patent Appl. No.: 10/562,707

Mäder K., Gallez, B., Liu K.J. and Swartz H.M., 1996. Noninvasive in vivo characterization of release processes in biodegradable polymers by low frequency electron paramagnetic resonance spectroscopy; Biomaterials (1996) 457-461

Medlicott, N.J. and Tucker, I.G., 1999. Pulsatile release from subcutaneous implants; Advanced Drug Delivery Reviews 38 (1999) 139-149

Mendelson, R.A., Finger, F.L. and Bagley, E.B., 1971. Die Swell and Recoverable Shear Strain in Polyethylene Extrusion; Journal of Polymer Science: Part C, NO. 35, 177-188 (1971)

Merkli, A., Tabatabay, C., Gurny, R. and Heller, J., 1998. Biodegradable Polymers for the Controlled Release of Ocular Drugs; Progress in Polymer Science., Vol. 23, 563-580, 1998

Mididoddi, P.K., Prodduturi, S. and Repka, M.A., 2006. Influence of Tartaric Acid on the Bioadhesion and Mechanical Properties of Hot-Melt Extruded Hydroxypropyl Cellulose Films for the Human Nail; Drug Development and Industrial Pharmacy, 32:1059-1066, 2006

Mididoddi, P.K. and Repka, M.A., 2007. Characterization of hot-melt extruded drug delivery systems for onychomycosis; European Journal of Pharmaceutics and Biopharmaceutics 66 (2007) 95-105

Milacic, V. and Schwendeman, S.P., 2014. Lysozyme Release and Polymer Erosion Behavior of Injectable Implants Prepared from PLGA-PEG Block Copolymers and PLGA/PLGA-PEG Blends; Pharmaceutical Research (2014) 31:436-448

Mishra, N. et al., 2008. Biodegradable polymer based particulate carrier(s) for the delivery of proteins and peptides. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 7(4), pp.240-251

Mollan, M., 2003. Historical Overview, Ghebre-Sellassie, I. and Martin C. (Eds.), Pharmaceutical extrusion technology, (Volume 133, pp. 1-18), New York, Marcel Dekker, Inc.

Moore, J.W. and Flanner, H.H., 1996. Mathematical comparison of dissolution profiles; Pharmaceutical Technology, vol. 20, pp. 64–74, 1996

Münstedt, H. and Schwarzl, F.R., 2014. Deformation and Flow of Polymeric Materials, DOI 10.1007/978-3-642-55409-4_11, © Springer-Verlag Berlin Heidelberg 2014

N

Negrín, C.M., Delgado, A., Llabres, M. and Evora, C., 2004. Methadone implants for methadone maintenance treatment. In vitro and in vivo animal studies; Journal of Controlled Release 95 (2004) 413-421

Ndindayino, F., Henrist, D., Kiekens, F., Van den Mooter, G., Vervaet, C., and Remon, J.P., 2002a. Direct compression properties of melt-extruded isomalt; International Journal of Pharmaceutics 235 (2002) 149-157

Ndindayino, F., Vervaet, C., Van den Mooter, G. and Remon, J.P., 2002b. Direct compression and moulding properties of co-extruded isomalt/drug mixtures; International Journal of Pharmaceutics 235 (2002) 159-168

O

Onishi, H., Takahashi, M. and Machida, Y. 2005. PLGA Implant Tablet of Ketoprofen: Comparison of in Vitro and in Vivo Releases; Biological and Pharmaceutical Bulletin 28(10) 2011-2015 (2005)

P

Park T.G., 1994. Degradation of poly(D,L-lactic acid) microspheres: effect of molecular weight; Journal of Controlled Release 30 (1994) 161-173

Patel, P.N., Smith, C.K. and Patrick, C.W., 2005. Rheological and recovery properties of poly(ethyleneglycol) diacrylate hydrogels and human adipose tissue; © 2005 Wiley Periodicals, Inc.

Patel, R.B., Solorio, L., Wu, H., Krupka, T. and Exner, A.A., 2010. Effect of injection site on in situ implant formation and drug release in vivo; Journal of Controlled Release 147(3) (2010) 350-358

Perez, C., Castellanos, I.J., Costantino, H.R., Al-Azzam W. and Griebenow K., 2002. Recent trends in stabilizing protein structure upon encapsulation and release from bioerodible polymers; Journal of Pharmacy and Pharmacology, 54: 301-313, 2002

Pitt, C.G., Gratzl, M.M., Kimmel G.L, Surles J. and Schindler, A., 1981. Aliphatic polyesters II. The degradation of poly(DL-lactide), poly(ε-caprolactone), and their copolymers in vivo; Biomaterials 1981; Vol. 2; 215-20

Q

Qian, H., Wohl, A.R., Crow, J.T., Macosko, C.W. and Hoye, T.R., 2011. A Strategy for Control of "Random" Copolymerization of Lactide and Glycolide: Application to Synthesis of PEG-b-PLGA Block Polymers Having Narrow Dispersity; Macromolecules 44(18): 7132-7140

Qian, F., Szymanski, A. and Gao, J., 2001. Fabrication and characterization of controlled release poly(D,L-lactide-co-glycolide) millirods; © 2001 John Wiley & Sons, Inc. Journal of Biomedical Materials Research 55: 512-522, 2001

Quintavalle, U., Voinovich, D., Perissutti, B., Serdoz, F., Grassi, G., Dal Col, A. and Grassi, M., 2008. Preparation of sustained release co-extrudates by hot-melt extrusion and mathematical modelling of in vitro/in vivo drug release profiles, European Journal of Pharmaceutical Sciences 33 (2008) 282-293

Quinten, T., Beer, T.D., Vervaet, C. and Remon, J.P., 2009. Evaluation of injection moulding as a pharmaceutical technology to produce matrix tablets; European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 145-154

R

Raiche, A.T. and Puleo, D.A., 2006. Modulated release of bioactive protein from multilayered blended PLGA coatings; International Journal of Pharmaceutics 311 (2006) 40-49

Ravivarapu, H.B., Burton, K. and DeLuca, P.P., 2000a. Polymer and microsphere blending to alter the release of a peptide from PLGA microspheres; European Journal of Pharmaceutics and Biopharmaceutics 50 (2000) 263-270

Ravivarapu, H.B., Lee, H. and DeLuca, P.P., 2000b. Enhancing Initial Release of Peptide from Poly(*d*,*l*-lactide-*co*-glycolide) (PLGA) Microspheres by Addition of a Porosigen and Increasing Drug Load

Rawat, A., Stippler, E., Shah, V.P. and Burgess, D.J., 2011. Validation of USP apparatus 4 method for microsphere in vitro release testing using Risperdal® Consta®; International Journal of Pharmaceutics 420 (2011) 198-205

Repka, M.A., Battu, S.K., Upadhye, S.B., Thumma, S., Crowley, M.M., Zhang, F., Martin, C. and McGinity, J.W. 2007. Pharmaceutical Applications of Hot-Melt Extrusion: Part II; Drug Development and Industrial Pharmacy, 33:1043-1057, 2007

Repka, M.A., Repka, S.L. and McGinity, J.W., 2002. Bioadhesive hot-melt extruded film for topical and mucosal adhesion applications and drug delivery and process for preparation thereof; US6375963 B1

Repka, M.A., Majumdar, S., Battu, S.K., Srirangam, R. and Upadhye, S.B., 2008. Applications of hot-melt extrusion for drug delivery; Expert Opinion on Drug Delivery (2008) 5 (12):1357-1376

Rothen-Weinhold, A., Besseghir, K., Vuaridel, E., Sublet, E., Oudry, N. and Gurny, R., 1999. Stability studies of a somatostatin analogue in biodegradable implants; International Journal of Pharmaceutics 178 (1999) 213–221

S

Samani, A., Zubovits, J. and Plewes, D., 2007. Elastic moduli of normal and pathological

human breast tissues: an inversion-technique-based investigation of 169 samples; Phys. Med. Biol. 52 (2007) 1565-1576

Samadi, N., Abbadessa, A, Di Stefano, A., van Nostrum, C.F., Vermonden, T., Rahimian, S., Teunissen, E.A., van Steenbergen, M.J., Amidi, M. and Hennink, W.E., 2013. The effect of lauryl capping group on protein release and degradation of poly(D, L-lactic-co-glycolic acid) particles; Journal of Controlled Release 172 (2013) 436-443

Santoveña, A., García, J.T., Oliva, A., Llabrés M. and Fariña, J.B., 2006. A mathematical model for interpreting in vitro rhGH release from laminar implants; International Journal of Pharmaceutics 309 (2006) 38-43

Seidlitz, A. and Weitschies, W., 2012. In-vitro dissolution methods for controlled release parenterals and their applicability to drug-eluting stent testing; Royal Pharmaceutical Society 2012 Journal of Pharmacy and Pharmacology, 64, pp. 969-985

Semmling, B., Nagel, S., Sternberg, K., Weitschies, W. and Seidlitz, A., 2013. Long-term stable hydrogels for biorelevant dissolution testing of drug-eluting stents; Journal of Pharmaceutical Technology & Drug Research

Sershen, S. and West, J., 2002. Implantable, polymeric systems for modulated drug delivery; Advanced Drug Delivery Reviews 54 (2002) 1225-1235

Shah, S.S., Cha Y. and Pitt C.G., 1992. Poly (glycolic acid-co-DL-lactic acid): diffusion or degradation controlled drug delivery?; Journal of Controlled release, 18(1992) 261-270

Shiah, J.G., Bhagat, R., Blanda, W.M., Nivaggioli, T., Peng, L., Chou, D. and Weber, D.A., 2011. Ocular implant made by a double extrusion process; Patent No.: US 8,034,370 B2, Appl. No.: 11/931,954, Assignee: Allergan, Inc., Irvine, CA (US)

Shuwisitkul, D. 2011. Biodegradable implants with different drug release profiles; Inaugural-Dissertation, Freie Universität Berlin

Siegel, S. and Winey, K., 2012. Drug-containing implants and methods of use thereof; Patent No.: US 8,329,203 B2, Appl. No.: 11/195,845, Assignee: The Trustees of

the University of Pennsylvania, Philadelphia, PA (US)

Siepmann, J., Siegel, R.A. and Siepmann, F., 2012. Diffusion Controlled Drug Delivery Systems in Fundamentals and Applications of Controlled Release Drug Delivery, J. Siepmann et al. (eds.); Advances in Delivery Science and Technology, DOI 10.1007/978-1-4614-0881-9_6, © Controlled Release Society 2012

Siepmann, J., Elkharraz, K., Siepmann, F. and Klose, D., 2005. How Autocatalysis Accelerates Drug Release from PLGA-Based Microparticles: A Quantitative Treatment; Biomacromolecules 2005, 6, 2312-2319

Steiner, R., 2003. Extruder Design, Ghebre-Sellassie, I. and Martin C. (Eds.), Pharmaceutical extrusion technology. (Volume 133, pp. 19-38). New York, Marcel Dekker, Inc.

Steendam, R., van Steenbergenb, M.J., Henninkb, W.E., Frijlinka, H.W. and Lerka C.F., 2001. Effect of molecular weight and glass transition on relaxation and release behaviour of poly(DL-lactic acid) tablets; Journal of Controlled Release 70 (2001) 71-82

Stevens, R.E., Gray, V., Dorantes, A., Gold, L. and Pham, L., 2015. Scientific and Regulatory Standards for Assessing Product Performance Using the Similarity Factor, f2; The AAPS Journal, Vol. 17, No. 2, March 2015(© 2015), DOI: 10.1208/s12248-015-9723-y

Stevenson, C.L., Rhodes, C.A. and Prestrelski, S.J., 2012. Delivery of Peptides and Proteins via Long Acting Injections and Implants in J.C. Wright and D.J. Burgess (eds.), Long Acting Injections and Implants, Advances in Delivery Science and Technology; DOI 10.1007/978-1-4614-0554-2_20, © Controlled Release Society, 2012

T

Tracy, M.A., Ward, K.L., Firouzabadian, L., Wang, Y., Dong, N., Qian, R. and Zhang, Y., 1999. Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro; Biomaterials 20 (1999) 1057-1062

V

Vert, M., 2005. Aliphatic Polyesters: Great Degradable Polymers That Cannot Do Everything; Biomacromolecules 2005, 6, 538-546

Vert, M., Li, S. and Garreau, H., 1990. More about the degradation of LA/GA-derived matrices in aqueous media; Journal of Controlled Release, 16 (1991) 15-26

Vogelhuber, W., Rotunno, P., Magni, E., Gazzaniga, A., Spruss, T., Bernhardt, G., Bouschauer, A. and Göpferich, A., 2001. Programmable biodegradable implants; Journal of Controlled Release 73 (2001) 75-88

W

Wang, J., Wang, B.M. and Schwendeman, S.P., 2002. Characterization of the initial burst release of a model peptide from poly(D,L-lactide-co-glycolide) microspheres; Journal of Controlled Release 82 (2002) 289-307

Wang, K., 2012. Chapter 4: Die Swell of Complex Polymeric Systems, Viscoelasticity – From Theory to Biological Applications; Edited by Juan de Vicente, InTech 2012

Wang, N., Wu, X.S., Li, C. and Feng, M.F., 2000. Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers: I. Synthesis and characterization; Journal of Biomaterials Science, Polymer Edition, Vol. 11, No. 3, pp. 301-318 (2000)

Wang, X., Sui, S., Yan, Y. and Zhang, R., 2010. Design and fabrication of PLGA sandwiched cell/fibrin constructs for complex organ regeneration; Journal of Bioactive and Compatible Polymers 25, 229-240

Weiler, W. and Gogolewski, S., 1996. Enhancement of the mechanical properties of polylactides by solid-state extrusion: I. Poly(d-lactide); Biomaterials 17 (1996) 529-535

Wischke, C. and Schwendeman, S.P., 2012. Degradable Polymeric Carriers for Parenteral Controlled Drug Delivery in Fundamentals and Applications of Controlled Release Drug Delivery, J. Siepmann et al. (eds.); Advances in Delivery Science and Technology, DOI 10.1007/978-1-4614-0881-9_8, © Controlled Release Society

2012

Witt, C., Mäder, K. and Kissel T., 2000. The degradation, swelling and erosion properties of biodegradable implants prepared by extrusion or compression moulding of poly(lactide-co-glycolide) and ABA triblock copolymers; Biomaterials 21 (2000) 931-938

Wright, J.C. and Burgess, D.J., 2012. Long Acting Injections and Implants; Advances in Delivery Science and Technology, DOI 10.1007/978-1-4614-0554-2_1, © Controlled Release Society, 2012

Wuthrich, P., Ng, S.Y., Fritzinger, B.K., Roskos, K.V. and Heller, J., 1992. Pulsatile and delayed release of lysozyme from ointment-like poly (ortho esters); Journal of Controlled Release, 21(1992) 19l-200

Y

Ye, M., Kim, S. and Park, K., 2010. Issues in long-term protein delivery using biodegradable microparticles; Journal of Controlled Release 146 (2010) 241-260

Young, C.R., Koleng, J.J. and McGinity, J.W., 2002. Production of spherical pellets by a hot-melt extrusion and spheronization process; International Journal of Pharmaceutics 242 (2002) 87-92

Z

Zhang, F. and McGinity, J.W., 2000. Properties of Hot-Melt Extruded Theophylline Tablets Containing Poly(Vinyl Acetate); Drug Development and Industrial Pharmacy, 26(9), 931–942 (2000)

Zhou, T., Lewis, H., Foster, R.E. and Schwendeman, S.P., 1998. Development of a multiple-drug delivery implant for intraocular management of proliferative vitreoretinopathy; Journal of Controlled Release 55 (1998) 281-295

Zolnik, B.S., Leary P.E. and Burgess D.J., 2006. Elevated temperature accelerated release testing of PLGA microspheres; Journal of Controlled Release 112 (2006) 293-300

Zolnik, B.S., Raton, J-L. and Burgess D.J., 2005. Application of USP Apparatus 4 and In Situ Fiber Optic Analysis to Microsphere Release Testing; Dissolution Technologies, May 2005

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7 List of Publications

Teodosic, J. and Bodmeier, R., 2013. Reduction of Initial Burst Release from Hot-Melt Extruded PLGA Implants; AAPS Annual Meeting and Exposition, November 2013, San Antonio, Texas, USA

Teodosic, J. and Bodmeier, R., 2014. Biodegradable high drug loading reservoir systems with controlled prednisone release; 9th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, March-April 2014, Lisbon, Portugal

Teodosic, J. and Bodmeier, R., 2014. Poly(lactide-co-glycolide) Implants Prepared by Hot-Melt Extrusion and Spray Coating; AAPS Annual Meeting and Exposition, November 2014, San Diego, California, USA

Teodosic, J. and Bodmeier, R., 2015. Biodegradable High Drug Loaded Reservoir Systems with Pulsatile Drug Delivery; AAPS Annual Meeting and Exposition, October 2015, Orlando, Florida, USA

CHAPTER

8 Curriculum Vitae

For reasons of data protection, the Curriculum vitae is not published in the online version