Development and Characterization of Hot-melt Extruded Biodegradable Poly(lactide-co-glycolide) Implants

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Declaration of Independence

Herewith I certify that I have prepared and written my thesis independently and that I have not used any sources and aids other than those indicated by me. This dissertation has not yet been presented to any other examination authority in the same or a similar form and has not yet been published.

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1 Introduction

1.1 Biodegradable Polymers

In the past decades parenteral drug delivery became more important since most newly discovered active pharmaceutical ingredients are listed in the Biopharmaceutical Classification System (BCS) in class II or IV, which are defined as poorly soluble but good permeable drugs for class II and both poorly soluble and poorly permeable drugs for class IV [1-3]. To overcome solubility and permeability issues with BCS class II/ IV drugs at gastrointestinal administration or to modify drug release to a prolonged release, these drugs are processed into various drug delivery systems (DDS) e.g., emulsions/ self-emulsifying systems, lipid based systems, and polymeric systems [4-6]. The use of biodegradable polymers that disintegrate over time during an application eliminates the need of a surgical removal of a beforehand applied non-biodegradable DDS after treatment. Non-biodegradable polymers that are commercially used include ethylene vinyl acetate, silicone, its poly(vinyl alcohol), and polyimide [7]. Due to their ability to disintegrate, biodegradable polymers are furthermore used as degradable plates and screws in orthopedic surgery as well as scaffold material of tissue and organ reparation [8]. In general, biodegradable polymers are classified as natural or synthetic polymers. They can be degraded by either hydrolysis or enzymatically [9]. Natural biodegradable polymers include collagen, gelatin, alginate, and chitosan while synthetic polymers include aliphatic polyesters (i.e. poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone), poly(p-dioxanone), and poly(ortho esters), polyurethanes, poly(amino acids), polyanhydrides and polyphosphazenes [10-12]. Since biodegradable polymers require properties such as not to cause toxic or inflammatory reactions after application into the human body, non- toxic degradation products which can be metabolized and excreted from the body, and suitable mechanical properties for the intended application [9, 13]. Most commonly used polymers in biodegradable DDS are PLA, PGA, and PLGA [7].

1.2 PLGA-based Drug Delivery Systems

Poly(lactic-co-glycolic acid) (PLGA) is the copolymer of poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) and is commonly used in drug delivery systems (DDS) due to its biodegradability and biocompatibility [14, 15]. These polyesters are the most investigated since one of the first suture materials, developed in the 1960's, was based on poly(glycolic acid) [9] while the first DDS with PLGA compound was patented in the early 1970's [16].

PLA, PGA, and PLGA consist of a hydrolysable aliphatic backbone (Figure 1) thus polymer degradation was generally observed by a hydrolyzation mechanism [15]. Although it was found that PLGA degradation is heterogenous for larger DDS, where water penetrates into the PLGA matrix and ester bond cleavage by hydrolysis starts from the center of the DDS, PLGA matrices generally undergo bulk erosion [8, 11, 17-19]. During the degradation of PLGA, ester bonds are randomly cleaved and the copolymer is separated into oligomers and monomers (Figure 2) [17, 20]. Ester cleavage results in a higher amount of carboxylic acid end groups thus decreasing the pH of the surrounding environment and accelerating further PLGA degradation, which is known as autocatalyzed acidic degradation [8].

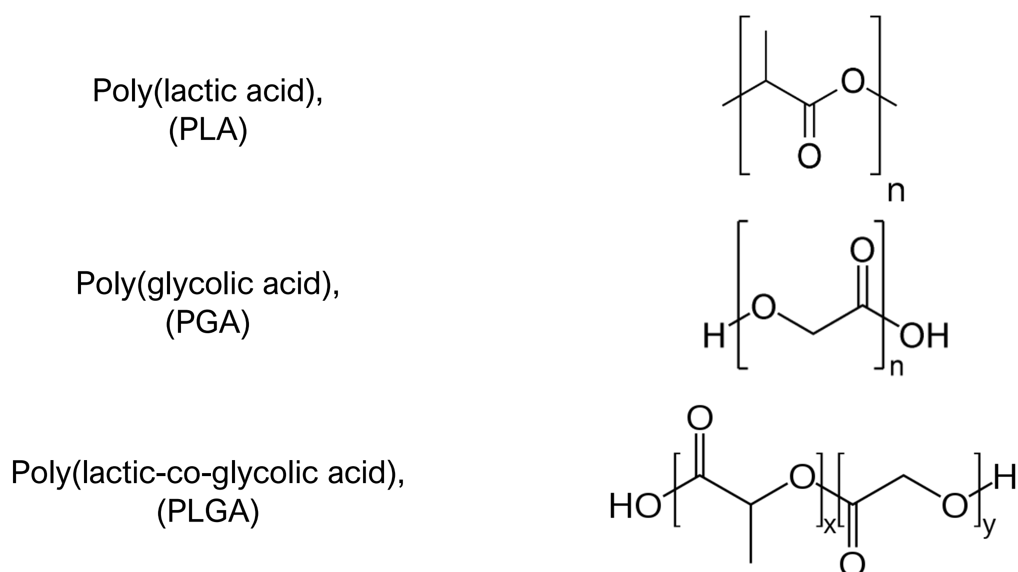

Figure 1 Structure of PLA, PGA, and their copolymer PLGA with x: number of units of lactic acids, and y: number of units of glycolic acids.

Drug release from most PLGA matrices is characterized with a triphasic release curve. A small burst phase in which the excess active pharmaceutical ingredient is released from the DDS's surface, followed by a lag phase and finally a release phase associated with the start of polymer degradation [21]. Several factors influence polymer degradation and thus the drug release from PLGA-based DDS. The main factor is the hydrophobicity of the polymer, characterized by lactic acid to glycolic acid (L:G) ratio, the polymer's average molecular weight, and designated acidic or ester end groups. Furthermore, characteristics of the incorporated drug as well as shape, porosity, and size of the PLGA-based DDS affect polymer degradation and thus drug release [17, 21, 22].

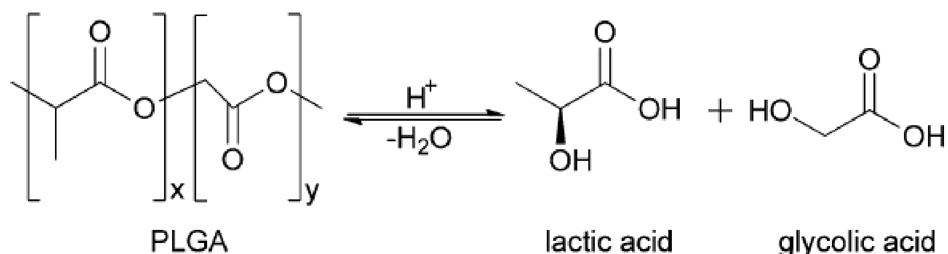

Figure 2 Hydrolysis of PLGA into PLA and PGA, adapted from [20].

To overcome poor drug solubility, amorphous solid delivery systems were introduced [23, 24]. A variety of preparation methods exist to obtain PLGA-based DDS. PLGA microparticles and nanoparticles can be prepared by solvent evaporation/ extraction processes, multiple emulsion process, phase separation, or spray drying [14, 25]. *In situ* forming DDS, such as microparticles (ISM) and implants (ISI), form after subcutaneous or intramuscular injection due to polymer precipitation and incorporation of the drug within the matrix at the site of injection after water penetration from the body tissue [26-28]. Solid biodegradable implants can be prepared using compression, compression- or injection molding, ram-extrusion, or hot-melt extrusion [14, 25, 29]. Although it is crucial to fabricate thermolabile drugs, hot-melt extrusion is still the most commonly used preparation method to obtain solid biodegradable implants since it is a single continuous process without the need for

organic solvents or surfactant additives [29-32]. Since various product patents expired in the last decade and generic products were developed, a variety of PLA- and PLGA-based DDS and medical devices are currently marketed worldwide and a selection is presented in Table 1.

Table 1 Examples of PLA/ PLGA-based DDS on the market, based on the Orange Book of the U.S. Food and Drug Administration, Rote Liste Online, and company websites [33, 34].

Product name	Drugs	DDS	Administration route	Duration of action
Bydureon BCise®, Astra Zeneca	Exenatide	Suspension/ Microparticles	Subcutaneous	1 week
Decapeptyl Gyn®, Ferring	Triptorelin acetate	Powder for suspension/ Microparticles	Subcutaneous/ intramuscular	1 month
Eligard®, Astellas	Leuprorelin acetate	Powder/ *In situ* forming implant	Subcutaneous	1 – 6 months
Enantone depot®, Takeda	Leuprorelin acetate	Powder for suspension/ Microparticles	Subcutaneous	1 – 3 months
Leuprorelin ratiopharm®, Ratiopharm	Leuprorelin	Implant	Subcutaneous	3 months
Ozurdex®, Allergan	Dexamethasone	Implant	Intravitreal	3 – 6 months
Profact®, Apogepha	Buserelin acetate	Implant	Subcutaneous	3 months
Risperdal Consta®, Janssen	Risperidone	Powder for suspension/ Microparticles	Intramuscular	2 weeks
Ziletta®, Flexion Therapeutics	Triamcinolone acetonide	Powder for suspension/ Microparticles	Intra-articular	12 weeks
Zoladex®, Astra Zeneca	Goserelin acetate	Implant	Subcutaneous	1 – 3 months

1.3 Hot-melt Extrusion

Extrusion is defined as the continuous process of pumping a deformable or even viscous material under pressure though a die resulting in a product of uniform shape. Hot-melt extrusion (HME) is the extrusion of raw material at elevated temperatures, which are over the glass transition temperature of the used polymers [32, 35]. Deriving from plastic and food industry, HME started growing attention in the 1970's for pharmaceutical applications [36, 37]. The number of patents issued and publications with preparation of drug delivery systems (DDS) using HME for pharmaceutical applications were steadily growing since various DDS could be prepared by HME (Table 2) [35, 38, 39]. One major advantage is that mixing of raw materials, melting, and processing to a final shape is combined in one continuous step which makes HME an attractive method to obtain polymeric DDS. Furthermore, HME is a solvent-free preparation method. The absence of water and most of oxygen inside the extruder, allows the processing of hydrolytically and oxidation labile drugs. Although the materials are processed at elevated temperatures, thermolabile compounds could be used due to a short residence time in the heated barrel [32]. Formulation preparation by HME allows to increase solubility and bioavailability of poorly water-soluble drugs thus making it an attractive method since over 40% of new molecular entities are poorly bioavailable and soluble [32, 35, 40].

Table 2 Examples of DDS prepared by hot-melt extrusion.

DDS	Published by
Implants	Ghalanbor et al., 2012 [30]
Transdermal/ Transmucosal	Crowley et al., 2004 [39]
Tablets	Zhang et al., 2000 [41]
Pellets	Young et al., 2002 [42]
Granules	Follonier et al., 1995 [43]

Nevertheless, disadvantages of HME might be that although raw material have a short residence time in the extruder barrel, thermolabile drugs such as proteins are complicated to process [29, 30]. Furthermore, the materials fed into the extruder through a hopper require good flow properties to assure a constant feed rate [32].

Ram extrusion

Hot-melt extrusion processes can be categorized into ram extrusion and screw-extrusion [35]. Ram extrusion is in contrast to HME with a screw-extruder one exception to what was described before since it is a discontinuous process. The mixed raw materials are fed into a cylinder and are heated during an induction time. After induction the molten or soften blend is pressed with a piston under high pressure through a die into extrudates (Figure 3). Due to the possibility of moving the piston inside the cylinder to the front of the die, a major advantage of ram extrusion is that smallest amounts of compounds can be processed with almost no loss thus high yielding [44]. The absence of shear forces thus shear heating allows processing of thermolabile compounds. Nevertheless, since ram extruders do not provide internal mixing, poor temperature homogeneity within

the extrusion barrel as well as the extrudates, ultimately resulting in lower homogeneity of extrudates, is a major disadvantage [35].

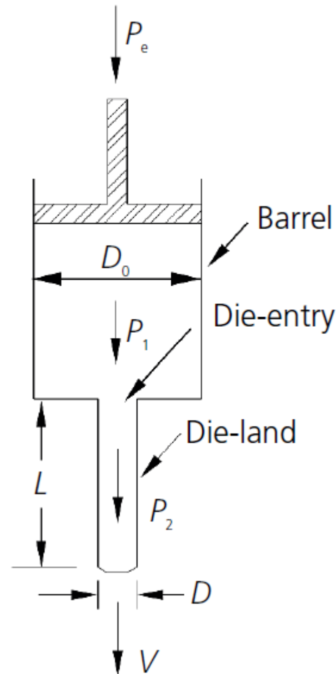

Figure 3 Schematic setup of ram extrusion, adapted from [45].

For the preparation of poly(lactide-co-glycolide) (PLGA) implants Ghalanbor et al. introduced a small lab-scale ram extrusion device consisting of a polypropylene syringe, functioning as the extruders cylinder, fixed in a self-made die (Figure 4) [29]. Small amounts of 0.5 – 1.0 g drug polymer blends are filled into the syringe, the whole device is then heated in an oven at desired temperatures for a given time, and the soften blend is then pressed with the piston through the die to obtain cylindrical extrudates.

Figure 4 Syringe- die device as a lab- scale ram extrusion introduced by Ghalanbor et al. (left) [29].

Screw extrusion

The most commonly and commercially used HME extruders in plastic-, food-, and pharmaceutical industry are screw extruders since the continuous process allows a high throughput and an up-scaling from formulation development to production can easily be done [32, 39, 40]. Screw extruders for HME are generally classified as single-screw extruders and multiple-screw extruders, while the focus on multiple-screw extruders is on twin-screw extruders.

While the setup of the extruders differs in the number of screws used for HME, the principle is the same for all types. Mixtures of active pharmaceutical ingredient and matrix former are introduced to the system through a feed hopper. Rotating screw(s), powered by an electrical and adjustable motor, transport the blend through the extruder barrel which can be heated to elevated temperatures. The soften blend is ultimately pressed through an outlet to obtain extrudates of a desired shape (Figure 5). Additionally, various monitoring sensors can be applied to the system to control process parameters such as torque, pressure, melt viscosity as well as screw speed and the barrel’s zone temperatures, which are crucial for the HME process [46].

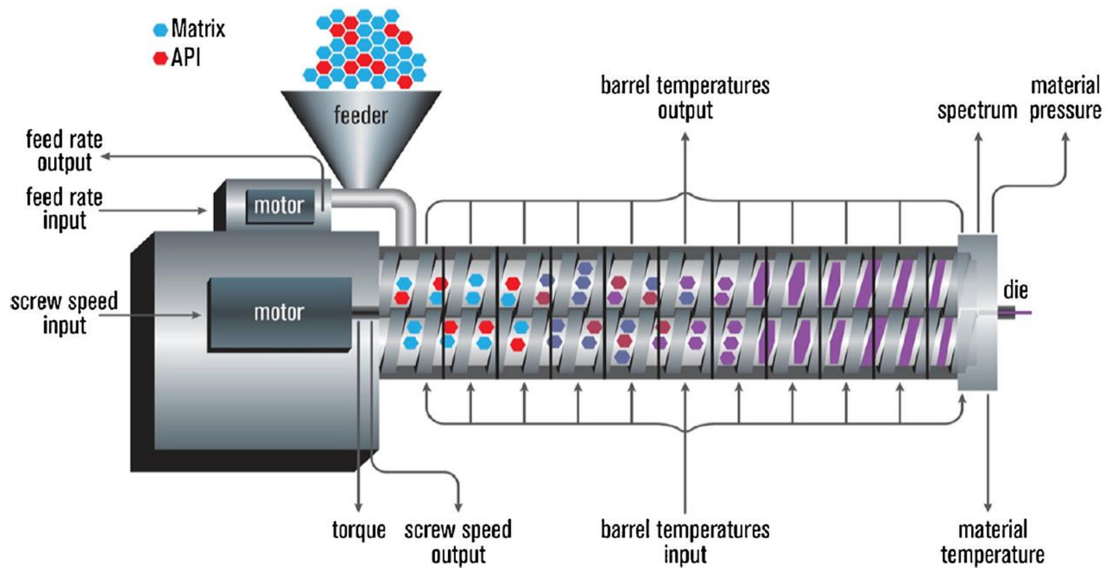

Figure 5 Schematic depiction of HME with a twin-screw extruder, adapted from [32].

Single-screw extruder

Since single-screw extruders provide simple maintenance, low investments and have been modified only slightly since their invention at the end of the 19th century, they are the most common screw-extruders used for HME [32, 35]. In general, feeding, conveying, melting, and pumping are the four main functions of a single-screw extruder. After feeding of raw material into the extruder, the continuously rotating screw conveys it along the heated barrel. The molten blend is then pressurized at the end of the barrel while pressed through the die into a desired shape (Figure 6). Output rates of single-screw extruders are mainly determined by the rotation speed of the screw when not performed with a mass flow regulating feeder. Due to their low costs and maintenance, single-screw extruders are favorable at productions with HME [32, 35].

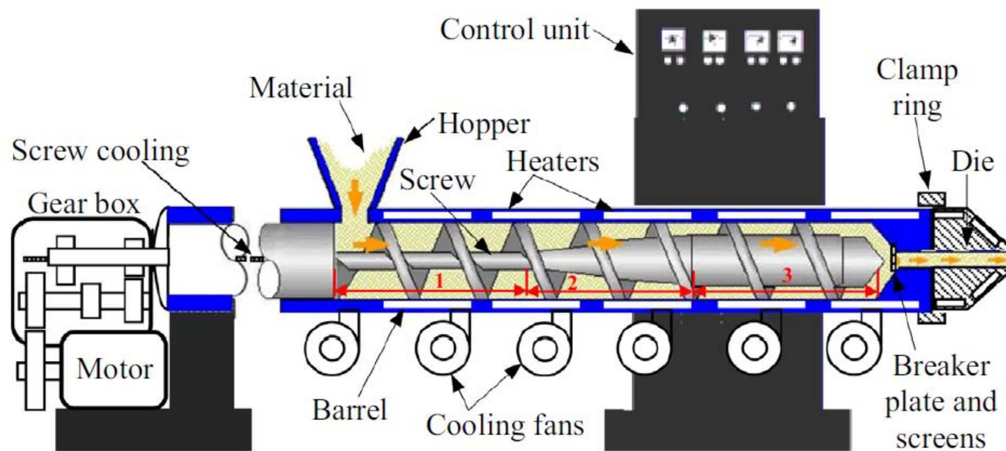

Figure 6 Schematic setup of a single-screw extruder with (1) solids conveying, (2) melting, and (3) melt conveying, adapted from [47].

Twin-screw extruder

In general, screw-extruders (Figure 7) decrease melt viscosity and provide good mixing of blends during HME due to higher shear forces from the rotating screws thus processing raw materials into more uniform and homogeneous extrudates compared to ram extruders [48]. In contrast to single-screw extruders, twin-screw extruders provide a higher mixing and kneading of raw materials while conveying inside the barrel is faster thus decreasing residence time of blends [32, 35]. In a twin-screw extruder two rotating screws are side by side and can be operated either in a co-rotating way, where both screws rotate in the same direction, or vice versa: counter-rotating. The latter method is only used when high shear forces are required for an HME process [49]. Since the counter-rotating screws convey the material by pressing it through the gap between the screws high pressure development during HME is inevitable thus limiting the screw speed and decreasing the output rate compared to co-rotating systems. Furthermore, the potential of incorporating air into the extrudate is higher than with co-rotating extruders [35].

Figure 7 Example of a twin-screw extruder, Haake MiniLab Compounder (Thermo Scientific, Karlsruhe, Germany), adapted from [50].

Screw designs of counter- and co-rotating extruders can either be intermeshing thus providing a self-cleaning mechanism and preventing material overheating or non-intermeshing [32, 35, 40]. Since non-intermeshing screw designs are only utilized when high viscous materials are processed or large volumes of volatile compounds have to be removed (through a separate opening), most co-rotating extruders are operated with intermeshing screws [35]. To overcome certain mixing-, dispersing-, and conveying issues and to adapt various formulations, most twin-screw extruders are equipped with modular screws, allowing to change screw designs individually to meet desired requirements [40]. Screw parts can therefore be interchangeable and designed to have either a higher mixing- and kneading- or higher conveying effect (Figure 8).

Figure 8 Kneading- and conveying elements of modular extruder screws, picture adapted from [51].

Overall HME with screw-extruders is a continuous process and with all its advantages applicable to the development and production of various pharmaceutical applications such as implants, transdermal and transmucosal drug delivery systems, tablets, pellets, and granules. Nevertheless, due to the high energy input required and the limitations of processing thermostable compounds, only a few products prepared by HME can be found on the market (i.e., Zoladex®, Astra Zeneca, and Ozurdex®, Allergan).

1.4 Drug Release Testing of Parenteral Dosage Forms

In the formation development and quality control of parenteral dosage forms drug release testing is an important tool to ensure an intended drug release profile [52-54]. Drug release testing of controlled/ extended release parenteral dosage forms is not standardized until today, since current apparatus for release testing of regulatory authorities, such as the United States Pharmacopeia (USP), European Pharmacopeia (EP), and Japanese Pharmacopeia (JP), are designed for transdermal and oral dosage forms [54-56]. Although it is found that a reciprocating cylinder apparatus as well as a flow-through cell, according to USP apparatus 3 and 4, are generally the best choice to test drug release from parenteral drug delivery systems (DDS), modifications to them are essential due to specific product requirements and purpose of the test (Figure 9) [52, 54].

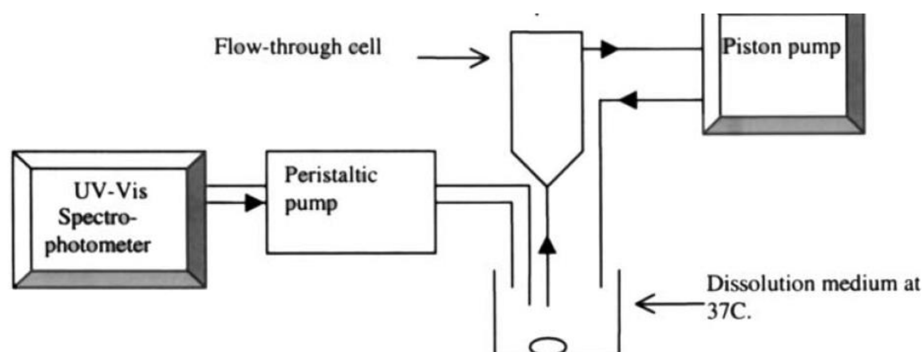

Figure 9 Schematic setup of the USP apparatus 4: flow-through cell, adapted from [57].

Most drug release tests for polymeric implants and microparticulate DDS are performed with the sample-and-separate-method, where the DDS are placed in vials containing release medium and samples are taken at determined time points [58]. In general, it is sought to develop a release test method simulating *in vivo* conditions. Hereby, release medium composition (i.e., pH, buffer capacity, and

osmolality) and temperature have to be considered [59, 60]. Furthermore, due to the long-term release tests of extended release DDS, microbial contamination could be compensated with the addition of a standard preservative that should neither interact with any formulation compound nor the pH of the release medium [52]. In the quality control of parenteral dosage forms real-time release tests can last up to weeks (e.g. different poly(lactic-co-glycolic acid) (PLGA) grades) or several months (e.g. poly(lactic acid) (PLA) based DDS), depending on the properties of formulation compounds [61]. It is therefore often required to accelerate the tests to study drug release from these dosage forms in a shorter time without the loss of information. This could generally be achieved by changing parameters such as temperature, solvent, medium pH, enzymes, surfactants, and agitation rate [58, 60, 62].

Enzymes

Although the enzymatic degradation of PLGA is discussed, the addition of enzymes in a release test thereof is only used for specific DDS e.g. porous PLGA foams [63]. Since drug release from PLGA implants and microparticles is mostly depending on polymer erosion and the polymer degradation is driven by the hydrolytic cleavage of the aliphatic backbone, the use of enzymes in the medium during release tests might not influence the drug release. In the USP general chapter <711> “dissolution”, the use of pepsin or pancreatin is only recommended for gelatin capsules and gelatin coated tablets to overcome crosslinking and avoid the failure of non-release from the formulation [64, 65].

Surfactants and other additives

For the release tests of water insoluble drugs many approaches for testing were investigated. To maintain sink conditions, complete exchange of the release medium or large volumes thereof were the general methods, but a large amount of buffer is required for the measurement [66, 67]. Another approach is the increase of solubility by using hydroalcoholic release media which dissolves the drug during the release test but lacks the relevance according to physiological environment [68-70]. The most common way for the release tests of water insoluble drug compounds is the addition of surfactants to the release medium. Above the critical micelle concentration, surfactants accelerate drug release by wetting, deflocculation and micellar solubilization [71, 72]. In the case of extended-release parenteral dosage forms e.g., lipid implants, surfactants can accelerate drug release by wetting, penetration of buffer and solubility increase of the active pharmaceutical ingredient [73, 74]. Furthermore, surfactants can stabilize release test systems with PLGA microparticles by preventing agglomeration [75, 76]. The addition of ethanol or acetonitrile can further accelerate the release by increasing porosity of the PLGA device [58, 77, 78].

pH of release medium

Acidic and basic pH of the release medium can affect the drug release from biodegradable implants and multiparticulate DDS by accelerating the non-enzymatic hydrolysis of PLGA backbone of the system [58, 62]. While basic pH of the release medium may result in a surface erosion of PLGA implants and microparticles, under acidic pH the degradation of PLGA follows bulk erosion [79-81].

Temperature

The use of elevated temperatures at release tests is a common practice to accelerate drug release and has already been studied for PLGA based dosage forms [60, 75, 82]. An increase in molecular mobility at higher temperatures results in an accelerated drug release by diffusion, especially over the glass transition temperature (T_g) [58]. Additionally, an increased polymer hydration and thus degradation could be observed [60]. In general, release tests could be shortened with good correlation to real-time release studies by using elevated temperatures, but it was recommended to use temperatures under the polymer´s T_g [83]. To determine the possibility of transferability from accelerated drug release at elevated temperatures to real-time release and vice versa, the Arrhenius equation is used (Equation 1) [84, 85].

$$k = A * e^{-E/RT} \qquad \text{Equation 1}$$

Where *k* is the release constant of a zero-order release kinetic, *A* is a constant, E is the activation energy, R the gas constant and T the absolute temperature. Release constants can be calculated using drug release data. The Arrhenius plot of the natural logarithm of k versus 1/T results in a negative linear correlation (Equation 2).

$$\ln(k) = -\frac{E}{R} * \frac{1}{T} + \ln(A) \qquad \text{Equation 2}$$

Prediction of real-time release

For the prediction of real-time release from accelerated drug release studies, the use of the mathematical model of Weibull function was recommended and already used [52, 58, 86]. It was considered the most powerful mathematical model to

describe release kinetics from extended-release dosage forms e.g., PLGA-based microparticles [87]. In addition, a variety of mathematical models for describing drug release kinetics for different dosage forms have been established e.g., the Higuchi model, the Hopfenberg model, the Cooney model, and the Baker model to name a few [88, 89]. They are categorized in empirical/ semi-empirical mathematical models and mechanistic realistic theories based on Fick´s second law of diffusion, including the consideration of polymer swelling, drug release, and/ or polymer degradation [90]. Complexity of those models however imparts their use since various mathematical equation requirements have to be met and it is not always possible to screen a formulation in the development of a DDS with a desired drug release profile. In formulation development it is often easier to establish a simple applicability map to estimate drug release from different polymer matrices [91].

1.5 Sterilization of pharmaceuticals

Sterilization is the method of making a product sterile, which is defined as an environment entirely free of microbes and/ or the capability of reproduction thereof [92]. It is used for parenteral drug delivery systems, ophthalmic dosage forms as well as external and internal medical devices [93-95]. Divided into three categories, sterilization methods are physical alteration, physical removal (e.g., filtration) and inactivation (e.g., irradiation) (Figure 10) [92, 96].

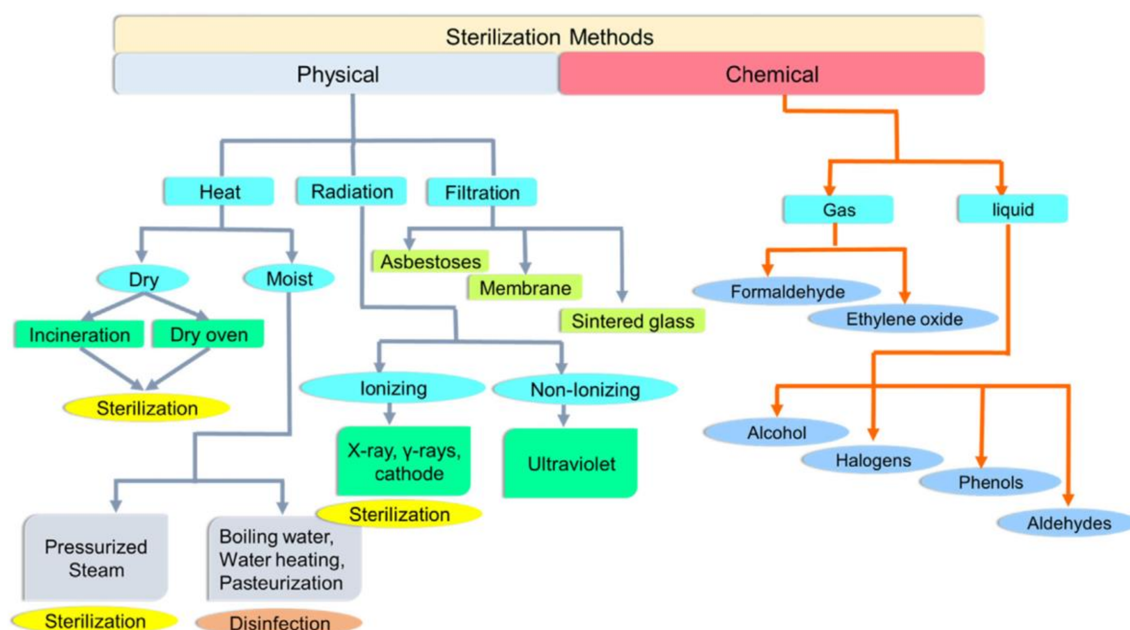

Figure 10 Categorization of sterilization methods, adapted from [92].

Heat sterilization

Dry heat sterilization is commonly used for heat-tolerant items i.e., glassware and syringes, since the method requires relatively high temperatures compared to other sterilization techniques (Table 3). It can be processed with a hot air oven, a conveyer oven, conducted heat or dry heat in a vacuum [97]. On the one hand, acquisition costs are low and dry heat is essential for condensate or water removal, but on the other hand, higher temperatures and exposure times limit

thermally sensitive materials and a complete heat-penetration to all device parts have to be ensured to guarantee sterilization effectiveness [98, 99].

Moist heat sterilization by an autoclave process is the exposure of the products to high temperatures in a saturated steam environment under pressure, where all product parts have to reach at least 121 °C after 15 min – 30 min [100]. It is one of the most reliable sterilization methods since heat transfer from the pressurized steam is more efficient than superheat or dry heat [101]. During the process, products are exposed to sterilization cycles comprising of heating, sterilization, and cooling phases (Figure 11). Under these conditions, structural and metabolic parts of microbes are destroyed e.g., denaturation of proteins and destruction of endotoxins, and are the main factors for an efficient sterilization [100].

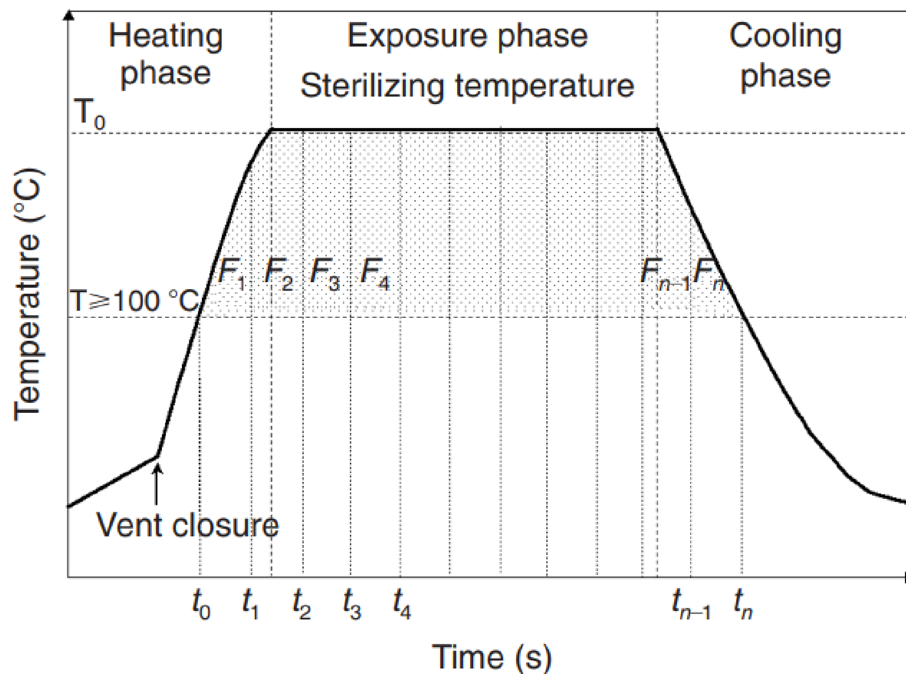

Figure 11 Example of a sterilization cycle in an autoclave, adapted from [101].

Chemical sterilization

The two most common chemical sterilization methods are the use of gaseous ethylene oxide at concentrations of 800 – 1200 mg/L and a temperature of 45 – 63 °C and formaldehyde at 70 – 75 °C with concentrations of 15 – 100 mg/L (Table 3). Both can be used for thermolabile compounds but come with several disadvantages e.g., toxic and carcinogenic residues and changes in the drug release from formulations (Table 4) [102, 103].

Filtration sterilization

Filtration methods can be categorized in sieving, adsorption and trapping in the filter´s matrix and two general kind of filters, depth filters and membrane filters, are used for filtration sterilization [92]. Sterilization effect is not given by inactivation but removal of microorganisms e.g., bacteria, yeast, mold and non-live particles [104]. They can be utilized for ophthalmic formulations, biological preparations and injections but are limited to the viscosity and size of the products (Table 3, Table 4).

Table 3 Examples of sterilization conditions, adapted from [92].

Sterilization method	Type	Conditions	Uses
Heat sterilization	Hot air oven	160 °C for 120min 170 °C for 60min 180 °C for 30min	Glassware, syringes
	Autoclave	121 °C for 15min	Glass, metal, rubber materials, sutures etc.
Chemical sterilization	Ethylene dioxide	800 – 1200 mg/L, 45 – 63 °C	Glassware, metal instruments, intraocular lenses
	Formaldehyde	15 – 100 mg/L, 70 – 75 °C	Vaccines
Irradiation	Gamma rays	2.5 – 50 kGy	Disposable medical equipment
Filtration	Membrane filters	0.22 µm, 0.2 µm, 1 mm pore size	Ophthalmic products, biological preparations, injections

Radiation sterilization

The application of high energy radiation e.g., gamma- and x-ray radiations, cause the inactivation of microorganisms by forming of radicals of hydrogen and hydroxyl and damaging cellular structures e.g., DNA and RNA [92, 105, 106]. It is recommended to use this method for products in the dry state, as radiolysis of water can occur in aqueous solutions and undesirable changes can occur in the formulations exposed to it. When it comes to the sterilization of PLGA implants, gamma irradiation was found to be a reliable sterilization technique since physicochemical properties of the polymer, stability, and drug release [107].

Table 4 Advantages and disadvantages of sterilization methods for ophthalmic formulations, adapted from [102].

Sterilization method	Advantage	Disadvantage	References
Autoclaving	Low cost	Chemical degradation, structural modification	[108]
Filtration	Thermosensitive drugs can be processed	Viscosity, size	[95, 109-111]
Gamma irradiation	Viscous material, drug and adjuvant thermally sensitive, no residue, effective against bacteria, yeast and fungus	Chemical degradation, free radical, rate of drug delivery, gas formation, high cost	[108, 112]
Gaseous ethylene oxide	Low cost, drug and adjuvant thermally sensitive	Toxic residue, cascade of oxidation, chemical change	[113]
High hydrostatic pressure	Bar-resistant nanoparticles (polymeric carriers)	Modifies adsorption, physical and chemical stability	[114]
Formaldehyde	Low cost	Toxicity (truncates proteins) and carcinogens, re-dispersion	[109, 115, 116]
Gas plasma	Low temperature, non-toxic	Oxidative, aggregation	[109, 114, 117]

1.6 Mechanical Properties of PLGA implants

Breaking force and tensile strength of solid dosage forms

Solid dosage forms have to maintain mechanical integrity in terms of production, packaging, and shipping to reach the patient intact without failure. Therefore, mechanical properties are commonly tested for the development and quality control (QC) of solid dosage forms like tablets to avoid breaking, capping etc. during these steps [118, 119]. The mechanical strength of tablets is referred to as tensile strength which is usually measured by pulling a specimen directly, but since brittle materials make these tests more difficult, tensile strength of tablets is measured indirectly [120]. The diametral compression test, which is known as indirect tensile strength test was developed in 1953 by two engineers [119, 121, 122]. To determine the maximum breaking force and tensile strength, tablets are diametrically compressed between two platens and the force needed until break is recorded, subsequently converted to tensile strength by calculation [118-120, 122, 123]. The breaking force itself could be used to compare compacted powders of the same size and shape while tensile strength is needed for a general comparison independent of dimensions.

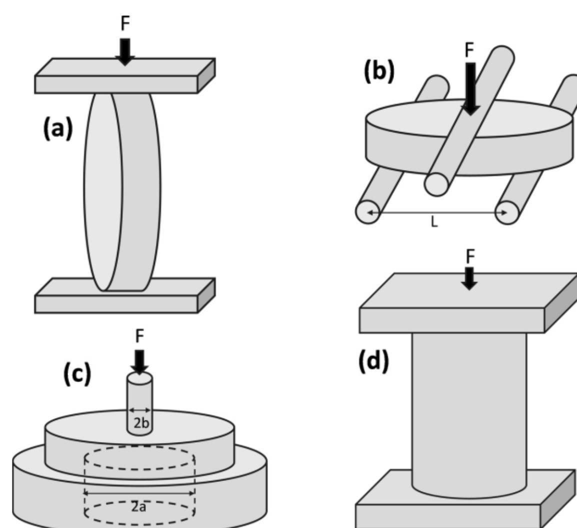

Figure 12 Exemplary setups for breaking force measurements of tablets; (A) diametral compression, (B) three-point bending test, (C) biaxial flexure test, and (D) uniaxial compressive test, adapted from [124].

Breaking force measurements

Since the mechanical strength is depending on the tablet's dimensions, first methods only apply to round, flat-faced compacts and calculations and testing methods had to be modified for the different shaped tablets. The United States Pharmacopoeia describes in monograph <1217> "Tablet Breaking Force" a second equation to calculate the tensile strength of cylindrical convex-faced tablets from breaking force measurements with diametral compression tests [125, 126]. Tensile strength calculation of elongated tablets from the breaking force using the same method was developed by Pitt et al. [119]. Due to the variety of tablets, not every shaped compact can be measured with the diametral compression test (Figure 12). 3-point bending tests as well as 4-point bending tests were recommended for the use of measuring the breaking force of capsuled shaped tablets, reviewed from Stanley et al. and also described in the USP <1217> for quality control in today's pharmaceutical industry. The majority of produced solid dosage forms are tablets and therefore best investigated in terms of mechanical integrity. Although sufficient mechanical properties of other dosage forms e.g., biodegradable implants for parenteral use, are in the same way important for quality control of manufacturing processes with regard to filling and packaging processes and in-use performance, investigations in terms of mechanical integrity can rarely be found.

Breaking force measurements of PLGA-based devices

First fracture force measurements of poly(lactic-co-glycolic acid) (PLGA) specimen could be found in the medical field of bone tissue engineering [127, 128]. Deriving from food industry, breaking force and compression measurements have gained importance in fields of mechanical integrity and quality control of medicinal products like internal fixations [129, 130]. Although metal insertions are mechanically strong and ceramics show good biocompatibility, a material which combines both characteristics would be

preferred [128]. Bone fixations made from poly(lactic)- and poly(glycolic) acids (PLA, PGA) and derivates thereof are used since they degrade over time and obviate the need of a second surgery to remove them from the patient's body (Figure 13) [10, 12]. Additionally, bones tend to fracture again after the removal of rigid bone fixations made from metal [131]. Furthermore, metallic insertions could cause disturbance in tissue growth, corrosion at the site of action leading to local irritation, a decreased heat and cold sensitivity as well as an interference with therapeutic and diagnostic radiation [132-134]. The degradation time needed for biodegradable fixations to ensure a full restoration of bone integrity can be adjusted by controlling the specimen´s PLGA, PLA, and PGA ratios and composition [135]. During this time, fixations have to maintain mechanical integrity despite degradation to relieve mechanical stress from the tissue and fulfill the intended use [136]. To test the mechanical integrity of medical PLGA devices 3-point-bending and 4-point-bending tests are commonly used as a liable measurement [132, 137].

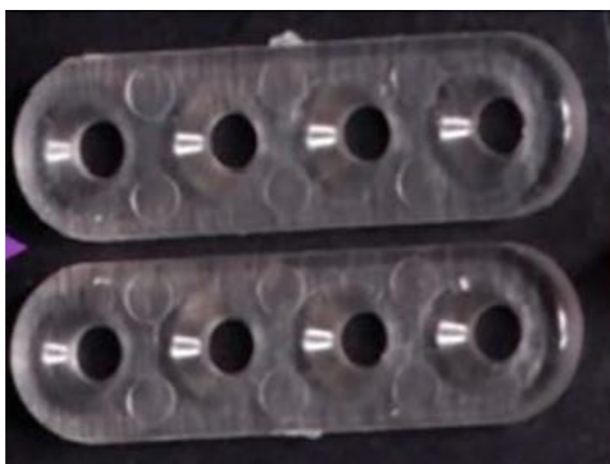

Figure 13 Bone fixations made of biodegradable PLGA, adapted from [138].

When it comes to pharmaceutical dosage forms based on PLA, PGA and PLGA, mechanical properties have only been investigated for a few. The mechanical integrity of PLGA-based films, measured using a puncture test, was found to be

highly dependent on the molecular weight and lactide content of the PLGA grade used in the formulation [139]. Only for compressed lipid implants, the mechanical properties were tested using a texture analyzer, similar to a crushing test, and found to have no relationship to drug release rates from these implants [140]. And although PLGA implants have already been tested for mechanical properties, published publications e.g. patents often lack empirical relationships and hence transferability [141]. Just like tablets, biodegradable implants have to maintain mechanical integrity during production, sterilization, packaging, and transport to arrive intact at the patient. Therefore, quality control of the mechanical properties is sufficient in the development and production of PLGA-based implants. As described before, beam shaped compacts are recommended to be measured by 3-point-bending or 4-point-bending tests to achieve accurate results [122].

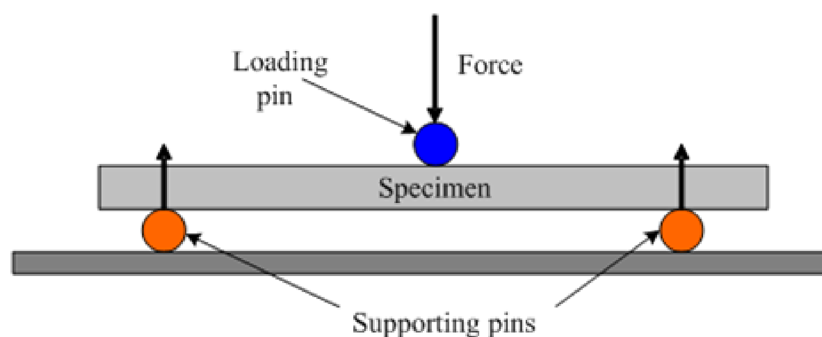

Figure 14 Setup of a three-point bending test, adapted from [142].

3-point-bending tests are commonly performed in the field of material science to obtain mechanical properties of beam shaped specimen since it is an easy-to-use method [143]. For the measurement the compact is placed on two outer supports and force is applied to the specimen's center to obtain flexural modulus and flexural strength (Figure 14) [144]. In the QC of biodegradable implants, it is necessary to observe the mechanical stability to maintain an intact product during development and processing. With a 3-point-bending test using a texture analyzer, implant's hardness (peak force at break), elongation, toughness (slope

of force-displacement curve) and energy required until break (area under the curve of force-displacement curve) can be measured. In this work, the influence of various formulation and process parameters on the mechanical properties (peak force at break, elongation, slope, and the energy) of PLGA implants prepared by hot melt extrusion was investigated using a 3-point-bending test.

1.7 Research Objectives

1. Investigation of drug release from PLGA-based implants prepared by hot-melt extrusion.
2. Establishing applicability maps of drug release from PLGA-based implants for the formulation development with optimized release curves.
3. Analytical method development of an accelerated *in vitro* drug release for PLGA-based implants.
4. Characterization of drug release influenced by preparation method, gamma irradiation and implant storage.
5. Characterization of mechanical properties of PLGA-based implants.

2 Materials and Methods

2.1 Materials

Active pharmaceutical ingredients and polymers

Brimonidine Tartrate (Carl Roth GmbH + Co KG, Karlsruhe, Germany), Dexamethasone, micronized (Fagron GmbH, Barsbüttel, Germany), Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 502 (PLGA 502), Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 502H (PLGA 502H), Poly(D,L-lactide-co-glycolide) 50:50, Resomer® RG 503H (PLGA 503H), Resomer® RG 504H (PLGA 504H), Poly(D,L-lactide-co-glycolide) 75:25, Resomer® RG 752S (PLGA 752S) (Evonik Industries AG, Essen, Germany)

Table 5 Properties of PLGA grades [145].

PLGA grade	Inherent Viscosity, dL/ g	Average molecular weight, kDa
Resomer® RG 502	0.16 – 0.24	7 – 17
Resomer® RG 502H	0.16 – 0.24	7 – 17
Resomer® RG 503H	0.32 – 0.44	24 – 38
Resomer® RG 504H	0.44 – 0.60	38 – 54
Resomer® RG 752S	0.16 – 0.24	7 – 17

Solvents and other excipients

Acetone (VWR International S.A.S., Fontenay-sous-Bois, France), Acetonitrile (VWR International S.A.S., Fontenay-sous-Bois, France), Calcium Chloride (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), Glacial Acidic Acid (Hedinger GmbH & Co. KG, Stuttgart, Germany), Methanol (VWR International S.A.S., Fontenay-sous-Bois, France), Sodium Chloride (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), Sodium Dihydrogen Phosphate Dihydrate (Merck KGaA, Darmstadt,

Germany), Sodium Hydroxide (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), deionized water (DI water, Berlin, Germany)

2.2 Methods

2.2.1 Preparation of brimonidine free base from brimonidine tartrate

In a 500 mL glass beaker an amount of 1 – 2 g brimonidine tartrate (*solubility in water:* 21.11 g/ L; *pKa:* 7.8) was dissolved in 75 – 150 mL DI water. Under stirring with a magnetic stirrer (IKA Labortechnik, Staufen im Breisgau, Germany), the solution´s pH was monitored with a pH-meter (Seven Multi, Mettler-Toledo International Inc., Columbus, USA). The starting pH of 3.5 – 3.6 was adjusted dropwise in short time intervals to pH 11 with a 1N aqueous sodium hydroxide solution ($NaOH_{aq}$; 2.0 g NaOH in 50 mL DI water) until the precipitation of brimonidine free base was complete, which was at a calculated pH 10.8 (Henderson-Hasselbalch, Equation 3). The suspension then was vacuum filtered and washed once with 0.01N $NaOH_{aq}$ and twice with DI water. With yields of 90%, the yellow precipitate was dried overnight in an oven (Heraeurus Deutschland GmbH & Co. KG, Hanau, Germany) at 40 °C, collected into a glass vial and stored in an orange gel desiccator under exclusion of light.

$$pH = pKa - \log\left(\frac{[salt]}{[free\ base]}\right) = 7.8 - \log\left(\frac{0.001}{0.999}\right) = 10.8 \qquad \text{Equation 3}$$

2.2.2 Preparation of PLGA implants by HME with syringe-die method

Hot-melt extrusion (HME) of biodegradable dexamethasone, brimonidine and combination implants was performed using a syringe-die method, comparable to a laboratory-sized ram extrusion, which was adapted from Ghalanbor et al. [29] and further developed (Figure 15). For the first, syringes were fixed on an inhouse-made die, heated in an oven (Heraeurus Deutschland GmbH & Co. KG, Hanau, Germany) at 140 °C for 5 – 15 min depending on the polymer and drug

loading, and the molten powder blend was forced through a 1.0 mm opening resulting in extrudate diameters of 0.9 – 1.1 mm. For the second, a luer lock cannula with the same diameter was equipped to the syringe, functioning as the ram extrusion die. Beforehand, 0.5 – 1.0 g blends of active pharmaceutical ingredient and PLGA were homogenized in a mortar with pestle and filled into a 1 mL luer lock syringe and compacted with the syringe´s piston. After extrusion, extrudates were collected, manually cut to implants of approximately 3 mm length and 1.0 – 1.5 mg weight and stored in a desiccator under light exclusion. Batches are listed in Table 6.

Table 6 Formulations prepared with syringe-die method.

Batch	Dexamethasone, w/w%	Brimonidine, w/w%	PLGA grade
1 – 3	-	10, 30, 50	502H
4 – 6	-	10, 30, 50	503H
7 – 9	-	10, 30, 50	502
10 – 12	-	10, 30, 50	752S
13 – 15	10, 30, 50	-	502H
16 – 18	10, 30, 50	-	503H
19 – 21	10, 30, 50	-	502
22 – 24	10, 30, 50	-	752S
25	50	-	752S/502H
26	50	-	752S/503H
27	50	-	752S/502
28	-	50	752S/502H
29	-	50	752S/503H
30	-	50	752S/502

Figure 15 Syringe-die device as a lab-scale ram extrusion introduced by Ghalanbor et al. (left) [29] and adapted lab- scale extrusion device with luer lock die (right).

2.2.3 Preparation of PLGA implants by HME with twin-screw extruders

Haake MiniLab Compounder

Batches of dexamethasone implants were prepared by hot-melt extrusion using a twin-screw MiniLab Compounder from Haake (HAAKE MiniLab Rheomex CTW5, Thermo Scientific, Karlsruhe, Germany). It was operated with two conical co-rotating screws at different settings. Batches prepared with HAAKE MiniLab were processed at 85 °C and 105 °C and screw speeds of 30 rpm and 120 rpm, respectively. Premixed blends of approximately 5.0 g were filled manually through a hopper into the preheated barrel and the molten blend was pressed through a 0.5 mm die, resulting in extrudate diameters of 0.49 – 0.51 mm. Extrudates were collected, manually cut into implants of approximately 6 mm length and 1.0 – 1.3 mg weight and stored in an orange gel silica-filled desiccator.

ThreeTec ZE9

Placebo batches and dexamethasone implants prepared with the Three-Tec ZE 9 extruder (ZE 9 Twin-screw Extruder, Three-Tec GmbH, Seon, Switzerland) were processed at 105 °C and a screw speed of 100 rpm. Powder blends of approximately 5.0 g were manually fed into the hopper. Modular co-rotating

screws with alternating mixing and kneading elements transported the blends through the barrel (Figure 16). Dies with diameters of 0.5 mm, 1.0 mm and 1.25 mm were attached to the end of the barrel. Extruded filaments were cooled down using a conveying belt, collected, and manually cut into implants of approximately 6 mm, 18 mm and 24 mm length and diameters of around 0.5 mm, 1.0 mm, and 1.25 mm. A list of batches is seen in Table 7.

Table 7 Batches prepared with twin-screw extruders.

Batch	Extruder	Temperature, °C Screw speed, rpm	Dexamethasone, w/w%	PLGA grade
31	Haake MiniLab	85 °C, 30 rpm	50	502H/ 502
32		105 °C, 120 rpm	50	502H/ 502
33	ThreeTec ZE9	105 °C, 100 rpm	20	502H
34			40	502H
35			50	502H
36			60	502H
37			20	502H/ 502
38			40	502H/ 502
39			50	502H/ 502
40			60	502H/ 502
Placebo			-	502H
Placebo			-	503H
Placebo			-	504H

Figure 16 Modular extruder screws of the ThreeTec ZE9.

2.2.4 Sterilization of PLGA implants

Sterilization of dexamethasone and brimonidine implants was performed by Co-60 gamma irradiation at Synergy Health Radeberg GmbH (Radeberg, Germany). Minimum and maximum dose were 27.2 kGy and 29.8 kGy, respectively. Batches sterilized by gamma irradiation are listed in Table 8.

Table 8 Batches sterilized by gamma irradiation.

Batch	Extruder	Dexamethasone, w/w%	PLGA grade
25	Syringe-die	50	752S/502H (1:1)
26		50	752S/503H (1:1)
27		50	752S/502 (1:1)
31	Haake MiniLab	50	502H/ 502 (3:1)
32		50	502H/ 502 (3:1)
37	ThreeTec ZE9	20	502H/ 502 (3:1)
38		40	502H/ 502 (3:1)
39		50	502H/ 502 (3:1)
40		60	502H/ 502 (3:1)

2.2.5 *In-vitro drug release*

For the drug release testing, implants were cut to length of approximately 6 mm with a scalpel, accurately weighed (1.0 – 1.3 mg) with a Mettler MT5 balance (Mettler Toledo, Gießen, Germany), and placed in 30 mL glass vials with screw cap (one implant per vial, n=3, if not otherwise stated), which were filled with 25 mL of 0.9% sodium chloride solution (saline). Closed glass vials were placed in a horizontal incubation shaker (Lauda-GFL, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at 37 °C and 80 rpm. At predetermined time points of approximately every 2 – 3 days, samples of 2 mL were taken with a pipette (Eppendorf Research® plus, Eppendorf SE, Hamburg, Germany) and replaced with fresh medium. Samples were cooled down to room temperature before further processing with the UV/ VIS-spectrophotometer. Release tests were finished when the implants degraded completely, or drug release ran into a plateau.

Drug concentrations of samples from implant release tests were measured using a UV/ Vis- spectrophotometer (Agilent 8453, Agilent Technologies Inc., Palo Alto, USA). Quartz cuvettes were filled with samples using a pipette and analyzed at the maximum wavelength of 242 nm with a single wavelength background at 400 nm for dexamethasone. Brimonidine base release was measured at a maximum wavelength of 250 nm with a single wavelength background at 450 nm. When the absorbance exceeded 2.0, measurements were done at the second peak wavelength of 320 nm for brimonidine. Concentrations were then calculated using a standard curve of the drugs in the release medium (Figure 17).

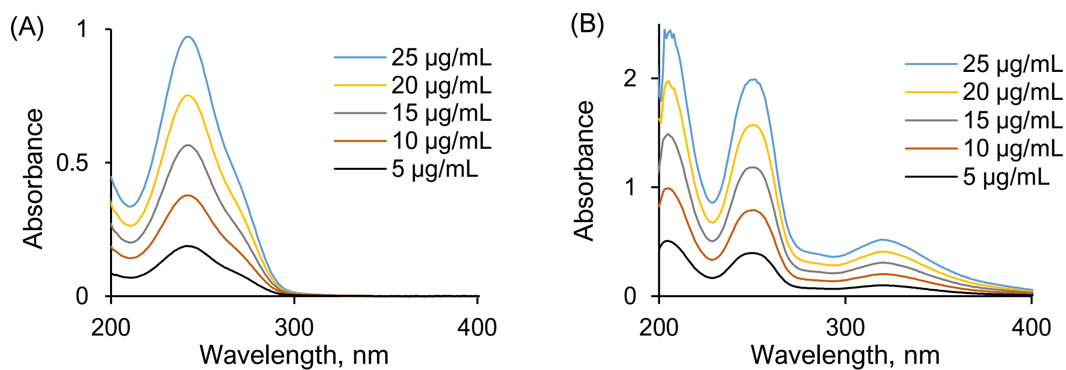

Figure 17 UV spectra of (A) dexamethasone and (B) brimonidine in saline.

Drug concentrations of samples from combination implants were measured and analyzed at maximum peak wavelengths of 247 nm and 320 nm for dexamethasone and brimonidine, respectively. A single wavelength background was set to 450 nm. Concentrations were then calculated using a standard curve of dexamethasone and brimonidine in a binary mixture of both drugs (Figure 18).

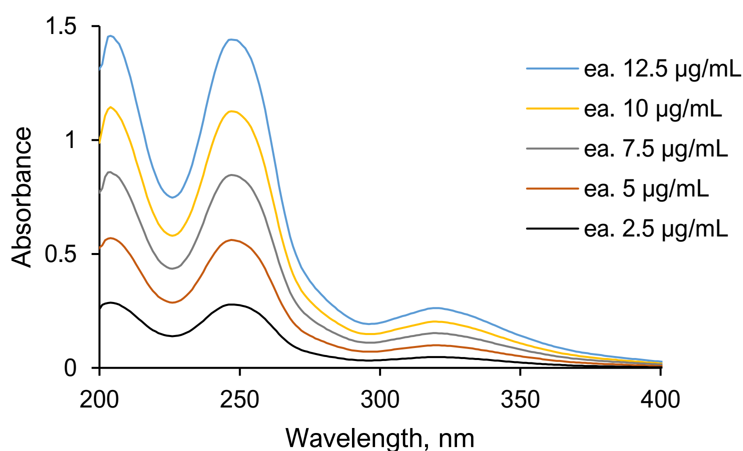

Figure 18 UV spectra of dexamethasone and brimonidine in a binary mixture.

Accelerated in-vitro drug release

Implants were cut to length of approximately 6 mm length, accurately weighed (1.0 – 1.3 mg) with a Mettler MT5 balance (Mettler Toledo, Gießen, Germany), and placed in 30 mL glass vials with screw cap (one implant per vial, n=3, if not otherwise stated). Glass vials were filled with 25 mL of either phosphate buffered saline pH 2 (PBS pH 2, USP) or phosphate buffered saline pH 12 (PBS pH12, USP). Closed glass vials were placed in a horizontal incubation shaker (Lauda-GFL, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at 80 rpm and temperatures of 37 °C, 45 °C or 65 °C. At predetermined time points, samples of 2 mL were taken with a pipette (Eppendorf Research® plus, Eppendorf SE, Hamburg, Germany) and replaced with fresh medium. Samples were cooled down to room temperature before further processing with the UV/ Vis-spectrophotometer. Release tests were finished when the implants degraded completely, or drug release of a compound run into a plateau.

2.2.6 Assay

HPLC method

Drug content of dexamethasone implants was determined using the USP method for dexamethasone assay. In a 10 mL volumetric flask accurately weighed implants (1.0 – 1.3 mg) were dissolved in 3 mL acetonitrile and DI water was added to volume (one implant per vial, n=10). Samples were analyzed by high performance liquid chromatography (HPLC) system (Shimadzu Deutschland GmbH, Duisburg, Germany) using a 250 x 4.0 mm RP-8 column at predetermined parameters (Table 9). For the stock solution, approximately 20 mg dexamethasone was dissolved in 20 mL methanol using a volumetric flask. 2 mL of stock solution was diluted to 20 mL with the mobile phase.

Table 9 Parameters for USP method of dexamethasone assay by HPLC.

Parameter	**Assay (USP)**
Column material	RP-8 (L7)
Column size, mm	250 x 4.0
Column temperature	ambient
Flow rate, ml/min	2.0
Mobile phase	Acetonitrile: Water (3:7)
Gradient	n.a.
Detection, nm	254
Sample solvent	Methanol (diluted with mobile phase)
Autosampler, °C	ambient
Injection volume, µl	15-30
Sample concentration	0.3 mg/ mL

UV/ VIS- method

Assay samples of implants analyzed with UV/ VIS- spectrophotometer (Agilent 8453, Agilent Technologies Inc., Palo Alto, USA) were prepared by dissolving one implant in 5 mL acetonitrile in a 10 mL volumetric flask and dilution to volume with DI water (n=3). Absorbance was measured at the maximum peak wavelength of 242 nm for dexamethasone with a single wavelength background at 400 nm and at a maximum peak wavelength of 250 nm for brimonidine with a single wavelength background at 450 nm. Drug contents were calculated using a standard curve. Concentrations of combination implant samples were measured at a wavelength of 320 nm for brimonidine and 247 nm for dexamethasone using standard curves in a binary mixture.

2.2.7 Incubation of PLGA implants

Desiccators were filled with a saturated sodium chloride solution and a saturated calcium chloride solution to obtain 75% RH and 30% RH over the solutions, respectively. Dexamethasone implants were placed on petri dishes in each desiccator and mechanical properties measurements (n=5) were performed every second day until equilibrium hydration of PLGA implants.

2.2.8 Water Content by Karl Fischer Titration

Water content of dexamethasone implants was determined with a C20 Coulometric Karl Fischer titrator (Mettler Toledo AG, Schwerzenbach, Switzerland). Accurately weighed implants were dissolved in glass vials in 1 mL of glacial acidic acid (99.5 %) (approx. 10 mg implants per vial). Aliquots of 150 µL (n=3) were injected to the titration vessel and titrated to the end point with Karl Fischer reagent (Hydranal Coulamat AD, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Water content was calculated based on weight of the injected samples.

2.2.9 Optical Light Macroscope

To determine implant dimensions and surface morphology an optical light macroscope (Inteq Informationstechnik GmbH, Berlin, Germany) with 20fold magnification was used. Length and diameter were measured using the instrument´s software Easy Measure after calibration.

2.2.10 Mechanical Properties of PLGA Implants

3-point bending tests were performed with a texture analyser TA.XTplus (Stable Micro Systems, Godalming, England) using a 5 kg load cell (n=10 and n=5).

Samples (PLGA implants of 6 mm and 18 mm length; 0.5 mm, 1.0 mm, and 1.25 mm in diameter) were placed horizontally on an implant holder over a 4 mm or 12 mm gap and force was applied with cylindrical probes of 2 mm, 3 mm, and 6 mm diameter to the implant´s center (Figure 19). Hardness (peak force), flexibility (elongation), toughness (slope) and energy required until break (area under the curve, AUC) were analyzed using the instrument´s software Exponent®. Software setup is listed in Table 10 and formulations analyzed in Table 11.

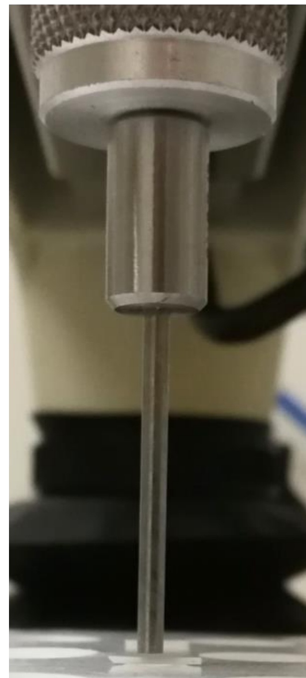

Figure 19 Exemplary measurement setup: 3-point bending test of PLGA implants with texture analyser TA.XTplus.

Table 10 Software setup for mechanical properties measurements of PLGA implants.

Parameter	Setup
Trigger mode	Button
Test speed	0.01 mm/ sec
Post- test speed	10 mm/ sec
Target mode	Distance
Break mode	Level
Break sensitivity	0.01 N
Break detect	Stop
Stop plot	Start position
Tare mode	Auto

Table 11 Batches of PLGA implants analyzed for mechanical properties.

Batch	Dexamethasone, w/w%	PLGA grade
35	50	502H
36	60	502H
37	20	502H/ 502 (3:1)
38	40	502H/ 502 (3:1)
39	50	502H/ 502 (3:1)
40	60	502H/ 502 (3:1)
Placebo	-	502H
Placebo	-	503H
Placebo	-	504H

3 Results and Discussion

3.1 Estimation of dexamethasone release from PLGA implants

As a first approach in formulation development, it is often easier to estimate drug release from a single graph rather than establishing complex mathematical models. An applicability map was therefore established from which one could easily decide which composition a dexamethasone formulation must have to deliver a desired dose over time. To create such a graph, dexamethasone release from different PLGA matrices was investigated in terms of the PLGA end groups, PLGA average molecular weight, lactic acid to glycolic acid ratio (L:G ratio) of PLGA grades, and drug loading of implants. Dexamethasone implants were prepared by hot-melt extrusion (HME) using the syringe-die method, a laboratory scale ram extrusion, adapted from Ghalanbor et al. [29].

3.1.1 Influence of PLGA type on dexamethasone release from implants

Acid end groups of PLGA make the implants less hydrophobic and tend to release the drug faster. The effect of molecular weight and drug loading were investigated for acid and ester terminated PLGA.

All implants prepared with PLGA 502H had a small burst phase of approximately 3% and a lag phase of only 7 days (Figure 20). After 4 – 5 weeks all implants released dexamethasone completely. Higher drug loadings of implants resulted in a faster dexamethasone release. Implants with 10% drug loading released dexamethasone within 19 days after the lag phase and implants with 30% and 50% drug loading released dexamethasone within 15 days and 11 days, respectively. One major reason for that is the preparation method of the implants. The syringe-die method could not incorporate the drug into the filaments as good as an extrusion with a twin-screw extruder (chapter 3.5.1), since no shear forces as well as no in-process mixing were present to homogeneously mix drug and

polymer. Hence, drug distribution within the implants was heterogenous and excess dexamethasone on implant surfaces produced a small burst phase within the first day of release tests.

After a burst phase, a lag phase of 10 days was observed for dexamethasone implants prepared with 503H followed by a release phase of approximately 20 days in 10% and 30% loaded implants, while implants containing 50% drug had an 18-day release phase (Figure 20). As with 502H implants, implants with higher drug content released faster than those with less. With an inherent viscosity of 0.32 – 0.44 dL/g (24 – 38 kDa) for 503H, its molecular weight and thus its hydrophobicity was higher than that of 502H with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa). Therefore, the onset of degradation and degradation itself was slowed down and lag and release phases were increased compared to 502H implants.

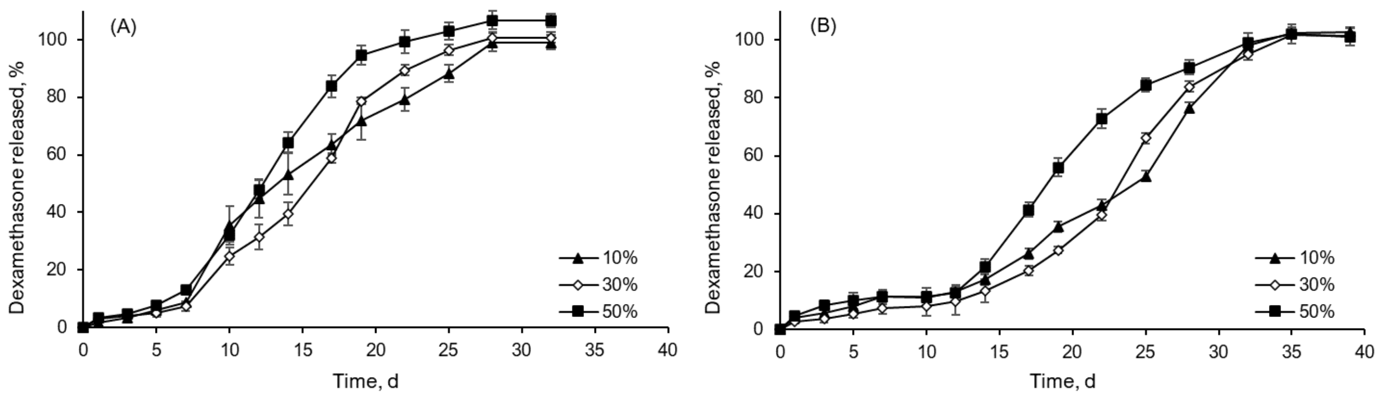

Figure 20 Influence of drug loading on dexamethasone release from (A) PLGA 502H implants and (B) PLGA 503H implants.

502 with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa) has a comparable average molecular weight to 502H but is ester terminated. The ester end capping for 502, compared to 502H, reduces the hydrophilicity of the polymer in almost the same proportion as increasing the molecular weight but keeping end groups acidic (502H vs. 503H). Dexamethasone release from 502 implants consisted also of an approximately 11-day lag phase, followed by a 20-day release phase (Figure 21). Again, implants with a 50% drug loading released dexamethasone faster than implants with less incorporated drug although the release curves appeared to be more similar.

To investigate the influence of the L:G ratio on dexamethasone release, implants were additionally prepared with 752S. The average molecular weight with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa) and ester end groups were comparable to 502, but L:G ratio was 75:25 for 752S compared to 50:50 for 502. This resulted in lag phases of 13, 23 and 40 days for formulations containing 10%, 30% and 50% dexamethasone, respectively (Figure 21). But the L:G ratio only affected the lag phase, once the polymer started to degrade, drug loading became the main factor influencing drug release. A 42-days release phase for 10% dexamethasone implants compared to 38 days and 31 days for 30% and 50% dexamethasone implants, respectively (Figure 22).

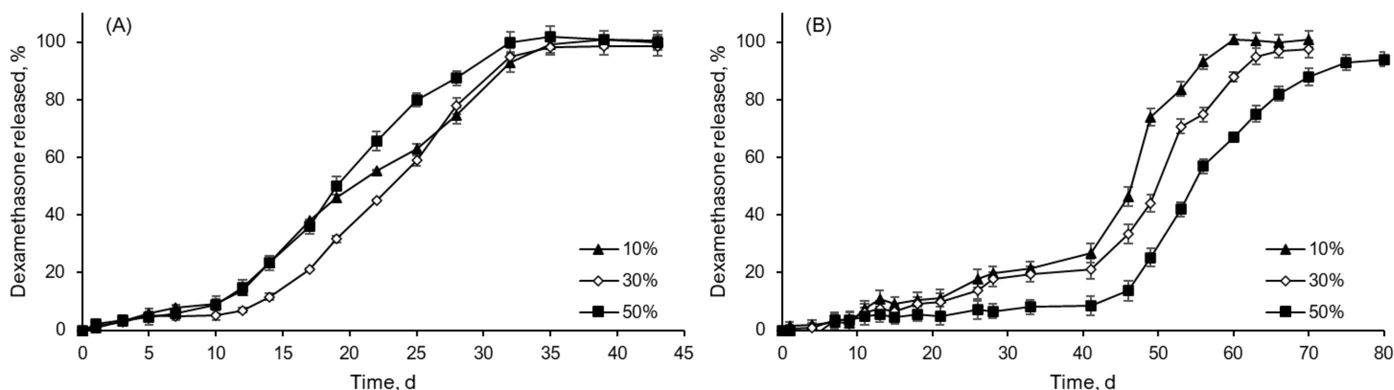

Figure 21 Influence of drug loading on dexamethasone release from (A) PLGA 502 and (B) PLGA 752S implants.

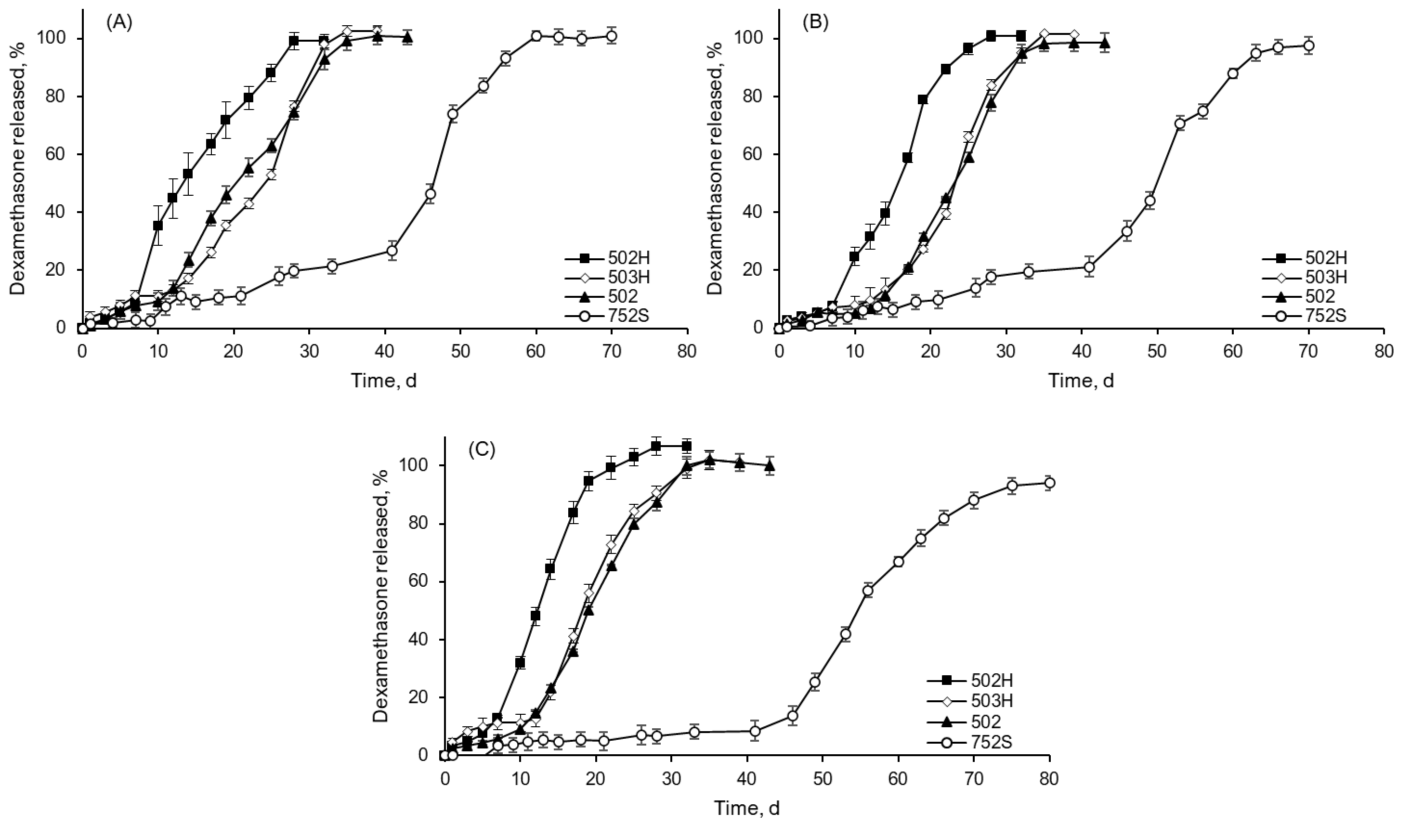

Figure 22 Comparison of dexamethasone release from different PLGA grade implants with drug loadings of (A) 10%, (B) 30% and (C) 50%.

3.1.2 Dexamethasone release from implants with PLGA mixtures

A mix of PLGAs within a formulation is important when it comes to modifying drug release. Both the lag phase and the release phase could be adjusted by using different PLGA grades. Dexamethasone release from implants with PLGA combinations (1:1) of 752S with 502H, 503H, and 502 were studied to see what changes in the release profiles occurred with respect to these release phases.

752S/ 502H and 752S/ 503H implants both had a lag phase of only 12 days (compared to 40 days from 50% dexamethasone 752S implants), indicating that the release phase was dictated by the acidic end groups of the 50:50 PLGA grades (Figure 23). The difference in polymer molecular weight was observed with the release period of approximately 16 days for 752S/ 502H implants and 20 days for 752S/ 503H implants. L:G was more rapidly degraded to oligomers and monomers by hydrolysis for lower molecular weight PLGAs. With 502 in the mixture with 752S, lag phase was reduced by 23 days from 40 to 17 days. The release phase of 18 days was in between those of 752S/ 502H and 752S/ 503H. Since the burst phase was similar for all implants, this phase was not influenced by formulation components but by the preparation method of implants, which was further investigated and described in chapter 3.5.1.

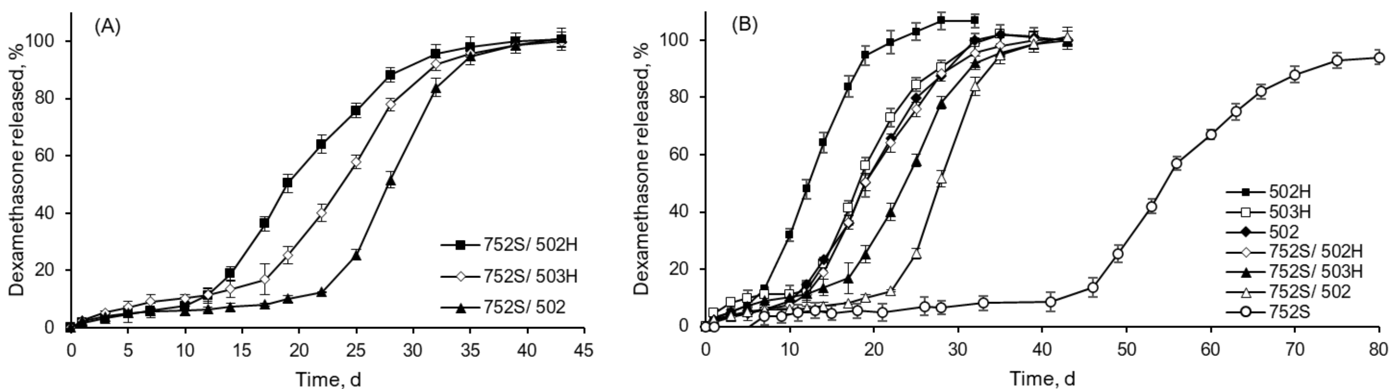

Figure 23 (A) Influence of polymer mixture on dexamethasone release from 50% loaded implants employing different mixtures of PLGA grades and (B) Comparison of dexamethasone release from implants with 50% drug loading.

3.1.3 Applicability map of biodegradable dexamethasone implants

In order to create an applicability map by which one could estimate dexamethasone release from PLGA implants, the drug release profiles were summarized in terms of lag phase, defined as the time for the dexamethasone concentrations in the release test medium to reach 10% of complete release (t_{10}), and the release phase, defined as the period between 10% and 90% of complete dexamethasone release (t_{90}-t_{10}) with respect to PLGA grade and drug loading (Table 12). In summary, dexamethasone release from 502H implants was characterized by a 7-day lag phase followed by 19-, 15- and 11-days release phase for 10%, 30% and 50% drug loading, respectively. Although the lag phase for 503H was 10 days and for 502 implants approximately 11 days, release of dexamethasone for both PLGAs was 20, 19 and 18 days for formulations with 10%, 30% and 50% dexamethasone content.

Table 12 Dexamethasone release data from PLGA implants as a function of PLGA grades and drug loading.

PLGA grade	Dexamethasone content, %	Release t_{10} (Time, d)	Release t_{90} (Time, d)	Release t_{90}-t_{10} (Time, d)
502H	10	7	26	19
	30	7	22	15
	50	7	18	11
503H	10	10	30	20
	30	10	29	19
	50	10	28	18
502	10	11	31	20
	30	12	31	19
	50	11	29	18
752S	10	13	55	42
	30	23	61	38
	50	40	71	31
752S/ 502H	50	12	28	16
752S/ 503H	50	12	32	20
752S/ 502	50	17	35	18

Plotting the release period t_{90}-t_{10} over lag phase t_{10} visualized the dexamethasone release from implants described before (Figure 24). The drug loading affected the release from 502H implants only in t_{90}-t_{10} while the lag time was the same irrespective of drug loading. Using 503H or 502 as biodegradable polymer, t_{10} and t_{90}-t_{10} were in short range to each other with all drug loadings observed. The biggest difference in lag phases were present for implants with 10% < 30% < 50% drug loading.

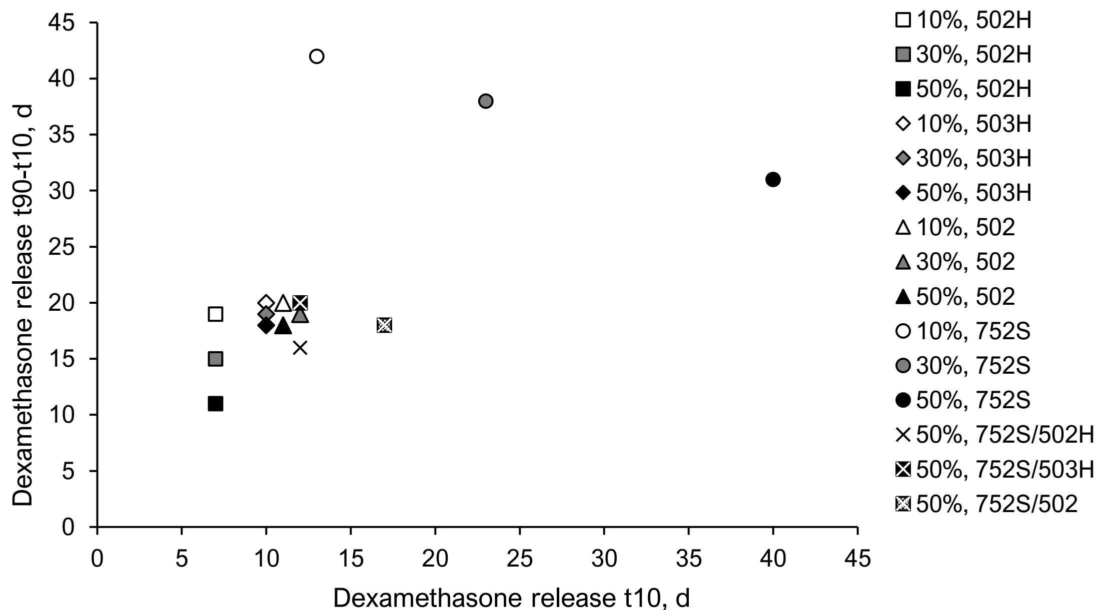

Figure 24 Applicability map of the influence of drug loading and PLGA grade on dexamethasone release time and release onset.

The assumption that dexamethasone release from implants with PLGA mixtures line in between the release from PLGA implants with a single polymer was confirmed by plotting t_{90}-t_{10} over t_{10} (Figure 24). With determination coefficients (R^2) of 0.993, 0.962 and 0.989 for implants with 752S/ 502H, 752S/ 503H and 752S/ 502, respectively, a good correlation could be observed. Furthermore, without the need to look at the single release curves, it was possible to conclude,

that for implants of 1:1 PLGA mixtures lag and release phase of dexamethasone was more affected by L:G ratios and molecular weight of the polymer.

3.1.4 Applicability map for the development of a biodegradable implant

To confirm the use of the applicability maps, the development of a PLGA implant with a desired dexamethasone release was checked. As proof-of-concept, the most common approved dexamethasone implant on the market was chosen, namely Ozurdex®, assigned by Allergan, Inc. (Irvine, CA, US). Dexamethasone release from Ozurdex is described with a lag phase of approximately 1 week and a release phase of 2 weeks [141]. Therefore, starting from the applicability map, the objective was to find a biodegradable dexamethasone formulation with a minimal lag phase and a release phase of approximately 14 days.

Based on Figure 24, independent of the drug loading, dexamethasone was released from 502H after 7 days, which was the earliest of all PLGA grades investigated. Release phases for 502H and 502 implants containing 50% dexamethasone were 11 days and 18 days, respectively. Since the use of two different PLGA grades within one formulation would result in the average release time of both polymers individually (502H: 7 days; 752S: 31 days, 752S/ 502H: 16 days) a formulation with a 502H/ 502 PLGA mixture was chosen to achieve a 14 day release (Table 13). The second option would be the use of 503H in the mixture with 502H (also with an average release time of 14.5 days), but to obtain a more consistent formulation in terms of polymer molecular weight, 502 was chosen.

Table 13 502H and 502 implant release phases and expected (average) release phase of 502H/ 502 implants.

Drug loading, %	DEX/502 t_{90}-t_{10}, d	DEX/502H t_{90}-t_{10}, d	Average t_{90}-t_{10}, d
10	20	19	19.5
30	19	15	17
50	18	11	14.5

Not only a shift of release periods but also on lag phases to a prolonged release from implants with 1:1 PLGA mixtures compared to single PLGA grade implants was observed. To obtain a short lag phase of approximately 7 days, it was assumed that a higher content of 502H was needed.

In summary, to develop a high drug loading dexamethasone implant with a short lag phase of no longer than 7 days and a release phase of approximately 14 days, 502H for the early onset of dexamethasone release and 502 to prolong the release in a ratio of 3:1 (to minimize the shift of the lag phase) were chosen for the formulation.

Dexamethasone implants with a drug load of 50% and even 60% and a polymer matrix of 502H/ 502 in a 3:1 ratio had a desired drug release with a lag phase of 7 days and a release phase of approximately 14 days (Figure 25). Therefore, it the applicability map for biodegradable dexamethasone implants was successfully implemented and used to develop a new formulation with a desired drug release.

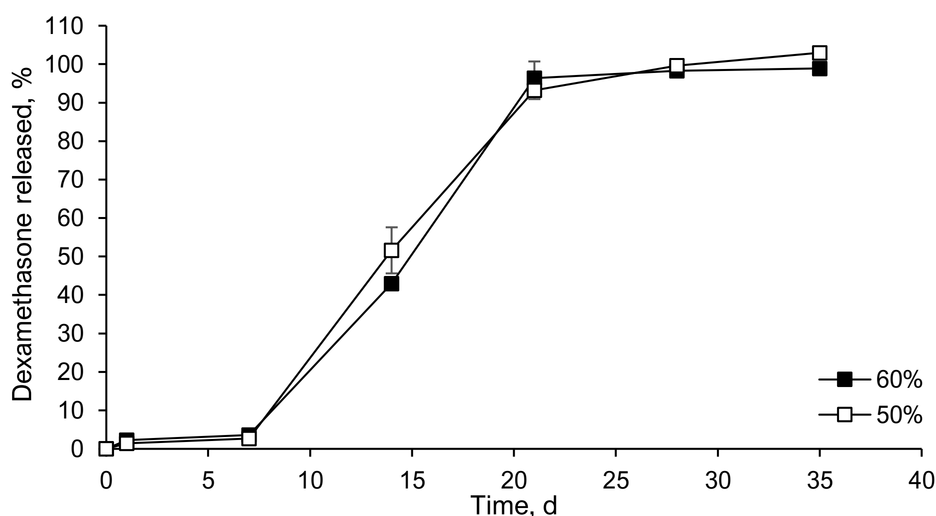

Figure 25 Dexamethasone release from proof-of-concept formulations.

3.1.5 Conclusions

The influence of poly(lactide-coglycolide) (PLGA) grade on drug release from biodegradable dexamethasone implants prepared with the syringe-die method were investigated in terms of PLGA end groups, PLGA average molecular weight, lactic acid to glycolic acid (L:G) ratio of PLGA grades, and drug loading of implants.

The lag phase of dexamethasone release from implants prepared with 502H, 503H, and 502 were 7, 10, and approximately 11 days, respectively, independent of the drug loading. Lag phases for dexamethasone release from 752S implants were 13 days, 23 days, and 40 days for formulations containing 10%, 30%, and 50% dexamethasone, respectively. The average molecular weight of 503H with an inherent viscosity of 0.32 – 0.44 dL/g (24 – 38 kDa) compared to 0.16 – 0.24 dL/g (7 – 17 kDa) for 502H increases the polymer hydrophobicity thus prolonging the lag phase of dexamethasone release from 7 days to 10 days due to the delayed start of hydrolytic PLGA degradation. 502 with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa) has a comparable average molecular weight to 502H but has been ester terminated. The ester end capping for 502, compared to 502H, reduces the hydrophilicity of the polymer in almost the same proportion as increasing the molecular weight but keeping end groups acidic (502H vs. 503H). The lag phase of dexamethasone was prolonged from 7 days to 11 days. A major influence on the lag phase of dexamethasone release is the PLGA L:G ratio. 752S has a comparable inherent viscosity to 502 but consist of a 75:25 L:G ratio instead of 50:50 for 502. Lag phases of dexamethasone release increased from 11 days for 502 implants to 13 days, 23 days, and 40 days for 752S implants due to an increased hydrophobicity. For all biodegradable implants, the release time after the lag phase was shorter for implants containing 50% dexamethasone compared to 30% dexamethasone implants and 10% dexamethasone implants .

Plotting the release period over lag phase visualized the dexamethasone release from PLGA implants in applicability maps which were then successfully used to

estimate drug release and to develop a biodegradable dexamethasone implant with a desired release curve.

3.2 Estimation of brimonidine base release from PLGA implants

From the marketed Ozurdex® implant, it was known that not only the intravitreal injection of the implant can cause local side effects, but also the application of the glucocorticoid itself resulted in a rise of intraocular pressure (IOP) for approximately 30% of all patients. In most cases, the treatment of increased intraocular pressure is carried out symptomatically with eye drops containing pressure-lowering agents such as brimonidine, which is a α_2-adrenoceptor-agonist and thus acting sympathomimetic. The application several times a day as well as side effects such as local irritation of the eye can however result in a decreased patient compliance [146]. Therefore, a formulation for a biodegradable implant for the application of brimonidine directly to the site of action was developed. The requirement for the implant was that it released the active substance to the same extent as dexamethasone. The increased IOP would be treated directly avoiding the application of eye drops.

To develop such a formulation brimonidine release from biodegradable PLGA implants prepared by hot-melt extrusion (syringe-die method) was investigated in terms of PLGA end groups, polymer molecular weight, L:G ratio, and drug loading. The release data were summarized in an applicability map, consisting of lag phase plotted against release phase, to develop the desired release implant as previously with dexamethasone implants.

3.2.1 *Influence of PLGA type on brimonidine release from biodegradable implants*

Brimonidine implants with PLGA RG 502H released the drug with a small burst phase after 1 day, followed by a lag phase, defined as the time at which drug release exceeded 10%, until day 5, and a release phase of 9 days (Figure 26). In contrast to dexamethasone implants, the release of brimonidine from 502H was independent of drug loading. Although implants containing 30% drug appeared to release brimonidine faster, the release times of all implants prepared were similar and have full release at 12 days.

In contrast to 502H implants brimonidine release from 503H implants was influenced by the drug loading. Implants with 10% drug load have a higher burst phase and a short lag phase of 3 days, while formulations with 30% and 50% brimonidine had lag phases of 7 days and 10 days, respectively (Figure 26). With an inherent viscosity of 0.32 – 0.44 dL/g for 503H (24 – 38 kDa), its molecular weight and thus its hydrophobicity was higher than that of 502H with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa). Once the PLGA began to degrade, the release of brimonidine accelerated with increasing drug loading. While implants with 10% drug had a release phase of 11 days, 503H implants with 30% and 50% brimonidine released the drug within 8 days and 6 days, respectively. When released into the medium, brimonidine base is usually present in ionized form [147], thus additionally accelerating PLGA degradation by acid catalysis of ester cleavage.

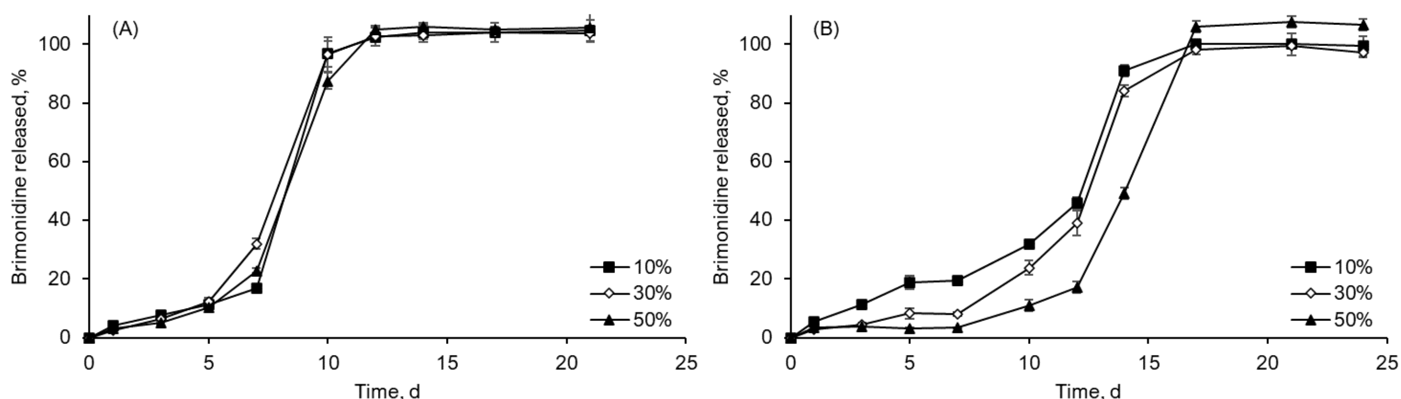

Figure 26 Influence of drug loading on brimonidine release from (A) PLGA 502H implants and (B) PLGA 503H implants.

502 with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa) had a comparable average molecular to 502H but has been ester terminated. The ester end capping for 502 reduced the hydrophilicity of the polymer compared to 502H and thus extended the lag phase to 10 days (Figure 27). Brimonidine was then released within 6 days for all formulations, regardless of drug loading and hence comparable to release from 502H implants.

To investigate the influence of the L:G ratio on brimonidine release, implants were prepared with 752S. The average molecular weight with an inherent viscosity of 0.16 – 0.24 dL/g and ester end groups are comparable to 502, but the L:G ratio is 75:25 for 752S compared to 50:50 for 502. This explained lag phases of 12 days for formulations containing 10%, 30% and 50% brimonidine (Figure 28). But the L:G ratio only affected the lag phase. Once the polymer started to degrade, drug loading again did not influence the drug release. As with previously investigated PLGA grades of similar molecular weight, all formulations released brimonidine within the same time, which was 12 days for 752S implants. Regarding the release of brimonidine from 502H and 502 implants, it was also assumed that drug would be released from 752S implants with 50% drug load at the same time with 10% and 30% formulations.

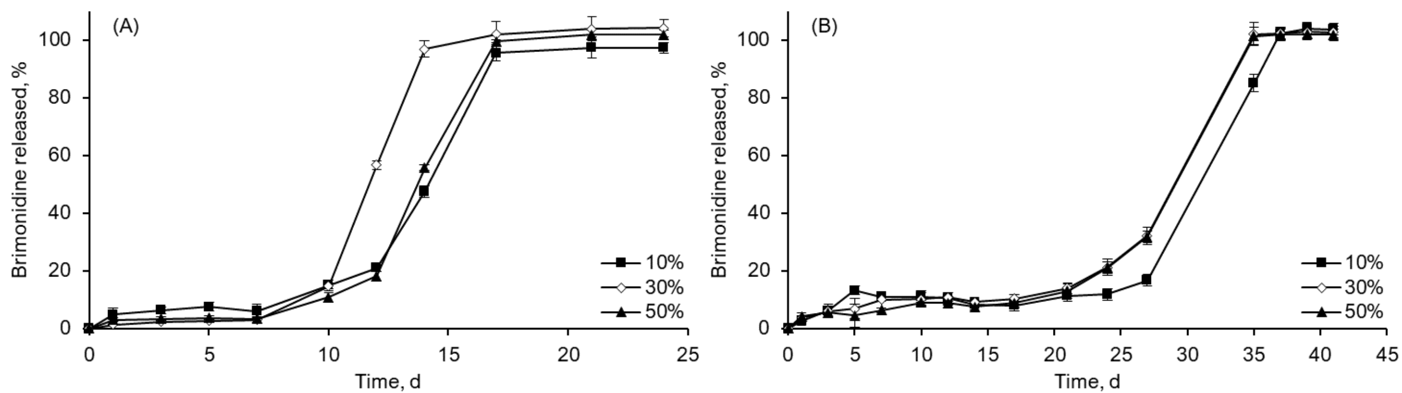

Figure 27 Influence of drug loading on brimonidine release from (A) PLGA 502 implants and (B) PLGA 752S implants.

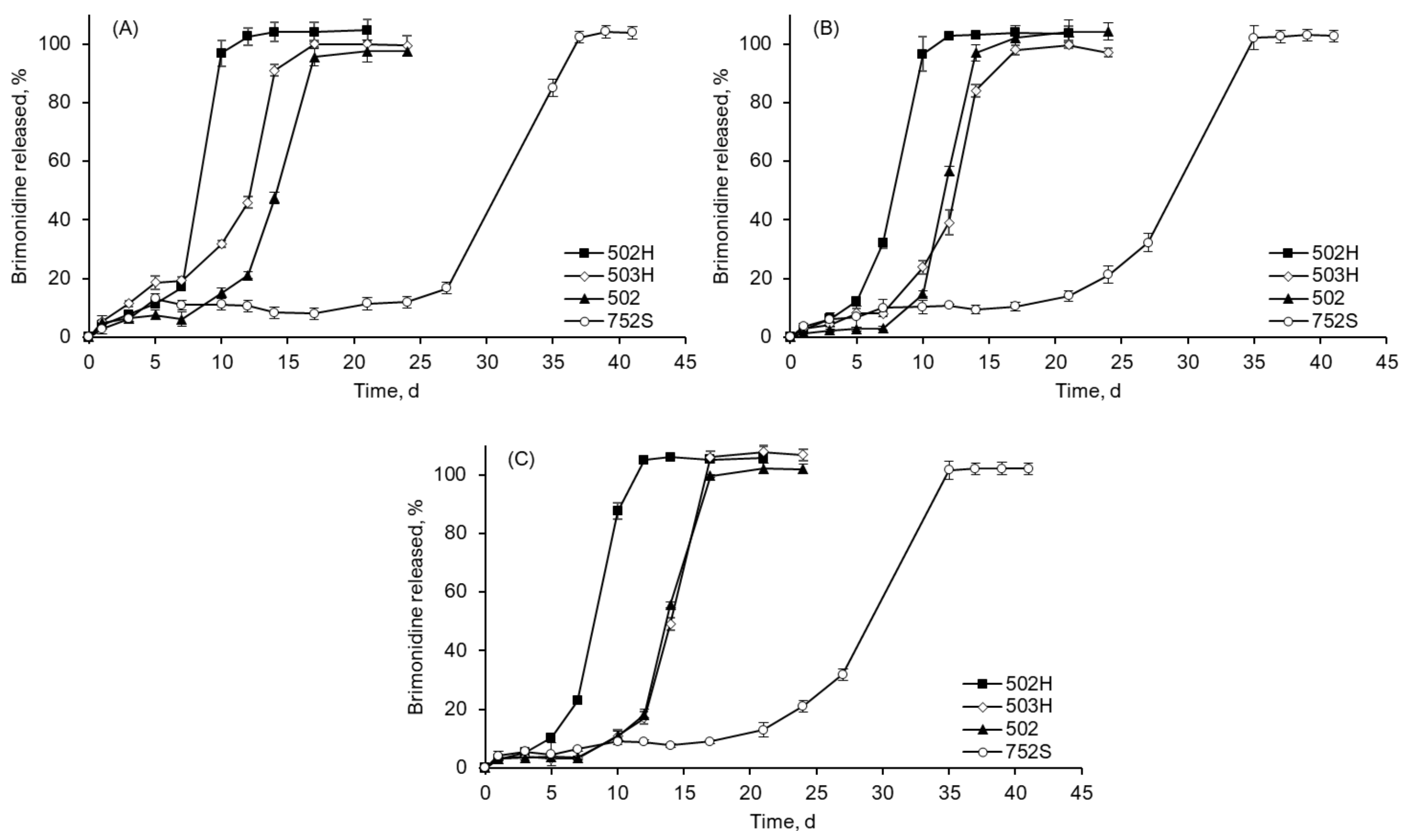

Figure 28 Comparison of brimonidine release from PLGA 502 and 752S implants with different drug loadings; (A) 10%, (B) 30% and (C) 50%.

3.2.2 *Brimonidine base release from implants with PLGA mixtures*

In Figure 29 the brimonidine release from implants containing PLGA blends of 752S with either 502H, 503H, or 502 is shown. The lag phase of brimonidine implants with a mixture of PLGA 752S and 502H was 9 days, indicating that the onset of drug release was dictated by the hydrophilicity of the acid terminated PLGA since the lag phases of individual 502H and 752S implants were 5 days and 24 days, respectively. The same was for 752S/ 503H and 752S/ 502 implants containing 50% brimonidine.

The release phase of 752S/ 502H was more influenced by the hydrophobicity of the 75:25 L:G ratio from PLGA 752S. While single 502H and 752S implants containing 50% brimonidine had release phases of 4 days and 12 days, respectively, implants with the PLGA mixture release the drug within 11 days.

Interestingly, the hydrophobic properties of the other PLGA grades appeared to have a synergistic effect when combined. Implants with blends of 752S/ 503H and 752S/ 502 released brimonidine in 13 days and 15 days, respectively, which was longer than the release phase of 752S implants containing 50% brimonidine (12 days).

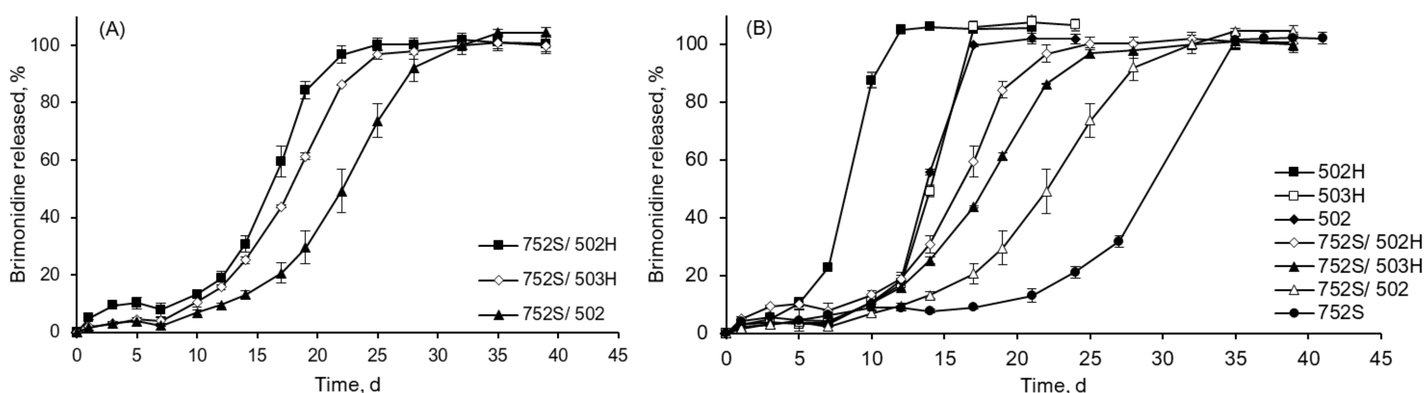

Figure 29 (A) Influence of polymer mixture on brimonidine release from 50% loaded implants employing different mixtures of PLGA grades and (B) Comparison of brimonidine release from implants with 50% drug loading.

3.2.3 Applicability Map of biodegradable brimonidine base implants

In summary, brimonidine release from 502H implants was characterized of a 5-day lag phase followed by a 4-day release phase independent of the drug loading. Brimonidine release from 503H implants appeared to be the only from all formulations investigated that was influenced by drug loading. Lag phases were 3 days, 7 days, and 10 days while release phases were 11 days, 8 days, and 6 days for implants containing 10%, 30% and 50% brimonidine, respectively. Ester end capping of PLGA resulted in an extended lag phase of 10 days for 502 implants versus 5 days for 502H implants. The release phase was also slightly increased to 6 days. An L:G ratio of 75:25 for PLGA 752S implants prolonged the lag phase to 21 – 24 days and the release phase to 12 days independently of the drug loading.

In order to create an applicability map by which one could estimate brimonidine release from PLGA implants, the observed drug releases from prepared formulations were summarized in terms of lag phase, defined as the time for the brimonidine concentrations in the release test medium to reach 10% of complete release (t_{10}), and release phase, defined as the period between 10% and 90% of complete brimonidine release (t_{90}-t_{10}) with respect to PLGA grade and drug loading (Table 14).

Table 14 Brimonidine release periods from PLGA implants with respect to PLGA grades and drug loading.

PLGA grade	Brimonidine content, %	Release t_{10} (Time, d)	Release t_{90} (Time, d)	Release t_{90}-t_{10} (Time, d)
502H	10	5	9	4
	30	5	9	4
	50	5	9	4
503H	10	3	14	11
	30	7	15	8
	50	10	16	6
502	10	10	16	6
	30	10	16	6
	50	10	16	6
752S	10	21	33	12
	30	21	33	12
	50	24	36	12
752S/ 502H	50	9	19	11
752S/ 503H	50	10	18	13
752S/ 502	50	12	25	15

Plotting the release period t_{90}-t_{10} over lag phase t_{10} visualized the brimonidine release from implants described before (Figure 30). With brimonidine release from 752S implants independently of the drug load, the release periods of 502H, 502, and 752S implants as well as 503H implants with 50% drug loading, had a good determination coefficient (R^2) of 0.995. The drug loading only influenced brimonidine release with a PLGA grade of higher molecular weight. Release periods of 503H implants had a determination coefficient of 0.999.

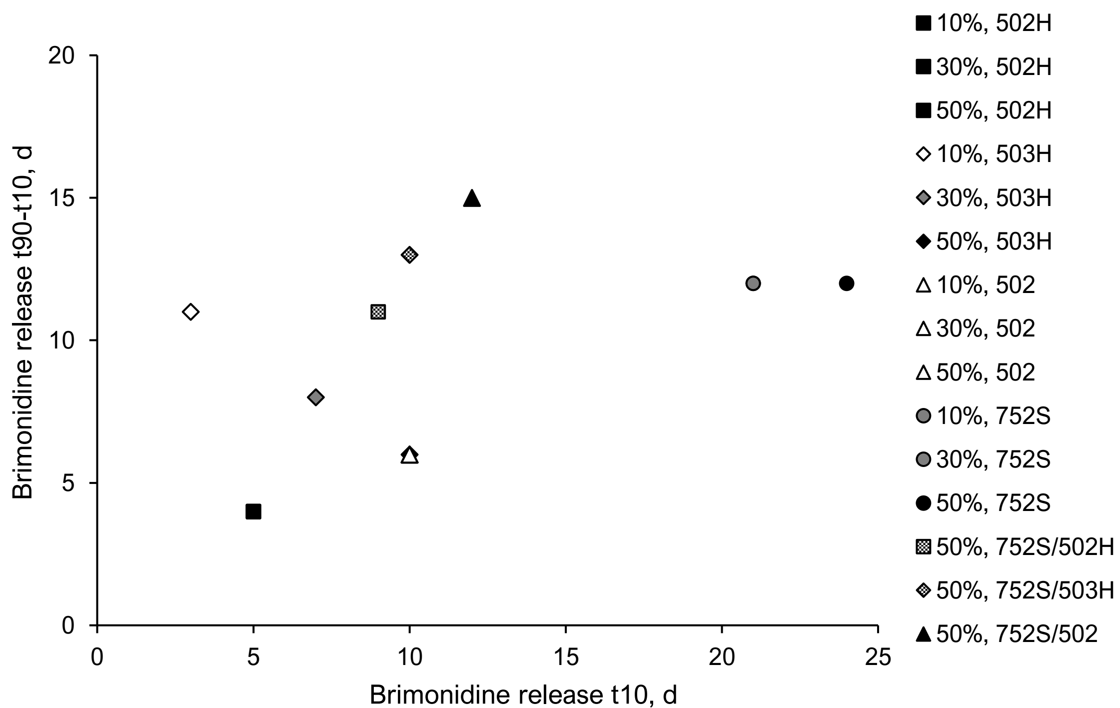

Figure 30 Applicability map of the influence of drug loading and PLGA grade on brimonidine release time and release onset.

In contrast to the dexamethasone release from implants with 1:1 PLGA mixtures, brimonidine release periods did not line up between the release periods of individual 50% drug implants. It was observed that the lag phase was more influenced by acid end PLGA groups while release phases were affected by the average L:G ratio and even in synergistic effect with the polymer molecular weight and ester end termination of PLGA.

3.2.4 Applicability Map for the development of a biodegradable implant

To overcome the increased IOP that is a side effect of an intravitreal dexamethasone implant, an attempt was made to develop a biodegradable implant containing brimonidine with the same release properties. Since IOP can only be observed as a side effect if dexamethasone has already been released, brimonidine was only required after dexamethasone release has started. Therefore, the objective was to develop a biodegradable brimonidine implant with a lag phase of over 7 days and a release phase of approximately 14 days or more.

Brimonidine release from PLGA implants with a 1:1 mixture of 752S/ 503H already met the required release with a 3-day longer lag phase and almost the same release time compared to the developed dexamethasone implant (Table 14, Figure 31). If a longer lag phase would be required, the 752S/ 502 implants, which start releasing brimonidine after 12 days, could be an alternative system.

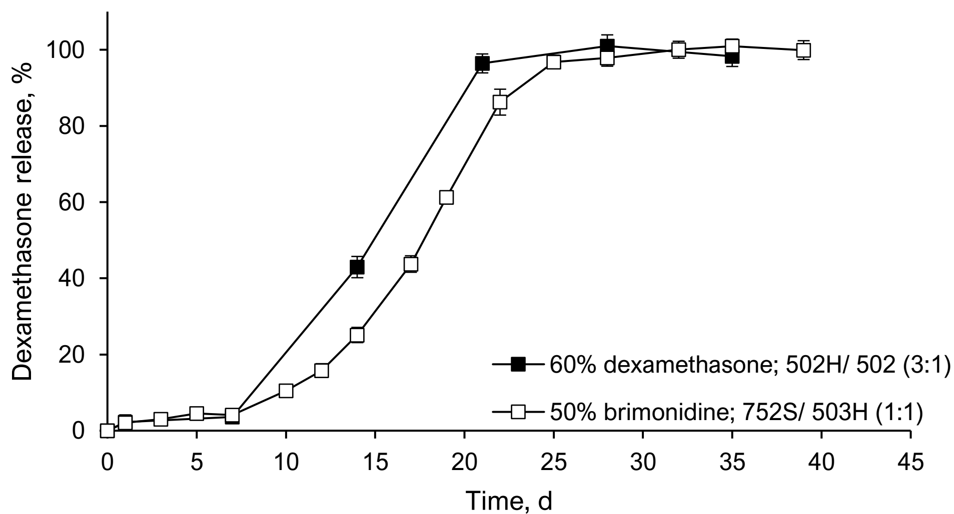

Figure 31 Brimonidine release from 752S/ 503H implants compared to dexamethasone release from 502H/ 502 implants.

While both formulations have a burst release of approximately 3% and a lag phase of 7 days, dexamethasone release from PLGA 502H/ 502 (3:1) implants was complete after 21 days and brimonidine release from PLGA 752S/ 503H (1:1) implants after 25 days (Figure 31). In a combination device of both drugs, it is preferred to release brimonidine slower than dexamethasone to overcome negative side effects e.g., increased intraocular pressure. This might also be achieved by increasing the lag phase of brimonidine release via increased contents of ester terminated PLGA.

3.2.5 Conclusions

Comparable to chapter 3.1, the influence of poly(lactide-coglycolide) (PLGA) grade on drug release from biodegradable brimonidine implants prepared with the syringe-die method were investigated in terms of PLGA end groups, PLGA average molecular weight, lactic acid to glycolic acid (L:G) ratio of PLGA grades, and drug loading of implants.

The lag phase of brimonidine release from implants prepared with 502H, 502, and 752S were 5 days, 10 days, and approximately 21 days, respectively, independent of the drug loading. In contrast to dexamethasone implants, lag phases for brimonidine release from 503H implants were 3 days, 7 days, and 10 days for formulations containing 10%, 30%, and 50% brimonidine, respectively. The average molecular weight of 503H with an inherent viscosity of 0.32 – 0.44 dL/g (24 – 38 kDa) compared to 0.16 – 0.24 dL/g (7 – 17 kDa) for 502H increases the polymer hydrophobicity resulting in the major influence for brimonidine release from PLGA implants. 502 with an inherent viscosity of 0.16 – 0.24 dL/g (7 – 17 kDa) has a comparable average molecular weight to 502H but has been ester terminated. The ester end capping for 502, compared to 502H, reduces the hydrophilicity of the polymer, and prolongs the lag phase from 5 days to 10 days for brimonidine implants. In contrast to dexamethasone implants, drug release from brimonidine implants prepared with 752S was not influenced by drug

loading. For all biodegradable implants prepared, the release time after the lag phase was independent of drug loading except for brimonidine implants prepared with 503H. Release times were 4 days, 6 days, and 12 days for implants prepared with 502H, 502, and 752S, respectively. 503H implants containing 10%, 30%, and 50% brimonidine released the drug within 11 days, 8 days, and 6 days, respectively.

Interestingly, the hydrophobic properties of the other PLGA grades appeared to have a synergistic effect when combined. Implants with blends of 752S/ 503H and 752S/ 502 released brimonidine in 13 days and 15 days, respectively, which was longer than the release phase of 752S implants containing 50% brimonidine (12 days).

3.3 Dexamethasone and brimonidine base combination implants

To avoid the intravitreal injection of a second implant after the application of a dexamethasone implant and thus reduce local side effects such as eye irritation due to the implantation through a needle, an approach to develop a biodegradable implant containing both dexamethasone and brimonidine was made. Although the desired drug release of the individual drugs were achieved with different PLGA grades and mixtures thereof, the drug release of combination implants (containing dexamethasone and brimonidine) was investigated with the individual PLGA grades in terms of PLGA molecular weight, L:G ratio and end groups. Biodegradable implants with both drugs and different PLGA grades were prepared by HME. A total dose of 60%, corresponding to 700 µg dexamethasone and 200 µg brimonidine per implant, was kept constant for all formulations studied.

Combination implants with acid terminated PLGA

Brimonidine release from combination implants prepared with 502H was faster compared to the release from single brimonidine/ 502H implants. A short lag phas of 1 – 2 days and a release phase of approximately 8 days was observed for the release of brimonidine (Figure 32). Interestingly, dexamethasone release from combination implants was incomplete for all implants prepared and tested. After a lag phase of 9 days, dexamethasone release proceeded to a maximum of approximately 24% at day 35 of release testing. After reaching its peak, dexamethasone release stopped, and the implant did not degrade anymore. To rule out errors in formulation preparation and the measurement or release method, PLGA implants were collected from the medium after release tests and the residual dexamethasone content was determined using the HPLC assay method for dexamethasone (USP). For combination implants with 502H, released

and residual dexamethasone contents summed up to 102.0% ± 2.3% for all implants. This confirmed that the release test method and measurements were correct and only dexamethasone release was incomplete.

Drug releases from combination implants with 503H were similar to 502H combination implants. Again, brimonidine release had a short lag phase, but release time was prolonged to approximately 13 days. As with 502H combination implants, dexamethasone release was also incomplete after 35 days of release testing (Figure 32). Released and residual dexamethasone content summed up was 95.2% ± 1.4% for all implants, again confirming a correct measurement and an incomplete release.

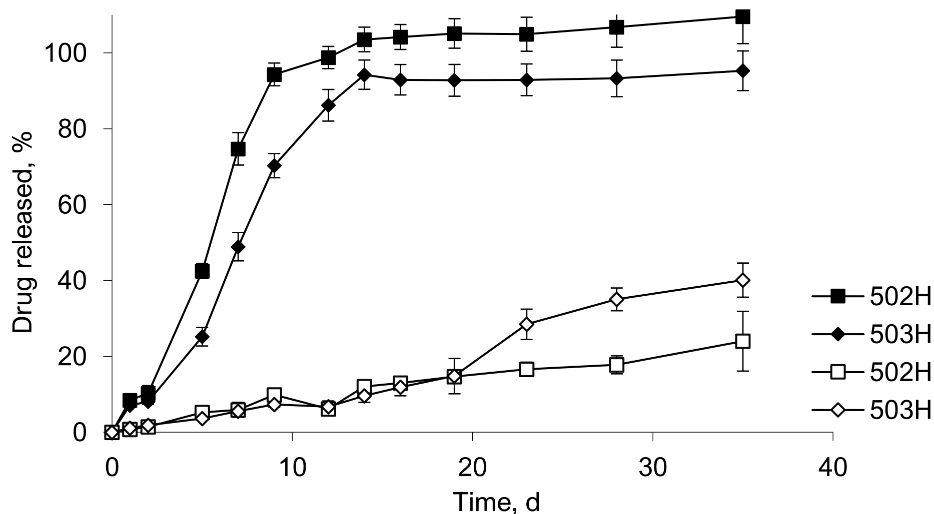

Figure 32 Brimonidine release (closed symbols) and dexamethasone release (open symbols) from combination implants with PLGA 502H and PLGA 503H in saline.

The release tests of combination implants in PBS pH 12 resulted in a complete release of both drugs. As described before, PLGA matrices tend to degrade via surface erosion when exposed to basic medium conditions, thus providing

direct drug release without lag phase (Figure 33). While brimonidine release from PLGA 502H implants was complete after 3 days, dexamethasone release was slower and complete after 6 days, which agrees with accelerated release studies carried out in chapter 3.4.1. Although the burst release for dexamethasone from 503H implants is higher than brimonidine release, complete drug was released after 8 days for both. The higher average molecular weight of 503H compared to 502H results in a slower drug release to a decreased degradation of the PLGA backbone.

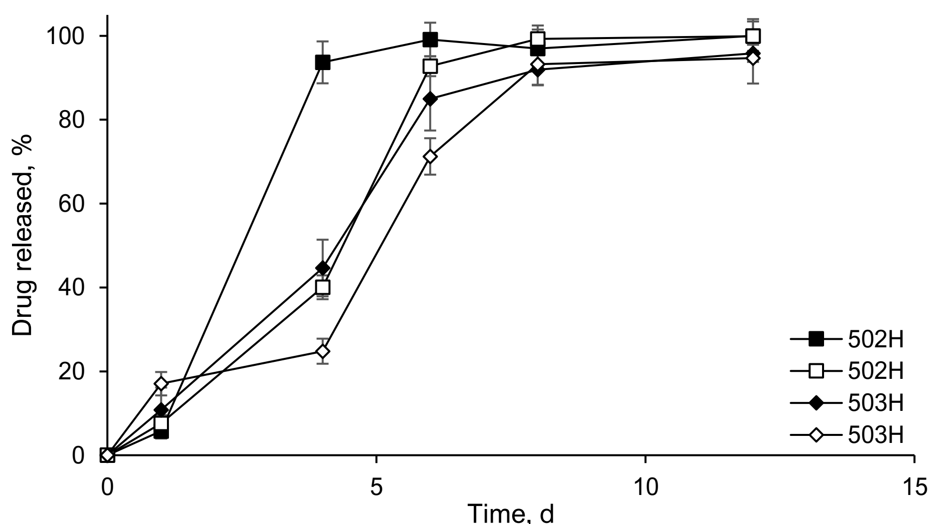

Figure 33 Brimonidine release (closed symbols) and dexamethasone release (open symbols) from combination implants with PLGA 502H and PLGA 503H in PBS pH 12.

Combination implants with ester terminated PLGA

Brimonidine release from 502 combination implants was similar to that of 502H combination implants (Figure 34). No lag phase and an 8-days release phase was observed. Also, the maximum of approximately 50% dexamethasone release was reached after 35 days. Residual and released dexamethasone content summed up to 96.5 ± 2.1 %.

As expected, dexamethasone release from 752S combination implants was incomplete again, reaching a maximum of approximately 24% after 35 days while released and residual dexamethasone content summed up to 98.4% ± 2.0% (Figure 34). Brimonidine release was a typical triphasic release from PLGA matrices. After a burst phase the lag phase of approximately 12 days was observed before complete release within 14 days.

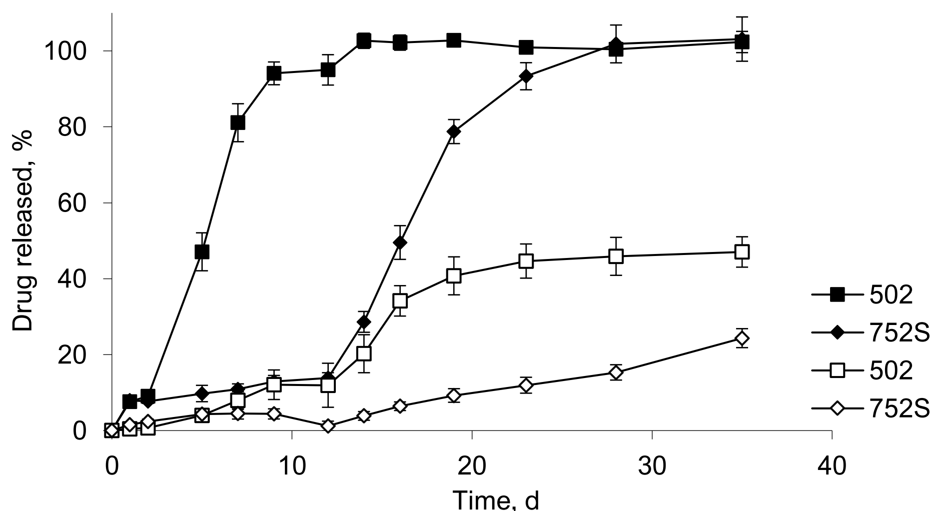

Figure 34 Brimonidine release (closed symbols) and dexamethasone release (open symbols) from combination implants with PLGA 502 and PLGA 752S in saline.

The release tests of combination implants in PBS pH 12 resulted in a complete release of both drugs (Figure 35). While brimonidine release from PLGA 502 implants was complete after 6 – 7 days, dexamethasone release was slower and complete after 8 days. During the lag phase of PLGA 752S implants, brimonidine and dexamethasone release was observed to approximately 35%. Brimonidine release was decreased from 28 days in saline to 21 days in PBS pH 12, while dexamethasone completely released within 25 days in PBS pH 12, only 25% released from 752S implants after 35 days of release testing in saline.

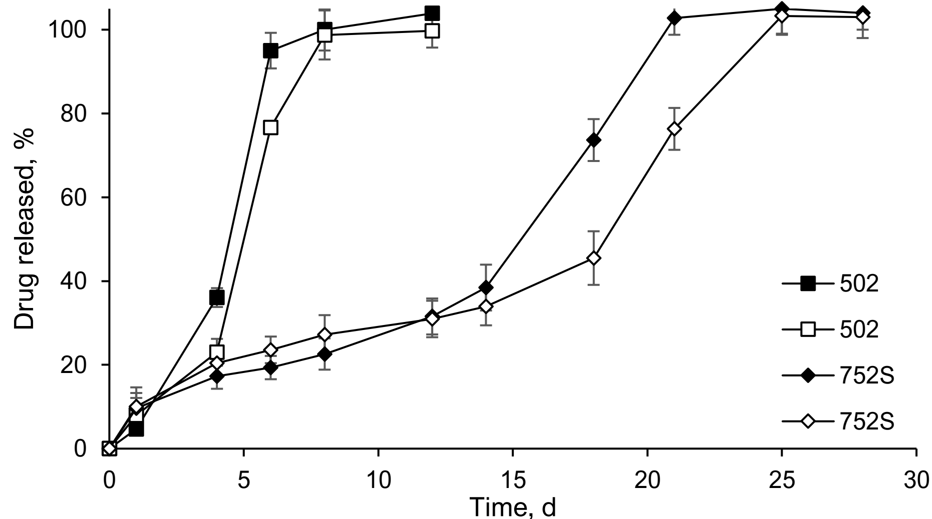

Figure 35 Brimonidine release (closed symbols) and dexamethasone release (open symbols) from combination implants with PLGA 502 and PLGA 752S in PBS pH 12.

Combination implants with PLGA mixtures

Although dexamethasone release from combination implants with PLGA mixtures was incomplete after 35 days of release testing, the amount released reached approximately 70% (Figure 36). Residual and released dexamethasone summed up to 97.2% ± 1.7% for all 752S/ 502 implants and 96.9% ± 1.3% for 752S/ 503H combination implants. While brimonidine release for 752S/ 502 implants was complete after 14 days, 752S/ 503H implants release brimonidine within 21 days. Regarding the combination implants with only one PLGA grade, it appeared that the lag phase was influenced by the PLGA L:G ratio and the release phase by the PLGA molecular weight, since implants containing 503H released both drugs slightly slower.

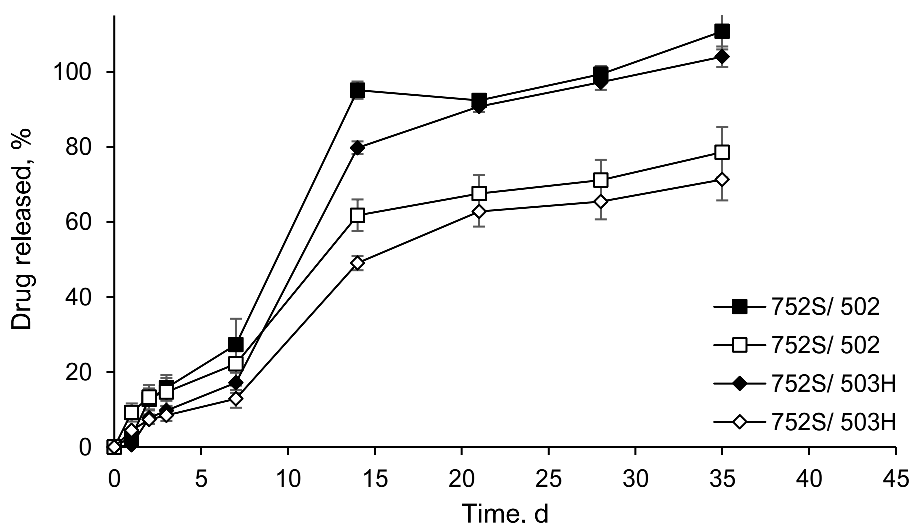

Figure 36 Brimonidine release (closed symbols) and dexamethasone release (open symbols) from combination implants with PLGA mixtures in saline.

Since the reason for an incomplete dexamethasone release was unclear, it was assumed that it is either an interaction of both drugs during the release test or due to the simultaneous incorporation into a PLGA matrix. Two single implants

containing either brimonidine or dexamethasone, both prepared with a PLGA mixture of 1:1 752S/ 502H, were therefore placed simultaneously into one vial during release test (Figure 37). Brimonidine release from the implant next to the dexamethasone implant was similar to the drug release of a single brimonidine 752S/ 502H implant (Figure 29). It was not affected by the presence of an implant releasing dexamethasone and vice versa (Figure 37). A lag phase of 9 days and a release phase of 11 days for the release of brimonidine and a 12-day lag phase followed by a 16-day release phase for the dexamethasone implant were according to the already release data of single implants (Table 12, Table 14). The reason for an incomplete dexamethasone release from combination implants was therefore not simultaneous release of both drugs and could be an interaction between both drugs and/ or PLGA during hot-melt extrusion. Unfortunately, this interaction was not further investigated in this work.

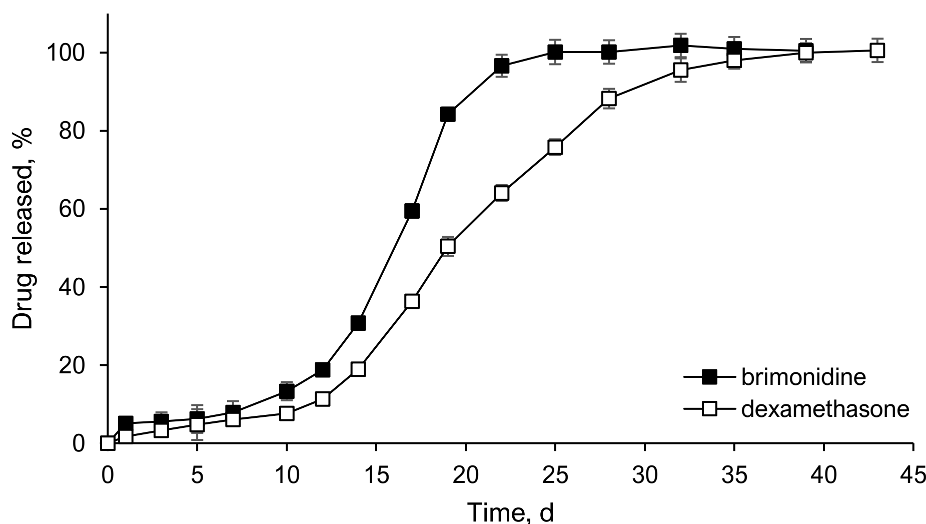

Figure 37 Simultaneous release test of single brimonidine 752S/ 502H and dexamethasone 752S/ 502H implants in one vial.

Conclusions

Although it was assumed, deriving from the results of chapter 3.1 and 3.2, that the incorporation of dexamethasone and brimonidine in the same PLGA matrix would not lead to an exact simultaneous release of both drugs, it was still the simplest way to combine both drugs within one implant. Using a mixture of PLGA grades, combination implants prepared with 752S/ 502 or 752S/ 503H showed promising results. Unfortunately, dexamethasone release from all combination implants prepared was incomplete after 35 days of release testing and implants were not completely degraded. Released and residual dexamethasone content summed up to 95% - 102%. When accelerating PLGA degradation with PBS pH 12 as release medium, dexamethasone and brimonidine release were complete after 7 days. The simultaneous drug release testing of single dexamethasone and brimonidine implants showed that both drugs did not affect each other's release. It was hence assumed that either a drug – drug or drug – drug – PLGA interaction occur during the preparation of combination implants with hot-melt extrusion but was not further investigated and should be considered in future projects.

Nevertheless, the combination of dexamethasone and brimonidine in a single biodegradable implant could be achieved through a co-extrusion process, which future projects should focus on.

3.4 Accelerated release of dexamethasone implants

Release tests for the biodegradable implants investigated in the previous chapters lasted for several weeks, depending on the PLGA grade and DDS properties e.g., drug loading, used for the formulation. Implants prepared with 752S, and a drug loading of 50% dexamethasone required 12 weeks for release testing. In order to obtain valuable information in a shorter amount of time, a reliable accelerated release test method was investigated and established. Since drug release from biodegradable implants was controlled by PLGA degradation, its acceleration was desired. This could be achieved by either a release test at elevated temperature or by adjusting the pH of the release medium [148]. PLGA degradation, i.e., ester cleavage by hydrolysis, could be acid- or base-catalyzed [149]. Therefore, dexamethasone release from implants with 50% drug loading and PLGA 502H or 502 was investigated in terms of temperature and release medium pH.

3.4.1 Influence of temperature and pH on dexamethasone release

Release tests of biodegradable implants at elevated temperatures resulted in an accelerated dexamethasone release from PLGA 502H implants with 50% drug loading. While 32 days were required to release dexamethasone in saline at 37 °C, it took only 14 days to achieve complete release at an elevated temperature of 45 °C from the 502H implants (Figure 38). Furthermore, drug release accelerated to only 3 days when using a release test temperature of 65 °C. Drug release was accelerated due to increased molecular and polymer mobility at temperatures above T_g (glass transition temperature) and release curves changed from the typical triphasic to a biphasic release without lag phase. Complete dexamethasone release was found to be 106.29% ± 2.8% and 100.82% ± 1.9% for implant release tests at 65°C in saline and PBS pH2, respectively. At a standard release test temperature of 37 °C, another way to catalyze PLGA degradation during measurement thus accelerating drug release was to catalyze it by using an acidic or basic release medium, thereby enhancing

ester cleavage of lactide to glycolide (L:G) bonds. Although PLGA degradation is known for an acidic autocatalytic effect thus assuming that an acidic pH of the release medium would enhance the degradation of these biodegradable implants, dexamethasone release from 502H implants was slightly slower when using PBS pH 2 (USP) compared to dexamethasone release in saline at temperatures of 37 °C and 45 °C (Figure 38). Since the microenvironmental pH at the autocatalytic PLGA degradation was found to be as low as pH 2 [8], lowering the pH of the release medium was not enhancing PLGA degradation.

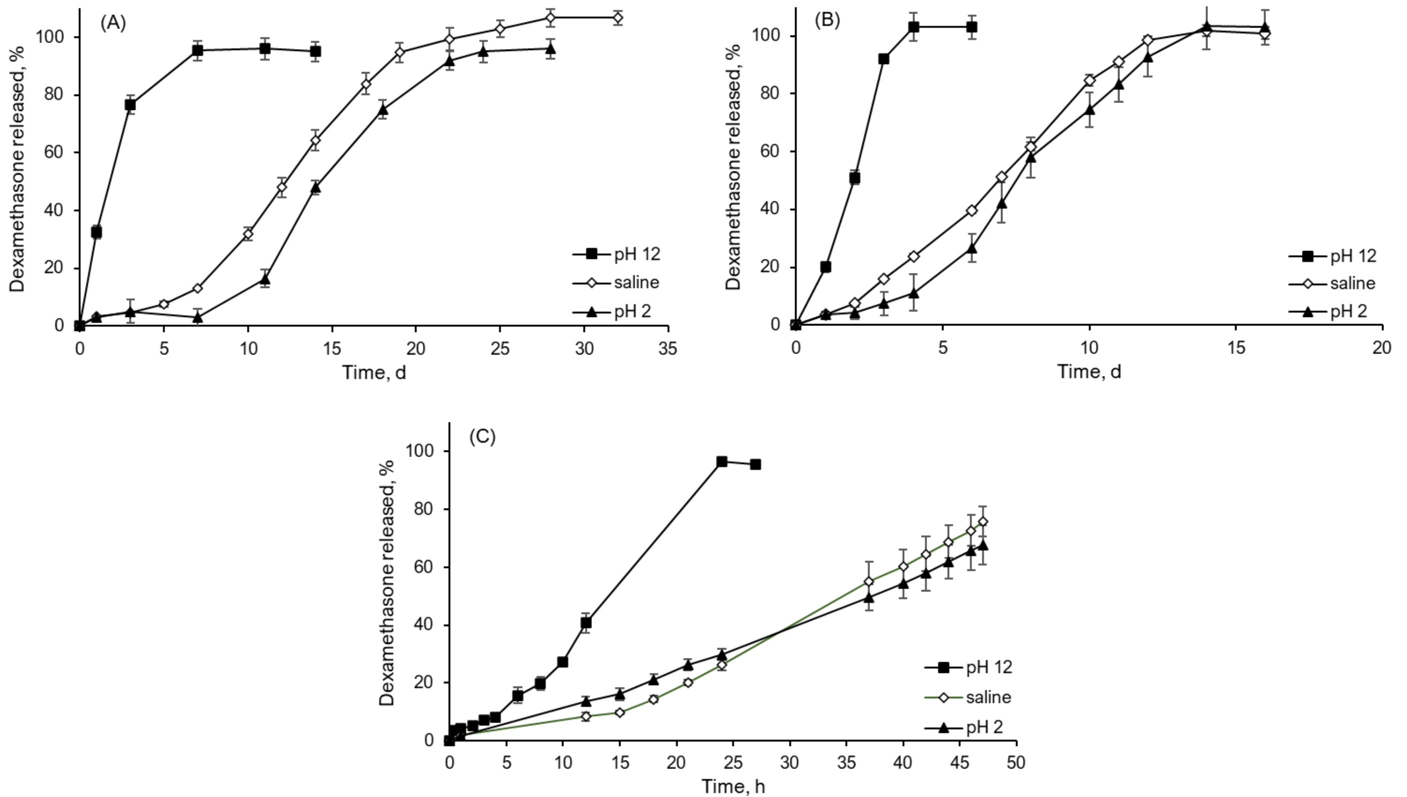

Figure 38 Influence of release medium pH on dexamethasone release from PLGA 502H implants with 50% drug loading at release test temperatures of (A) 37 °C, (B) 45 °C and (C) 65 °C.

At a release test temperature of 65 °C, dexamethasone release in saline was similar to the release in PBS pH 2. Only the use of a basic PBS pH 12 (USP) resulted in an accelerated PLGA degradation thus dexamethasone release. The implants directly started to release the drug without a lag phase and were completely degraded after 10 days, 4 days, and 24 hours of release testing at temperatures of 37 °C, 45 °C, and 65 °C, respectively. Under basic conditions, PLGA degradation and thus dexamethasone release from biodegradable implants begins directly without any lag phase [79]. Unfortunately, the lag phase is an important piece information that might get overlooked when using PBS pH 12 (Figure 39).

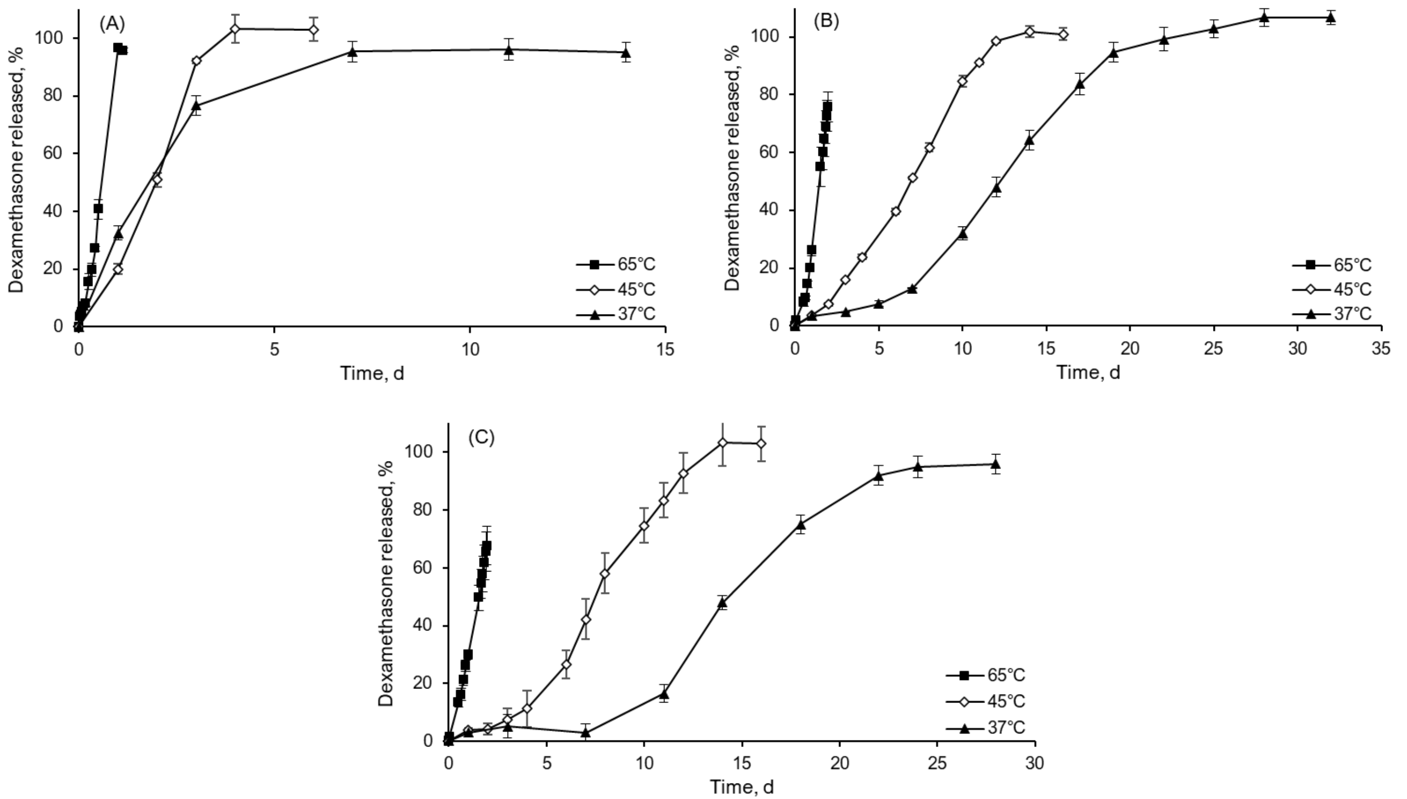

Figure 39 Influence of release test temperature on dexamethasone release from PLGA 502H implants with 50% drug loading in (A) PBS pH 12 (USP), (B) saline, and (C) PBS pH 2 (USP).

Based on Figure 39 zero order release kinetic constants for dexamethasone release after the lag phase were calculated from the slopes of drug release (cumulative %) versus release time (days) for PLGA 502H implants in PBS pH 12, saline, and PBS pH 2 and are presented in Table 15.

Table 15 Calculated release constants of dexamethasone release from PLGA 502H implants in PBS pH 12, saline, and PBS pH 2 at different release test temperatures.

	Release constant k, $days^{-1}$		
Temperature, °C	pH 12	saline	pH 2
37	25.01	6.98	6.36
45	36.01	9.77	10.49
65	108.17	50.12	37.79

The data was then plotted according to the Arrhenius equation to determine a numerical relationship between dexamethasone release rates at different temperatures (Figure 40). The logarithm of the release constant k versus 1/T, where T is the absolute temperature, resulted in a linear correlation of dexamethasone release rates at 37 °C, 45 °C, and 65 °C for all release media used. The goodness of fit (R^2) were 0.99, 0.98, and 0.99 for release tests in PBS pH 12, saline, and PBS pH 2, respectively. Therefore, accelerated release tests of biodegradable implants at elevated temperatures are suitable to obtain release data with good correlation to real-time release. Nevertheless, the use of PBS pH 12 is not recommended since the information of lag phases at drug releases from PLGA implants is lost even at standard temperatures of 37 °C.

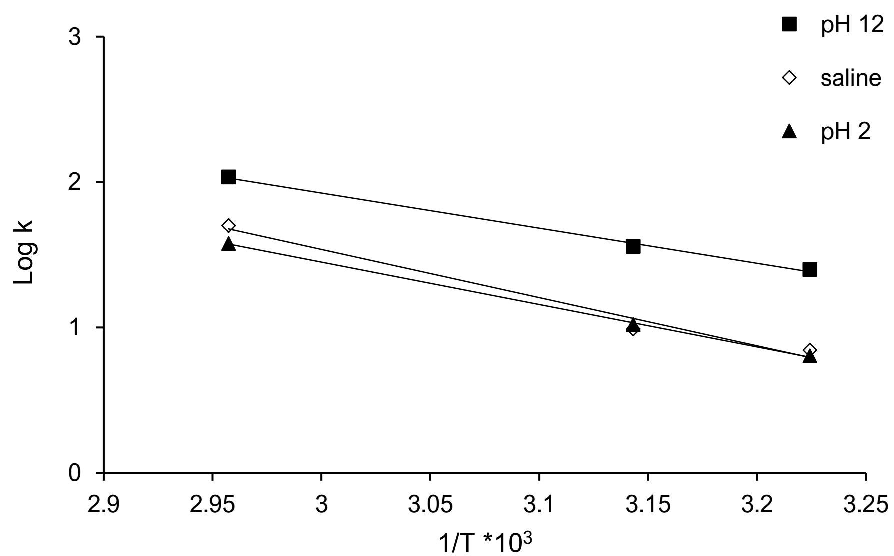

Figure 40 Arrhenius plot of the rate of dexamethasone release from PLGA 502H implants with 50% drug loading as a function of temperature at 37 °C, 45 °C, and 65 °C in different release media.

Ester end PLGA 502 implants

Similar to 502H implants under accelerated release test conditions, dexamethasone release from ester end implants was already affected by the increase of temperature at the release test, as seen for drug release in saline (Figure 41). Within 2 weeks, dexamethasone was already completely released. Again, the use of PBS pH 12 resulted in a faster release of dexamethasone without lag phase and within 1 week. Although no lag phase was observed for implants in PBS pH 12, 502 implants released dexamethasone more slowly than 502H implants. This difference could also be seen in saline and PBS pH 2 at 37° C as well as at 45 °C. Because release tests at 45 °C for biodegradable implants took half the time when performed at 37 °C, this is a suitable method to obtain fast results of drug releases and to compare different formulation approaches.

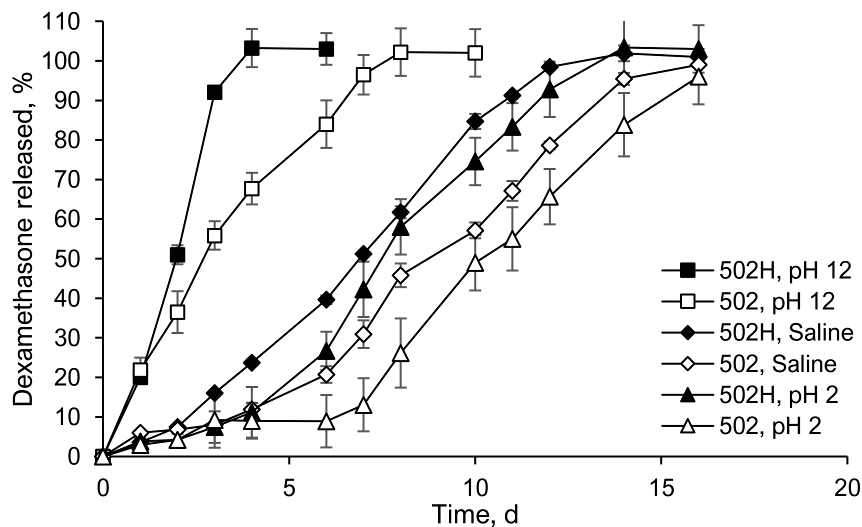

Figure 41 Comparison of acid end PLGA and ester end PLGA release under accelerated conditions at 45 °C.

3.4.2 Accelerated release tests applied to proof-of-concept formulation

After investigating release medium of different pH and elevated temperatures to obtain a reliable accelerated release test method, following parameters were chosen for release tests. Although the use of PBS pH 12 resulted in the fastest PLGA degradation thus drug release, release curves lacked the information of lag phases for all implants at 37 °C, 45 °C, and 65 °C. Since an acidic microenvironment during PLGA degradation autocatalyzed its erosion, dexamethasone release from implants in PBS pH 2 was not further accelerated *in vitro*. The most reliable accelerated method was at an elevated temperature of 45 °C using saline as release medium.

Applied to 50% dexamethasone implants developed with a 3:1 mixture of PLGA 502H and 502, drug release was accelerated from 4 weeks to 2 weeks for release tests at 37 °C and 45 °C, respectively (Figure 42). As with other formulations tested before, burst, lag phase and release phase could be observed at elevated temperature and can be compared to real-time release, making the accelerated release test at 45 °C a method to obtain drug release data in half the time required.

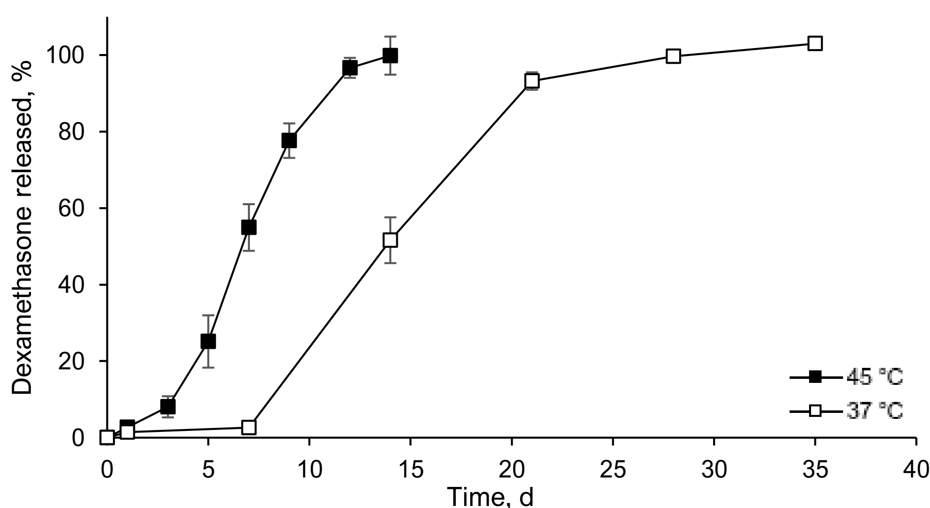

Figure 42 Comparison of dexamethasone release from release tests at 37 °C and accelerated release at 45 °C from 502H/ 502 (3:1) implants.

3.4.3 *Accelerated release of brimonidine base implants*

Brimonidine release at accelerated release test temperature of 45 °C was almost three times faster than at standard temperature of 37 °C (Figure 43). Correlation coefficients of 0.95 and 0.99 for drug releases at 37 °C and 45 °C were observed from brimonidine implants with 752S/ 502H and 752S/ 502, respectively. Furthermore, at 45 °C it was already observed that brimonidine release from implants with the PLGA mixture of 752S/ 502 had a longer lag and release phase compared to 752S/ 502H, indicating that differences in formulations can already be evaluated after drug release testing under accelerated conditions.

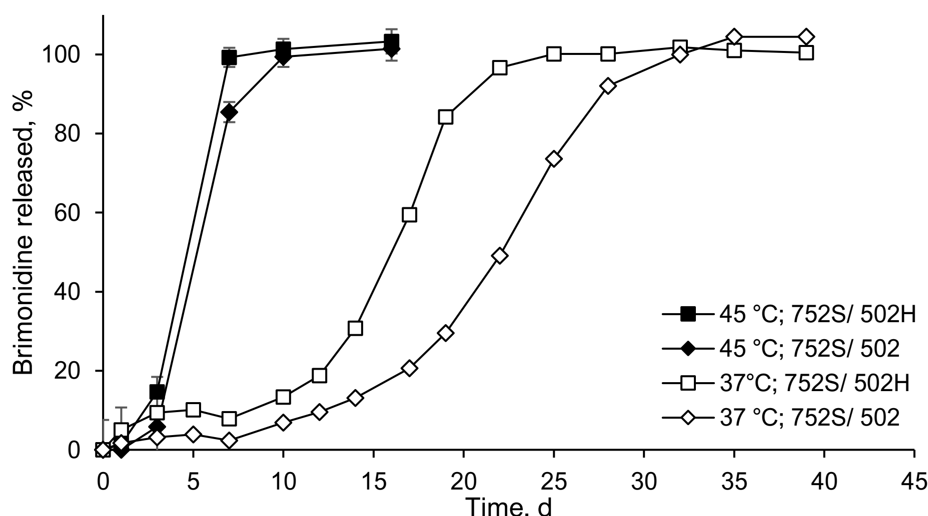

Figure 43 Brimonidine release from implants with PLGA mixtures of 752S/ 502H and 752S/ 502 at 37 °C and 45 °C.

3.4.4 Conclusions

The change of the release medium from aqueous sodium chloride (0.9%, saline) to phosphate buffered saline pH 2 (PBS, USP) did not accelerate PLGA degradation and thus dexamethasone release from the implants. Since the microenvironmental pH also decreases to approximately pH 2 during the autocatalytic degradation of PLGA, a decrease in release medium pH does not improve the drug release to the desired acceleration. The use of PBS pH 12 (USP) provides an accelerated PLGA degradation through basic catalyzed hydrolysis. PLGA implants degraded completely after 10 days, 4 days, or 24 hours when PBS pH 12 was used at temperatures of 37 °C, 45 °C, and 65 °C, respectively. Unfortunately, lag phases of dexamethasone release could not be observed due to the rapid PLGA degradation. This makes it difficult to compare different formulations or estimate drug releases under standard release test conditions.

The best conditions for accelerated release tests of dexamethasone implants was an elevated temperature of 45 °C in the standard release medium, saline. By increasing the temperature at, dexamethasone release from PLGA implants took only half the time of drug release at 37 °C while still be able to observe differences between formulations. Linear correlations with the Arrhenius plot proved that drug release profiles can be generated at elevated release temperatures and transferred to real-time release.

3.5 Influence of processing conditions, sterilization and storage on dexamethasone release

3.5.1 Influence of hot-melt extrusion method and process parameters on dexamethasone release from PLGA implants

In addition to PLGA grades themselves, many factors influence the drug release from biodegradable PLGA implants. One major is the preparation method. There are different types of hot-melt extrusions, like ram extrusion, hot molding, single-screw extrusion, or double-screw extrusion. In this work three different methods were used to prepare PLGA implants.

First was the syringe-die method, a lab scale ram extrusion adapted from Ghalanbor et al. [29], which was a device that required only a small amount of approximately 0.2 – 1 g drug/ PLGA blend to extrude filaments in a short time. The disadvantage of the method is that not only the blend required a good homogenization before extrusion, but also an in-process mixing was not possible since the molten blend inside the syringe during extrusion was pressed through the die with a piston. The second method used was the hot-melt extrusion with the Haake MiniLab Compounder, which was a twin-screw extruder with two co-rotating, conical screws. The third extrusion method was the hot-melt extrusion with a ThreeTec ZE9 twin-screw extruder, which was equipped two modular screws and three individually warmed heating zones. While both extruders required a minimum of 5.0 g of the blend, the ThreeTec ZE9 incorporated the drug slightly better into the PLGA matrix due to the use of modular screws, which could be equipped with alternating conveying and kneading screw modules.

Comparing biodegradable 502H/ 502 (3:1) implants containing 50% dexamethasone prepared with the Haake MiniLab Compounder and ThreeTec ZE9, the largest difference in dexamethasone release was observed at the burst phase (Figure 44). While implants showed a higher burst release of approximately 7% – 8% when extruded with the Haake MiniLab, implants from the ThreeTec ZE9 showed only 1 – 2% burst, indicating that dexamethasone was more present on the implant surface thus was incompletely incorporated while extrusion with the Haake MiniLab. During the lag phase, no dexamethasone was released for implants prepared with the ThreeTec ZE9 while implants from the Haake MiniLab released dexamethasone to an amount of approximately 10% within 7 days. Since more drug was available incorporated in implants from the ThreeTec ZE9 thus further accelerating dexamethasone release at the start of PLGA degradation by pore formation, the release phase was approximately 2 days shorter than the dexamethasone release phase from implants prepared with the Haake MiniLab.

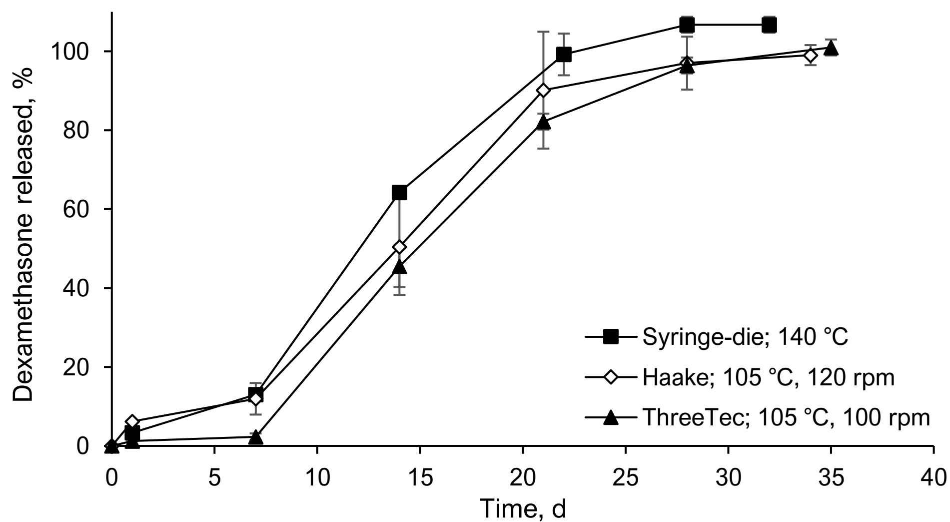

Figure 44 Influence of extrusion method on dexamethasone release from PLGA 502H/ 502 (3:1) implants.

The preparation of dexamethasone implants with the syringe-die method affected the drug release similar to the preparation of implants with the Haake MiniLab when it comes to burst and lag phase. Although for implants with PLGA 502H/ 502 the burst phase was only approximately 4%, other formulations prepared with the syringe-die method often showed higher bursts after 1 day of release testing (chapter 3.1.1, chapter 3.2.1). During the lag phase, dexamethasone was released to approximately 10%. The release phases for implants prepared with the ThreeTec ZE9 as well as the syringe-die method were comparable, making the latter a reliable method for screening PLGA formulations.

Not only the extruder used to prepare biodegradable implants but also extrusion parameters itself influence the final DDS thus drug release from the implants. 502H/ 502 implants prepared with the Haake MiniLab at 105 °C and a screw speed of 120 rpm released dexamethasone slightly faster than implants prepared at 85 °C and a screw speed of 30 rpm (Figure 45). Hot-melt extrusion with the Haake MiniLab at higher extrusion parameters results in an uneven surface of the extrudates, known as the “sharkskin effect” (Figure 46) [150]. The increased surface are led the implant to a slightly faster PLGA degradation thus dexamethasone release.

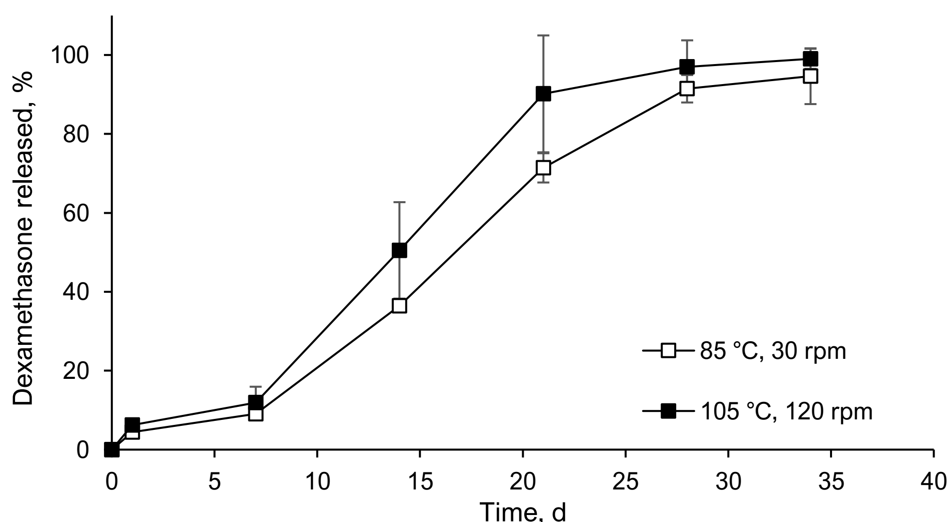

Figure 45 Influence of extrusion parameters on dexamethasone release from 502H/ 502 (3:1) implants, prepared with Haake MiniLab.

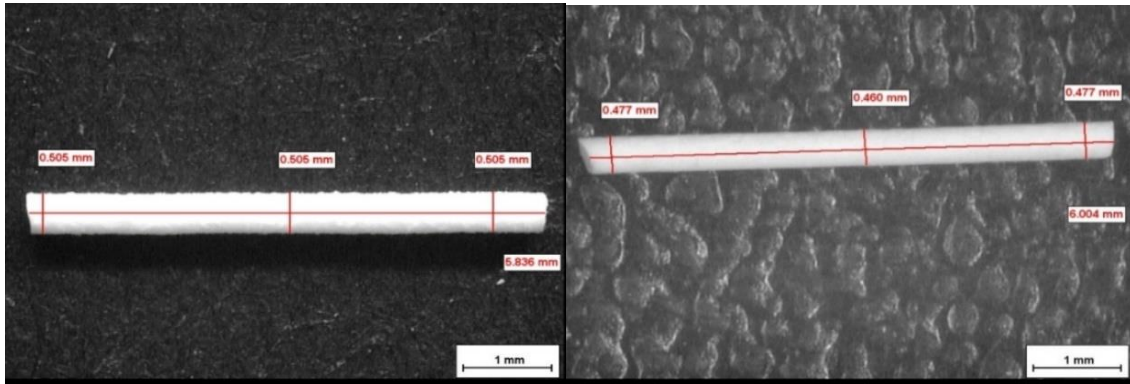

Figure 46 Morphological appearance of dexamethasone implants prepared at 105 °C and 120 rpm (left, sharkskin effect), and at 85 °C and 30 rpm (right) with Haake MiniLab.

3.5.2 Influence of implant sterilization and storage on dexamethasone release

Since biodegradable intravitreal implants have to be sterile and one part of their preparation is sterilization by gamma irradiation after hot-melt extrusion and cutting. Furthermore, after filling the applicators and packaging, DDS have to meet their release specifications for years to meet a proper shelf life. Dexamethasone release from PLGA implants was therefore investigated in terms of implant sterilization by gamma irradiation and storage time at room temperature in a silica gel desiccator.

Compared to dexamethasone release after hot-melt extrusion, 502H/ 502 implants with 50% dexamethasone showed an increased burst release from 4% to approximately 10% after 1 day of release testing for implants prepared with Haake MiniLab at 85 °C and a screw speed of 30 rpm when stored for three years (Figure 47). Gamma irradiated and 3 years stored implants had a similar release curve. During the lag phase drug was released to 20% compared to only 10% after extrusion. Although dexamethasone is slightly released during the lag phase, the characteristic sigmoidal release curve is observable. Nevertheless, a 3-year storage time might be not acceptable for implants prepared with this extruder and extrusion parameters since the dexamethasone release is almost 7 days faster than the release directly after hot-melt extrusion.

After 3 years of storage and gamma irradiation, 502H/ 502 implants with 50% dexamethasone prepared with the Haake MiniLab at 105 °C and a screw speed of 100 °C showed a burst release of approximately 10% compared to 4% burst release after hot-melt extrusion. The following lag phase was only slightly observable for sterilized implants. For both implants, dexamethasone was released during that phase to approximately 20% and 30% for stored and sterilized implants, respectively. Again, a 3-year shelf life would not be acceptable for implants prepared with this extruder and extrusion parameters since the

release curves differ from dexamethasone release directly after hot-melt extrusion.

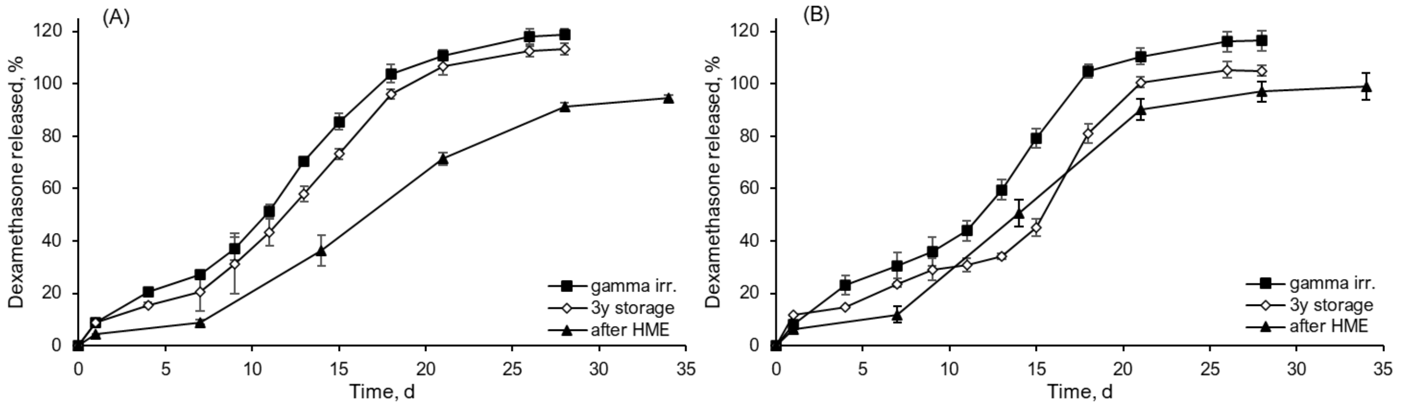

Figure 47 Influence of 3 years storage and gamma irradiation on dexamethasone release from implants prepared with Haake MiniLab at (A) 85 °C and 30 rpm, and (B) 105 °C and 120 rpm.

For implants containing 20% or 40% dexamethasone and a mixture of 3:1 502H/ 502 prepared with the ThreeTec ZE9 gamma irradiation and a 2-year storage resulted in a comparable release curve to dexamethasone release after hot-melt extrusion (Figure 48). Although a slight dexamethasone release during the lag phase of approximately 10% was observed, the typical triphasic release from PLGA implants was still given and comparable to the zero-point data. Sterilization of implants did not affect dexamethasone release since release curves of stored and sterilized implants were similar. Although drug release from implants containing 20% dexamethasone had a slightly faster release compared to implants after extrusion, a storage time of 2 years with gamma irradiated implants is for both drug loadings is reasonable and acceptable since the dexamethasone release was similar from implants directly after hot-melt extrusion.

The same was observed for 502H/ 502 (3:1) implants containing 50% and 60% dexamethasone prepared with the ThreeTec ZE9. Although a slight dexamethasone release could be seen during the lag phase, release curves did not differ from those of implants directly after hot-melt extrusion, indicating that gamma irradiation and a 2-year storage does not influence the drug release of implants prepared with this extruder and extrusion parameters.

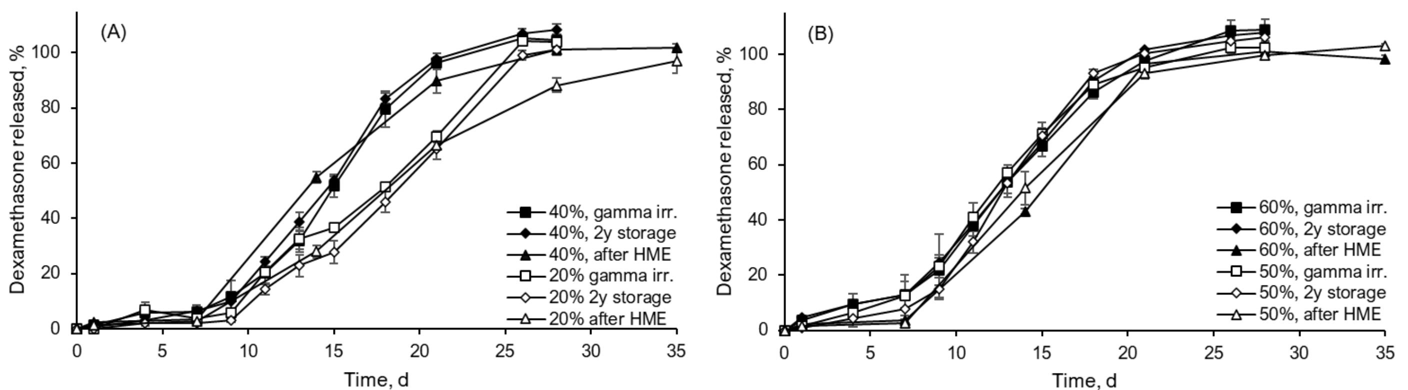

Figure 48 Influence of 2 years storage and gamma irradiation on dexamethasone release from implants prepared with ThreeTec ZE9 at 100 °C and 100 rpm.

Gamma irradiation on dexamethasone implants with PLGA mixtures of 752S/ 502H and 752S/ 503H did not influence the drug release compared to dexamethasone release from implants directly after hot-melt extrusion (Figure 49). Although it was assumed that the same could be true for all formulations, gamma irradiation decreased the lag time of dexamethasone release from implants prepared with 752S/ 502 from 22 days to 18 days.

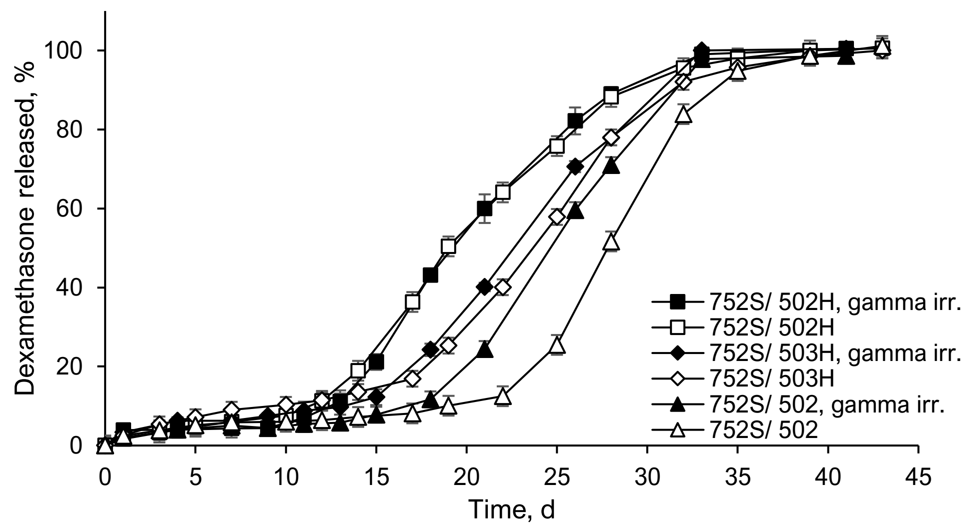

Figure 49 Influence of gamma irradiation on dexamethasone release from implants with PLGA mixtures prepared with the ThreeTec ZE9.

3.6 Mechanical properties of biodegradable implants

Sufficient mechanical properties of biodegradable implants for parenteral use are important for manufacturing processes regarding filling and packaging processes as well as in-use performance. They are needed for cutting the implants to the proper length, loading implants into an applicator, and injecting to the site of action (respectively ejecting from applicators). Implants analyzed by a three-point bending test with a texture analyzer TA.XTplus (Figure 50). For each measurement an implant was placed over a gap and force was applied with a probe to the implants center until break.

The aim of this study was to characterize the mechanical properties of biodegradable implants in terms of hardness (peak force at failure), flexibility (elongation), toughness (slope), and energy required until break (area under the curve, AUC) (Figure 51). The influence of drug loading, molecular weight of polymer matrix, implant dimensions such as length and diameter and moisture content on mechanical properties were determined.

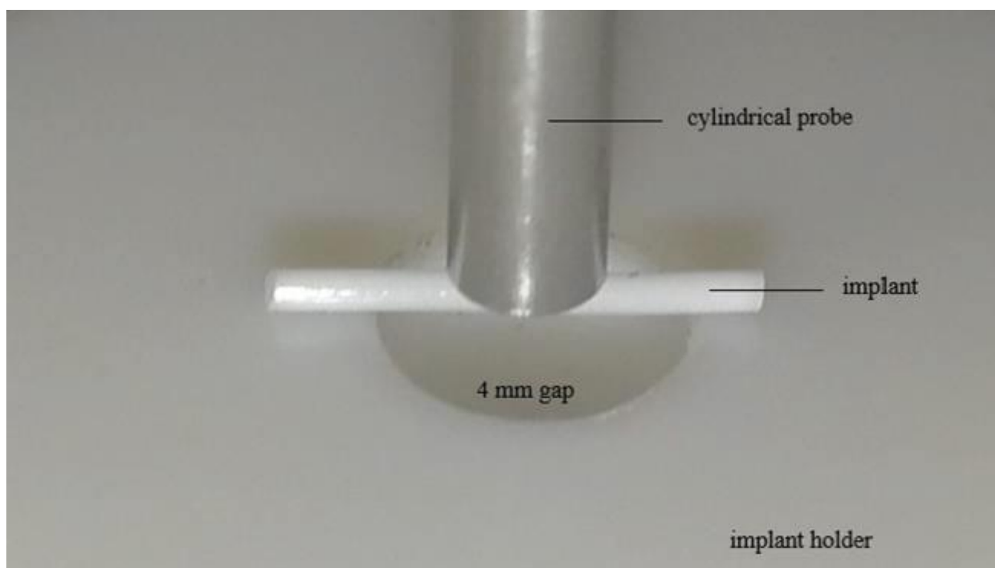

Figure 50 Three-point bending test measurement setup to determine mechanical properties of PLGA implants.

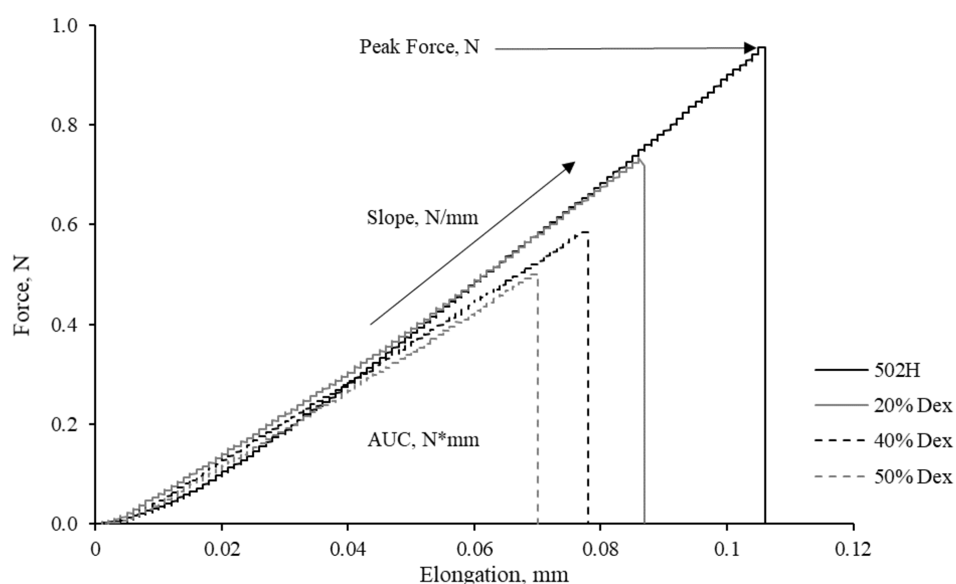

Figure 51 Exemplary measurements of mechanical properties of PLGA implants.

3.6.1 Influence of probes and gap size on mechanical properties test method

A three- point bending test was chosen to avoid the need for complex apparatus structures. Furthermore, while it was possible to calculate the tensile strengths of PLGA implants using the chosen method ([122]), this was not required as there was no need to compare DDS of different shape. Since implant dimensions were kept constant, the correlation of directly measured physical parameters to formulation contents was investigated to find a simple method for quality control of PLGA implants.

To find a measurement setup, dexamethasone implants (50 % drug load, PLGA 502H/ 502 [3:1]) were placed over a gap of 4 mm and 12 mm and force was applied to the implants center with cylindrical probes of different diameter (Table 16). Implant dimensions were approximately 0.5 mm in diameter and 18 mm in length (to overcome the gap of 12 mm).

Table 16 Influence of gap and probe size on mechanical property parameters (n = 10).

Gap size, mm	Cyl. diameter, mm	Peak Force, N	Elongation, µm	Slope, N/mm	AUC, N*mm
4	2	0.491 ±0.036	65 ±4	7.38 ±0.35	0.016 ±0.002
	3	0.651 ±0.041	68 ±3	9.51 ±0.69	0.021 ±0.002
12	2	0.133 ±0.006	261 ±10	0.50 ±0.04	0.017 ±0.001
	3	0.151 ±0.011	216 ±17	0.60 ±0.05	0.014 ±0.002
	6	0.187 ±0.013	198 ±14	0.93 ±0.04	0.018 ±0.001
Correlation (12 mm gap)		0.99	-0.87	0.99	0.51

With increasing cylinder diameter, the peak force at failure also increased with a correlation coefficient of 0.99 as well as the slope of the force/ elongation curves with a correlation of 1.00 (Figure 52, Table 16). In contrast, elongation decreased with a correlation of 0.87. Implants broke earlier with larger cylinder diameters. Since peak forces and slopes increase and elongation decreases with larger cylinder diameters, AUC remains the same over all measurements since only setup parameters were changed and the implant formulation as well as implant dimensions were kept constant.

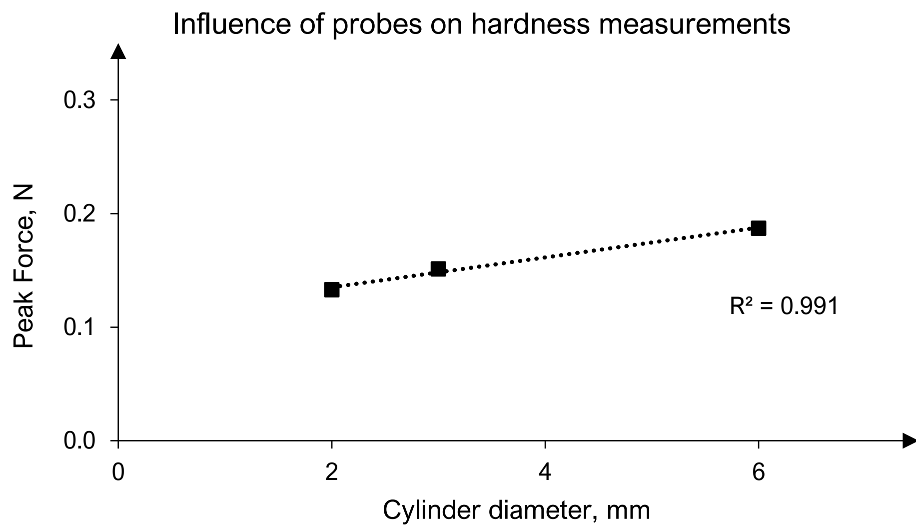

Figure 52 Influence of probe diameter on peak force of 18 mm PLGA implants.

3.6.2 Influence of implant size on mechanical properties of dexamethasone implants

Not only the setup but also the difference in shape of implants influenced the measurements of mechanical properties of PLGA implants. A batch of dexamethasone implants of approximately 0.5 mm in diameter with 60% drug loading and a PLGA mixture of 502H/ 502 in a ratio of 3:1 was extruded with a twin-screw extruder and cut to 6 mm, 12 mm, 18 mm, and 24 mm. Although the same formulation was analyzed, the difference in implant length was measurable for all parameters but one. With increasing length, peak force at break and AUC also increased, while elongation decreased, and the slope remained the same. Because the force applied to the implant center was distributed through a larger PLGA network with longer implants, a higher force was required to break them. The unchanging slope may indicate the measurement of the same formulation (Table 17).

Table 17 Influence of implant length on mechanical properties of dexamethasone implants (60% drug loading, 502H/ 502 (3:1)) (n = 5).

Implant length, mm	Peak Force, N	Elongation, µm	Slope, N/mm	AUC, N*mm
6	0.39 ±0.02	63 ±6	6.47 ±0.26	0.011 ±0.001
12	0.40 ±0.02	64 ±3	6.34 ±0.40	0.012 ±0.001
18	0.42 ±0.05	67 ±6	6.29 ±0.39	0.013 ±0.001
24	0.45 ±0.02	65 ±2	6.95 ±0.57	0.014 ±0.002
Correlation coefficient	0.99	0.55	0.59	0.97

To determine the change of mechanical properties with respect to implant diameter, three placebo batches of PLGA 502H were prepared with diameters of approximately 0.5 mm, 1.0 mm, and 1.5 mm.

In contrast to the change in implant length, in which the axial force distribution was changed, an increase in the implant diameter led to a higher radial force distribution during the measurements. Therefore, all parameters increased with increasing diameter of 502H implants in good correlation (Table 18).

Table 18 Influence of implant diameter on mechanical properties of PLGA implants (n = 5).

Implant diameter, mm	Peak Force, N	Elongation, µm	Slope, N/mm	AUC, N*mm
0.5	0.89 ±0.013	110 ±7	8.88 ±0.74	0.09 ±0.007
1.0	2.94 ±0.11	140 ±13	22.05 ±1.15	0.19 ±0.021
1.25	6.01 ±0.31	233 ±16	25.74 ±0.51	0.66 ±0.023
Correlation coefficient	0.95	0.89	0.99	0.85

As the developed biodegradable implants only had a length of approximately 6 mm, the gap size of 4 mm was chosen for measurements. A cylindrical probe with a 2 mm diameter was also preferred to the 3 mm cylinder, as the latter could cause problems with the measurements due to the lack of clearance between probe and the gap wall. All measurements in the following chapters were performed with the chosen setup parameters and implants of approximately 0.5 mm in diameter and 6 mm in length.

3.6.3 *Influence of drug loading on mechanical properties of dexamethasone implants*

Consistent negative correlations between drug loading and measured parameters confirm the assumption that an increase in drug loading results in an decrease in hardness, flexibility, and toughness of implants (Table 19, Figure 53).

The more drug loaded into the PLGA matrix, the more brittle the implants became (Figure 53). This must be considered when designing a medical device such as an applicator, since an implant with high drug load would hence be more likely to break at packaging or injection (respectively ejection from the applicator). Furthermore, the measurement of all four parameters was a suitable tool in quality control of biodegradable implants with respect to drug loading.

Table 19 Influence of drug loading on mechanical properties of PLGA 502H implants (n = 5).

Drug loading, %	Peak Force, N	Elongation, µm	Slope, N/mm	AUC, N*mm
0	0.89 ±0.13	110 ±7	8.88 ±0.74	0.09 ±0.007
20	0.70 ±0.07	80 ±4	8.36 ±0.43	0.06 ±0.004
40	0.58 ±0.04	70 ±5	7.80 ±0.30	0.04 ±0.003
50	0.49 ±0.05	70 ±4	7.37 ±0.56	0.03 ±0.002
60	0.41 ±0.06	63 ±5	7.49 ±0.21	0.012 ±0.001
Correlation coefficient	-0.99	-0.94	-0.98	-1.00

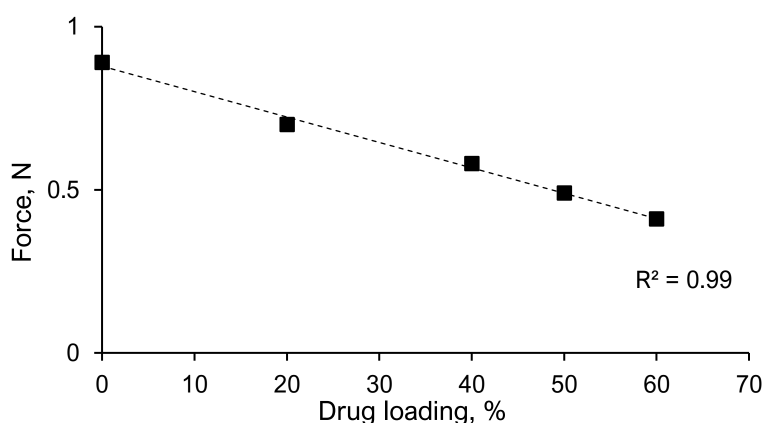

Figure 53 Influence of drug loading on hardness of dexamethasone 502H implants.

3.6.4 Influence of polymeric molecular weight on mechanical properties of PLGA implants

Placebo batches of different molecular weight (PLGAs 502H, 503H and 504H) were prepared by hot- melt extrusion, cut to 6 mm length, and analysed using the method described before. Inherent viscosities of Resomer® 502H, 503H, and 504H were 0.16 – 0.24 dL/g (7 – 17 kDa), 0.32 – 0.44 dL/g (24 – 38 kDa) and 0.44 – 0.60 dL/g (38 – 54 kDa), respectively.

As the molecular weight increased, the hardness and flexibility of implants also increased. Peak force, elongation and AUC were positively correlated with an increase in molecular weight (Table 20, Figure 54). Therefore, peak force, elongation and AUC should be considered as the main parameters to characterize different molecular weight polymer matrices.

Table 20 Influence of polymers MW on mechanical properties of PLGA implants (n = 5).

PLGA grade	Peak Force, N	Elongation, µm	Slope, N/mm	AUC, N*mm
502H	0.89 ±0.13	110 ±7	8.88 ±0.74	0.09 ±0.007
503H	1.79 ±0.30	610 ±151	3.26 ±0.34	0.61 ±0.05
504H	2.69 ±0.33	650 ±43	4.13 ±0.35	0.65 ±0.25
Correlation coefficient	1.00	0.90	-0.78	0.90

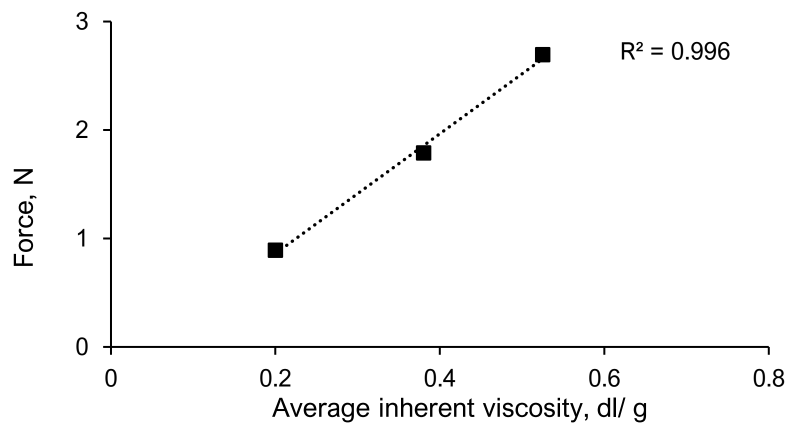

Figure 54 Influence of polymer molecular weight on hardness of placebo PLGA implants.

3.6.5 Influence of water content on mechanical properties of dexamethasone implants

Dexamethasone implants of 6 mm length were prepared by hot- melt extrusion and stored for 7 days in a silica gel desiccator, at 30% relative humidity (RH), and 75% RH. Implants stored at 30% RH and 75% RH reached plateau in fracture forces after 1 day and 7 days, respectively, indicating an equilibrium hydration (Figure 55). Relative water contents determined by Karl-Fischer titration were 0.9%, 2.9% and 5.6% for implants stored in a silica gel desiccator, at 30% RH and 75% RH, respectively.

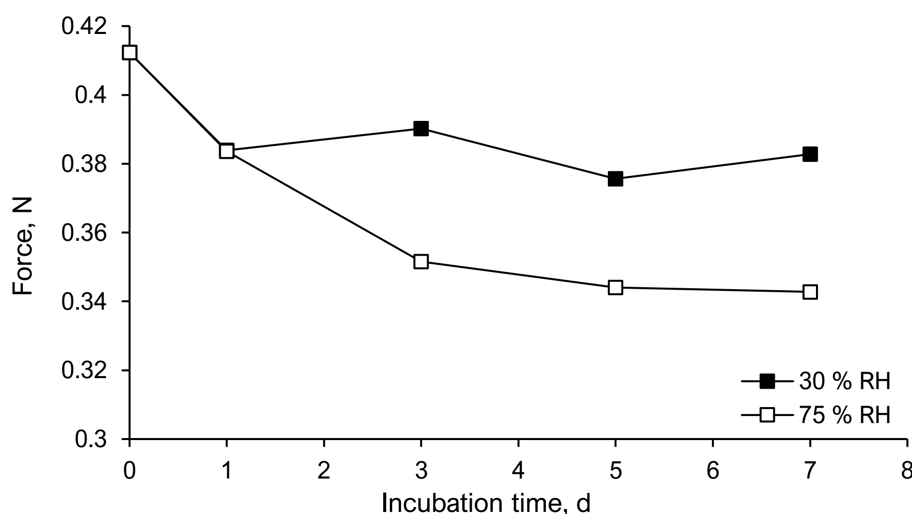

Figure 55 Peak force over hydration time of dexamethasone implants (60 % drug loading, PLGA 502H).

Contrary to the assumption that measured parameters increased with increasing moisture content, hardness, elongation, toughness, and AUC decreased with increasing hydration (Table 21). Instead of softening during hydration, dexamethasone implants became more brittle and less flexible, indicating a start of polymer degradation or pore forming.

Table 21 Influence of implant water content on mechanical properties of 60% dexamethasone 502H implants (n = 5).

Storage conditions	Water content, wt%	Peak Force, N	Elongation, µm	Slope, N/mm	AUC, N*mm
Silica gel desiccator	0.91	0.41 ±0.028	63 ±5	7.49 ±0.34	0.012 ±0.001
30% RH	2.88	0.38 ±0.021	60 ±4	6.32 ±0.14	0.010 ±0.001
75% RH	5.64	0.34 ±0.033	58 ±4	5.93 ±0.59	0.009 ±0.001
	Correlation coefficient	-1.00	-0.99	-0.93	-0.99

3.6.6 Conclusions

It could be shown that it is possible to measure and characterize the mechanical properties of biodegradable PLGA implants with a texture analyzer in terms of drug loading, polymer molecular weight, implant dimensions and moisture content by implementing an easy-to-use three-point bending test method. Especially peak forces, but also elongations, and AUC are sufficient parameters to describe differences in formulation and dimensional properties while the slope of the curves have no beneficial correlations in terms of molecular weight and length of PLGA implants. Without the necessity of a complex method setup or converting measured parameters into tensile strengths, this method could be a simple alternative for the quality control of biodegradable implant formulations.

4 Summary

The influence of three different extrusion methods as well as extrusion temperature and screw speed on drug release from PLGA-based implants were investigated. Comparing biodegradable implants prepared with the Haake MiniLab under extrusion parameters of 85 °C/ 30 rpm and 105 °C/ 120 rpm, an increase in processing temperature and screw speed resulted in extrudates of uneven surface with a so-called “sharkskin effect”. The increased surface area led to a higher burst release of drug and ultimately resulted in a shorter release phase due to an increased PLGA-degradation. Biodegradable implants prepared with the ThreeTec ZE9 at a similar extrusion temperature of 105 °C/ 100 rpm had a lower burst and an even surface morphology. Due to the absence of mixing elements in ram-extrusion processes, the syringe-die method had a poor drug incorporation and thus a high burst release. Nevertheless, drug release phases from implants prepared with the syringe-die method were comparable to those of implants prepared with the ThreeTec ZE9, making it an attractive tool for formulation screening due to low processing times, small amounts of formulation blends needed, and the comparability to implants prepared with the ThreeTec ZE9. Therefore, the syringe-die method as a screening tool for hot-melt extruded implants was utilized in 3.1 and 3.2.

For the establishment of an applicability map the influence on dexamethasone release from PLGA-based implants was investigated in terms of PLGA end groups, PLGA lactic acid to glycolic acid (L:G) ratio, polymer’s average molecular weight, and drug loading (chapter 3.1). Dexamethasone release from implants follows the typical drug release curve of PLGA-based drug delivery systems (DDS). A small burst release from excess drug on the implant’s surface, followed by a lag phase and the release phase, which is designated to the start of polymer degradation. Lag phases of dexamethasone release from implants prepared with 502H, 503H, and 502 were independent of the drug loading, while lag phases for dexamethasone release from 752S implants were influenced by drug loading. The

release time after the lag phase was shorter for 752S implants containing higher drug loadings. Plotting the release phase over the lag phase, dexamethasone release was visualized in an applicability map. This applicability map was successfully utilized to develop a biodegradable dexamethasone implant with a desired release consisting of a lag phase of maximum 7 days and a release phase of approximately 14 days. This could be achieved by the preparation of a dexamethasone implant with 50%/ 60% drug loading and a PLGA mixture of 502H/ 502 in a 3:1 ratio.

Next, a formulation of a biodegradable implant for the application of brimonidine base was developed. The requirement for the implant was that it released the active substance to the same extend as dexamethasone was released from the already developed implant in order to possibly enable a simultaneous injection. Brimonidine release from biodegradable PLGA implants was investigated in terms of PLGA end groups, polymer molecular weight, L:G ratio, and drug loading. Release data was again collected in an applicability map, containing lag phase plotted against release phase, to develop the desired release implant as previously with dexamethasone implants. However, a biodegradable implant was successfully developed that released brimonidine from PLGA implants with a 1:1 mixture of 752S/ 503H with a 3-day longer lag phase and almost the same release time compared to the developed dexamethasone implant.

Combination implants with both drugs released brimonidine within several days, but dexamethasone release was incomplete for all formulations. Since simultaneous release from single dexamethasone and brimonidine implants was complete for both drugs, a drug – drug or drug – drug – PLGA interaction was assumed but not further investigated. Nevertheless, a combination implant containing both drugs could be possible using alternative preparation methods like co-extrusion.

In order to achieve a reliable release test method to obtain drug release curves from biodegradable implants after a short time period, an accelerated release test method was established by investigating the influence of temperature and pH of

the release medium on dexamethasone release from PLGA implants. The change of the release medium from aqueous sodium chloride (0.9%, saline) to phosphate buffered saline pH 2 (PBS, USP) did not accelerate PLGA degradation and thus dexamethasone release from the implants. The use of PBS pH 12 (USP) provides an accelerated PLGA degradation through basic catalyzed hydrolysis. PLGA implants degraded completely at temperatures of 37 °C, 45 °C, and 65 °C. Unfortunately, lag phases of dexamethasone release could not be observed at pH 12 due to the rapid PLGA degradation. This makes it difficult to compare different formulations or estimate drug releases under standard release test conditions. The best conditions for accelerated release tests of dexamethasone implants herein were at an elevated temperature of 45 °C in the standard release medium, saline. By increasing the temperature at release tests, dexamethasone release from PLGA implants took only half the time of drug release at 37 °C while still be able to observe differences between the formulations.

Dexamethasone release from 502H/ 502 implants was investigated in terms of implant sterilization by gamma irradiation and storage time at room temperature in a silica gel desiccator. The storage time of 2 years for implants prepared with the ThreeTec ZE9 resulted in a slight dexamethasone release during the lag phase but was declared as acceptable since the drug release was still similar to those of implants directly after HME. Dexamethasone release from implants prepared with the Haake MiniLab Compounder significantly changed after 3 years of storage. Although the typical sigmoidal release curve was still seen, the burst release increased, dexamethasone was released during the lag phase, and finally the release phase was decreased. Overall, a 2-years storage of gamma irradiated, PLGA-based dexamethasone implants was acceptable when it comes to drug release.

Ultimately, the mechanical properties of biodegradable PLGA implants were investigated in terms of drug loading, molecular weight of polymer matrix, implant dimensions such as length and diameter and moisture content. It was possible to measure and characterize the mechanical properties of biodegradable PLGA

implants with a texture analyzer in terms of drug loading, polymer molecular weight, implant dimensions and moisture content by implementing an easy-to-use three-point bending test method. Especially peak forces, but also elongations, and AUC are sufficient parameters to describe differences in formulation properties while the slope of the curves have no beneficial correlations in terms of molecular weight and length of PLGA implants. Without the necessity of a complex method setup or converting measured parameters into tensile strengths, this method could be a simple alternative for the quality control of biodegradable implant formulations.

5 Zusammenfassung

Der Einfluss von drei verschiedenen Extrusionsmethoden sowie Extrusionstemperatur und Schneckengeschwindigkeit auf die Wirkstofffreisetzung aus PLGA-basierten Implantaten wurde untersucht. Beim Vergleich biologisch abbaubarer Implantate, die mit dem Haake MiniLab unter Extrusionsparametern von 85 °C/ 30 U/min und 105 °C/ 120 U/min hergestellt wurden, führte eine Erhöhung der Prozesstemperatur und der Schneckengeschwindigkeit zu Extrudaten mit unebener Oberfläche und einem sogenannten „Haifischhauteffekt". Die vergrößerte Oberfläche führte zu einer höheren Burst-Freisetzung und letztendlich zu einer kürzeren Freisetzungsphase aufgrund eines erhöhten PLGA-Abbaus. Biologisch abbaubare Implantate, die mit dem ThreeTec ZE9 bei ähnlichen Extrusionsparametern von 105 °C/ 100 U/min hergestellt wurden, hatten einen geringeren Burst und eine gleichmäßige Oberflächenmorphologie. Aufgrund des Fehlens von Mischelementen bei Ram-Extrusionsprozessen kam es beim Syringe-die-Verfahren zu einer schlechten Wirkstoffeinarbeitung und damit zu einer hohen Burst-Freisetzung. Dennoch war die Wirkstofffreisetzung von Implantaten, die mit der Syringe-die-Methode hergestellt wurden, mit denen von Implantaten vergleichbar, die mit dem ThreeTec ZE9 hergestellt wurden, was es aufgrund der geringen Verarbeitungszeiten, der geringen Mengen an benötigten Formulierungsmischungen und der Vergleichbarkeit zu einem attraktiven Werkzeug für das Formulierungsscreening macht auf Implantate. Daher wurde in 3.1 und 3.2 die Syringe-die-Methode als Screening-Tool für heißschmelzextrudierte Implantate verwendet.

Zur Erstellung einer Applicability Map wurde der Einfluss auf die Dexamethasonfreisetzung aus PLGA-basierten Implantaten im Hinblick auf PLGA-Endgruppen, das Verhältnis von PLGA-Milchsäure zu Glykolsäure (L:G), das durchschnittliche Molekulargewicht des Polymers und die Wirkstoffbeladung untersucht (Kapitel 3.1). Die Freisetzung von Dexamethason aus Implantaten folgt

der typischen Freisetzungskurve von PLGA-basierten Drug Delivery Systems (DDS). Eine kleine, stoßartige Freisetzung des überschüssigen Arzneimittels auf der Oberfläche des Implantats, gefolgt von einer Verzögerungsphase und der Freisetzungsphase, die dem Beginn des Polymerabbaus zuzuordnen ist. Verzögerungsphasen der Dexamethasonfreisetzung aus mit 502H, 503H und 502 präparierten Implantaten waren unabhängig von der Wirkstoffbeladung, während Verzögerungsphasen der Dexamethasonfreisetzung aus 752S Implantaten durch die Wirkstoffbeladung beeinflusst wurden. Die Freisetzungszeit nach der Verzögerungsphase war bei 752S Implantaten mit höheren Wirkstoffbeladungen kürzer. Durch Plotten der Freisetzungsphase über der Verzögerungsphase wurde die Freisetzung von Dexamethason in einer Applicability Map visualisiert. Diese Applicability Map wurde erfolgreich genutzt, um ein biologisch abbaubares Dexamethason Implantat mit einer gewünschten Freisetzung zu entwickeln, die aus einer Verzögerungsphase von maximal 7 Tagen und einer Freisetzungsphase von etwa 14 Tagen besteht. Dies konnte durch die Herstellung eines Dexamethason Implantats mit 50%/ 60% Wirkstoffbeladung und einer PLGA-Mischung aus 502H/ 502 im Verhältnis 3:1 erreicht werden.

Als nächstes wurde eine Formulierung für ein biologisch abbaubares Implantat mit Brimonidin entwickelt. Die Anforderung an das Implantat bestand darin, dass es den Wirkstoff im gleichen Maß freisetzte wie Dexamethason aus dem bereits entwickelten Implantat, um möglicherweise eine gleichzeitige Injektion zu ermöglichen. Die Freisetzung von Brimonidin aus biologisch abbaubaren PLGA-Implantaten wurde im Hinblick auf PLGA-Endgruppen, Polymermolekulargewicht, L:G-Verhältnis und Wirkstoffbeladung untersucht. Die Freisetzungsdaten wurden in einer Applicability Map dargestellt, die die Verzögerungsphase gegen die Freisetzungsphase aufzeichnete, um das gewünschte Implantat wie zuvor bei Dexamethason Implantaten zu entwickeln. Es wurde erfolgreich ein biologisch abbaubares Implantat entwickelt, das Brimonidin aus PLGA-Implantaten mit einer 1:1-Mischung aus 752S/503H, einer um 3 Tage längeren Verzögerungsphase

und nahezu der gleichen Freisetzungszeit im Vergleich zum entwickelten Dexamethason-Implantat freisetzt.

Kombinationsimplantate mit beiden Arzneimitteln setzten Brimonidin innerhalb weniger Tage frei, die Freisetzung von Dexamethason war jedoch bei allen Formulierungen unvollständig. Da die gleichzeitige Freisetzung beider Arzneimittel aus einzelnen Dexamethason- und Brimonidin Implantaten vollständig war, wurde eine Arzneimittel-Arzneimittel- oder Arzneimittel-Arzneimittel-PLGA-Wechselwirkung angenommen, aber nicht weiter untersucht. Dennoch könnte ein Kombinationsimplantat, das beide Medikamente enthält, durch alternative Herstellungsmethoden wie die Co-extrusion möglich sein.

Um eine zuverlässige Testmethode zur Freisetzung von Arzneimitteln aus biologisch abbaubaren Implantaten nach kurzer Zeit zu erhalten, wurde eine Testmethode zur beschleunigten Freisetzung entwickelt, bei der der Einfluss von Temperatur und pH-Wert des Freisetzungsmediums auf die Dexamethasonfreisetzung aus PLGA-Implantaten untersucht wurde. Der Wechsel des Freisetzungsmediums von Kochsalzlösung (0,9 %, Saline) zu phosphatgepufferter Kochsalzlösung pH 2 (PBS, USP) beschleunigte den PLGA-Abbau und damit die Dexamethasonfreisetzung aus den Implantaten nicht. Die Verwendung von PBS pH 12 (USP) sorgte für einen beschleunigten PLGA-Abbau durch basisch katalysierte Hydrolyse. PLGA-Implantate wurden bei Temperaturen von 37 °C, 45 °C und 65 °C vollständig abgebaut. Leider konnten bei pH 12 aufgrund des schnellen PLGA-Abbaus keine Verzögerungsphasen der Dexamethasonfreisetzung beobachtet werden. Dies macht es schwierig, verschiedene Formulierungen zu vergleichen oder die Freisetzung von Arzneimitteln unter Standardbedingungen für Freisetzungstests abzuschätzen. Die besten Bedingungen für Tests zur beschleunigten Freisetzung von Dexamethason Implantaten lagen hier bei einer erhöhten Temperatur von 45 °C im Standard Freisetzungsmedium Saline. Durch die Erhöhung der Temperatur bei Freisetzungstests dauerte die Freisetzung von Dexamethason aus PLGA-

Implantaten nur halb so lange wie bei 37 °C, während dennoch Unterschiede zwischen den Formulierungen beobachtet werden konnten.

Die Dexamethason Freisetzung aus 502H/502 Implantaten wurde im Hinblick auf die Sterilisation des Implantats durch Gammabestrahlung und die Lagerzeit bei Raumtemperatur in einem Kieselgel-Exsikkator untersucht. Die Lagerungszeit von 2 Jahren für mit dem ThreeTec ZE9 präparierte Implantate führte zu einer leichten Dexamethasonfreisetzung während der Lag-Phase, wurde jedoch als akzeptabel eingestuft, da die Wirkstofffreisetzung immer noch derjenigen von Implantaten direkt nach HME ähnelte. Die Dexamethason Freisetzung aus Implantaten, die mit dem Haake MiniLab Compounder präpariert wurden, veränderte sich nach dreijähriger Lagerung deutlich. Obwohl die typische sigmoidale Freisetzungskurve immer noch zu sehen war, nahm die Burst-Freisetzung zu, Dexamethason wurde während der Verzögerungsphase freigesetzt und schließlich wurde die Freisetzungsphase verringert. Insgesamt war eine zweijährige Lagerung gammabestrahlter, PLGA-basierter Dexamethason Implantate im Hinblick auf die Wirkstofffreisetzung akzeptabel.

Letztendlich wurden die mechanischen Eigenschaften biologisch abbaubarer PLGA-Implantate im Hinblick auf die Wirkstoffbeladung, das Molekulargewicht der Polymermatrix, die Implantat Abmessungen wie Länge und Durchmesser sowie deren Feuchtigkeitsgehalt untersucht. Durch die Implementierung einer benutzerfreundlichen 3-point-bending Testmethode war es möglich, die mechanischen Eigenschaften biologisch abbaubarer PLGA-Implantate mit einem Texture Analyzer zu messen und zu charakterisieren. Insbesondere Bruchkräfte, aber auch Ausdehnungen und AUC sind ausreichende Parameter, um Unterschiede in den Formulierungseigenschaften zu beschreiben, während die Steigung der Kurven keine vorteilhaften Korrelationen hinsichtlich des Molekulargewichts und der Länge von PLGA-Implantaten aufweist. Ohne die Notwendigkeit eines komplexen Setups oder der Umrechnung gemessener Parameter in Tensile Strength könnte diese Methode eine einfache Alternative für die Qualitätskontrolle biologisch abbaubarer Implantat Formulierungen sein.

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