Optimization of *in vitro* test conditions for PLGA-based drug delivery systems

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To my Family

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1. Introduction

1.1 Parenteral controlled release drug delivery systems

Morphine was the first official parenteral injected drug induced in 1867. Soon after, the number of parenteral formulations has been increased dramatically. The intravenous, subcutaneous, intramuscular, intraperitoneal, and intrathecal routs are examples of parenteral administration. However, the major administration routes of parenteral controlled release systems are subcutaneous and intramuscular. Products such as oil solutions (D. B. Larsen, Joergensen, Olsen, Hansen, & Larsen, 2002; D. B. Larsen, Parshad, Fredholt, & Larsen, 2002), emulsions (Collins-Gold, Lyons, & Bartholow, 1990; Florence & Whitehill, 1982), liposomes (Sharma & Sharma, 1997), micelles (Alkan-Onyuksel, Ramakrishnan, Chai, & Pezzuto, 1994), implants (Ueno, Refojo, & Liu, 1982) and microparticles (Herrmann & Bodmeier, 1995) are identified as parenteral controlled release drug delivery systems. In comparison to conventional oral dosage forms these systems can maintain the drug in the desired therapeutic range for days, weeks, months, and for some products, even years after one administration and offer several advantages including:

- Increase of bioavailability: Biopharmaceuticals, such as proteins and peptides, are large hydrophilic compounds administered via parenteral injections to circumvent their inherent instability in the gastro-intestinal tract as well as their low permeability across biological membranes (Frokjaer & Otzen, 2005). Another growing group of pharmaceuticals often requiring administration by injection is low-molecular-weight hydrophobic drugs, which also have low oral bioavailability (Christian Wischke & Schwendeman, 2008). Administration by injection leads to discomfort for the patient.
- Long release period: The use of controlled release formulations enables the frequency of injections to be reduced, which improve the patient's compliance, especially those who require daily or long-term treatment and reduce the need for follow-up care.
- Constant drug plasma concentration: Another advantage of controlled release formulations is that they result in a more constant plasma concentration of the drug, which is better kept within the therapeutic window. Frequent administrations often result in rises and falls in the concentration. Too high a concentration can cause unwanted side effects, while too low a concentration results in the loss of therapeutic effect. This means that lower total doses can be reduced with the controlled release formulations (Johnson et al., 1996).

- Localized delivery of drug: The product can be administrated directly at the site where drug action is needed and hence systemic exposure of the drug can be reduced.

The major Issues during application of injectable drug delivery systems are pain and tissue damage at the injection site, which decrease the patients' compliance.

1.1.1 Polymeric controlled release systems

The development of polymeric controlled release system introduced a new concept in drug administration. These systems are less complicated and with high stability. Encapsulation in the polymer carrier eliminates the degradation of drugs; moreover, the release profile of the drugs can be controlled by properly choosing polymers.

Polymers used in parenteral controlled release systems can be grouped into two main categories: non-biodegradable and biodegradable polymers. The first polymeric controlled release devices is a reservoir system based on non-biodegradable polymer silicone rubber (Folkman, Long, & Rosenbaum, 1966). The disadvantages of such system lay in that firstly, surgical removal of drug-depleted delivery systems of non-biodegradable polymers is difficult and painful and non-removal may pose toxicological problems; secondly, although this diffusion controlled delivery system is an excellent tool of modulating drug release, is largely dependent on polymer permeability and drug characteristics. The basic mechanism in non-degradable devices being diffusion, drugs which have either high molecular weight (7500) or poor solubility in polymer are not amenable to diffusion controlled release (Jalil & Nixon, 2008; Sinha & Trehan, 2003). To overcome these problems, biodegradable polymers for sustained release parenteral drug delivery systems began to develop in early 1970s. When compared to non-biodegradable polymers, they have the improved biocompatibility and are degraded in the body, This avoids the need for surgical removal and thus improves the patient acceptance (Danckwerts & Fassihi, 2008).

1.1.2 Biodegradable polymers:

Biodegradable polymers commonly contain chemical linkages such as esters, anhydrides, amides, peptides and glycosides. These polymers degrade *in vivo* either enzymatically or non-enzymatically to biocompatible and non-toxic byproducts. These can be further metabolized or excreted via normal physiological pathways. Biodegradable polymer not only have been

extensively used in controlled delivery systems, but also extended to medical devices (Leenslag, Pennings, Bos, Rozema, & Boering, 1987), wound dressing (Hubbell, 1996), and for fabricating scaffolds in tissue engineering (F. Shi, Gross, & Rutherford, 1996).

Biodegradable polymers are calcified as natural or synthetic (Mishra et al., 2008). The investigation of natural biodegradable polymer as drug carrier has been concentrated on proteins and polysaccharides (Table 1.1) (Mohanty, Misra, & Hinrichsen, 2000). Natural biodegradable polymers are attractive because they are natural products of living organisms, readily available, relatively inexpensive and capable of multitude of chemical modifications (Sinha & Trehan, 2003).

Table 1.1 Nature biodegradable polymers

Proteins	Globulin, Gelatin, Collagen, Casein, Bovine serum albumin, Human serum albumin
Polysaccharide	Starch, Cellulose, Chitosan, Dextran, Alginic acid

Synthetic biodegradable polymers have gained more popularity than natural biodegradable polymers. The major advantages of synthetic polymers include high purity of the product, more predictable, uniformity and free of concerns of immunogenicity. Furthermore, synthetic polymers provide with a wider range of mechanical properties and degradation rate. In the past years, there are numerous biodegradable polymers synthesized. Most of these polymers contain labile linkages in their backbone such as esters, orthoesters, anhydrides, carbonates, amides, urethanes, etc. The synthesis, biodegradability, and application of these polymers have been well reviewed **(Table 1.2)** (Gombotz & Pettit, 1995; J. H. Park, Ye, & Park, 2005; Winzenburg,

Schmidt, Fuchs, & Kissel, 2004). Although some of the biodegradable materials have been approved for the use in medical devices (Middleton & Tipton, 2000), biodegradable polymers such as poly(lactic acid), poly(glycolic acid) and their copolymers (PLGA, Figure 1.2) (Holland, Tighe, & Gould, 1986) are the most commonly used polymers as drug release controlling matrices in approved parenteral products due to their excellent biocompatibility and biodegradability and mechanical strength (J. M. Anderson & Shive, 2012; Athanasiou, Niederauer, & Agrawal, 1996; Jain, 2000). In addition, other polymers such as poly(ε-caprolactone and polyphosphoesters are being investigated for drug and gene delivery (Zhao, Wang, Mao, & Leong, 2003). Poloxamers, copolymers of polyethylene oxide and polyoxypropylene, are another interesting class which provided a wide range of applications in pharmaceutical and biomedical field and were investigated for drug delivery (Kwon & Okano, 1999).

Table 1.2 Synthetic biodegradable polymers

Polyesters: Poly(glycolic acid), Poly(lactic acid), Poly(lactic-co-glycolic acid), Poly(caprolactone)
Polyanhydrides
Polyorthoesters
Polyurethanes
Tyrosine-derived polycarbonates
Polyphosphazenes

1.2 Biodegradable polyesters based on lactic and glycolic acid

1.2.1 Synthesis of PLGA

According to the biodegradable polymer classification, homopolymers poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) as well as mixtures thereof, poly(lactic-co-glycolic acid) (PLGA) are categorized as synthetic, bulk eroding, linear, aliphatic poly(α-esters).

Polymers and copolymers of lactic and glycolic acids can be prepared in two ways: by a direct polycondensation reaction of lactic acid and glycolic acid (Figure 1.1), resulting in polymers of low molecular weight or by a ring opening polymerization of the cyclic diesters (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid (Figure 1.2). The ring opening polymerization yields the polymers of high molecular weight (> 10,000 g/mol) and of better mechanical properties (Gentile, Chiono, Carmagnola, & Hatton, 2014; Qian, Wohl, Crow, Macosko, & Hoye, 2011; Silva, Cardoso, Silva, Freitas, & Sousa, 2015; N. Wang, Wu, Li, & Feng, 2000). Furthermore, this method allows a better control of the molecular weight distribution (polydispersity) and the end group functionality of the (co-)polymer (Jain, 2000).

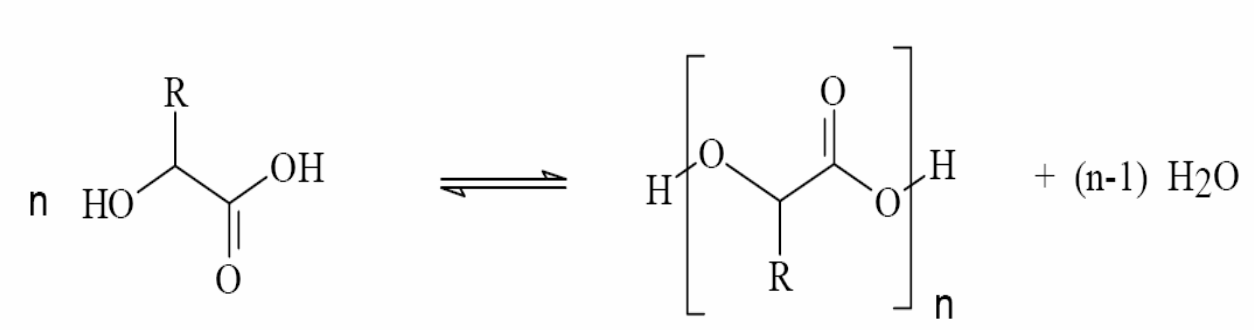


Figure 1.1 Synthesis of poly(lactide) by direct polycondensation

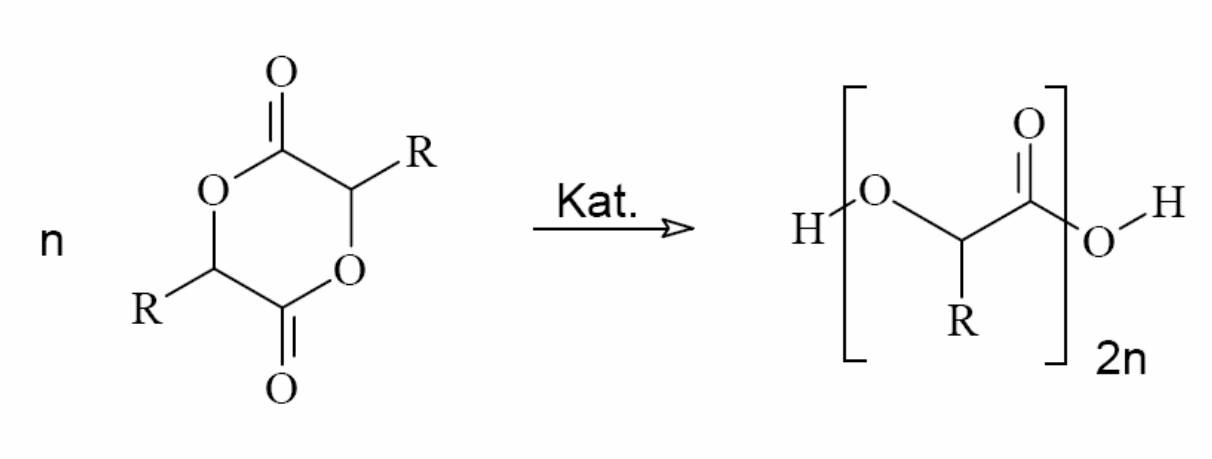


Figure 1.2 Synthesis of poly(lactide) by ring-opening polymerization

The selection of the reactants and the synthesis conditions will determine the physicochemical properties of the resulting polyesters, such as hydrophilicity, mechanical strength, glass transition and crystallinity (Gilding & Reed, 1979; Miller, Brady, & Cutright, 1977; Omelczuk & McGinity, 1992).The characteristics, which can be used to describe the final polymers include the weight or number averaged molecular weight, the polydispersity, the ratio of lactic and glycolic acid monomers, the ratio of D- and L-lactic acid monomers and the endgroup functionality. Although rarely specified, in random copolymers the segment length of monomeric repeat units of a product is important since short block lengths avoid the formation of crystalline domains in the polymer, which provides homogeneous controlled release matrices (Shard, Clarke, & Davies, 2002).

1.2.2 Polymer erosion

The term erosion is used for the loss of material from the polymer bulk (Achim Göpferich, 1996). This process requires the degradation of the polymer into soluble oligomers or monomers, but the focus is not on the single chain's properties but on those of the bulk, e.g. mass, outer dimension or mechanical stability. Two basic mechanisms are distinguished: Surface erosion and bulk erosion (Figure 1.3). However, pure forms of the described erosion processes are rare.

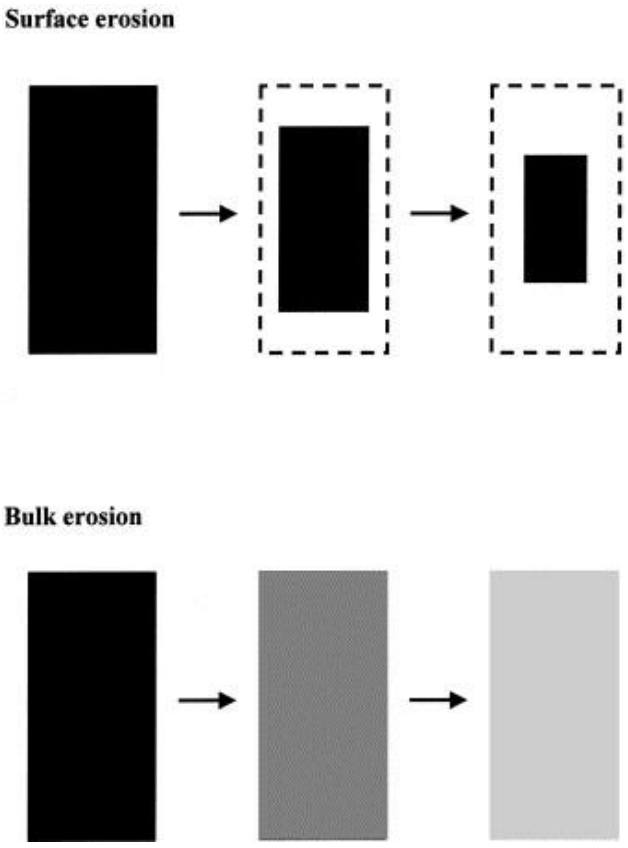


Figure 1.3 Schematic illustration of the changes a polymer matrix undergoes during surface erosion and bulk erosion (J. Siepmann & Göpferich, 2001)

1.2.2.1 Surface erosion

Surface erosion is characterized by a degradation and mass loss process localized merely at the polymer's surface (Achim Göpferich, 1996). Surface erosion is linked to the precondition that the agent needed for degradation, e.g. water, cannot penetrate the polymer bulk. Hence, only polymer chains at the surface of the bulk can be cleaved and transferred into soluble oligomers or monomers. Not until their removal, the polymer chains of the next layer can be degraded. This mechanism explains for the usually observed very constant rate of weight loss (based on the surface area) and the continuous changes in the outer dimensions of the polymer bulk throughout the entire erosion process. The mechanical properties of the remaining polymer bulk do not change within the complete erosion process. Surface erosion can be found with many polyorthoesters.

1.2.2.2 Bulk erosion

Bulk erosion is characterized by uniformly distributed degradation throughout the polymer bulk (Achim Göpferich, 1996). Ideally, the probability of chain cleaving is evenly distributed within the complete bulk. As long as the polymer chains are still insoluble, they cannot leave the bulk. Every polymer showing bulk erosion therefore usually retains most of its mass over quite a long time before finally showing a fast mass loss. This loss is correlated with the loss of the now soluble shortened polymer chains that leave the bulk's structure. Different to the mass, the mechanical properties continuously change during the erosion process. Bulk erosion requires the agent forcing chain degradation to penetrate faster into the bulk than a thought 'front of degradation'. In most biodegradable polymers, water is this agent as it allows hydrolytic cleavage of the polymer chains. Thus, water permeability of the bulk determines whether a polymer undergoes bulk erosion. The degradation rate of a bulk-eroding polymer is relatively independent of its shape, as long as no additional factors are involved. As many polyesters show autocanalization effects during their degradation, accelerating the further degradation, different diffusion pathways for the leaving of formed acidic degradation products can change the degradation behavior. This can explain e.g. the different reported degradation times of PLGA scaffolds *in vitro* and *in vivo* as well as of samples of different sizes (Lichun Lu et al., 2000; Vert, Li, & Garreau, 1992). It has also been shown, that the ratio between amorphous and crystalline parts of semicrystalline polymers can change during erosion. As crystalline regions within a polymer bulk usually have lower water permeability, their degradation rate tends to be slower than that of amorphous parts. Therefore, the relative amount of crystalline regions within the bulk will increase during degradation process, affecting e.g. the rate of the further degradation and the bulk's mechanical properties (Pitt, Chasalow, Hibionada, Klimas, & Schindler, 1981). The same principle has to be applied to co-polymers with larger blocks (M.-H. Huang, Li, Hutmacher, Coudane, & Vert, 2006).

1.2.3 Degradation of PLGA

Polyesters like PLA and PLGA are bulk-eroding polymers and degrade by randomly hydrolysis of the functional groups in an aqueous environment, without significant contribution of enzymes, which requires the presence of water. The cleavage of ester bond linkages yields carboxylic end groups and hydroxyl groups. The formed carboxylic groups then could catalyze and accelerate the hydrolysis of other ester bonds, a phenomenon referred as autocatalysis. Degradation of the polyesters leads to polymers of shorter chain length and below a critical molecular weight of about 1050-1150 Da (T. G. Park, Yong Lee, & Sung Nam, 1998), the oligomers can dissolve in the aqueous surrounding medium and diffuse out of the matrix. The end products of the degradation are lactic acid (pKa 3.85) and glycolic acid (pKa 3.83), which are both non-toxic and excreted via the lungs (after incorporation in the tricarboxylic acid cycle) or the urine.

1.3 Release mechanisms of PLGA-based drug delivery systems

There are three release mechanisms for drug molecules to be released from a PLGA-based DDS, which are illustrated in Figure 1.4:

- diffusion through water-filled pores (diffusion controlled)
- diffusion through the polymer (diffusion controlled)
- due to polymer erosion (erosion controlled)

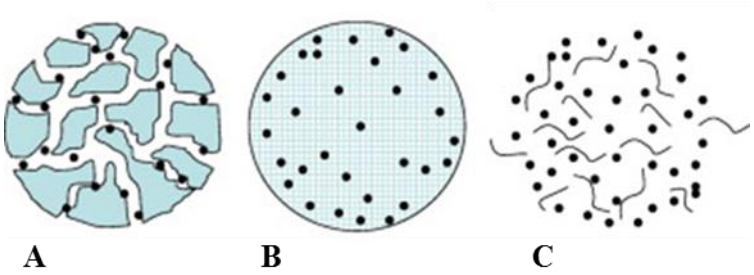


Figure 1.4 The release mechanisms: (A) diffusion through water-filled pores, (B) diffusion through the polymer and (C) erosion (Fredenberg, Wahlgren, Reslow, & Axelsson, 2011)

Diffusion through water-filled pores are the most common way of release, as the encapsulated drug is a protein or a peptide, which are too large and too hydrophilic to be transported through the polymer phase.

Diffusion through the polymer phase may occur when the drug is small and hydrophobic (Raman, Berkland, Kim, & Pack, 2005). However, the drug must enter the water phase, either at the surface or in the pores inside the DDS, before being released.

The encapsulated drug may also be released without any transport due to dissolution of the polymer, i.e. erosion. Erosion also creates pores, thus increasing the rate of diffusion. However, there is a difference between erosion leading to drug release without drug transport, and erosion that increases the rate of drug transport. The latter has been reported as a release mechanism countless times, at least after a lag period, which is often described as diffusion-controlled release (Alexis, Venkatraman, Rath, & Boey, 2004; Goraltchouk, Scanga, Morshead, & Shoichet, 2006; Lam, Duenas, Daugherty, Levin, & Cleland, 2000; L. Wang, Venkatraman, & Kleiner, 2004)

In general describing the release mechanism of PLGA-based DDS is not sometimes problematic, due to the complexity of the system it is not always clear which of the processes is dominating, and in a chain of processes that leads to drug release it is not obvious which one is the rate-determining process

1.3.1 Factors influencing drug release from PLGA-based drug delivery systems

1.3.1.1 Water uptake

Water is absorbed by the polymer immediately upon immersion in water or administration *in vivo*. The rate of water absorption, or hydration of the DDS is rapid compared to drug release (Batycky, Hanes, Langer, & Edwards, 1997; Blasi, D'Souza, Selmin, & DeLuca, 2005). Water absorption is a pore-forming process. These pores are too small for drug transport during the early stage of this process; however with increasing of the number and size of water-filled pores, a porous connected network allowing drug release is formed (Mochizuki, Niikawa, Omura, & Yamashita, 2008).

1.3.1.2 Hydrolysis

Polymer degradation and hydrolysis starts immediately upon contact with water and subsequent decrease in molecular weight. The polymer becomes less hydrophobic with decreasing molecular weight, and at 1100 Da the oligomers become water soluble (T. G. Park, 1994).

Several factors, material but also formulation properties can affect the degradation kinetics. The degradation rates of the polyester are influenced by parameters such as the initial weight average molecular weight, the hydrophobicity (lactic acid > glycolic acid monomers), the degree of crystallinity (e.g., increased in PGA and L-PLA) and the glass transition temperature of the amorphous phase.

The hydrophobicity of capped PLGA, which is esterified with an alkyl alcohol, is higher than for PLGA with free carboxyl groups (uncapped PLGA). Accordingly, uncapped PLGA degrades faster than capped PLGA (Tracy et al., 1999). Another factor affecting the degradation of the polyesters is the enantiomeric composition of the polymer (de Jong et al., 2001). Two stereoisomeric forms of PLA are commercially available, optically active L-PLA and racemic D,L-PLA. L-PLA is a semicrystalline material (isotactic), whereas D,L-PLA is amorphous (Gilding & Reed, 1979). However, during hydrolysis degradation products of D,L-PLA can crystallize and thus further degrade at a lower degradation rate (Li, Garreau, & Vert, 1990). The decrease of the molecular weight of PLGA and PLA follows a pseudo-first order kinetic (A Göpferich & Tessmar, 2002), which reflects a random chain scission process.

The pH has an important effect on the hydrolysis rate of polyesters. PLA and PLGA have a stability optimum at pH 4 - 5 and are hydrolyzed under acid and base catalysis (de Jong et al., 2001).

The cleavage of an ester bond linkage yields a hydroxyl and a carboxyl group and the formation of carboxylic acids during degradation of the polyesters can accelerate the hydrolysis of other ester bonds (Shenderova, Burke, & Schwendeman, 1999). This autocatalytic phenomenon is known to cause heterogeneous degradation inside PLGA matrices (Li & McCarthy, 1999) i.e. faster degradation at the center of the PLGA matrix than at the surface. This effect becomes more pronounced with increasing dimensions of a DDS (Dunne, Corrigan, & Ramtoola, 2000) as

the acid gradient increases, but heterogeneous degradation has also been reported in particles and films with dimensions as small as 10 μm (T. G. Park, 1995). As consequence of autocatalysis, bimodal molecular weight distributions can be found in size exclusion chromatograms of samples from degradation studies.

1.3.1.3 Erosion

Hydrolysis of the polymer backbone is the initiation of the erosion process, which is a series of events, including a decrease in the molecular weight, a decrease in glass transition temperature with decreasing M_w, a loss of mechanical properties and finally, a loss of mass via the dissolution of small polymer fragments (Lyu, Sparer, & Untereker, 2005).

Dissolution of polymer degradation products and erosion create pores. Small pores, formed by water absorption consequently grow during polymer erosion, and eventually coalesce with neighboring pores to form fewer, larger pores (Batycky et al., 1997). Small pores may also be closed (Kang & Schwendeman, 2007). This phenomenon is related to the mobility of the polymer chains, and their ability to rearrange (Yamaguchi et al., 2002). The mobility of polymer chains depends on the glass transition temperature (T_g).

1.4 PLGA-based drug delivery systems

Biodegradable delivery systems based on PLGA can be in the form of solid implants, microparticles or delivery systems that form in situ. Polymer based drug delivery systems can be classified into two types: reservoir-based systems, and monolithic matrix systems (Figure 1.5).

In reservoir-based systems, the drug reservoir is enclosed within insoluble polymer. The drug releases through the rate-controlling porous polymeric membrane. Monolithic matrix systems are similar to reservoir-based systems, but in this case, the drug is dispersed or dissolved within a polymer matrix. The drug release can be diffusion, swelling, and/or erosion controlled. Compared to reservoir systems, matrix systems are easier to be manufactured because they are homogeneous in nature and they are also safer since a mechanical defect of the reservoir device rather than matrix device may cause dose dumping (X. Huang & Brazel, 2001). However, if

polymer matrix is non-degradable, the constant release profile is difficult to be achieved with matrix system (Fung & Saltzman, 1997).

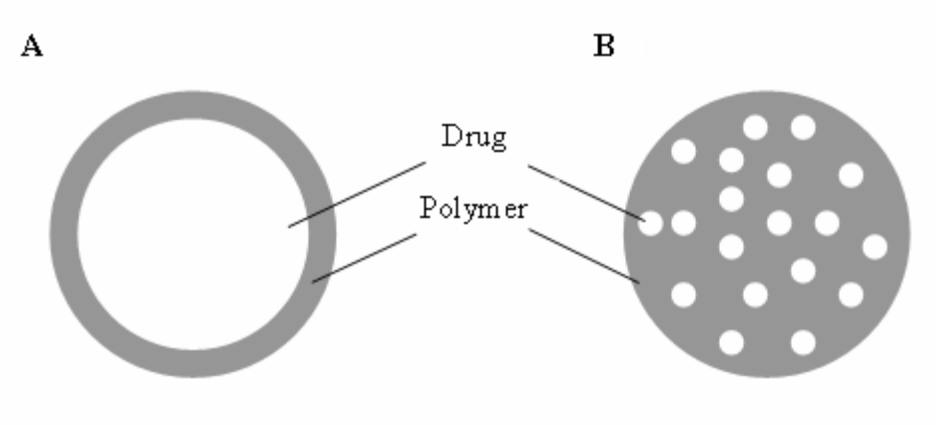


Figure 1.5 Polymeric delivery systems; (A) Reservoir systems; (B) Matrix systems

1.4.1 Biodegradable implants

Four PLGA based implants delivering small peptides and low molecular weight drug are available on the market (Table 1.3). The solid implants for controlled release of drugs are usually cylindrical polymer matrices (rods), which can be on the millimeter to centimeter scale, facilitating large loadings of active materials. More complicated three-dimensional implant structures, such as tubes, scaffolds or other structural supports, which are of special interest for tissue engineering applications, can be fabricated as well. Manufacturing techniques include solvent casting (T. G. Park, Cohen, & Langer, 1992), extrusion (Zhang, Wyss, Pichora, Amsden, & Goosen, 1993), melt compression, injection molding (Sundback, Hadlock, Cheney, & Vacanti, 2003), compression molding (Schliecker et al., 2004) and freeze drying (Hsu et al., 1996). The major disadvantage of these delivery systems is their limited patient acceptance, due to the required painful administration into the subcutaneous tissue by surgical intervention or insertion using large-bore needles (trocar).

1.4.1 Biodegradable microparticles

Microparticles are spherical, polymeric carrier particles of a size between 1 and 1000 μm, which contain drug either in form of a reservoir (microcapsules) or dissolved / dispersed in the polymer

matrix (microspheres). PLGA microparticles can be prepared by different microencapsulation techniques including solvent extraction/evaporation processes, phase separation (coacervation) and spray drying (Jain, 2000). A choice of the technique depends on the nature of polymer, the drug, the intended use and the duration of therapy. However, solvent evaporation with emulsification is the most often used technique, at least in the laboratory scale, due to possibility in adaption for drugs of different physicochemical properties (C Wischke & Schwendeman, 2012). The polymer solution containing the drug (in solution or dispersion) is emulsified in an external phase. The internal solvent is removed by partition into the external phase and/or by evaporation (O'Donnell & McGinity, 1997). Oil-in-water (O/W) and oil-in-oil (O/O) emulsion techniques have been applied to produce microparticles using solvent evaporation. The conventional O/W solvent evaporation is appropriate for lipophilic drugs, for instance steroids. For water-soluble drugs, peptides and proteins, low encapsulation efficiency is frequently observed. A double emulsion (W/O/W) technique has been introduced in order to circumvent the problems relating to water-soluble substances (Jaraswekin, Prakongpan, & Bodmeier, 2007; Schwach et al., 2003).The preparation of PLA/PLGA microparticles by coacervation is a complex method in which the resulting microparticles frequently agglomerate since the method lacks any stabilizers or emulsifiers (Jain, 2000). A drug in the form of a solution or particles is dispersed into the polymer solution. Subsequently, the coacervation of the polymer is induced by a phase separation inducing agent. Soft coacervate droplets are hardened using another nonsolvent of the polymer, such as hexane. Large amounts of solvents are required in the coacervation process, and residual solvents are a concern for this process. Compared to solvent evaporation and coacervation, spray drying is more rapid, easier to scale up, and less dependent on factors inherent in the drugs and polymers. In the spray drying method a PLA/PLGA solution with a dissolved or dispersed drug is sprayed though the nozzle of a spray dryer to form microparticles. Dichloromethane and ethyl acetate are useful to prepare the polymer solution. The microparticles from this method are sometimes not spherical; the formation of fibers or irregular-shaped particles could be found when using this technique (Jain, 2000; Schwach et al., 2003).

Preferentially, microparticles have a size of less than 250 µm (J. H. Park et al., 2005), which allow injection through smaller needles after re-dispersing them in a suitable aqueous medium. The applicability of smaller needles reduces pain during administration and thus improves the

patient comfort. The more convenient administration compared to implants makes microparticles an attractive biodegradable drug delivery system. However, their manufacturing is technically challenging.

Table 1.3 Examples of marketed PLGA-based drug delivery systems

Product	**Therapeutic**	**Dosage form**	**Company**	**Indication**	**Administration rout**
Arestin®	Minocycline hydrochloride[1]	Microparticle	OraPharma	Periodontal disease	Subgingival
Atridox®	Doxycycline hyclate[1]	In situ forming implant	Tolmar	Chronic adult periodontitis	Subgingival
Bydureon®	Exenatide[2]	Microparticle	Zeneca	Type II diabedes mellitus	Subcutaneous
Decapeptyl®	Triptorelin acetate[2]	Microparticle	Ferring	Prostate cancer	Subcutaneous
Eligard®	Leuorolide acetate[2]	In situ forming implant	Astellas	Prostate cancer	Subcutaneous
Enantone®	Leuprolide acetate[2]	Microparticle	Takeda	Prostate cancer, endometriosis	Subcutaneous
Enantone® Gyn	Leuprolide acetate[2]	Microparticle	Takeda	Prostate cancer, endometriosis	Subcutaneous

Lupron® Depot	Leuprolide acetate[2]	Microparticle	Abbvie	Prostate cancer	Intramuscular
Leuprone® HEXAL®	Leuprolide acetate[2]	Implant	Hexal	Prostate cancer	Subcutaneous
Nutropin® Depot	Somatropin, recombinant human growth hormone[3]	Microparticle	Genentech	Short stature	Subcutaneous
Ozurdex®	Dexamethasone[1]	Implant	Allergan	Macular edema, retinal vein occlusion, uveitis	Intravitreal
Pamorelin® LA	Triptorelin embonate[2]	Microparticle	Ipsen Pharma	Prostate cancer	Intramuscular
Profact Depot®	Buserelin acetate[2]	Implant	Sanofi-Aventis	Prostate cancer, endometriosis	Subcutaneous
Risperdal® Consta	Risperidone[1]	Microparticle	Janssen/Alkermes	Schizophrenia	Intramuscular
Sandostatin® LAR	Octreotide acetate[2]	Microparticle	Novartis	Acromegaly, carcinod	Intramuscular

				syndrome	
Somatuline® LA	Lanreotide acetate[2]	Microparticle	Ipsen	Acromegaly	Intramuscular
Suprecur® MP	Buserelin acetate[2]	Microparticle	Sanofi-Aventis	Prostate cancer	Subcutaneous
Trelstar™ Depot	Triptorelin pamoate[2]	Microparticle	Watson	Prostate cancer	Intramuscular
Zoladex®	Goserelin acetate[2]	Implant	Astra Zeneca	Prostate cancer	Subcutaneouse

[1] low molecular weight drug

[2] peptide

[3] protein

1.5. *In Vitro* Drug Release Testing of Parenteral Dosage Forms

In vitro release studies are generally performed to accomplish one or more of the following aims (Burgess, Hussain, Ingallinera, & Chen, 2002; L. Lachman, H. Lieberman, 1986):

- As an indirect measurement of drug availability, especially in preliminary stages of product development
- Quality control to support batch release and to comply with specifications of batches proven to be clinically and biologically effective
- Assess formulation factors and manufacturing methods that are likely to influence bioavailability
- Substantiation of label claim of the product
- As a compendial requirement

Since the introduction of the rotating basket apparatus (USP 1) as the first standardized apparatus for *in vitro* dissolution testing in the USP in 1970, dissolution testing has gone through major changes, including the design of new apparatus and the introduction of more biorelevant testing conditions.

An *in vitro* release profile reveals fundamental information on the structure (e.g., porosity) and behavior of the formulation on a molecular level, possible interactions between drug and polymer, and their influence on the rate and mechanism of drug release and model release data (Washington, 1990). Over the last few years, regulatory activity in *in vitro* dissolution testing has become even more important with regard to the establishment of *in vitro-in vivo* correlations (IVIVC) and for the evaluation of scale-up and post-approval changes. Such information facilitates a scientific and predictive approach to the design and development of sustained delivery systems with desirable properties.

However, this evolution of methods has mainly focused on the oral route of administration. Recently, the number of products that are delivered via non-oral routes of administration has greatly increased the number of marketed products and the interest in controlled release parenteral products has multiplied. The reasons for the delivery via alternative routes such as the parenteral administration include advanced targeting strategies, as well as the increasing number of new drug entities that cannot be successfully delivered via the oral route of administration due to various reasons such as instability in the gastrointestinal tract, adverse reactions upon systemic exposure, patient compliance, and accessibility to specific organs or local sites of the body, etc. Although a sizable amount of research has focused on the parenteral controlled drug delivery systems, very little attention has been devoted to the development of an *in vitro* release technique (Seidlitz & Weitschies, 2012).

Unlike controlled release oral formulations, there are no regulatory standards for parenteral controlled delivery systems. Also, the current United States Pharmacopeia (USP) apparatus for *in vitro* release testing was designed mainly for oral and transdermal products and is not directly applicable for parenteral products administered subcutaneously or intramuscularly. For example, concerns with using USP apparatuses 1 (basket) and 2 (paddle) include sample containment, large volume of media required for testing, and sampling procedure. USP apparatuses 5 (paddle

over disc), 6 (cylinder), and 7 (reciprocating holder) were designed for the transdermal route and do not offer any advantages for parenteral delivery systems such as microparticles. Additionally, drawbacks of USP apparatuses 3 (reciprocating cylinder) and 4 (flow-through cell), designed for extended-release oral dosage forms, include evaporation (reciprocating cylinder) and filter blockage along with polymer migration leading to variable flow rates (flow-through cell).

The dosage forms applied to deliver these new drug entities are as diverse as the sites of delivery. Depending on the intended therapeutic action, controlled release parenterals can be administered intravenously, intramuscularly, subcutaneously or intra-articularly, can be implanted into tumor tissue, ocular or peri-ocular tissue, teeth, bone, blood vessels or inserted into other natural passages/ conduits such as the esophagus. The target of drug delivery can either be local structures or the entire organism. The dosage forms include, but are not limited to, monoliths such as rods, lipophilic solutions, disperse systems such as microspheres, nanoparticles, liposomes, emulsions, and suspensions, in-situ forming gels or solids, cements, wafers, and coated medical devices such as drug-eluting stents and drug-eluting pacing leads. Reviews have been published on the technologies used for many of these dosage forms (Kreye, Siepmann, & Siepmann, 2008; Packhaeuser, Schnieders, Oster, & Kissel, 2004; Y. Shi & Li, 2005; Yasukawa, Ogura, Kimura, Sakurai, & Tabata, 2006).

From this representative but incomplete listing it is evident that specialized *in vitro* release test systems are necessary to address the diversity of the dosage forms and their sites of application. This diversity may be one of the reasons why currently there is no standard compendial dissolution test method for controlled release parenterals in the United States Pharmacopeia (USP), in the European Pharmacopoeia (Ph. Eur.) or in the Japanese Pharmacopoeia (JP). A number of workshop reports have been published stating the need for regulatory guidance on this issue and highlighting some of the approaches used at present (Brown et al., 2011; Burgess, Crommelin, Hussain, & Chen, 2004; Martinez, Rathbone, Burgess, & Huynh, 2008; Siewert et al., 2003).

The current methods that are used most often for *in vitro* dissolution testing of parenteral dosage forms are mostly noncompendial, although they sometimes include USP apparatus designed for other routes of administration. They are categorized into three general groups: sample and

separate methods, dialysis membrane-based methods and flow-through or continuous flow methods (Kastellorizios and Burgess 2012; Seidlitz and Weitschies 2012a) (Figure 1.6). The impact of the experimental conditions used for drug release measurements from PLGA parenteral depot systems have been reported in the literature, but not yet fully understood.

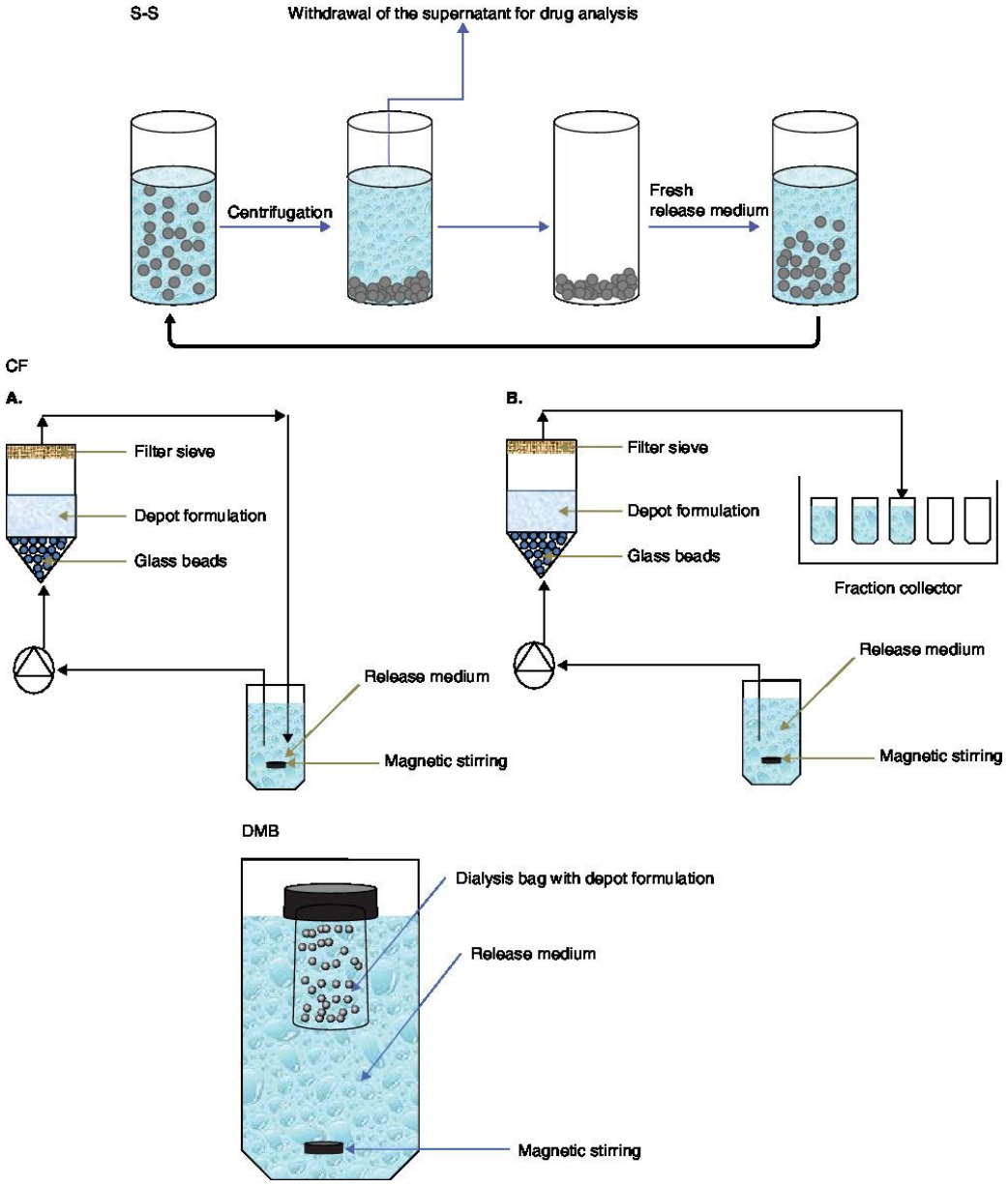


Figure 1.6 Basic principles of three different types of in vitro release sketched as examples of a sample and separation method (S-S), a closed (A) and an open (B) continuous flow method (CF) and a dialysis membrane-based technique (DMB) (C. Larsen, Larsen, Jensen, Yaghmur, & Østergaard, 2009)

1.5.1 Sample and separate method

Sample and separate method has been widely used as in vitro release testing for parenteral formulations. When using this method, the formulation is typically placed in a vial, tube, or beaker containing the release media. Media selection is based on drug solubility and stability over the duration of the release study (e.g., phosphate buffer pH 7.4). The closed system is left at constant temperature 37°C. Modifications of the basic technique to study drug release include size of container, use of agitation, and sampling methods.

Container size: Container selection depends on the volume of dissolution media necessary to maintain sink conditions without compromising the sensitivity of the assay for the activity being studied. For example, *in vitro* release studies have been performed in tubes or vials when small volumes (<10 mL) are used (Takada, Kurokawda, Miyazaki, Iwasa, & Ogawa, 1997; Volland, Wolff, & Kissel, 1994; Yang, Chia, & Chung, 2000) and bottles or Erlenmeyer flasks when larger volumes (100-400 mL) (Jeong et al., 2003; Liu, Kuo, Sung, & Hu, 2003) of media are required.

Type, extent, and use of agitation: Once suspended in media, microparticles may be subjected to continuous or intermittent agitation for the duration of the release study. Agitation of microspheres using a paddle was reported to prevent aggregation of microspheres, which significantly reduced the release rate from rifampicin microspheres (Bain, Munday, & Smith, 2008). Continuous agitation may be provided by using a magnetic stirrer at a fixed speed (Negrín, Delgado, Llabrés, & Évora, 2001), wrist shaker rotating at 360° (Murty, Goodman, Thanoo, & DeLuca, 2003), incubator shaker (Latha, Lal, Kumary, Sreekumar, & Jayakrishnan, 2000), shaking water bath (Kim & Burgess, 2008; Mi et al., 2003; Yen, Sung, Wang, & Yoa-Pu Hu, 2001), tumbling end-over-end (Liggins & Burt, 2001), or high-speed stirring/revolution of bottles (Latha et al., 2000). In some cases, the media contents were kept static during incubation at 37°C (T. G. Park et al., 1998).

Sampling technique: Drug release is monitored at intermittent intervals by separating the particles from the bulk media either by filtration or centrifugation. Filtration of media contents is accomplished using membrane filters having a size that can filter polymer fragments followed by analysis of supernatant (Liu et al., 2003; Yen et al., 2001). Centrifugation of media contents is

also widely used and may be followed by sampling of the supernatant (Jiang, Woo, Kang, Singh, & DeLuca, 2002; Lacasse et al., 1997; T. G. Park et al., 1998) or analysis of remaining drug in the microspheres as with etoposide (Schaefer & Singh, 2002), peptides such as vapreotide, a somatostatin analog (Blanco-Príeto, Campanero, Besseghir, Heimgatner, & Gander, 2004), leuprolide, a luteinizing hormone releasing hormone analog (Byung Ho Woo et al., 2002), and thyrotropin- releasing hormone (TRH) (Toshiro, Hiroaki, Yusuke, Yasuaki, & Hajime, 1991), because of instability in the release media. As an alternative to filtration or centrifugation of microparticles, Bodmeier *et al*. (Bodmeier & McGinity, 1987) allowed the media contents to settle before sampling the supernatant. The volume of supernatant withdrawn depends on drug solubility and stability, assay sensitivity, and maintenance of sink conditions. For poorly water-soluble drugs, such as paclitaxel, all of the release media (10 mL) was withdrawn at each analysis followed by replacement with the exact volume sampled (Ruan & Feng, 2003). A similar procedure was adopted for interferon-α where low loading (1.1%) necessitated the removal of 3-ml supernatant from the release media (4 mL) (Diwan & Park, 2003). For drugs such as amoxicillin, which are unstable in media, complete withdrawal of supernatant was achieved by centrifugation followed by analysis of remaining native drug in microspheres and supernatant (Kim & Burgess, 2008).

Buffer replacement: Buffer replacement is necessary to maintain sink conditions post sampling. In some cases, total buffer replacement is necessary to prevent the accumulation of drug degradation products in solution (Murty et al., 2003). For samples subjected to filtration, buffer replacement is accomplished by 'back-washing' as reported by (Hickey, Kreutzer, Burgess, & Moussy, 2002). For centrifuged samples, buffer replacement is generally followed by resuspension of microparticles (Wei, Pettway, McCauley, & Ma, 2004).

Advantages and disadvantages: This technique provides a direct and reasonably accurate assessment of *in vitro* release.

However, permanent aggregation of microspheres during filtration and/or centrifugation is a major concern and may lead to lower release rates (Bain et al., 2008). To minimize effects of agitation, surfactants have been used (D'Souza, Faraj, & DeLuca, 2005; Shameem, Lee, Deluca, & Street, 1999) and/or intermittent shaking of media contents was performed (Ravivarapu, Lee,

& DeLuca, 2000). Sampling is another major issue, especially when filtration or centrifugation is used. Small-sized particles (<10 mm) lead to filter clogging when polymer degradation and dissolution occur. Loss in volume because of filtration during sampling and buffer replacement is a concern when the amount of release media is small. Sampling by filtration cannot be used with drugs that bind to the filter. Centrifugation followed by analysis of the supernatant is an alternative to filtration. However, time to sediment increases as the particles start degrading. Also, redispersion of the degraded particles is difficult. Because release studies for these extended release dosage forms could run into months, total buffer replacement is sometimes necessary to maintain sink conditions. This is very difficult to accomplish if filtration or centrifugation is used as the sampling technique.

Furthermore, the separation step has to be fast enough not to influence the release profile. Alternatively, the microparticles could be recovered at periodic intervals and remaining drug analyzed (Schaefer & Singh, 2002). This destructive technique requires a large amount of microparticles and is not an attractive option to study release.

A comparison of the outcomes of dissolution testing using a sample and separate method with and without agitation has been published (D'Souza & DeLuca, 2005). The results showed a distinct deceleration of release in the unstirred setup and emphasized the need to establish standardized and reproducible hydrodynamics. In a different study the impact of two different types of agitation (horizontal shaking and stirring in an USP 2 paddle apparatus) on release from microspheres was evaluated, revealing immense differences depending on the type of agitation (Bain et al., 2008). Apparently, the shaking movement was not sufficient to prevent microsphere aggregation and resulted in markedly slower release. Due to a lack of standardization of such parameters, it is often difficult – if not impossible – to compare the results obtained with slightly diverging sample and separate methods. In an adapted sample and separate method proposed liposomes were embedded in a donor compartment, an agarose gel in the bottom of glass vials, which was topped with a liposome-free agarose layer to separate the dosage form from the acceptor media above. According to those authors, the method was suitable to permit the perfusion of released proteins while retaining the liposomes (Peschka, Dennehy, & Szoka, 1998).

1.5.2 Flow-through method

The idea of using flow-through methods for dissolution testing was introduced in the 1960s, almost simultaneously by Baun et al. and Langenbucher (Baun & Walker, 1969; Langenbucher, 1969). This concept is represented in the USP, Ph.Eur., and JP as the flow-through cell (USP 4). The chamber typically consists of a conical lower and a cylindrical upper part and is perfused by dissolution media from bottom to top. The monographs of USP, Ph. Eur., and JP describe different types of cells, a implant cell (diameter 22.6 mm) and a powder cell (diameter 12.0 mm). The Ph. Eur. additionally describes a cell for lipophilic solid dosage forms such as suppositories, which was designed to separate dissolved drug from the molten vehicle. Other noncompendial cells have been introduced. The perfusion can either be performed in an open system, with fresh media supplying the sample chamber the whole time, or in a closed loop of recirculating media. These two options enable adaptation of media volume over a wide range to ensure sink conditions. Violation of sink conditions inside the cell might however occur in spite of large media volumes, if the release from the dosage forms very fast compared with the media replacement in the cell as determined by the flow rate.

Pumps and flow rates: The flow-through cell is typically operated at a flow rate of 16 mL/min, alternatively the monographs of the USP, the Ph. Eur. and JP suggest flow rates of 4 or 8 mL/min. It has to be kept in mind, though, that the flow rate of 16 mL/min was chosen to be consistent with the compendial setups for the basket and paddle apparatus, so that approximately one liter of dissolution media flows past the formulation in one hour, however the volume typically used in apparatus 1 and 2, and does not necessarily represent biorelevant flow conditions and volumes for parenterals (Iyer, Barr, & Karnes, 2006). Modifications of the USP apparatus 4 have been used to assess drug release from parenteral formulations. A variety of setups, pumps and flow rates have been reported in the literature and are stated below.

Constant flow of media is achieved by using a peristaltic (Cortesi, Esposito, Menegatto, Gambari, & Nastruzzi, 1994; Vandelli, Rivasi, Guerra, Forni, & Arletti, 2001; Wagenaar & Müller, 1994), syringe (Aubert-Pouëssel et al., 2004; Aubert-Pouëssel, Bibby, Venier-Julienne, Hindré, & Benoît, 2002; Cheung, Kuba, Rauth, & Wu, 2004; Cortesi et al., 1994; Kılıçarslan & Baykara, 2003; Yüksel, Dinç, Onur, & Baykara, 1998) or high-performance liquid

chromatography (HPLC) (Longo & Goldberg, 1985) pump. In most cases, a lower flow rate resulted in incomplete release probably because of slower rates of hydration and dissolution of the polymer and drug, respectively. Conversely, cumulative release greater than 85% was obtained with higher flow rates. Hydration of the polymer matrix is the most important factor governing the release from microparticulate delivery systems. Once the polymer is hydrated, drug release occurs as a result of a combination of diffusional and erosional processes. This suggests that flow rate is an important parameter in the assessment of drug release when the flow-through method is used. Another parameter to be considered is the volume of buffer, which depends on drug solubility and assay sensitivity. In the event that buffer is being recirculated, it is important that sink conditions be maintained by replacing part or the entire buffer.

In general, the lower conical part is filled with glass beads to avoid turbulence at the media inlet as the diameter increases. The media outlet at the top of the cell is typically equipped with a filter to prevent undissolved material from leaving the cell. In this context, dissolution testing of formulations containing very small particles may pose a problem in the flow-through setups, since filter resistance increases with pore size reduction, which may then lead to considerable back-pressure inside the cells.

As with the sample and separate technique, media selection is based on drug solubility and stability over the duration of the release study.

During the experiments the flow-through cell is placed in a water bath at 37°C and the samples are withdrawn from the stirred media container (closed system) or collected at the media outlet (open system).

A major limitation of the apparatus is that the implant is directly placed in the flow of the medium. This is not a fully representation of the *in vivo* environment.

Advantages and disadvantages: The flow-through method attempts to simulate the *in vivo* environment by constantly circulating a small volume of media through immobilized microparticles to hydrate the particles and cause dissolution and diffusion of the drug. A major advantage of this method is that samples can be continuously and conveniently sampled and analyzed along with buffer replacement because of the automated process. Disadvantages with

this procedure include variation in the flow rate due to clogging of the filter (because of polymer degradation) leading to high-pressure buildup in the system. Also, low flow rates are achieved with the types of filters used (membrane and ultrafilters) and seem to be responsible for low rate and extent of drug release from microsphere formulations. Zolnik et al. studied the effect of hydrodynamics inside the flow-through apparatus (Zolnik, Leary, & Burgess, 2006). The pulsatile flow inside the flow-through cell was measured quantitatively using magnetic resonance imaging (MRI). It was found that the flow field inside the dissolution cells was, at most operating conditions, heterogeneous, rather than fully developed laminar flow, and characterized by re-circulation and backward flow. A model implant was shown to be contacted by a wide distribution of local velocities as a function of position and orientation in the flow cell. The use of 1 mm beads acted as a distributor of the flow but did not suffice to ensure a fully developed laminar flow profile, furthermore it was found that the conditions offering more uniform flow profiles are operation at lower flow rates, using the wider cell, using ballotini and placing the implant vertically.

Another possibly critical parameter in flow-through setups is the placement of the dosage form. It has been shown for implants that the positioning may have an influence on the hydrodynamics in the cells as well as on release (Morihara, Aoyagi, Kaniwa, Katori, & Kojim, 2002; Shiko, Gladden, Sederman, Connolly, & Butler, 2011).

Rawat et al. investigated the suitability of the modified USP apparatus 4 for possible compendial adaptation for drug release testing of microspheres (Rawat, Stippler, Shah, & Burgess, 2011). The robustness and reproducibility of method was tested using commercially available risperidone PLGA-based microparticles. Risperidone release was not affected by flow rate as well as by minor variations in the method such as amount of microspheres, flow-through cell size, and size of glass beads. However, the significant difference in release was observed by slight variation in temperature.

Just recently a new *in vitro* release method for dispersed systems, such as nanosuspensions, liposomes and emulsions, was introduced combining the use of a dialysis membrane mounted on a custom made adapter with the flow-through cell (Bhardwaj & Burgess, 2010). The authors of that study were able to show that the novel method was able to discriminate between three

different liposome formulations. By contrast, no discrimination was possible with the dialysis and reverse dialysis sac methods.

1.5.3 Dialysis Methods

Originally, the dialysis technique was used to study drug release from oily parenteral depot solutions (D. B. Larsen, Joergensen, et al., 2002; D. H. Larsen, Fredholt, & Larsen, 2000; Schultz, Møllgaard, Frokjaer, & Larsen, 1997) and suppositories (Lootvoet, Beyssac, Shiu, Aiache, & Ritschel, 1992), particulate-based injectable formulations of poorly water-soluble drugs (Parshad, Frydenvang, Liljefors, Cornett, & Larsen, 2003), and liposomes (Saarinen-Savolainen, Järvinen, Taipale, & Urtti, 1997). More recently, this technique has been used to study drug release from a variety of particulate systems for topical preparations (Parsaee, Sarbolouki, & Parnianpour, 2002), oral suspensions (Bodmeier, Chen, Tyle, & Jarosz, 1991), submicron emulsions (Levy & Benita, 1990), and intranasal (Martin, Bandi, Shulz, Roberts, & Kompella, 2002) delivery. Other novel dosage forms where the dialysis technique has been used include nanoparticles (Heiati, Tawashi, Shivers, & Phillips, 1997; Jeon, Jeong, Jang, Park, & Nah, 2000; Leo, Cameroni, & Forni, 1999; Peracchia et al., 1997), implants (Dash, Haney, & Garavalia, 1999), and micelles (La, Okano, & Kataoka, 1996).

The first reports on the use of dialysis methods for dissolution experiments were published in the 1960s for solid oral dosage forms (Barzilay & Hersey, 1968; Marlowe & Shangraw, 1967). In those cases, the membranes were inserted into the dissolution setup to separate the sample from undissolved formulation, e.g. granule particles and insoluble excipients. Dialysis methods consists of a small donor compartment (5 – 8 mL) separated from a large acceptor compartment (1000 mL) by a dialysis membrane, providing a driving force for drug transport to the outside and maintaining sink conditions. Both chambers are filled with dissolution media, heated to 37°C and the acceptor compartment is agitated. Common modes of agitation include a horizontal shaker (Nastruzzi, Esposito, Cortesi, Gambari, & Menegatti, 2008; J Siepmann, Faisant, Akiki, Richard, & Benoit, 2004) or using the USP paddle apparatus (Faisant, Siepmann, Oury, et al., 2002; Faisant, Siepmann, & Benoit, 2002) under agitation. Media selection is based on drug solubility and stability over the duration of the release study. Various modifications of the basic technique have been employed to assess drug release and are described below.

The most commonly reported setups utilize a dialysis bag **(Figure 1.7)** (Faisant, Siepmann, Oury, et al., 2002; Faisant, Siepmann, & Benoit, 2002; Jeon et al., 2000; Lee et al., 2002; Leo et al., 1999; Nastruzzi, Pastesini, et al., 2008; Nastruzzi, Esposito, et al., 2008; Peracchia et al., 1997; Prabhu, Sullivan, & Betageri, 2008; J Siepmann et al., 2004; Juergen Siepmann, Faisant, & Benoit, 2002; J. Wang, Wang, & Schwendeman, 2004) where dosage form is introduced into the bag that is sealed and placed in a vessel containing buffer. Such setups may be unrealistic, since the contact of the dosage form with aqueous media may influence the release rate controlling principle, e.g. the dissolution of the polymer carrier as reported by Nie et al. (Nie, Hsiao, Pan, & Yang, 2011).

As an alternative to the static placement of the donor compartment inside the acceptor compartment, a rotating dialysis **(Figure 1.7)** cell model was introduced for parenterals based on a model originally proposed for suppositories (C. Larsen et al., 2008; Pedersen, Østergaard, Larsen, & Larsen, 2005).

In vitro release of calcitonin (MW 3600) from microspheres using both the sample and separate method with agitation and dialysis bag (MWCO 12-14 kDa) showed complete release with both methods, with release being slower with the dialysis technique but more reproducible (Prabhu et al., 2008). In another report, the tube method showed slower release when compared to a dialysis bag (MWCO12-14 kDa), which was selected to study the *in vitro* release of ^{125}I-bovine calcitonin from PLGA microspheres, as it offered more advantages over the tube method (Diaz, Llabrés, & Évora, 1999). *In vitro* release of two proteins, carbonic anhydrase (MW 31 kDa) and bovine serum albumin (MW 66 kDa) from PLGA microspheres from a 'dialysis bag (MWCO 3.5 kDa)' was compared to the sample and separate method (T. G. Park, Lu, & Crotts, 1995). Both proteins were shown to be stable and active in the supernatant and microspheres when the dialysis method was used. It was believed that the dialysis bags permitted a constant pH because water-soluble oligomers, from polymer degradation, were removed, resulting in slower polymer degradation and greater stability of the protein. In addition, dialysis bags simulated the *in vivo* environment and retained sink conditions better than the tube method. The findings of these studies, however, should be interpreted with caution. In the aforementioned studies, the volume used for studying release from the tube method was equal to the volume added to the dialysis bag. However, total volume of media used in the dialysis method 69 vs. 10 mL (Prabhu et al.,

2008), 80 vs. 1 mL (Diaz et al., 1999), and 2000 vs. 4 mL (T. G. Park et al., 1995) was much larger than with the tube method. The low volumes (1-10 mL) used with the tube method would not be able to provide adequate buffer capacity, leading to build up of acidic degradation products resulting in peptide/protein instability in the outer media and in the acidic microenvironment of the microspheres.

The model parameters of dialysis method that can be varied include type/mode of agitation, ratio between donor and acceptor cell volumes, and molecular mass cutoff value of the dialysis membrane. However, the molecular mass cut-off value of the dialysis membrane and the membrane surface area are key parameters when characterizing these models. MWCOs has a board range, MWCOs selected for *in vitro* release studies should be high enough so that it doesn't limit the drug diffusion. In some cases, achievement of equilibration with the outer media was slow owing to the small membrane surface area available for drug passage. Slow equilibration limits an accurate analysis of initial drug levels in formulations where the burst release is high. In order to overcome this problem Float-a-Lyzer **(Figure 1.7),** a commercially available dialyzer with a large membrane surface area can be used. D'Souza et al. investigated the suitability of dialyzer for parenteral *in vitro* test using 1-month leuprolide PLGA microspheres. Float-a-Lyzer was capable of accurately assessing a low initial burst release (D'Souza & DeLuca, 2005).

This membrane was also stable to elevated temperatures, which implied that it could be used for a short-term release study in which high temperatures are used.

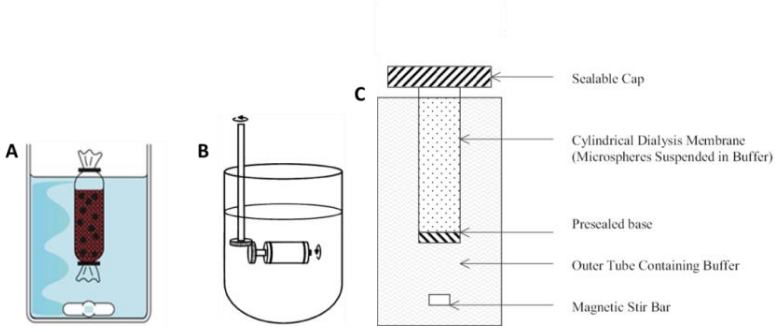


Figure 1.7 Basic principles of three different types of dialysis membrane-based technique, (A) a dialysis bag (B) a rotating dialysis (Schultz et al., 1997) and (C) Float-a-Lyzer (D'Souza & DeLuca, 2005)

Advantages and disadvantages: The dialysis method is attractive because sampling and media replacement are convenient because of physical separation of the microparticles from the outer media by a dialyzing membrane.

A major problem with this method is a possible violation of sink conditions if diffusion through the membrane is the rate determining step. To overcome this problem, a 'reverse dialysis' method has been proposed by Chidambaram et al. (Chidambaram & Burgess, 1999). In this setup, the dosage form is placed directly in a large chamber containing the dissolution media and the samples are withdrawn from microdialysis sacs immersed into the chamber, thus reversing the volume ratio between donor and acceptor compartment.

Furthermore achievement of equilibration with the outer media is slow and would limit an accurate analysis of initial drug levels in formulations where the burst effect is high (Janusz W Kostanski & DeLuca, 2000). However, this issue was addressed by using dialysis bags (more surface area) where initial release over 24 h was about 88% (J W Kostanski, Thanoo, & DeLuca, 2000).

Another disadvantage is that the time to equilibrate is prolonged if the bulk media is not stirred (formation of unstirred water layer). In such situations, it is recommended that the outer media be agitated to minimize unstirred water layer effects and to prevent accumulation of polymer degradation products, especially when the formulation contains a protein (T. G. Park et al., 1995). Also, this technique cannot be used if the drug binds to the polymer or membrane (Kinget, Bontinck, & Herbots, 1979). However, because of the ease of sampling and the possibility of total buffer replacement, this method seems to be an attractive option to study drug release from microparticles and other particulate dosage forms.

Disadvantages with the dialysis technique are a cumbersome setup procedure for dialysis bags (Diaz et al., 1999) and membrane at one end of a tube (Byung H Woo et al., 2001). This can be addressed by using a commercially available dialyzer (D'Souza & DeLuca, 2005) having a large surface area. Additionally, the regenerated cellulose membrane is stable up to 60°C, which would allow its use in short-term elevated temperature studies (D'Souza et al., 2005; Shameem et al., 1999). Also, the membrane may be washed and reused after each experiment, which would render it cost effective.

Several concerns are taken into consideration when perform a test for *in vitro* drug release of parental controlled drug delivery system. These tests are often run over a long time period (e.g., several weeks to months). Due to a long duration of a drug release, the evaporation of the release medium and the microbial contamination should be prevented. Suitable preservatives may be added to prevent microbial contamination. Standard preservatives, including cetylammonium bromide, benzalkonium chloride, parabens, phenol derivatives, mercury salts, and sodium azide along with appropriate concentrations to be used, are listed in many pharmaceutical textbooks. The selection has to be based on criteria such as compatibility with the active pharmaceutical as well as other formulation ingredients and the pH of the test medium. Issues with these compounds include their ionization properties, physicochemical interactions, or analytical interferences. The composition of the medium should take into consideration the osmolarity, pH, and buffer capacity of the fluids at the site of administration, which are usually assumed to represent the condition of plasma or the physiological fluid but with lower buffer capacity.

It has to be mentioned that sink conditions do not always prevail at the site of administration *in vivo*. Nevertheless, sink conditions should be used for standard *in vitro* dissolution testing to see the release profile of the dosage form that would occur if no restrictions to distribution away from the site of application were present (Burgess et al., 2002; D'Souza & DeLuca, 2006; Washington, 1990). According to the USP, sink conditions are maintained when the volume of medium equals at least three-times that required to form a saturated solution of the drug substance. It has been suggested that the experimental concentration should be kept below 10% of the concentration at saturation (Washington, 1990). Care must be taken when non-aqueous solvents or solubilizing agents are added to the dissolution media to obtain sink conditions as the release profiles may be greatly influenced by the concentration of the additives (Washington, 1990).

Moreover, the stability of active ingredients or drugs in the release medium is important. A drug has to be stable in the *in vitro* condition at the determined temperature and at pH of the release medium along the *in vitro* drug release test. However, the main challenges with this type of dosage form are to determine the appropriate duration of the test and the times at which samples are to be drawn in order to characterize the release profile adequately. While too long sampling

interval can lead to misunderstand drug release manners from implants, excessively frequent sampling is a waste of time.

Over the past decade, there have been attempts to compare *in vitro* test methods to study drug release from parenteral drug delivery systems. Nastruzzi et al. studied the release of bromocriptine mesylate from commercial Parlodel LA® microspheres using dialysis tubes and a flow-through cell method and also compared the reproducibility between the two *in vitro* tests (Nastruzzi, Esposito, et al., 2008). In the dialysis method, a dialysis tube containing 20 to 25 mg microspheres was placed into 100 ml of 50 mM citrate buffer pH 3.5, whereas in the flow-through cell method, 20 mg microspheres were packed in a column (45 × 9 mm) filled with 3 ml of 50 mM citrate buffer pH 3.5 with a flow rate of 0.12 ml/min. Very different release rates were obtained, although the overall shapes were similar. Greater release of drug with a longer time to plateau occurred with the dialysis technique, whereas with the flow-through cell, the time to reach the plateau was shorter, but the amount released was smaller.

Conti et al. assessed release from indomethacin PLA microspheres using the USP dissolution test apparatus, rotating bottle apparatus, shaker incubator, and a recycling flow-through cell. The following test conditions were employed to assess *in vitro* release in buffer (Conti, Genta, Giunchedi, & Modena, 2008):

- USP XII paddle dissolution test apparatus: 1000 mL buffer at 100 and 200 rpm
- Rotating bottle apparatus: 100 mL buffer at 29 rpm
- Shaker incubator: 100 mL buffer at 60 and 120 strokes/min
- Recycling flow-through cell: 1000 mL buffer at a flow rate of 17 and 33 mL/min

For the indomethacin PLA microspheres, drug release was fastest with the recycling flow-through cell with similar release profiles obtained using the USP dissolution XXII apparatus, shaker incubator, and rotating bottle apparatus. Results from experiments with bromocriptine mesylate and indomethacin microspheres were similar in that *in vitro* release with the flow-through cell was faster. Studies on *in vitro* release of spray-dried rifampicin microspheres formulated using a blend of R104 polymer (D,L-PLA, MW 2000) with R202H (D,L-PLA, MW 9000) were performed using bottles shaken horizontally in a water bath and with the USP dissolution apparatus (Bain et al., 2008). Briefly, about 10 mg of microspheres was added to a

bottle containing 100 ml dissolution medium with horizontal shaking at 1.5 Hz, whereas in the USP XXII test method, release of 50 mg microspheres was assessed in 500 ml dissolution medium agitated at 100 rpm at 37°C. The drug release was significantly faster with the USP paddle apparatus. Because the ratio of microspheres to dissolution media was the same, faster release with the USP paddle apparatus was attributed to the greater degree of agitation with the paddle, which prevented the microspheres from forming aggregates at the base of the vessel. When agitation was employed, the microspheres remained as individual particles and were continually suspended in the media, resulting in faster release.

In recognition of the need for a standard *in vitro* release method, a series of national and international workshops on quality assurance and performance of sustained and controlled release parenterals have been conducted in recent years (Burgess et al., 2004, 2002; Siewert et al., 2003). The issues addressed in addition to methodology were apparatus, outcomes, parameters necessary for method development, and *in vitro-in vivo* correlation (IVIVC) for sustained release parenteral dosage forms. The resulting publications included important guidelines for novel or special dosage forms, including implants, injectable microparticles formulations, and liposomes.

These delivery systems were categorized as those dosage forms requiring more work before a method can be recommended. Some of the concerns in need of resolution included evaporation, prevention of microbial contamination, osmolarity, pH, and buffer capacity of the media for these extended release formulations, as the time to conduct *in vitro* studies would encompass weeks or months.

For batch release testing and product development studies it is crucial to use the finished product for the *in vitro* studies. With parenteral products, additional production steps such as sterilization are often performed following the actual manufacture of the dosage form. These may influence release behavior. For example, gamma sterilization may induce polymer degradation, leading to changes in the average polymer molecular weight and the glass transition temperature and in turn to changes in the release rate (Martinez et al., 2008). Besides changes in the polymer during sterilization, the impact of the sterilization procedure on drug stability needs to be examined.

Also, the procedure with which the dosage form is applied may have an influence on its release profile. Consider, for example, the surface area that is formed after application of a depot intramuscularly or subcutaneous injection (Hirano, Ichihashi, & Yamada, 1982; Weng Larsen & Larsen, 2009). Even if such parameters will inevitably vary *in vivo*, the dissolution test design should enable careful evaluation of their influence on release and relevant conditions should be standardized in the *in vitro* setup. Some different approaches to deal with in-situ forming gels in an *in vitro* test have been described briefly in a review by Larsen et al. (C. Larsen et al., 2009).

1.5.4 Accelerated release testing:

While real time *in vitro* release tests are necessary to gain a mechanistic understanding of drug release and to help in formulation design, accelerated tests for controlled release parenterals are essential for quality control purposes since real time release tests are of the order of weeks to months (product specific). Currently, research is focused on shortening the time span of *in vitro* release experiments with the aim of providing a quick and reliable method for assessing and predicting drug release (Shameem et al., 1999; Siewert et al., 2003). Accelerated tests should be predictive of real time tests and they should be designed as early as possible in the development process in order to accrue sufficient data to underline the relationship between real time and accelerated tests. For commercial dosage forms that release drug for 30- 90 days or even longer, accelerated or short-term release provides the potential for conducting an *in vitro* release test in a matter of days rather than months. Release testing of these dosage forms at 37°C would require the addition of preservatives and impose certain limitations on the *in vitro* method, such as stability and compatibility of the components of the release device, like tubings and membranes. Therefore, a short-term release test might even be more reliable for quality-control purposes. In addition, short-term studies can provide a rapid assessment of formulation and processing variables that affect drug release from the delivery system, especially in the developmental stages. These short-term studies can be performed by accelerating one or more conditions employed in a real-time *in vitro* release study. There are several methods to accelerate release, including elevated temperature, altering pH, use of surfactants, solvent. Upon acceleration the mechanism of release may however change, as for example reported by Zolnik et al. (Zolnik et al., 2006). When employing higher temperatures to accelerate release, particle aggregation may pose a problem with disperse formulations (Shameem et al., 1999). Multiphasic release including

an initial burst, which is often observed with modified release parenteral dosage forms, is often impossible to adequately monitor in an accelerated release test. Therefore, an additional real-time test of the initial phase of release is often advisable (Iyer et al., 2006; Martinez et al., 2008).

As with the real-time *in vitro* release study, the method should be simple, reproducible under the conditions of study, inexpensive, and applicable to biodegradable microsphere formulations that have varying duration of action *in vivo*.

1.5.5 More realistic release test systems for parenteral products:

Dissolution tests may be modified to include biorelevant conditions by adapting media (including composition, volume and temperature, see above) or apparatus design. With regard to specialized apparatus, systems have been introduced for solid oral dosage forms that simulate certain aspects of physiological gastrointestinal passage, such as physicochemical and enzymatic conditions, hydrodynamic shear stress or mechanical stress in the stomach and/or the

Intestine (Garbacz et al., 2008)**.**

With parenteral products that are circulated through the vascular system with the blood, the media volume and composition are most likely the main physiological factors to be reflected in an *in vitro* test system. In this case, the apparatus may have less influence on release if standardized and reproducible hydrodynamic conditions are achieved, and general principles of dissolution testing are respected.

For parenteral products that are administered into tissue or confined spaces of the body, a different situation may arise, in which the apparatus is also of greatest importance. Depending on the surroundings, the release behavior of the dosage form and the physicochemical properties of the released substances, sink conditions may not prevail locally at the site of release. Assuming a diffusion layer model, this may lead to a deceleration of release from the dosage form due to a decrease in concentration gradient. Also, the mass transport in living human tissue will be very different compared with stirred media. The inclusion of basic physical properties representative of the situation *in vivo* may be a first approach to adapting dissolution tests as it will be even more complicated to include biological phenomena such as active transport or tissue reaction. In

the following section, attempts to reflect such physical conditions in *in vitro* systems will be summarized.

1.5.5.1 Subcutaneous Injection Site Simulator (Scissor):

Kinnunen et al. developed a novel *in vitro* system, termed Scissor (Subcutaneous Injection Site Simulator) **(Figure 1.8)** (Kinnunen et al., 2015). Sirius Scissor is designed to mimic the stresses a biopharmaceutical experiences when transitioning from formulation conditions to a subcutaneous environment, in order to understand about the physical behavior of parenteral drugs in the period immediately post-administration.

Scissor uses a dialysis-based injection chamber, which can incorporate various concentrations and combinations of acellular extracellular matrix (ECM) components at the subcutaneous injection site. This chamber is immersed in a container of a bicarbonate-based physiological buffer that mimics the subcutaneous injection site and the infinite sink of the body. Such an arrangement allows for real-time monitoring of the biopharmaceutical within the injection chamber, and can be used to characterize physicochemical changes of the drug and its interactions with ECM components. Movement of a biopharmaceutical from the injection chamber to the infinite sink compartment simulates the drug migration from the injection site and uptake by the blood and/or lymph capillaries. This system models environmental changes that a biopharmaceutical could experience as it transitions from conditions of a drug product formulation to the homeostatic state of the hypodermis following subcutaneous injection. Scissor is designed to recreate, in an *in vitro* setting, dynamic events that could affect the fate of a biopharmaceutical formulation delivered by subcutaneous injection *in vivo*. This transition involves changes in pH, temperature, ionic components (with a loss of excipients), and transient pressure changes due to volume introduction. The major drawback of this method is that it’s very complicated and expensive. Furthermore, this method follow up factors that may affect the bioavailability of biopharmaceutical after it is released from dosage form and does not consider factors influencing dosage form and drug release from dosage form *in vivo*.

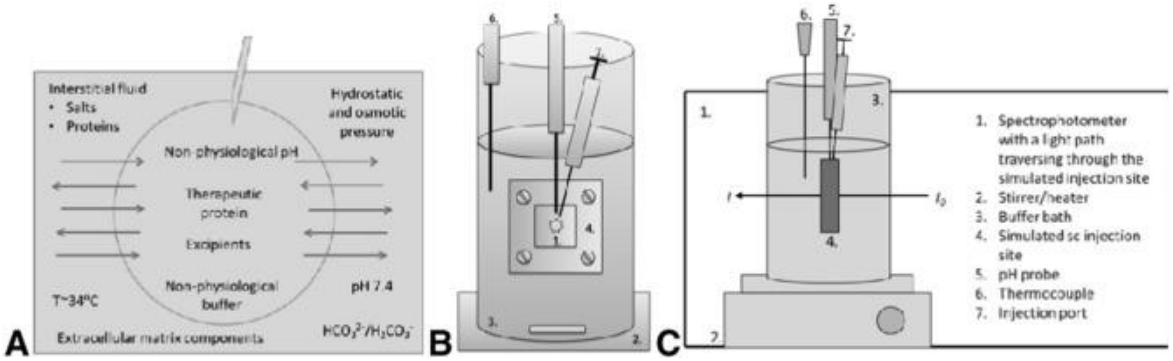


Figure 1.8 Dynamic events in the hypodermis following the injection of a biopharmaceutical formulation can be modeled using a modified dialysis system. A) Diagram of components and events denoting the exchange of small molecules (salts, sugars, and other excipients), buffer (transition to a bicarbonate-based environment), equilibration of temperature and pressure, and potential interaction with extracellular matrix (ECM) components. B) Cartoon depicting arrangement of positioned probes to continuously monitor pH inside and outside of the sample injection chamber and positioning of light path (crenulated circle) in a large solute bath chamber that emulates the infinite sink of the body. C) Cartoon depicting arrangement of injection chamber/infinite sink compartment in a single-beam spectrophotometer that allows for temperature control and stirring of the large bath contents. The numbering in B) and C) follows the legend shown in C) (Kinnunen et al., 2015).

1.5.5.2 Capillary bioreactor device:

Iyer et al. introduced a method for release testing of a biodegradable monolithic rod implant designed for subcutaneous implantation. This method is based on the idea of placing the implant, as well as glass beads simulating barriers formed by cells, into the extra-capillary space of a capillary bioreactor device **(Figure 1.9)** (Iyer, Barr, Dance, Coleman, & Karnes, 2007). The capillary bioreactor included 50 polyether sulfone capillaries (diameter 0.5 µm, pore size not reported), which were perfused by media at a flow rate of 1 ml/min. As the media was pumped into the reactor, the extra-capillary space also filled with media. The system was operated in a closed loop and the amount of drug released was determined from samples collected from the media reservoir. Release with a modified flow-through cell which was used as a reference method was faster, with an approximate doubling of the amount released after 90 days compared with the capillary device. Those authors reported a linear one-to-one correlation of the results

obtained using the capillary device with *in vivo* data. However, it has to be mentioned that even though release was examined for 90 days only approximately 3% of the drug load had been released during that time. The placement of the dosage form in between the capillaries and glass beads is expected to be of great importance for this setup and slight changes in the arrangement might lead to large deviations in the release behavior. Also, it remains to be investigated whether glass beads are feasible of simulating properly the barriers formed by cells of the subcutaneous tissue.

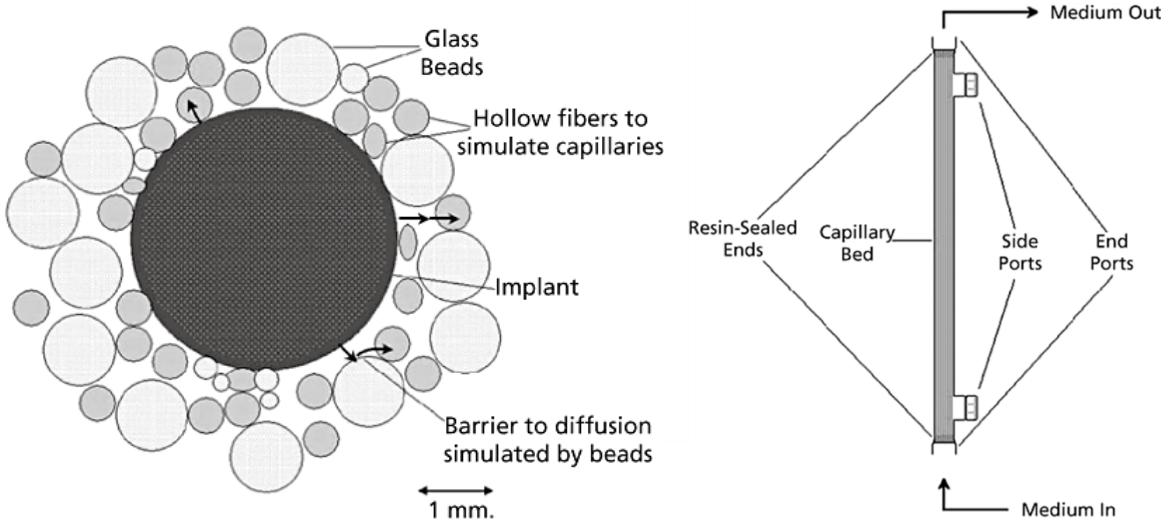


Figure 1.9 Capillary bioreactor device (left) top view with the implant positioned in the center and (right) transverse section (Iyer, Barr, Dance, et al., 2007)

1.5.5.3 Using agarose gel as a dissolution test:

A different approach to simulate tissue implantation based on a hydrogel compartment has been proposed for monolithic implants and microparticles (Allababidi & Shah, 1998; Hoang Thi et al., 2010). The gels were chosen for their ability to mimic tissue according to their rheological properties and water content, and used as acceptor compartments (Allababidi & Shah, 1998; Hoffman, 2002). In comparison with stirred media, the convective transport in the hydrogel is minimized but diffusion of small molecules is not hindered. This setup is thus more representative of the situation *in vivo*. The embedding of the dosage form in the gels (agar or

agarose) was slightly different in the different studies. In the case of the microparticles, a hole was created in the center of the gel into which a suspension of the particles was added. The monolithic implants were either placed in a hole in the gel and topped with an additional gel layer or completely surrounded by gel, as depicted in **Figure 1.10** (Allababidi & Shah, 1998; Hoang Thi et al., 2010). This could be a very important difference, since in the first two settings at least one surface of the drug containing compartment is in contact with a nondiffusible compartment (apparatus wall and in case of the microparticles also the air above the particle suspension). The change in contact area is expected to influence release. Upon implantation *in vivo* all surfaces are expected to be in contact with tissue and therefore the complete surrounding of the dosage form with the gel is the most favorable approach. In all setups samples of the hydrogel were withdrawn at predetermined time points at various distances from the product and drug content was determined. Thus, these methods also enabled the evaluation of spatial distributions within the gels. In this context it should be mentioned that sink conditions should be maintained in the overall system by providing a suitable amount of gel even though local violations of sink conditions near the implant may occur *in vitro* as well as *in vivo*. Hoang Thi et al. compared the results of their method, comprising agarose gels, to release testing in agitated vials using an orbital shaker plate system at 100 rpm/min (Hoang Thi et al., 2010). Release from all tested formulations was faster using the incubation method. The authors explained this difference with the high concentration gradients at the interface between dosage form and media as well as an observed fragmentation of the implant matrix in the agitated vials. (Gasmi et al., 2016) employed an incubation method for reference. In that case, however, the release was slower in agitated vials (horizontally shaken at 80 rpm/min) compared with the gel method. Those authors attributed this deceleration of release to the prevention of local pH change by acidic polymer fragments released in the stirred setup, whereas in the gel the pH underwent a local decrease in pH resulting in autocatalysis of further polymer degradation. Allababidi et al. on the other hand were not able to detect significant differences in cumulative release from their model implant using their agar gel method and a sample and separate method used as a control (horizontal shaker at 60 rpm/min) (Allababidi & Shah, 1998). Those results illustrated that the outcomes of dissolution testing can be very different for quite similar methods depending on which factors are controlling release from the dosage form.

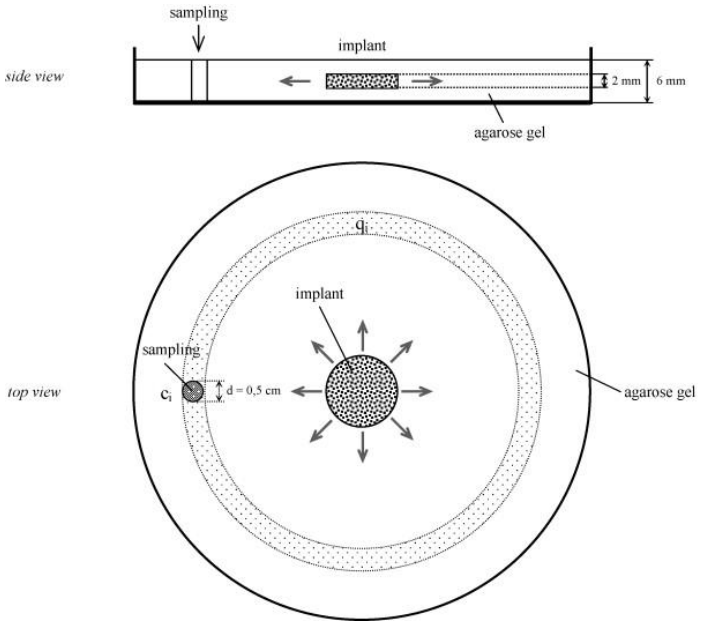


Figure 1.10 Schematic illustration of the experimental setup used for drug release measurements into agarose gels (ci = drug concentration in the withdrawn gel sample; qi = drug amount in the corresponding concentric ring) (Hoang Thi et al., 2010)

However, it has to be mentioned that even though the agar gel has a similar composition of extracellular matrix, it cannot simulate the real environment of the dosage forms after administration. Since the living tissues exhibit very different mass transport phenomena, *in vivo* extracellular matrix consist of two phases: a gel phase containing primarily collagen, elastin, and polysaccharides and a fluid phase made up of water and dissolved proteins in contrast to agar gel, which consist only of a gel phase and doesn't have any movement.

1.6 *In vivo* conditions at the site of implantation:

In general, when a product is administered into tissue, it will be in direct contact with the cells of the respective tissue and interstitial fluid, the drug delivery system is in a lipophilic/hydrophilic environment. After release from the dosage form the drug will be released into the extravascular interstitial tissue fluid.

In case of intramuscular or subcutaneous instillation, the drug is released into the extravascular interstitial tissue fluid. The structural characteristics of interstitial space are similar in all tissues, ultrastructural studies suggest that the interstitium is comprised of two phases: a gel phase containing primarily collagen, elastin, and polysaccharides (e.g., hyaluronate, glycosaminoglycans); and a fluid phase made up of water and dissolved proteins (Chary & Jain, 1989). The glycosaminoglycans are polyanionic polysaccharides that are charged at physiological pH (7.4) and are bound covalently to a protein backbone to form immobilized proteoglycans. The proteins present in the interstitial space are qualitatively the same as those present in plasma, although quantitatively, they are present in lower concentrations. This results in the interstitial colloid osmotic pressure (COP1) being less than that in plasma (Aukland & Reed, 1993). Although the components of interstitium are principally the same in all tissues, their relative amounts vary greatly (Aukland & Reed, 1993). It is believed that a network of endogenous macromolecules effectively reduces the distribution volume such that the interstitial space acts in a size exclusion manner, excluding very large molecules, and thereby affecting their interstitial occupancy (Bert, Mathieson, & Pearce, 1982; Watson & Grodins, 1978).

The movement of fluid in tissues under normal and pathological conditions is generally explained in terms of Starling's hypothesis. This hypothesis suggests that fluid is filtered at the arterial end of a vessel and mainly reabsorbed at the venous end. This fluid movement presumably leads to convective transport of macromolecules in the interstitium. The active agent is released into the tissue fluid and has to traverse the interstitium to reach a blood capillary or a lymphatic vessel by restricted diffusion in the gel phase and by convection and free diffusion in the fluid phase or possibly via transporters until reaching a blood capillary or a lymphatic vessel. Drug absorption from intramuscular and subcutaneous sites of depot injection may share some gross common features (Medlicott, Waldron, & Foster, 2004). Absorptive processes that may occur on injection into muscle or subcutaneous sites are presented in **Figure 1.11**

The size exclusion-like properties of the interstitium significantly reduce diffusion of plasma proteins and other macromolecules in the extracellular matrix. Compared with the interstitial fluid drainage by the lymph, the rate of filtration and reabsorption of fluid across the vascular capillaries is ~ 10-fold higher. Thus, drug molecules (with molecular masses below ~ 2 kDa), which are capable of entering blood and lymph capillaries at comparable rates, will be cleared

predominantly by the blood vessels (Porter, Edwards, & Charman, 2001). On the other hand, macromolecules above ~ 16 kDa as well as particulates are preferentially removed from the tissue by the lymphatics (Zuidema, Kadir, Titulaer, & Oussoren, 1994). The contribution of lymphatic uptake of macromolecules to the overall absorption process seems to be of most importance in relation to the subcutaneous injection site because only a few lymph vessels are located in muscle tissues (B. E. Ballard, 1968).

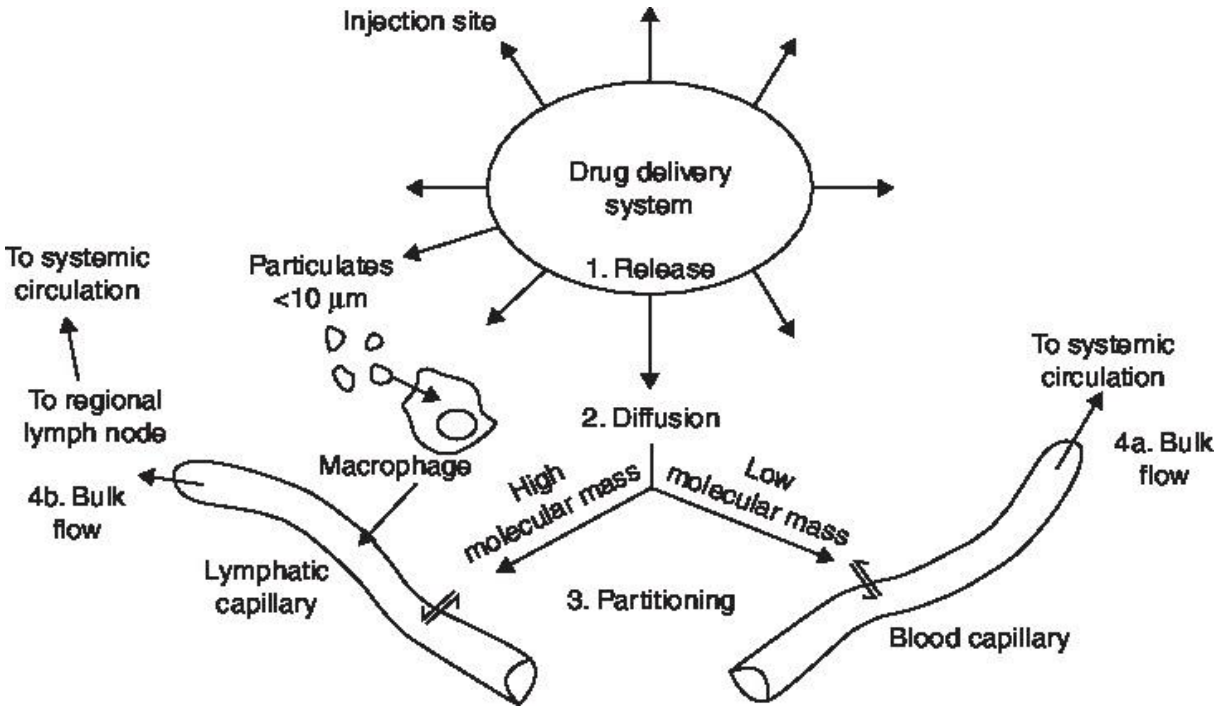


Figure 1.11 Possible pathways for absorption of drugs from controlled release parenteral dosage forms at intramuscular or subcutaneous sites (C. Larsen et al., 2009)

Normal blood flow to subcutaneous tissue is 1.5–2.5 mL/100 g/min (Benet, 1990; Enevoldsen, Simonsen, Stallknecht, Galbo, & Bulow, 2001) the blood flow to intramuscular tissue is higher. However, tissue blood flow is subject to high variability such as exercise, pathology diurnal changes, body position, etc. and may be of crucial importance to the resulting plasma profiles if release from the dosage form is not the rate-limiting factor. Estimation of the various rate constants would require an *in vivo* input rate, obtained through intravenous administration. In

addition, an estimation of drug concentrations at the interstitial site, possibly involving microdialysis studies would also provide useful information regarding disposition of the drug.

In sustained release parenterals, however, the ratelimiting step in the absorption is controlled by the delivery system, thereby reducing potential inter-injection and interpatient variability resulting from differences in injection site perfusion (Medlicott et al., 2004).

An additional barrier to diffusion is provided by cells, depending on the lipophilic character of the drug, it may be partitioned into the adipose cells, and be released back into interstitial fluid as a function of $-k$D, the re-distribution rate. This could lead to formation of a localized depot, represented by the drug concentration, C_a, in the tissue.

Also, when administering parenteral dosage forms that deliver over very long periods of time, changes of the surrounding tissue, as for example inflammation and formation of fibrous capsules due to foreign body reaction, have to be taken into account (F. D. Anderson, Archer, Harman, Leonard, & Wilborn, 1993).

The tissue reaction (intensity as well as duration of the inflammatory and wound healing processes) is dependent on the size, shape, and chemical and physical properties of the biomaterial (J. M. Anderson & Shive, 2012).

The host responds at the site of parenteral drug administration include acute inflammation, chronic inflammation, granulation, foreign body reaction and fibrosis (Keselowsky et al., 2007). The formation of fibrous capsules around drug delivery may take place over days to weeks and for more long-acting depots, this event may contribute to the overall variability of *in vivo* drug performance (F. D. Anderson et al., 1993). Also, the fact that microparticles < 10 µm may be phagocytosed or engulfed by macrophages and foreign body giant cells in the inflammatory and healing responses may affect variability.

Therefore, biological factors to consider when developing an *in vitro* release test in case of intramuscular or subcutaneous instillation include:

- the intramuscular or subcutaneous environment, its configuration, foreign body response such as immune system cells and encapsulation;

- the *in vivo* release mechanism of the drug delivery system (e.g., diffusion, erosion or osmosis);
- *in vivo* uptake of the drug and
- the complex interaction between the host and the drug delivery system.

The biological environment surrounding the formulation is not static, and it may not be possible to capture some of the biologically relevant reactions through the use of *in vitro* systems, such as inflammation with consequent cellular recruitment and fibrosis capsule formation.

Also, when the formulation is liquid like oil vehicle or in situ implants, differences in the interfacial area between the surface of the depot and the aqueous tissue fluid, which cause variability in the overall apparent release rate, may arise from less predictable spreading (oils and gels) or microparticles aggregation phenomena taking place at both subcutaneous and intramuscular injection sites.

1.7 *In vitro–in vivo* correlation of parenteral controlled release drug delivery systems:

The development of suitable *in vitro* release models (for quality control as well as formulation development purposes) is a critical activity, which, preferably, should be initiated in the early depot design phase. These efforts should ideally lead to the establishment of an *in vitro–in vivo* correlation (IVIVC) (C. Larsen et al., 2009). An IVIVC imparts *in vivo* validation to the *in vitro* dissolution test, which can then be used as a surrogate for bioequivalence testing. In addition, more meaningful dissolution specifications can be set using the concept of an IVIVC (Uppoor, 2001). A Guidance document was issued by the Food and Drug Administration in an effort to: (a) reduce the regulatory burden by decreasing the number of biostudies needed to get approval and maintain an extended release product on the market and (b) set dissolution specifications that are more meaningful clinically. The ultimate goal is that demonstration of valid IVIVCs would allow many of the biostudies that are generally required for major manufacturing changes to be replaced by simple *in vitro* dissolution tests. The ideal approach to IVIVC modeling is to develop one IVIVC model for the total plasma profile, but other approaches might also be pursued (Byung H Woo et al., 2001). Importantly, the development of a true IVIVC requires that a mathematical model describes the *in vitro–in vivo* relationship for two or more formulations showing different release characteristics (Young, Farrell, & Shepard, 2005). When a meaningful IVIVC has been established, it can be used as a surrogate for bioequivalence and for minimizing

the number of bioequivalence studies to be performed during drug product development (Uppoor, 2001). Four categories of correlations (A–D) have been described in the guidance. A Level A correlation represents a point-to-point relationship, generally linear, between *in vitro* dissolution rate and the *in vivo* input rate. A Level B correlation involves the principles of statistical moments. The mean *in vitro* dissolution time is compared either to the mean residence time or the mean *in vivo* dissolution time. This is not considered to be a point-to-point correlation and because a number of different *in vivo* curves will produce similar mean residence time (MRT) values, this cannot be considered discriminatory for different formulations. A Level C IVIVC represents a single point relationship between a dissolution parameter (such as percent dissolved at a particular time) and a pharmacokinetic parameter of interest, e.g. the area under curve (AUC). However, it does not reflect the complete shape of the plasma concentration curve, which is the critical factor that defines the performance of extended release products. A Level C correlation, although useful to screen and rank-order formulations in animal models during drug development, cannot be used for biowaivers or bioequivalence. The fourth category, D, is a multiple Level C correlation and it represents a relationship between one or more pharmacokinetic parameters, and the amount of drug dissolved, at multiple points of time on the dissolution profile (Iyer et al., 2006).

At best, the *in vitro* model may mimic the *in vivo* conditions to such an extent that a level A IVIVC can be established. This usually requires that drug release from the depot be the rate-limiting step in the absorption process and that the drug release mechanism is the same *in vitro* and *in vivo*. In this case, performance of *in vitro* release tests might be accepted instead of an *in vivo* bioequivalence study (biowaiver) for the evaluation of minor changes in the composition or manufacturing process of an already marketed product.

The lack of true IVIVCs can most likely be ascribed to barriers related to intrinsic drug properties or the inability to design *in vitro* methods sufficiently capable of mimicking the *in vivo* conditions at the injection site.

Often, it has been emphasized that *in vitro* release model development should be based on knowledge of the *in vivo* drug release mechanism (Burgess et al., 2004, 2002; Martinez et al., 2008). So far A lot of investigation has been done on the effect of the pH, ionic strength and

temperature of the release medium, and agitation and flow rate (in continuous flow methods) on the resulting drug release kinetics from PLGA-based formulations (Faisant, Akiki, Siepmann, & Benoit, 2006; Rawat, Bhardwaj, & Burgess, 2012). However, very little is known on the impact of *in vivo* conditions on the drug release mechanisms.

In general, with increasing duration of therapeutic activity, two or more *in vivo* events may contribute to the overall drug release characteristics. Thus, it is suggested that the probability for successful development of a suitable *in vitro* release model (for batch control and IVIVC), so to speak, is inversely proportional to the intended duration of action of the formulation.

Although parenteral controlled drug delivery systems have been used for intramuscular and subcutaneous administration for several decades, the *in vivo* drug release mechanism(s) are far from fully elucidated.

In vivo phenomena which are difficult to simulate using an *in vitro* release model include exact composition of tissue, non-predictable host responses, spreading and dispersion of the liquid formulation (oil vehicle and in situ implant) at the injection site.

Zolnik et al. investigated two PLGA microsphere formulations, with different polymer molecular weights to determine whether an *in vitro* and *in vivo* relationship could be established for dexamethasone release (Zolnik & Burgess, 2008). A USP apparatus 4 was used for *in vitro* testing. The *in vivo* release kinetics and pharmacodynamic effects of dexamethasone were evaluated using a Sprague Dawley rat model. The *in vitro* release from both formulations followed the typical triphasic profile of PLGA microspheres (initial burst release, followed by a lag phase and a secondary zero-order phase). The *in vivo* release profiles differed in that the lag phase was not observed and drug release rates were faster compared to the *in vitro* studies. It is speculated that the lack of lag phase *in vivo* may be a result of different PLGA degradation mechanisms *in vivo* as a consequence of the presence of enzymes as well as other *in vivo* factors such as interstitial fluid volume, and local pH. A linear *in vitro–in vivo* relationship was established after normalization of the time required to reach plateau for the in vitro and *in vivo* data and the *in vitro* release data were predictive of the *in vivo* release.

It was also reported that release rate from polylactic acid (PLA) intrascleral implant was *in vivo* faster than *in vitro.* The faster *in vivo* versus *in vitro* release rate may have reflected the decrease in pH inside the implant that occurred while it was imbedded in the eye. This drop in pH enhanced the autocatalysis occurring at the center of the polymer matrix. Furthermore, a burst release, which was seen *in vitro*, did not occur *in vivo* (Okabe et al., 2003).

The drug release of commercially available risperidone PLGA-based microparticles (Risperdal® Consta) was investigated t *in vitro* and *in vivo* (Rawat et al., 2011). A modified USP apparatus 4 was used for *in vitro* testing. The *in vivo* release kinetics and pharmacodynamic effects of risperidone were evaluated using human model and microspheres were placed in the intramuscular space. The *in vivo* profile of commercial Risperdal® Consta microspheres differed from the real-time *in vitro* profile and was faster initially and then slower after approximately 30 days. This effect is considered to be due to differences in the *in vivo* conditions such as small interstitial volume, low pH and immune response.

Risperidone implant was investigated to determine whether an *in vitro* and *in vivo* relationship could be established for drug release (Amann, Gandal, Lin, Liang, & Siegel, 2010; Rabin et al., 2008). A sample and separate method was used for *in vitro* testing. The *in vivo* release kinetics and pharmacodynamic effects of risperidone were evaluated using a Sprague Dawley rat model and implants were placed in the subcutaneous space. A level B correlation yielded between *in vitro* and *in vivo* data.

The authors confirmed that mass loss (microsphere weight) and PLGA molecular weight decreased at a faster rate *in vivo*. It was hypnotized that the absorption of acidic oligomers *in vivo* is faster, which were probably not as soluble under *in vitro* conditions. Faster enzymatic degradation *in vivo* was also stated as a reason for higher mass loss (Heya et al., 1994; Tracy et al., 1999).

1.8 Application of the described release setups to implants:

Implants are usually solid polymeric devices with a drug load and a release mechanism that ensures the amount of drug being delivered per time unit throughout their residence time in the body. They may be biodegradable and may require medical assistance for insertion and removal

if necessary. They may either act locally or systemically. The residence time varies from days to years. The difference from the medical devices is that the drug release, rather than the physical effect, is their primary purpose.

The majority of the dissolution tests reported for implants have been performed using incubation methods comparable with the sample and separate setup without subsequent filtration or centrifugation (Ghalanbor, Körber, & Bodmeier, 2010, 2012, 2013). Advantages of this method include ease of handling, the lack of need for specialized apparatus and the large number of experiments that can be conducted simultaneously. Furthermore, when incubating implants in closed vials or tubes, evaporation is not an issue and complete media changes can easily be performed by transferring the implant to a new container. Care has to be taken, however, to ensure that sink conditions are not violated at any time with these typically small volume setups.

The flow-through method also represents a feasible approach to dissolution testing of implants. The most striking advantage of using the flow-through cell is that the flow conditions can be adapted to the flow conditions present at the site of *in vivo* release. The impact of different flow rates on release from implant has been studied. As already described above, the system can be operated over a wide range of media volumes, which can easily be adapted with each experiment to assure sink conditions. Noncompendial cells have been introduced: for example a low volume implant cell with an inner diameter of 6 mm and a cell volume of 1 ml, which reduces the total minimum volume of medium to approximately 15 mL (Looney & Cqrporation, 1996). Evaporation can also be controlled by using sealed media containers.

As reported previously, the *in vivo- in vitro* correlations of parenteral controlled drug delivery systems are usually poor. A major limitation of all methods for dissolution testing of implants is that the dosage form is placed directly in the dissolution media. This does not represent the *in vivo* environment, in which the implant is typically in contact with cells and tissue fluids. Until now, the effect of mechanical properties of the tissue has not been studied and very little studies are done about the effect of the lipophilic environment of the tissue on the formulation and drug release from biodegradable implants.

1.9 Objectives

The purpose of this work was:

- to evaluate the swelling of the implant *ex vivo* and to quantify the tissue pressure acting on PLGA-based matrix implants *ex vivo*
- to design a new *in vitro* test to mimic mechanical properties of the tissue for solid parenteral DDS and to investigate the effect of restricted swelling on drug release from PLGA-implants
- to apply biphasic release test model as a new biorelevant model for drug release from PLGA-implants and investigate its effect on risperidone release from PLGA-based implants

2. Materials and Methods

2.1 Materials

2.1.1 Model drugs

Risperidone (Wuxi Jida Pharmaceutical Co. Ltd, China); Theophylline (BASF AG, Ludwigshafen, Germany) and Ibuprofen (BASF AG, Ludwigshafen, Germany).

Table 2.1 Solubility of model drugs in phosphate buffer pH 7.4 at 37°C

Drugs	Solubility (mg/mL)	Physicochemical properties
Risperidone	0.334	Basic
Theophylline	12.700	Non-ionic
Ibuprofen	7.000	Acidic

2.1.2 Polymers

Poly (lactide-co-glycolide) (PLGA) Lakeshore 5050 DLG 1A, Lakeshore 5050 DLG 2A, (Evonik, Kirschenallee, Darmstadt, Germany); PLGA Resomer® RG 502H, PLGA Resomer® RG 503H (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany).

Table 2.2. Inherent viscosity of PLGA

Polymers	Inherent viscosity (dl/g) (1)
5050 DLG 1A	0.05 – 0.15
5050 DLG 2A	0.15 – 0.25
PLGA RG 502H	0.16 – 0.24
PLGA RG 503H	0.32 – 0.44

(1) Obtained from the measurement of 0.1% solution in chloroform at 25°C and provided by Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany

2.1.3 Other Excipients

Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide (NaOH), sodium azide (Merck KGaA, Darmstadt, Germany); hydrochloric acid (HCl) (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), tetrahydrofuran (Carl Roth GmbH & Co. KG, Karlsruhe, Germany); magnesium stearate (Baerlocher GmbH, Unterschleissheim, Germany), 1-octanol, blue dextran (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), olive oil (Lipoid GmbH, Ludwigshafen, Germany), Flowlac® 100 (Meggle GmbH, Wasserburg, Germany), the spin label 4-(methylamino)-2-ethyl-5,5-dimethyl-4-pyridine- 2-yl-2,5-dihydro-1H-imidazol-1-oxyl (MEP) (Magnettech GmbH, Berlin, Germany).

2.2 Methods

2.2.1 Preparation of PLGA implants using hot melt extrusion

For *ex vivo* and *in vitro* studies powder blends of PLGA (Lake shore 5050 DLG 2A) and the blue dextran were manually mixed in a mortar with a pestle. Blue dextran was used in order to make the implants visible and to extract them easier from the tissue. Cylindrical implants were prepared by hot melt extrusion using a co-rotating conical twin screw extruder (HAAKE MiniLab Rheomex CTW5, Thermo Electron) at 80°C and a 1.5 mm extrusion die. Cylindrical matrices were obtained with a diameter of 1.5 – 2.5 mm. The implants were cut into 2.5 - 4 mm length. All experiments were performed at least in triplicate.

For biphasic studies polymer blends of PLGA (Lake shore 5050 DLG 1A) and the model drug risperidone were mixed in a 9:1 and 7:3 ratio w/w in a mortar with a pestle. 1 ml polypropylene syringes (LUER LOK™, B-D®, Singapore) were filled with approximately 1g of properly mixed formulation blends. The syringes were placed in a self-built die, fixed and heated at 105°C in an oven for 10 min. The molten blends were extruded manually, producing cylindrical matrices with diameters of 1.1 – 1.2 mm. The matrices were cut into 3-5 mm length.

2.2.2 Preparation of PLGA implants using direct compression

For risperidone, three different PLGA implants were prepared with polymer blends of PLGA (Lake shore 5050 DLG 1A, 502H and 503H), whereas for ibuprofen and theophylline implants, only PLGA 503H was used. In all cases, PLGA and drug were mixed in a 9:1 ratio w/w in a mortar with a pestle. Magnesium stearate was added as lubricant to each blend (0.5% wt). Implants were prepared by manually compressing the powder mixture into 8 mm flat faceted implants with an instrumented single punch tableting machine (EK0, Korsch AG, Berlin, Germany). The implants were characterized with regard to their dimensions and hardness (Multicheck, Erweka GmbH, Heusenstamm, Germany). Additionally, implants containing only drug (10%) and lactose as filler were prepared to study drug diffusion through the device.

Implants containing nitroxyl radical MEP and PLGA were prepared for EPR measurement. The nitroxyl radical MEP was dissolved in ethanol, mixed in a mortar with a pestle with polymer, and dried overnight, thus achieving a final concentration of 5 mmol/kg powder.

2.2.3 Restricted swelling device

A novel device was fabricated to prevent the swelling of formulations described results and discussion in section 3.B.2.4.

2.2.4 Adsorption test of the restricted swelling device

A solution of the drug in distilled water (2.76%) was filtered three times through a glass filter of the device. The concentration of the solution was determined using UV/VIS spectrophotometer (Shimadzu UV HP 8453, Shimadzu Japan) before and after filtration.

2.2.5 Diffusion through the restricted swelling device

Implants containing only drug (10%) and Flowlac 100 used as filler were placed in the device and the release study was carried out by the method previously described in section 2.2.7. Sink conditions were maintained throughout the study time.

2.2.6 Drug extraction from implants

To quantitatively detect the actual drug loading of implants, 2 mL of 2M NaOH was added to vials each containing an accurately weighed implant. After 24 hours when the implants were completely dissolved, 3 mL of 2M HCl was added to the vials, in order to dissolve the drug. The solution was analyzed using UV/VIS spectrophotometer (Shimadzu UV HP 8453, Shimadzu Japan) with a Peltier thermostated cell holder (Agilent 8453, Agilent Technologies Inc., Palo Alto, USA) equipped with UV-Chemstation biochemical analysis software at 277 nm, for risperidone implants, and with single wavelength background correction at 400 nm.

2.2.7 *In vitro* drug release study

Implants prepared using direct compression were placed in the restricted swelling devices, which were placed in the 120 mL custom-made glass bottles filled with phosphate buffer pH 7.4. Unrestricted implants were placed directly in the 120 mL custom-made glass bottles filled with 110 mL phosphate buffer pH 7.4. The release study was carried out under sink conditions. These bottles were incubated in a vertical position in the horizontal shaker (80 rpm, 37 °C; Gesellschaft für Labortechnik mbH, Burgwedel, Germany). At predetermined time points, 5 mL samples were withdrawn and replaced with fresh release medium. The drug amount in the release medium was quantified UV-spectrophotometrically at 277 nm for risperidone, 222 nm for ibuprofen and 271 nm for theophylline (Shimadzu UV HP 8453, Shimadzu Japan).The test was performed in triplicate (n = 3). The drug release of restricted implants was compared to that of unrestricted ones.

Implants prepared using hot melt extrusion were placed in Duran® 50 mL glass bottles filled with phosphate buffer pH 7.4. These bottles were incubated in a vertical position in the horizontal shaker (80 rpm, 37 °C; Gesellschaft für Labortechnik mbH, Burgwedel, Germany). At predefined sampling points, 5 mL of release sample was withdrawn and was replaced with fresh release medium. Testing was performed in triplicate (n = 3). In monophasic system, 60 mL phosphate buffer pH 7.4 and in biphasic system containing octanol, 45 mL phosphate buffer pH 7.4 and 15 mL 1-octanol were used as dissolution media. Drug concentrations in release samples were quantified in both phases spectrophotometrically at 277 nm for risperidone (Shimadzu UV HP 8453, Shimadzu Japan). For release study in biphasic system containing olive oil, at

predetermined times implants were taken out of the release medium and the remaining amount of drug in implants was determined using extraction method.

2.2.8 Degradation study and molecular weight determination

At predetermined time points, implants were withdrawn from release medium, vacuum dried for 48 h, and dried formulations were incubated in 2 mL tetrahydrofuran for five hours. The turbid samples were centrifuged at 17000 rpm for 15 minutes (HeraeusTM BiofugeTM, Thermo Fisher Scientific Inc., Waltham, MA, USA) to remove the particles and the supernatant was analyzed for the molecular weight distribution of the remaining polymer by gel permeation chromatography. Gel permeation chromatography (GPC) analysis was carried out using Shimadzu (Shimadzu, Tokyo, Japan) LD-10 liquid chromatograph equipped with degasser, pump, auto-injector and column oven in combination with Viscotek triple detector (TDA-300, Viscotek, Malvern Instruments Ltd., Malvern, UK) operated in double mode (differential refractive index, viscosimetry). A column with a linear range from 1200 g/mol to 18,000 g/mol (Mesopore 7.5 μm x 300 mm; Varian Inc., Darmstadt, Germany) and tetrahydrofuran were used as stationary and mobile phases, respectively. The sample concentration was 20 mg/ml with the corresponding injection volumes of 25 μl. Column and detector were operated at 30 °C and the flow rate was 1 ml/min. A refractive index (RI) detector was used to determine the molecular weights of PLGA, which was obtained from polystyrene standards with peak molecular weights of 1,260 g/mol, 2,360 g/mol, 4,920 g/mol, 9,000 g/mol, 19,880 g/mol (Varian Inc., Darmstadt, Germany). Data acquisition was performed using Omnisec software (Viscotek, Malvern Instruments Ltd., Malvern, UK).

2.2.9 *In vitro* and *ex vivo* experiments

For the *in vitro* experiment, implants were incubated in 60 mL of 0.1 M phosphate buffer pH 7.4, at 37°C. For the *ex vivo* experiments, implants were implanted into turkey breast, the small incision in the tissue was closed using tissue glue and they were incubated in either 1 mL (to avoid tissue dehydration) or in 60 mL (analogue to the *in vitro* release set-up) of 0.1 M phosphate buffer pH 7.4 containing 1% sodium azide as preservative at 37°C. Samples were taken at predetermined points for analysis. At each point, all implants were removed from old tissue and implanted into a fresh turkey breast under *ex vivo* experiments. For both conditions,

samples were taken at predetermined points and the swelling, water uptake and shape changes of the implants were assessed.

2.2.10 Determination of tissue force *ex vivo*

The tissue force applied to the implant *ex vivo* was determined with a balloon catheter in fresh turkey breast. For this purpose, a 3 mL Luer Lock syringe was filled with water and connected to a balloon catheter (OTW PTA balloon catheter). The plunger was placed in contact with the probe of a texture analyzer (Stable Micro System®, Vienna Court, UK) and the injection force was measured as a function of the plunger displacement (**Figure 2.1**). The displacement was set to 10, 15, 17.5, 20 and 25 cm and the injection speed was 15 mm/min. To find the appropriate test settings leading to a linear relationship between injection force and simulated tissue force (0 N, 10 N, 20 N), the force reached after a certain displacement was measured with the balloon catheter in air (blank) and with a weight of 1 and 2 kg placed on the balloon. Once the appropriate settings were determined, the balloon catheter was inserted in fresh turkey breast in order to measure the force applied to the implants *ex vivo*. The injection force in this *ex vivo* experiment (average of at least 3 measurements) was used to calculate the force applied to the formulation *ex vivo*.

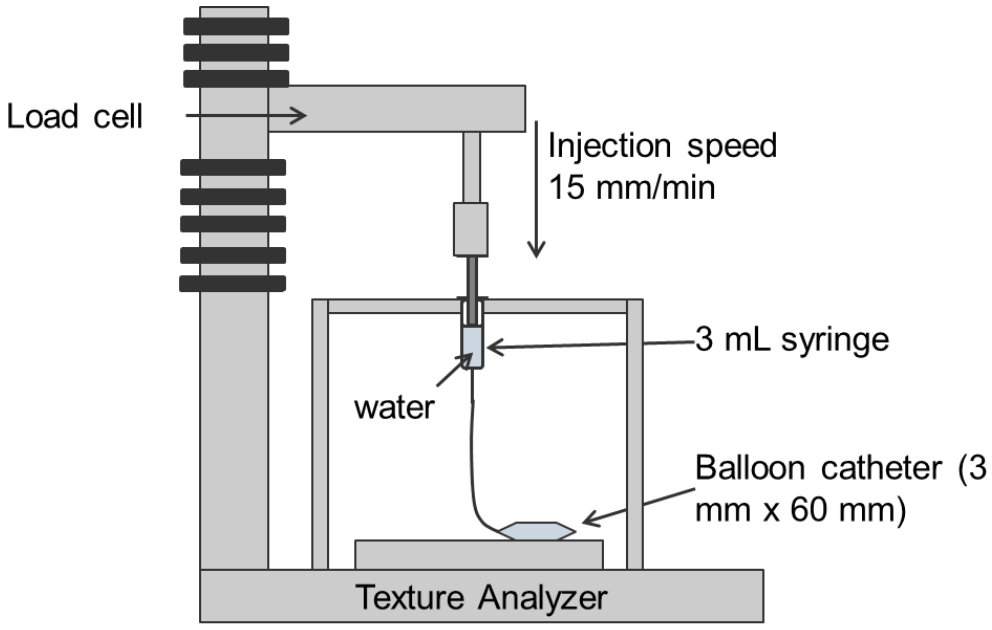


Figure 2.1 Illustration of the setup for injection force measurement

2.2.11 Differential scanning calorimetry (DSC)

DSC-studies (Mettler Toledo 822e) of implants after extrusion and after 1 day of release (~10mg) were recorded using a heating rate of 20°C /min under nitrogen atmosphere from -20°C to 80°C cooled to -20°C and heated again to 180°C at a rate of 5°C/min. Each thermogram obtained was normalized for sample weight and the glass transition temperature was determined using the Stare Software.

2.2.12 Water content of the turkey breast

Hydration and dehydration of tissue was studied by monitoring the water content of the turkey breast after incubating it alone or in 60 mL of PBS at 37°C over a period of 5 days. Water uptake of turkey breast meat was quantified by weight gain at predetermined time points (Equation 2.1).

$$\text{Water uptake (\%)} = \frac{(W_{ti} - W_0)}{W_o} \times 100 \quad (2.1)$$

W_{ti}: weight wet at time t_i

W_0: initial weight

2.2.13 Water uptake study

Water uptake of the implants was determined by their weight gain during the incubation time (Equation 2.2). At predetermined time points (t_i), the implants under both, restricted and unrestricted conditions, were taken out of the release medium, in *in vitro* and *ex vivo* studies the implants were taken out of the incubation medium or extracted from the turkey breast meat and weighed after the excess of water at the surface had been removed with filter paper. The wetted samples were then dried under vacuum to constant weight. The water uptake study was done for 12 days for implants under restricted versus unrestricted condition, 11 days for the implants in monophasic versus biphasic studies and 7 days for the implants in *in vitro* versus *ex vivo* studies, thereafter handling of the formulations was not possible.

$$\text{Water uptake (\%)} = \frac{(weight\ wet - weight\ dry)}{weight\ dry} \times 100 \quad (2.2)$$

2.2.14 Diameter increase

To study the swelling of the implants, the diameter of the implant was determined in its initial dry form (D_0) and at predetermined time points (D_{ti}) during the incubation time. Implants were taken out of the release medium and their diameter was determined using a macroscope. The diameter growth was calculated as followed (Equation 2.3):

$$\text{Diameter increase (\%)} = \frac{(D_{ti} - D_0)}{D_0} \times 100 \quad (2.3)$$

To study the swelling of the implants, the surface area of the implant was determined in its initial dry form (A_0) and at predetermined time points (A_{ti}) (Equation 2.4) over seven days. The implants were removed from the incubation medium (*in vitro*) or turkey breast (*ex vivo*). The diameter and length of implants were measured using a macroscope.

$$\text{Surface area change (\%)} = \frac{(A_{ti} - A_0)}{A_0} \times 100 \quad (2.4)$$

2.2.15 Determination of implant morphology by optical macroscope

Implant morphology, changes of the implant's shape and size were investigated using a macroscope (Inteq Informationstechnik GmbH, Berlin, Germany). The implants were observed and size was measured at predetermined time points before incubation and during the drug release study. The images were recorded by an image analysis software (EasyMeasure, Inteq Informationstechnik GmbH, Berlin, Germany).

2.2.16 PLGA mass loss

To study the mass loss of the implants, the weight of the formulations was determined in its initial dry form (W_0) and then at predetermined time points (W_{ti}) (Equation 2.5). The formulations were removed from the release medium and dried in the vacuum oven to constant weight (dry weight) (Heraeus oven VT 5042 EKP, Hanau, Germany coupled with a chemistry hybrid pump, Vacuubrand GmbH, Wertheim, Germany). The mass loss study was done for 12 days for the implants under restricted versus unrestricted condition and 13 days for the implants in *in vitro* versus *ex vivo* studies. Thereafter handling of the formulations was not possible.

$$\text{Mass loss (\%)} = \frac{(W_{ti} - W_0)}{W_0} \times 100 \quad (2.5)$$

W_0: initial weight

W_{ti}: dry weight at time t_i

2.2.17 Determination of µpH using EPR

In order to prepare matrix implants for EPR imaging experiments, the nitroxyl radical MEP was dissolved in ethanol and blended with polymer, thus achieving a final concentration of 5 mmol/kg powder. Implants were prepared as described in section 2.2.2.

At predetermined time intervals, restricted and unrestricted implants were withdrawn from the incubation medium, carefully placed on a Teflon plate and EPR spectra were recorded. After the measurements, the implants were placed back into the device and in the incubation medium. After 16 days, no more solid signals were detectable indicating that the spin probes migrated with the solvent outside the implants

The spin label 4-(Diethylamino)-2-ethyl-5,5-dimethyl-2-pyridine-4-yl-2,5-dihydro-1H-imidazol-1-oxyl was chosen based on its pK_a. Nitroxide spin probe was calibrated in citrate and phosphate buffer solutions in order to cover a pH range between 2.2 and 7.6. EPR spectra were recorded as the first derivative of the absorption signal, and the hyperfine splitting a_N was measured as the distance between the low-field ($m_I = +1$) and central ($m_I = 0$) lines. The measurements of the hyperfine splitting constant a_N of spin label with different pH values served as calibration curve. A sigmoidal Boltzmann was used to fit the data. The determination of accurate pH was possible in the range from 2.2 to 7 with this spin label

EPR spectra were recorded at a frequency of 9.4 GHz using an X-band ERS 220 spectrometer (ZWG, Berlin, Germany) with a custom-made tomography extension, equipped with a rectangular resonator. The EPR parameters used were as follows: microwave power, 2 mW; modulation amplitude, 0.1 mT; scan width, 10 mT; maximum gradient, 4.27 T/m; scan time per projection, 10 s; 95 projections with 512 points per projection; image matrix, 256 × 256 points. Images were obtained from the recorded set of EPR spectra by means of deconvolution and image reconstruction.

3. Results and Discussion

A

Investigation of the effect of tissue pressure on the shape, swelling and water uptake of PLGA-based matrix implants and quantification of tissue pressure acting on the formulation after implantation

3.A.1 Background

Advances in the development of controlled release parenteral formulations were made during recent years to overcome issues with patient acceptance and compliance related to multiple injections in the treatment of some chronic diseases. Furthermore, sustained or controlled parenteral delivery offers opportunities like reduced side effects as a result of long-lasting constant drug plasma levels and localized delivery in the body (Burgess et al., 2004; Rawat et al., 2012). Polymers used in parenteral controlled release systems are divided into two main categories: non-biodegradable and biodegradable polymers. PLGA has been used to a wide extent as biodegradable polymer in drug delivery systems. The reasons for the widespread use of PLGA are its biodegradability, its biocompatibility, and the fact that PLGA have been approved for parenteral use by regulatory authorities. A disadvantage of this polymer is that drug release from formulations containing PLGA *in vivo* differs often from *in vitro*. In order to improve IVIVC, *in vivo* factors, influencing PLGA formulations must take into consideration.

PLGAs absorb a large amount of water and are prone to swell. The degradation starts with the water uptake. During degradation, the molecular weight of the polymer decreases and it becomes more hydrophilic and polymer chains become more mobile, which further promotes polymer swelling (Fredenberg et al., 2011).

However after implantation of a PLGA drug delivery system in the body, the formulation is in contact with tissue cells (Iyer et al., 2006). The tissue applies a force on the formulation, which could lead to restricted swelling of the implant and change of its shape. Furthermore, water uptake *in vivo* could be decreased as well due to the limited amount of fluid, which leads to restricted swelling and ultimately affects the drug release kinetics.

Although different methods have been used to determine the mechanical properties of both muscle tissue and subcutaneous tissue (Aratow et al., 1993; Arda, Ciledag, Aktas, Aribas, & Köse, 2011; R. E. Ballard et al., 1998; Crenshaw, Karlsson, Gerdle, & Friden, 1997; Hospital, Imagine, Memorial, Jobe, & Angeles, 2010; H. T. Leong, Ng, Leung, & Fu, 2013; Samani & Plewes, 2004; Winters et al., 2009; Yungher, Wininger, Barr, Craelius, & Threlkeld, 2011), still only little is known about the force applied to the formulation after injection or implantation in the body. It was found that shear elasticity of muscle tissue is two times higher than

subcutaneous tissue and it increases significantly during active contraction (H. T. Leong, Ng, Leung, & Fu, 2013). In this study muscle tissue was used. In muscle, large homogeneous regions exist; such regions are in soft tissues very rare. Subcutaneous tissues are often very soft, have a high deformability and are composed of heterogeneous regions (Samani, Bishop, Luginbuhl, & Plewes, 2003). Therefore, the handling of subcutaneous tissue was problematic.

3.A.2 Evaluation of PLGA implants after implantation in turkey breast

Swelling is the increase of volume of material due to absorption of a solvent. Swelling is dependent on the amount of liquid material that can be absorbed and the amount of space available. In order to study these two factors, 3 experiments were designed. As control, in an *in vitro* experiment implants were placed in release medium, where an excess of space and water were available for swelling and implants were able to swell to their maximal degree. To decrease the available space (which could also simulate *in vivo* condition/ mechanical properties of tissue) implants were placed in fresh turkey breast (*ex vivo* experiment). To study how the amount of water available for the polymeric matrix affects polymer swelling, turkey breast was incubated with 60 mL phosphate buffer and with a minimal amount of phosphate (1 mL) buffer to avoid tissue dehydration at 37°C. In order to make sure in both cases different amount of water was available for implants, turkey breast was incubated with 60 mL phosphate buffer and without buffer and the water uptake of tissue was studied first. The results obtained in these studies are shown below.

3.A.2.1 Determination of the amount of water in the turkey breast

In order to determine the amount of water in the turkey breast when it was incubated at 37°C with and without phosphate buffer, water uptake of the tissue was monitored over 5 days.

Water uptake in the turkey breast when incubated in 60 mL of phosphate buffer was ~20% after 24 hours, whereas in the absence of phosphate buffer it lost ~ 3% of its weight in this time **(Figure 3.A.1).** In both experiments, the weight of the tissue then stayed constant until day 5. Therefore, for the *ex vivo* experiment 1 mL of phosphate buffer was added in order to prevent the weight loss of the turkey breast and to maintain the hydration state of the tissue.

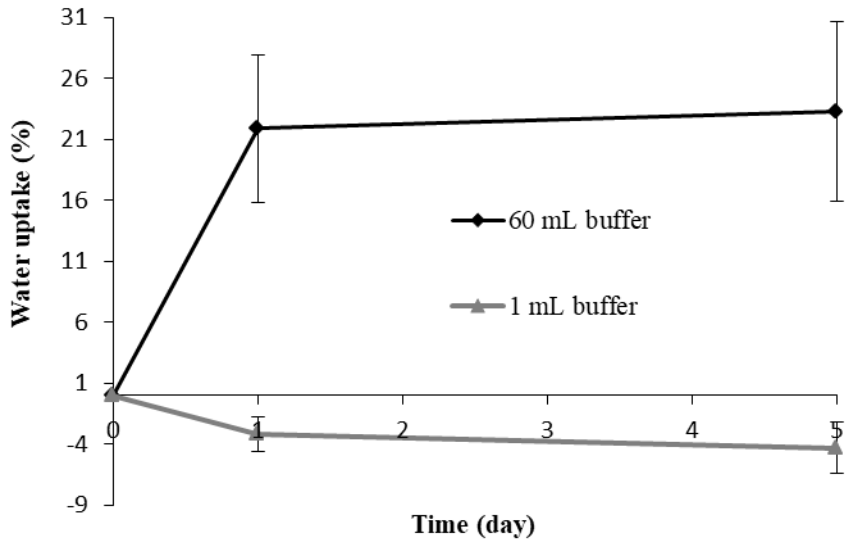


Figure 3.A.1 Water uptake of the turkey breast meat in phosphate buffer pH 7.4 at 37°C (n=3 +/- SD)

During the *in vitro* experiment the implants swelled and their surface area increased linearly over a period of 5 days. The percentage surface area increased up to 95% at day 5 (**Figure 3.A.2).** Macroscopic pictures confirmed the implant swelling, its increase in size and the unchanged cylindrical shape; nonetheless, the ends of the implant were deformed (**Figure 3.A.3)** because they could freely swell in the liquid medium. In contrast, under *ex vivo* conditions, the swelling was restricted. In both cases, the percentage surface area remained around 15% without fluctuating considerably during the complete experiment and it increased only to a maximum of approximately 23% (**Figure 3.A.2)**. These differences could be related to the restricted space in the turkey breast and the tissue pressure, which counteracted the expansion of the implants. The implants remained still cylindrical during the first five days but their ends changed minimally and rounded out. Afterwards they lost their cylindrical shape, hampering the determination of their surface area (**Figure 3.A.3)**. The reason for the complete deformation after day 5 was that the molecular weight of the implant' polymer decreased during degradation (Lu, Garcia, & Mikos, 1999). As a consequence, the implants became soft during the erosion phase (Wu & Ding, 2004, 2005) and the tissue pressure was high enough to prevent the implant' swelling and to enhance its deformation.

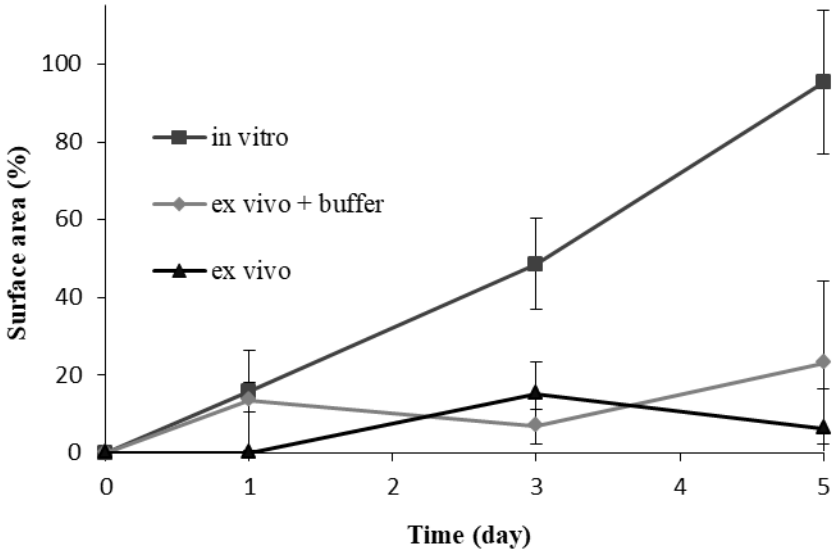


Figure 3.A.2 Surface area of PLGA-implants *ex vivo* versus *in vitro* in phosphate buffer pH 7.4 at 37°C (n=3 +/- SD)

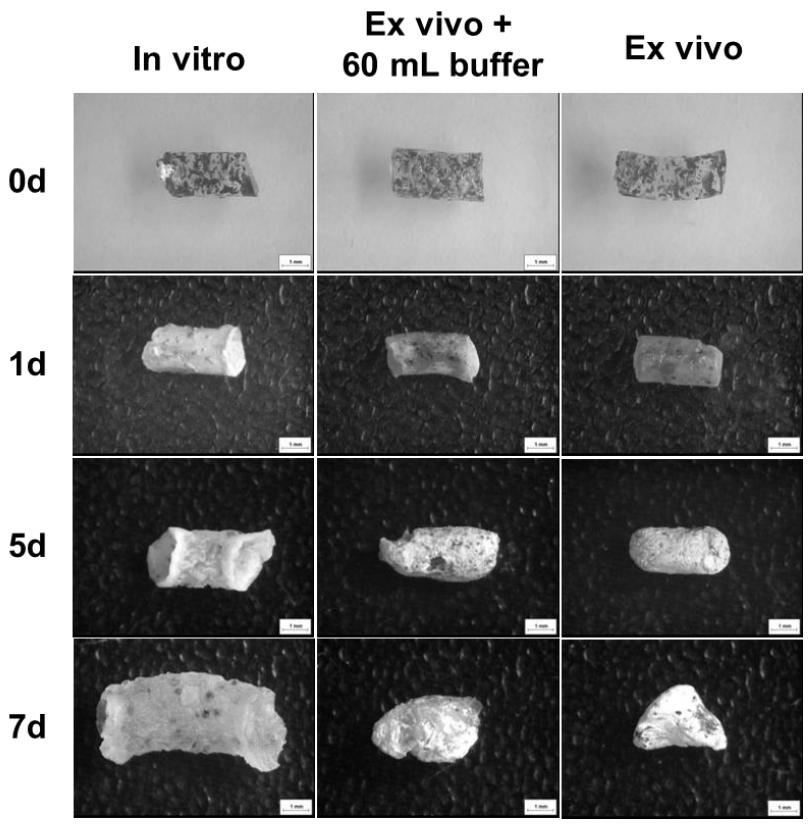


Figure 3.A.3 Macroscopic pictures of implants during *in vitro* and *ex vivo* incubation

Under *in vitro* conditions, the implants took up water and swelled, the water uptake increased linearly over a period of 7 days. The percentage surface area increased up to 107% at day 7 (**Figure 3.A.4)**. Whereas in the case of the *ex vivo* experiment with 1 mL buffer, which correlates better with the *in vivo* situation, the water uptake increased at a very slow rate and only to a maximum of 20% at day 7 (**Figure 3.A.4)**. The water uptake by the implant was low because available water was mainly taken up by the tissue in order to maintain its internal moisture content, and besides, the mechanical properties of the tissue prevented polymer swelling. Under *in vitro* conditions and *ex vivo* with 1 mL buffer, the water uptake correlated with the observed swelling behavior.

Ex vivo experiments with 60 mL buffer had a different behavior. There was no difference in water uptake of implants for both *ex vivo* experiments until day 3. After day 3, the water uptake of implants under *in vitro* conditions and *ex vivo* with 60 mL buffer increased at about the same rate (**Figure 3.A.4)**. Because the tissue contained higher amount of water under *ex vivo* conditions with 60 mL buffer compared to *ex vivo* experiment with 1 mL buffer (**Figure 3.A.1**). However, the water uptake in *ex vivo* experiment with 60 mL buffer was tendentially lower than the one under *in vitro* conditions, since the fraction of water absorbed by the tissue was available for the implant to swell; furthermore, the mechanical properties of the tissue could have also played a role.

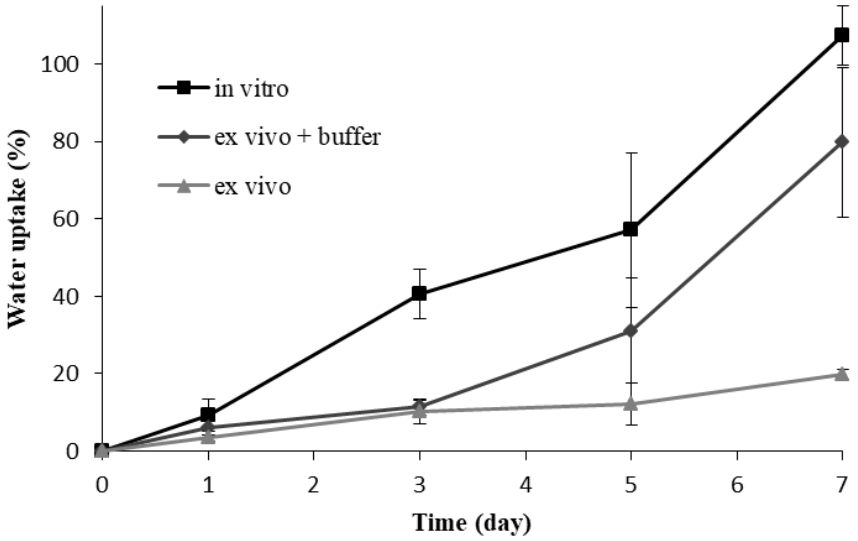


Figure 3.A.4 Water uptake of PLGA-implant *ex vivo* versus *in vitro* in phosphate buffer pH 7.4 at 37°C (n=3 +/- SD)

3.A.3 *Ex vivo* quantification of tissue pressure acting on PLGA-based matrix implants

Previous experiment showed a restricted swelling of implants under *ex vivo* conditions caused by limited physiological space and the mechanical properties of the tissue. In this experiment, the tissue force applied to the implant after implantation was determined with a balloon catheter and turkey breast meat model.

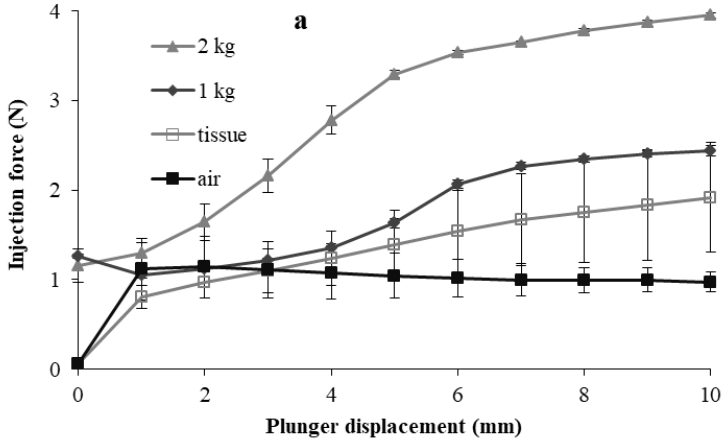
3.A.3.1 Method development

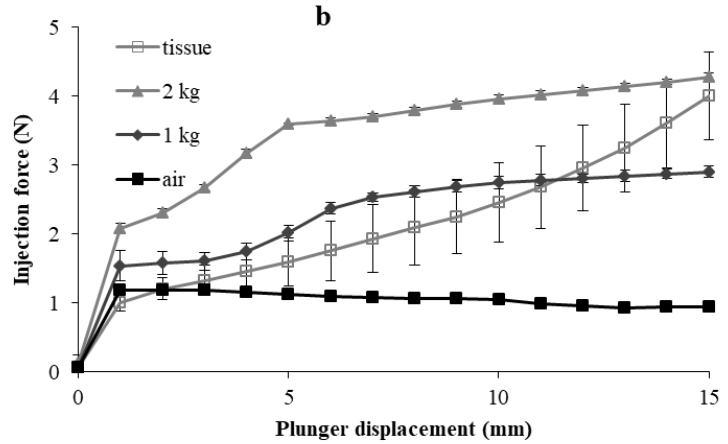
Injection speed, type of syringe and the size of the balloon catheter were the investigated parameters for the development of the method. With an injection speed of 15 mm/min, a 3-mL Luer Lock syringe and an OTW PTA balloon catheter 6 mm x 60mm results with good reproducibility were obtained (data not shown).

3.A.3.2 Determination of the force during injection of water into the balloon catheter

When the balloon catheter was exposed to air, the force which had to be applied to the syringe plunger during the injection of different amounts of water was divided into the three following phases: plunger-stopper breakloose force (PBF) followed by the dynamic glide force (DGF) (Cilurzo et al., 2011), and resistance force thereafter. The initial (0-5 mm plunger displacement) was related to the force required to initiate the movement of the plunger, which is the PBF (Cilurzo et al., 2011). PBF reached the maximum force (F_{max}) within 1 mm plunger displacement and then decreased, indicating that the highest value of force was required to promote the plunger motion (Cilurzo et al., 2011). This maximum value was followed by a plateau from 5 to 20 mm plunger displacement (second phase) indicating the sustained movement of the plunger to expel the content of the syringe into the balloon catheter and to fill and expand it with a constant force: the DGF (Cilurzo et al., 2011); i.e. once water started to flow through the needle and fill the balloon, the force remained almost constant in the second part of the profile. During the third part from 20 to 25 mm plunger displacement, the force increased rapidly because the balloon was completely filled with water and expanded to its maximum value. Pressing more water increased the pressure resistance of the balloon walls, which increased the force. In this phase pressure resistance of the balloon walls was measured **(Figure 3.A.5).**

When a weight (1 kg or 2 kg) was placed on the balloon catheter, the PBF increased with increasing weight and the shape of the curve changed: the initial peak disappeared and the force increased continuously as a function of plunger displacement **(Figure 3.A.5).**This was attributed to the weight placed on the balloon, which counteracted the movement of the plunger in this phase. However, DGF increased only slightly as a function of plunger displacement, indicating that the force needed for sustained movement of the plunger and filling the balloon catheter, increased only slightly. The reason for the minimal change of DGF might be that the force was applied only from one direction and not from all directions like in an *in vivo* situation. Consequently, the balloon catheter was able to expand to the sides.





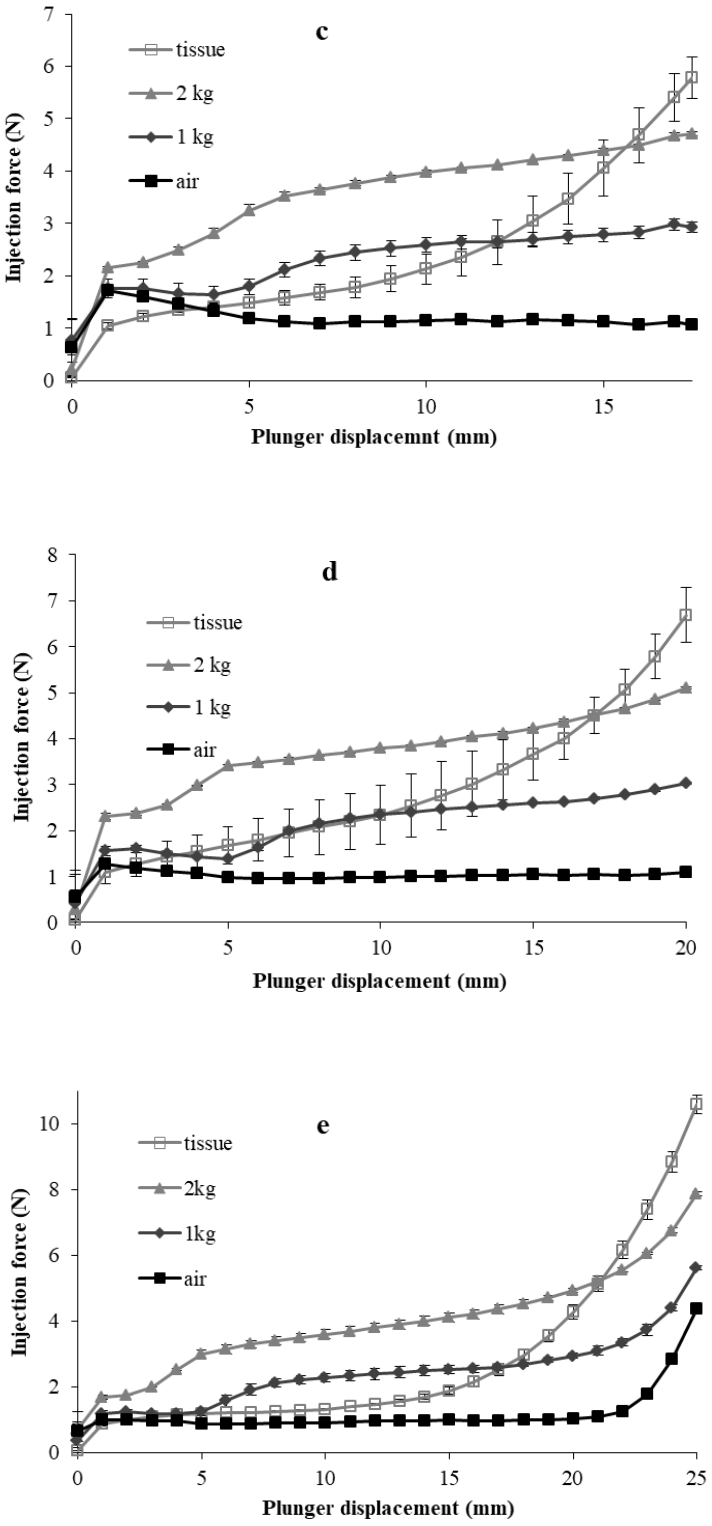


Figure 3.A.5 Force during injection of water into the balloon catheter as a function of plunger displacement: (a) 10 mm (b) 15 mm (c) 17.5 mm (d) 20 mm (e) 25 mm (n=3 +/- SD)

Once the balloon catheter is placed into the turkey breast, the force vs. plunger displacement profile of the balloon catheter exhibited a different trend. The PBF disappeared completely and the injection force increased exponentially during the plunger displacement (**Figure 3.A.6)**. In this case, the tissue surrounded completely the balloon catheter, contrary to the weights placed on the balloon, which was unidirectional. Due to the elastic properties of the tissue, during the expansion of the balloon, a resistance force toward the balloon walls was provided from all directions, which increased with higher balloon expansion. This led to the exponential profile of DGF vs. displacement **(Figure 3.A.6)**. For 25 mm plunger displacement, the shape of the profile remained unchanged however, the measured injection force was reduced and the resistance of the balloon catheters walls decreased (**Figure 3.A.6)** most probably due to a weakening of the balloon catheter walls throughout the experiments (i.e. same balloon catheter was used).

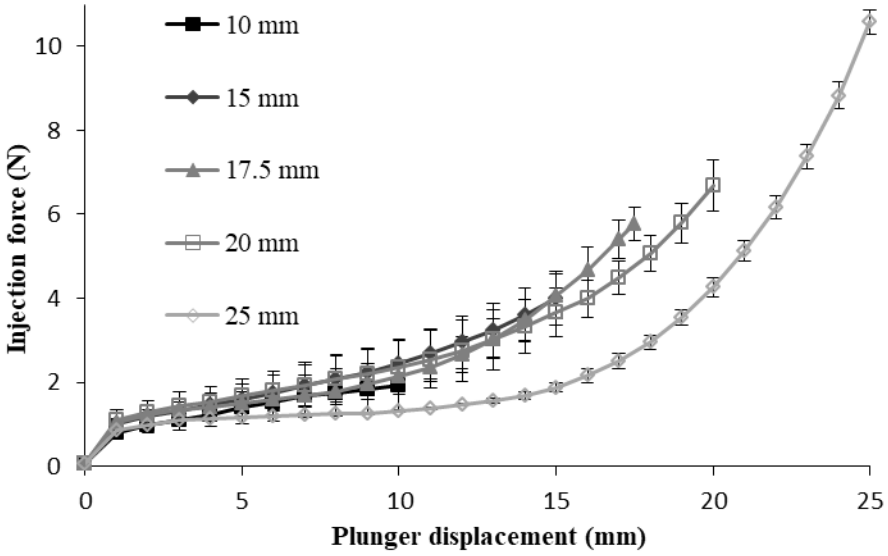


Figure 3.A.6 Injection force as a function of plunger displacement when the balloon catheter was placed into the turkey breast (n=3 +/- SD)

3.A.3.3 Relationship between the injection pressure and the pressure needed to expand the balloon catheter

The injection force while the balloon catheter was filled with water and expanded was measured with the texture analyzer (**Figure 3.A.6)**. The injection pressure was calculated taking into

account the surface area of the syringe plunger A (m²) and the measured injection force F (N) (Equation 3.1)

$$P = \frac{F}{A} \quad (3.1)$$

Furthermore, it is possible to calculate the pressure *ex vivo* using the Poiseuille equation (Equation 3.2), which describes the pressure drop in an incompressible and Newtonian fluid in laminar flow flowing through a narrow tube such as a hypodermic needle.

$$\Delta P\ (p2 - p1) = \frac{8\eta LQ}{\pi r^4} \quad (3.2)$$

Where $\Delta P\ (P2 - P1\ )$ is the pressure difference between the beginning and the end of the tube (Pa), Q is a flow rate (mm^3/s), η is a dynamic viscosity of the fluid (Pa s), L is the length of the tube (mm) and r is the radius of the tube (mm). Parameters involved in the Poiseuille equation are shown in **Figure 3.A.7.**

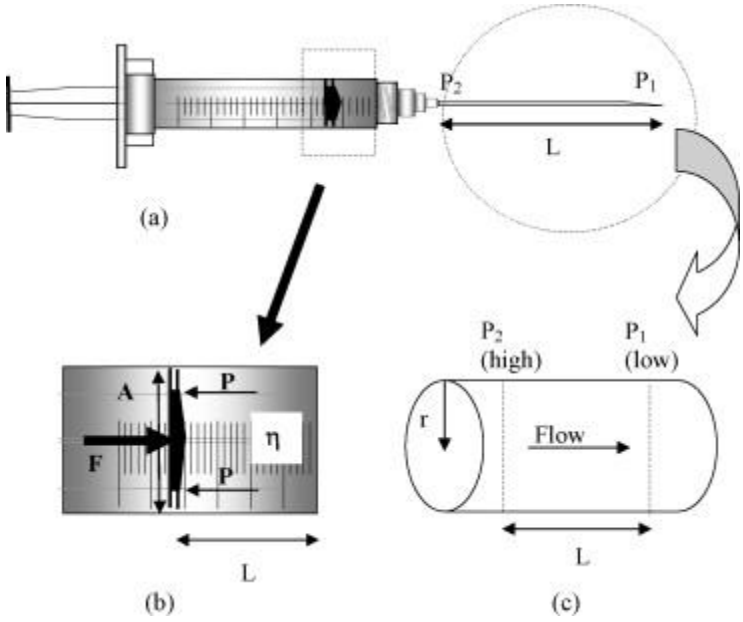


Figure 3.A.7 Illustration of syringe and some parameters related with injection force; (a) component of syringe connected with a needle; (b) force applied on syringe plunger and (c) pressure difference between the beginning and the end of needle (Rungseevijitprapa & Bodmeier, 2009)

Due to the complex shape of the syringe connected to the balloon catheter, the calculation of the pressure *ex vivo* with the measurement of the injection pressure was not possible. It was possible to measure the injection force applied on the syringe plunger to fill the balloon catheter with water via a syringe. Thus, the force applied to the balloon catheter *ex vivo* could be quantified, if a relationship between injection force and the force applied to the balloon catheter could be established. In order to find a relationship between injection force and force applied to the balloon catheter, the injection force was measured while the balloon catheter was in air and under a known weight (1 kg and 2 kg). The measurement was run with different plunger displacements, since different degree of expansion of the balloon catheter could be obtained. The injection force reached after injecting a certain amount of water into the balloon catheter (at the end of the plunger path) was plotted against the force applied to the balloon catheter, which was 0, 10 and 20 N, when the balloon catheter was in air, 1 kg and 2 kg weight was placed on the balloon catheter, respectively. A linear relationship was found between the injection force and the force applied to the balloon catheter for all tested plunger displacements and an appropriate equation was determined for each plunger displacement (**Figure 3.A.8).** The injection force increased linearly to the increase of the weight on the balloon catheter (**Figure 3.A.8).** The injection force of the 25 mm plunger displacement was higher, most likely because at 25 mm plunger displacement, additional force was required to overcome the pressure resistance of the balloon walls (**Figure 3.A.8).**

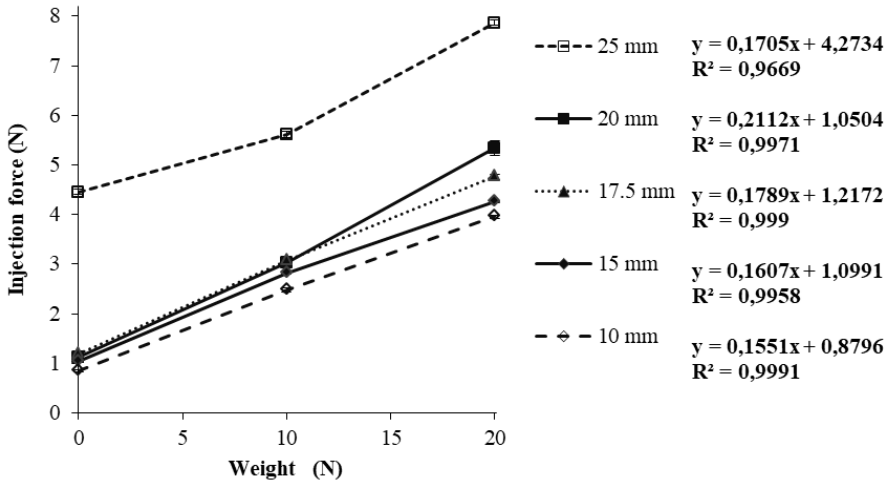


Figure 3.A.8 Correlation between injection force and the force applied to the balloon catheter (n=3 +/- SD)

After the appropriate equation was determined, the balloon catheter was inserted into a fresh turkey breast and the injection force was measured with the same plunger displacements, in order to measure the tissue force applied to the formulation *ex vivo*. The calculated force *ex vivo* was between 5.7 – 37.9 N for a plunger displacement between 10 and 25 mm.

The force applied to the balloon catheter *ex vivo* increased with increasing plunger displacement (**Figure 3.A.9).** This was caused by the mechanical properties of the tissue, which counteracted the expansion of the balloon catheter, as explained before.

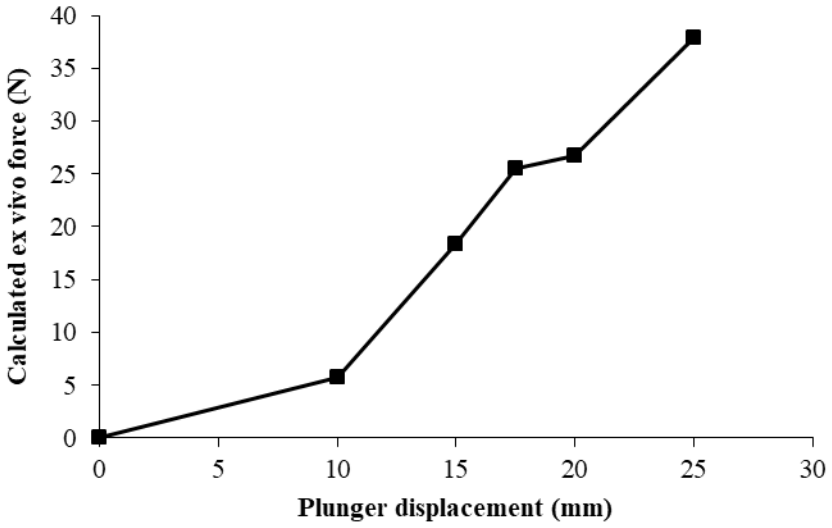


Figure 3.A.9 The calculated *ex vivo* force as a function of plunger displacement

For determination of the tissue elasticity, the measured force is usually plotted against displacement (Samani et al., 2003). However, in this study the injection force was related to plunger displacement, and the force *ex vivo* was related to the surface area of the balloon catheter. In order to determine the relationship between the force applied to the balloon catheter *ex vivo* and the surface area of the balloon catheter, the diameter of the balloon catheter was determined under a microscope after it was filled with a certain amount of water and the surface area was calculated.

Table 3.A.1 Determination of the diameter of balloon catheter using a microscope

Amount of water filled into the balloon catheter (mL)	Balloon diameter (mm)	Calculated surface area (cm^2)
0.60	2.10	8.19
0.90	2.52	9.92
1.05	2.71	10.70
1.20	2.77	10.96
1.50	2.83	11.19

The force applied to the balloon catheter *ex vivo* increased exponentially with increasing surface area of the balloon (**Figure 3.A.10).** This result is in agreement with the force-displacement data obtained from soft tissue elastic modulus study reported elsewhere (H.-T. Leong et al., 2013; Samani et al., 2003).

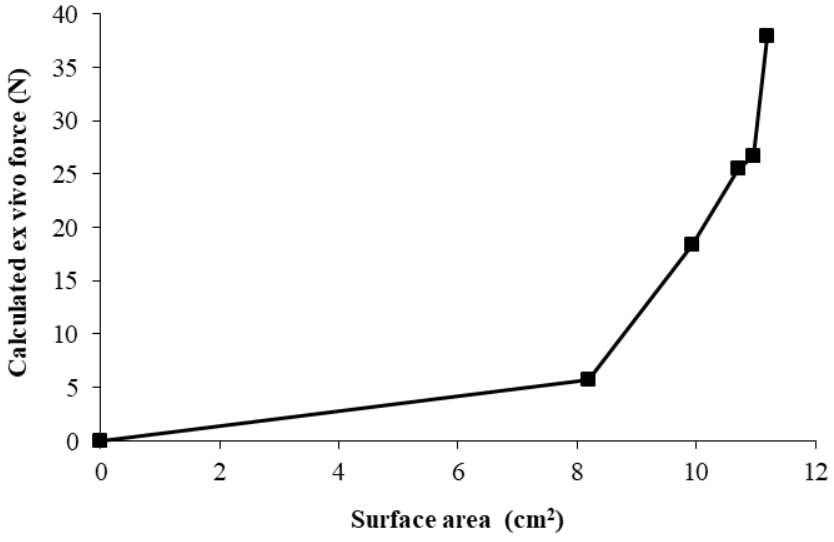


Figure 3.A.10 The calculated *ex vivo* force as a function of surface area of the balloon catheter

The goal of this study was to find out the pressure *ex vivo*. The pressure is the force applied to the surface of the balloon catheter, when it is placed into the turkey breast. The *ex vivo* force and the surface area of the balloon catheter was calculated for different plunger displacements. Thus it was possible to calculate the pressure *ex vivo* for different plunger displacements.

The pressure applied to the balloon catheter *ex vivo* increased with increasing plunger displacement (**Figure 3.A.11).** That shows that although the tissue is flexible, the physiological space for implant' swelling is limited and if the formulation in the tissue expands the pressure increases exponentially and counteracts the expansion of the formulation.

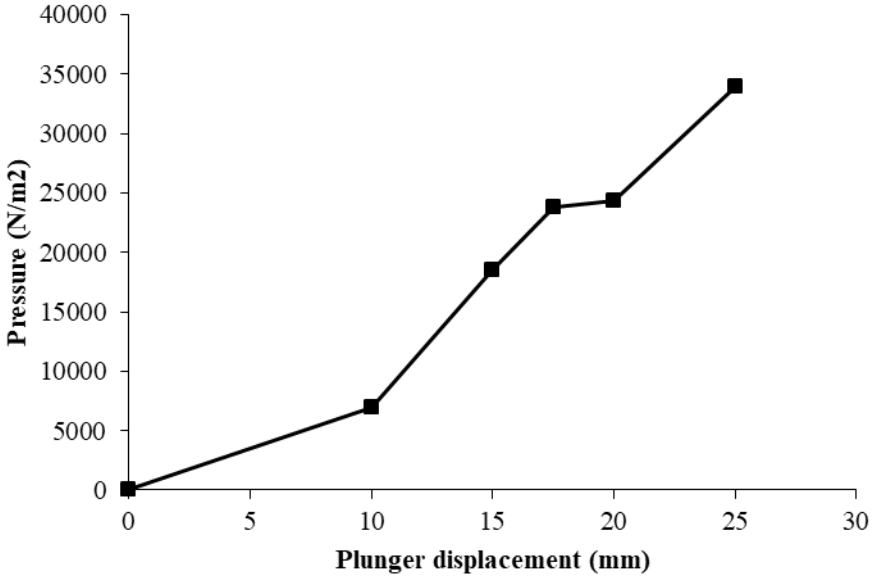


Figure 3.A.11 The calculated pressure *ex vivo* as a function of plunger displacement

3.A.4 Conclusions

Implants were incubated either in phosphate buffer (*in vitro*) or implanted in turkey breast (with 60 mL or with 1 mL additional buffer). The experiment showed that surface area and water uptake of implants were decreased under *ex vivo* conditions compared to *in vitro*, which led to restricted swelling. The surface area of implants was very similar for both *ex vivo* experiments. There was also no difference in the water uptake of implants for both *ex vivo* experiments over three days, but in the subsequent erosion phase, when the implants became soft, the water uptake was higher in the *ex vivo* experiment with 60 mL phosphate buffer. It can be concluded that the

mechanical properties of the tissue play a more important role for the restriction of swelling and the shape change of implants in comparison to the reduced amount of water. However, the amount of water in the matrix can influence polymer degradation and erosion and must also be taken into consideration.

Furthermore, a new method - using a texture analyzer and a balloon catheter - was developed to quantify the *ex vivo* force applied to an implanted object and to calculate the created pressure in the tissue. It illustrated that the *ex vivo* force and pressure increased with increasing expansion of the object, due to tissue resistance. That proves that although the tissue is flexible, if the formulation in the tissue expands the mechanical properties of the tissue counteract the expansion of the formulation.

This method could help to better predict the behavior of drug delivery systems and the drug release after implantation and to understand differences observed between *in vitro* and *in vivo* experiments

B

Design of a new *in vitro* test to mimic mechanical properties of the tissue for solid parenteral drug delivery systems and to investigate the effect of restricted swelling on drug release from PLGA-implants

3.B.1 Background

The release rate of the incorporated drug from the dosage forms is very important for the performance of parenteral controlled drug delivery systems. However, no standard *in vitro* release test methods have been established yet that correlate to *in vivo* drug release. Currently, a large spectrum of different *in vitro* release models is applied to monitor drug release from extended release parenteral dosage forms, including the use of sample and separate with or without agitation, dialysis-based membranes, the USP apparatus 2 (paddle method), and the modified USP apparatus 4 (flow-through cell method) (D'Souza & DeLuca, 2006). A major drawback of almost all of these commonly used *in vitro* release tests is that the formulations are exposed only to release medium, which does not mimic the physiological environment. The main administration routes for controlled release parenteral formulations are subcutaneous and intramuscular (J. M. Anderson & Shive, 2012; Katakam, Ravis, & Banga, 1997). When a PLGA-based implant is completely surrounded only by release medium, it can swell in all directions, differently to *in vivo*, where less water is available and the formulation is also in contact with tissue cells (Iyer et al., 2006). In previous study, it was shown that the tissue applies a force on the formulation, which leads to restricted swelling of the implant and change of its shape. Furthermore, water uptake *in vivo* was also decreased due to the limited amount of fluid, which leads to restricted swelling and ultimately affects the drug release kinetics. The aim of this study was to simulate the limited physiological space in an *in vitro* test, and to investigate the effect of restricted swelling on drug release from PLGA-based implants.

3.B.2 Simulation of the limited physiological space in an in vitro test

3.B.2.1 Air tight apparatus

In order to increase the air pressure, a glass bottle was equipped with a bike valve and a pump was used to insert air into the glass bottle (**Figure 3.B.1**). The glass bottle could withstand the pressure of 2 bars for 24 hours. The implant was placed in a test tube filled with 10 mL phosphate buffer and this was placed in the glass bottle. It was incubated at 37°C after it was pumped with air. Every day the implant was taken out to measure the swelling degree of the

implants using light microscope. The advantage of this method was that the agitation in both case under atmospheric condition and 2 bar of pressure was the same.

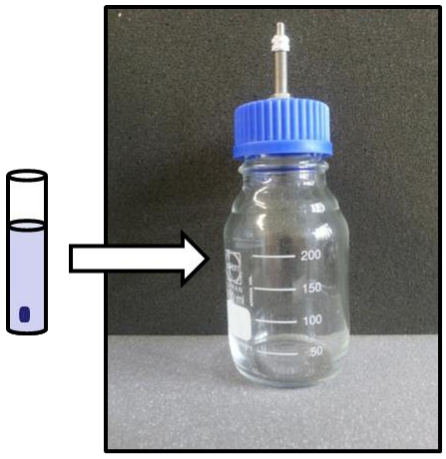


Figure 3.B.1 Schematic representation of the air tight apparatus

However, no significant difference in the swelling degree of implants at atmospheric pressure (~1 bar) and under a higher pressure (~2 bar) was observed. The reason was that the pressure is applied only to the surface of release medium and when PLGA swells, the volume of Implant increased but the overall volume did not change (**Figure 3.B.2**).

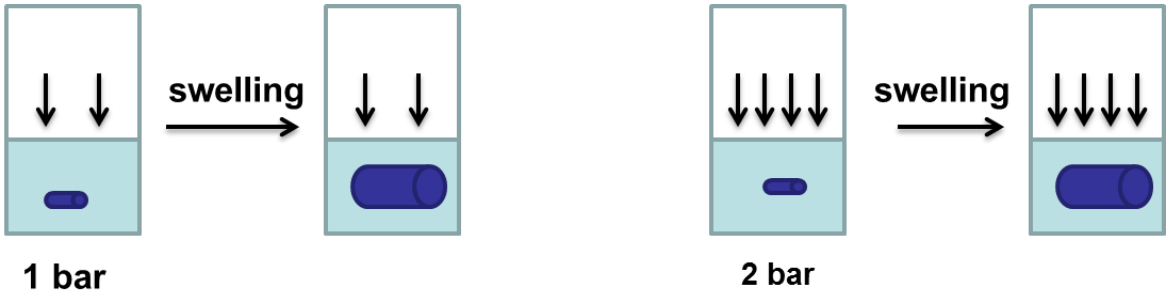


Figure 3.B.2 Schematic representation of the swelling study under atmospheric conditions and 2 bar of pressure

3.B.2.2 Modified continuous flow method

In this method, implant was set between two pieces of sponges, which were placed in a syringe filter. The closed filter and release medium reservoir were placed in a 37°C water bath, Phosphate buffer was pumped constantly through the system **(Figure 3.B.3)**.

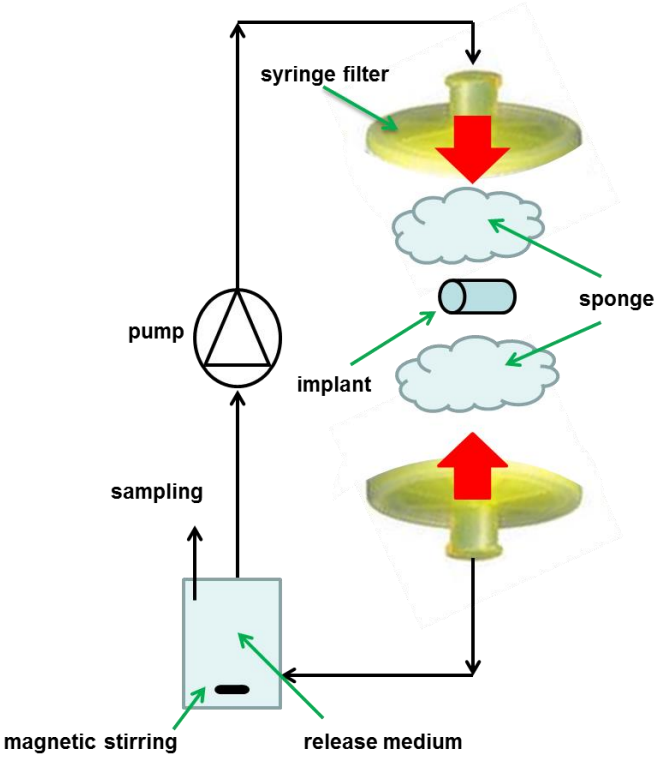


Figure 3.B.3 Schematic representation of the modified continuous flow method

After 1 day the shape of implant changed losing its cylindrical form, which was caused by the pressure applied only from both sides. In addition, the pressure applied couldn't be determined, and the implant stuck to the sponge becoming impossible to study the implant swelling.

3.B.2.3 Modified sample and separate method

In this method, the implant was placed in a glass bottle field with phosphate buffer and polystyrene or glass beads, which prevented the swelling. The glass bottle was incubated at 37°C (**Figure 3.B.4**).

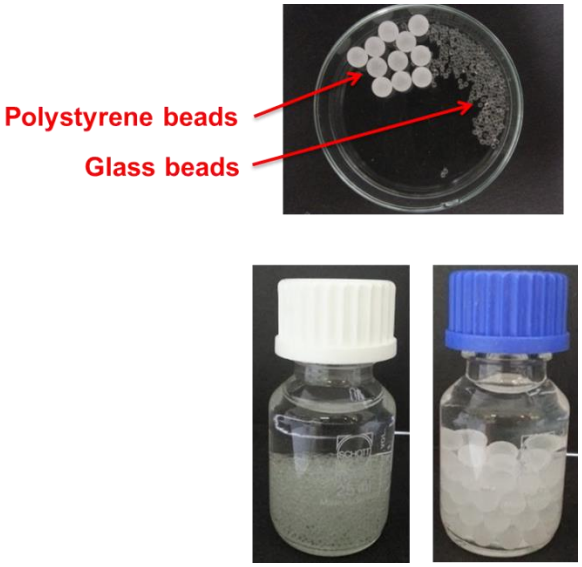


Figure 3.B.4 Schematic representation of the modified sample and separation method

Glass beads and polystyrene beads were different in size and weight and could simulate different pressure and agitation of the *in vitro* test.

Polystyrene beads could not prevent the swelling because they were floating and the free space between them was too large. Glass beads were able to restrict the swelling. However, the implants stuck to the beads (**Figure 3.B.5**).

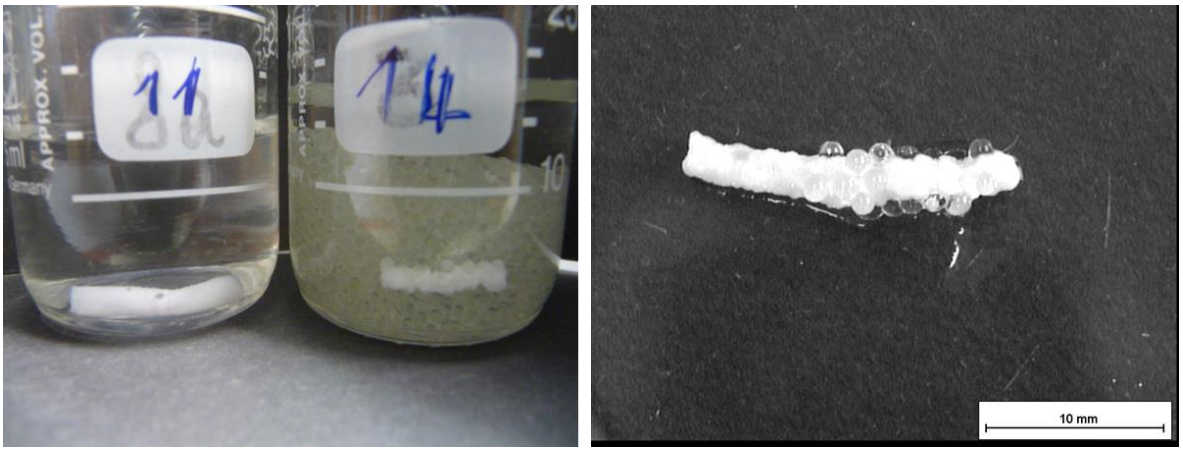


Figure 3.B.5 Implant in absence and present of glass beads (left), observation of implant taken out from the modified sample and separate method (right)

3.B.2.4 Restricted swelling device

A novel device was fabricated to prevent the swelling of formulations by placing it in a fixed geometric space where the formulation fitted exactly in. An implant (Ø 8 mm) was placed in a rubber ring with a hole (Ø 8 mm) fixed between two glass filters with a pore size between 160-250 µm to ensure the diffusion of drug and degradation products of PLGA from the device (**Figure 3.B.6**).

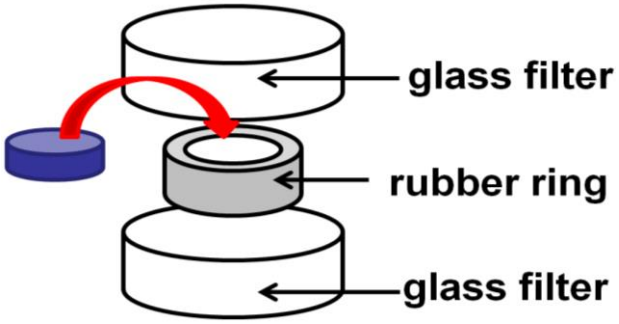


Figure 3.B.6 The restricted swelling device

3.B.2.4.1 Adsorption and diffusion test of the restricted swelling device

Less than 3% of drug was adsorbed by the filter after filtering the sample solution 3 times (**Figure 3.B.7**). Thus, drug adsorption was not significant and would not have a significant influence on the drug release results.

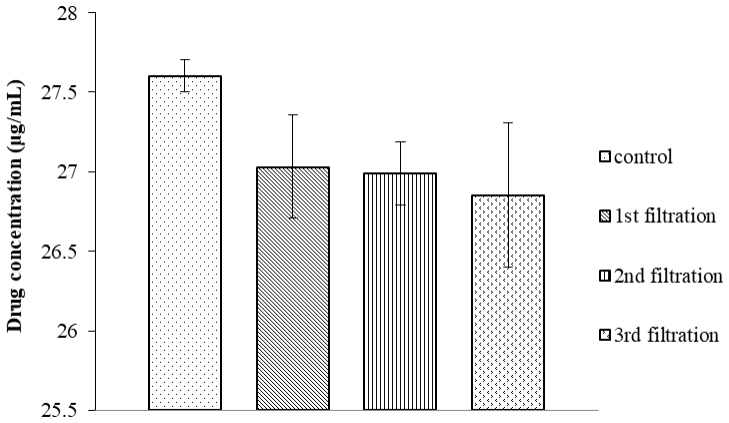


Figure 3.B.7 Adsorption test by filter with pore size 160-250 µm (n=3 +/- SD)

The pore size of the glass filter was between 160 and 250 µm, therefore complete diffusion of drug out of the device was expected. However, the test showed that the diffusion rate of drug through glass filter was slower than the control (**Figure 3.B.8**). Slower wettability of implant in the device might have caused reduced diffusion rate. The speed of drug passage through all 3 devices was not the same (**Figure 3.B.8**): diffusion rate of one device was faster than the other two devices. This could be explained by different distribution of pore size of filters. Nevertheless, the diffusion rate was fast enough, so that the drug release was controlled by the polymer matrix and not by the device.

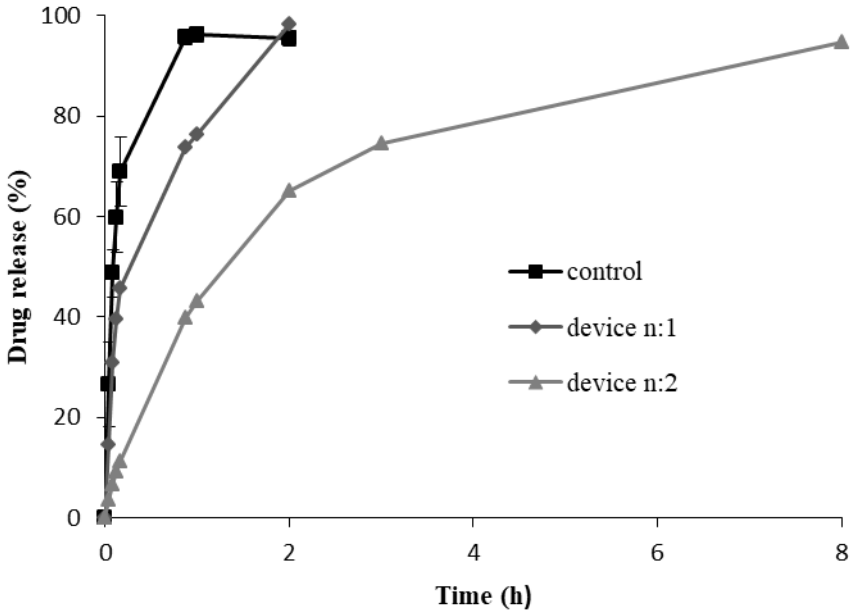


Figure 3.B.8 Diffusion through glass filters with pore size 160-250 µm in phosphate buffer pH 7.4 at 37°C (n=3 +/- SD)

3.B.3 The effect of restricted swelling on drug release from PLGA-based implants

3.B.3.1 Diameter increase of implants prepared with PLGA of different molecular weights

Very little is known about the effect of PLGA swelling on drug release. It is usually assumed that the swelling effect of PLGA is not pronounced. However, Gasmi et al. suggested that the swelling kinetics of the PLGA microparticles can play a decisive role in the control of drug

release (Gasmi, Danede, Siepmann, & Siepmann, 2015). Since swelling and diameter increase are related to each other, in this study, diameter increase and the release of risperidone from implants containing PLGA of different molecular weights (7, 10 and 25 kDa) was measured as a function of time.

For this purpose, PLGA implants were prepared with uncapped, 50:50 lactic acid: glycolic acid monomer ratio of different molecular weights in order to keep crystallinity constant.

The diameter increase of implants containing PLGA with molecular weight of 7 kDa, 17 kDa and 25 kDa was approximately 130%, 97% and 21% respectively at day 8 (**Figure 3.B.9**). Water was absorbed by the polymers from the beginning of the incubation and PLGA degradation started immediately upon contact with water **(Figure 3.B.14)**. This led to a decrease in molecular weight **(Figure 3.B.15)**. Erosion of the polymer started when the dissolved polymer degradation products were able to diffuse into the release medium (A Göpferich & Tessmar, 2002; Tracy et al., 1999). The water uptake and erosion onset was faster with lower molecular weight than with higher molecular weight polymer, which led to a faster swelling (**Figure 3.B.9**). Implants prepared with different polymers had about same diameter increase at the erosion onset but with a different rate, caused by their differences in molecular weight.

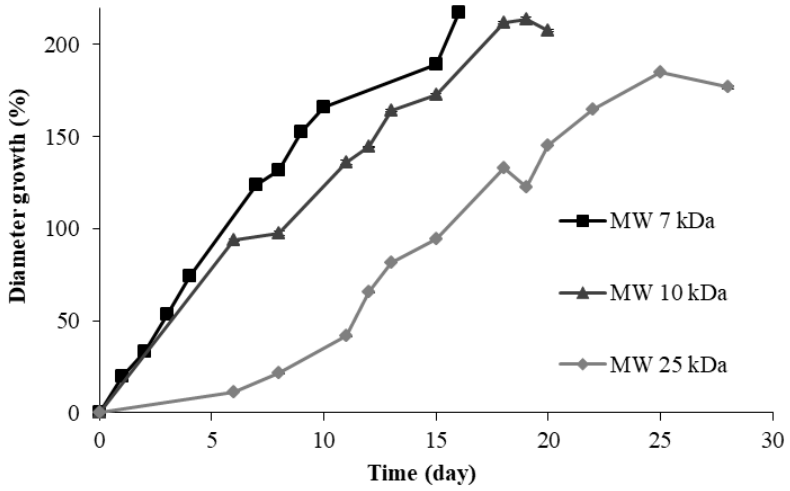


Figure 3.B.9 Diameter increase of PLGA implants in phosphate buffer pH 7.4 at 37°C (n=3 +/- SD)

3.B.3.2 Release of implants with different PLGA molecular weight

The release profile of risperidone (**Figure 3.B.10)** from implants containing PLGA showed a typical multiphasic pattern like also seen with microparticles (Rawat et al., 2011)**.**

As expected, there was no difference in drug release in the burst phase, since the drug loading for all three formulations was the same. The higher the molecular weight of the PLGA was, the longer was the lag phase (**Figure 3.B.10).** Implants containing PLGA with molecular weights of 7 kDa, 10 kDa and 25 kDa exhibited a lag phase until days 3, 6 and 11 respectively, because PLGA with higher molecular weight required more time for the erosion to take place (Zolnik & Burgess, 2008). In all cases, most of the risperidone was released during the polymer erosion phase, its release rate during this phase decreased with increasing PLGA molecular weight.

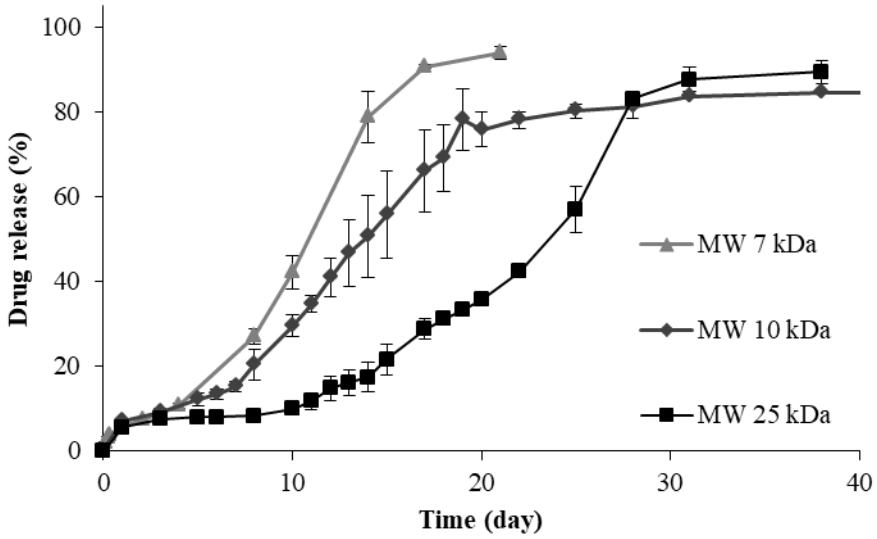
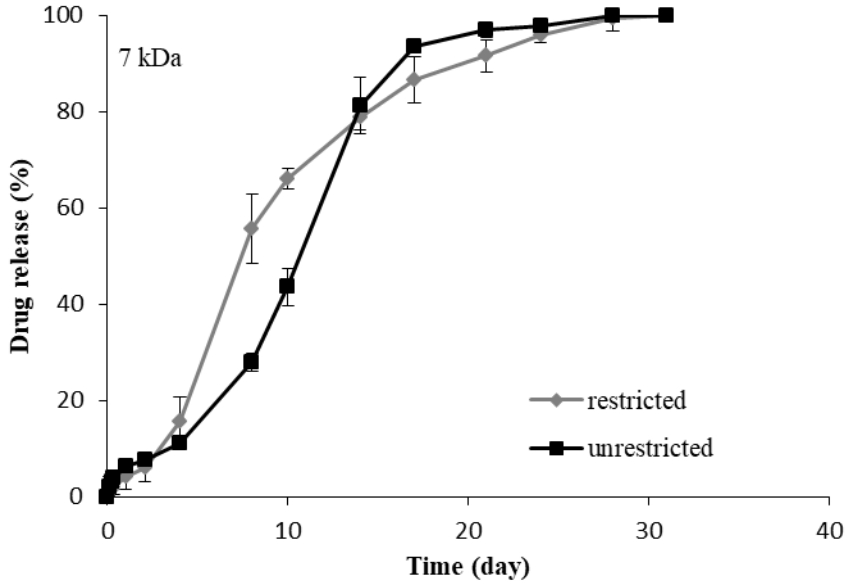


Figure 3.B.10 Effect of Polymer molecular weight on risperidone release from PLGA implants containing 10% w/w drug in phosphate buffer pH 7.4 at 37°C (n=3 +/−SD)

3.B.3.3 Release comparison of implant under restricted and unrestricted conditions

In order to study the effect of polymer swelling on risperidone release, drug release under restricted and unrestricted conditions was performed.

In both systems, the implants showed the same typical multiphasic release profile. As expected, the drug release during diffusion and lag phase was slightly lower for restricted implants compared to unrestricted, due to the lower wettability of implants in the device. However, the difference was not pronounced, because risperidone has a low solubility and it's release is mainly driven by polymer erosion. Once the erosion phase started, i.e. day 3, 6 and 11 for PLGA with molecular weight of 7 kDa, 10 kDa and 25 kDa respectively, the drug release rate in the device was first faster and then became slower than the unrestricted implants. (**Figure 3.B.11**). In all cases, the drug release slowed down after 80% drug released. A similar result was observed by Rawat et al, who studied the *in vivo* and *in vitro* release profiles of commercially available risperidone microparticles (Risperdal® Consta). The *in vivo* profile was initially faster than the real-time *in vitro* profile and then slower after approximately 30 days (Rawat et al., 2012). They assumed that a faster *in vivo* release during the lag phase is due to the accumulation of acidic PLGA degradation products. Hence, the difference in drug release in the erosion phase can be explained by the difference in polymer degradation, which may be caused by different water uptake and swelling degree of the implants in the two systems.



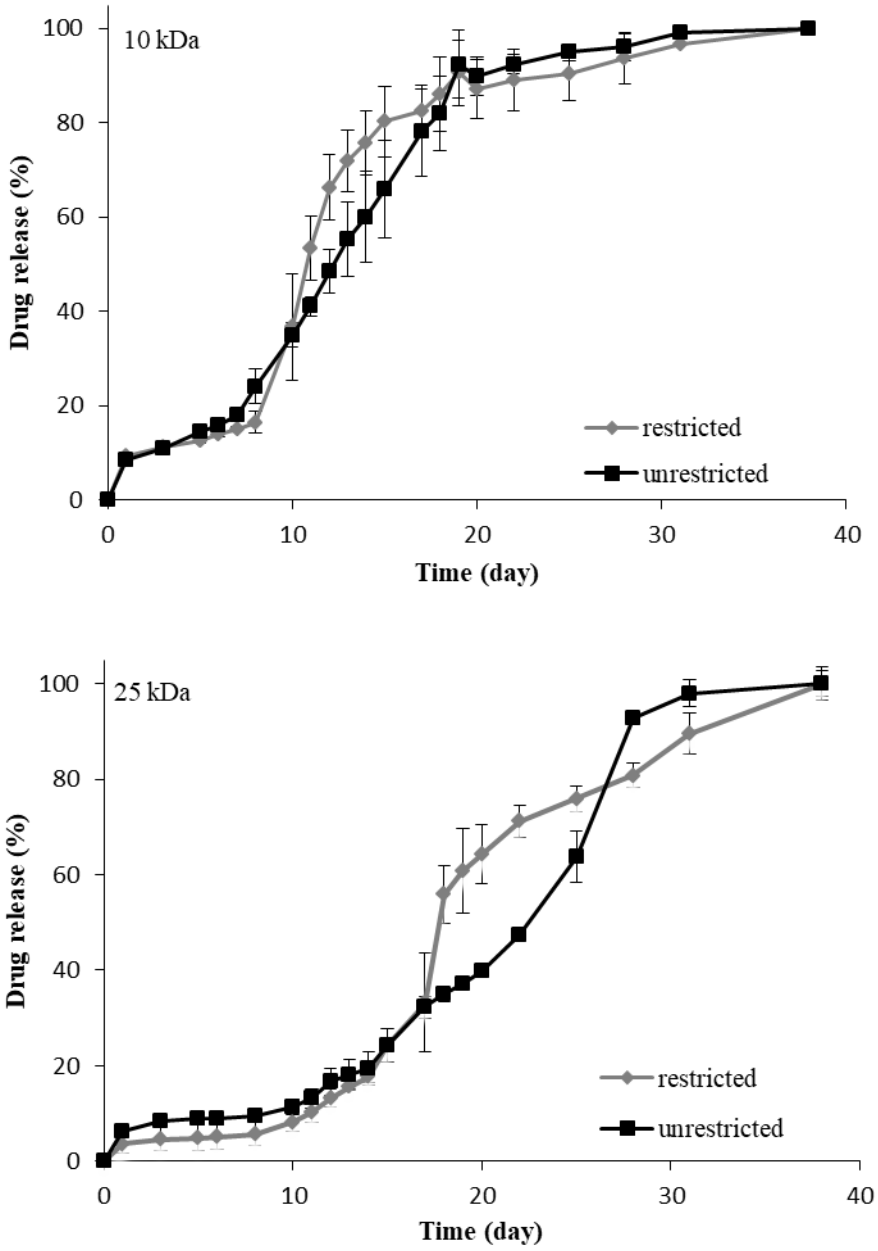


Figure 3.B.11 Risperidone release of restricted and unrestricted implants containing PLGA with a molecular weight of 7 kDa, 10 kDa and 25 kDa with 10% w/w drug in phosphate buffer pH 7.4 at 37°C (n=3 +/−SD)

Since the risperidone release pattern for PLGA with three different molecular weights under both, restricted and unrestricted conditions, was the same, only the polymer 503H (MW of 25 kDa) was chosen for further studies.

Risperidone is a basic drug and catalyze PLGA degradation (Souza, Faraj, Dorati, & Deluca, 2015), therefore an acidic drug and nonionic drug were chosen as model drugs as well.

Ibuprofen and theophylline both showed a triphasic release profile from PLGA implants consisting of an initial burst release, a diffusion phase followed by a lag phase and an erosion-controlled release phase thereafter (**Figure 3.B.12 and 3.B.13**).

The initial release of ibuprofen and theophylline was around two times higher from the unrestricted implants than from the restricted ones. Furthermore, ibuprofen release from the restricted implants occurred mainly during the erosion phase with 80% of drug being released, whereas only 20% of drug was released during the diffusion and lag phase (until day 10). In contrast, for unrestricted implants, approximately half of the drug content was released during the diffusion phase and lag phase, and the rest was released during the erosion phase (**Figure 3.B.12**). In the case of theophylline release from the device only 5% of the drug was released during the diffusion and the lag phases, while for the unrestricted implants 32% of drug was released during these stages (**Figure 3.B.13**). In conclusion under restricted condition for ibuprofen and theophylline, the release was mainly driven by PLGA erosion.

These differences could be attributed to the decreased swelling of implants in the device and reduced water uptake, which led to the decreased drug release during the diffusion controlled phase. This difference of drug release in the diffusion and lag phases were more pronounced for ibuprofen and theophylline compared to risperidone due to their higher solubility. The enhanced PLGA degradation for restricted implants allowed the formation of bigger pores and spaces from where the drug was released, and thus, increased the release rate in the erosion phase in comparison to the unrestricted polymer implants; these implants were able to swell and to create water filled pores for drug diffusion, which increased the drug release in the diffusion controlled phase.

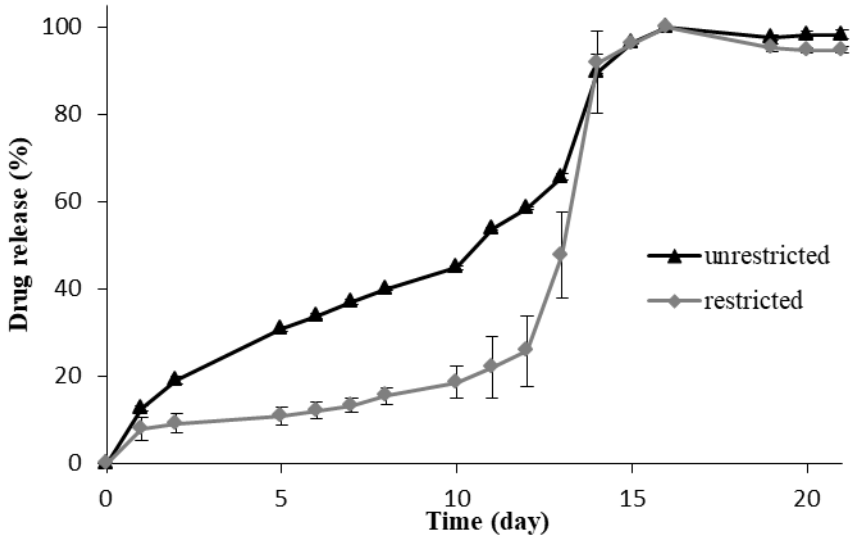


Figure 3.B.12 Ibuprofen release of restricted and unrestricted implants containing PLGA with a molecular weight of 25 kDa with 10% w/w drug in phosphate buffer pH 7.4 at 37°C (n=3 +/−SD)

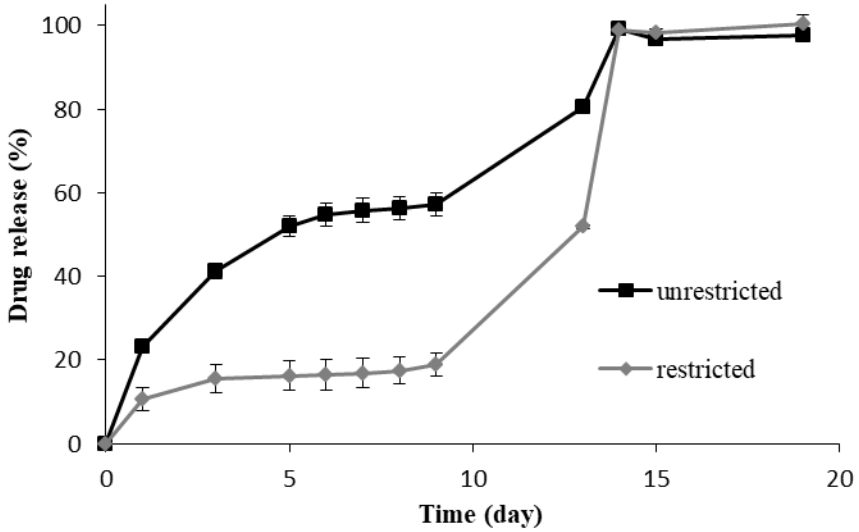
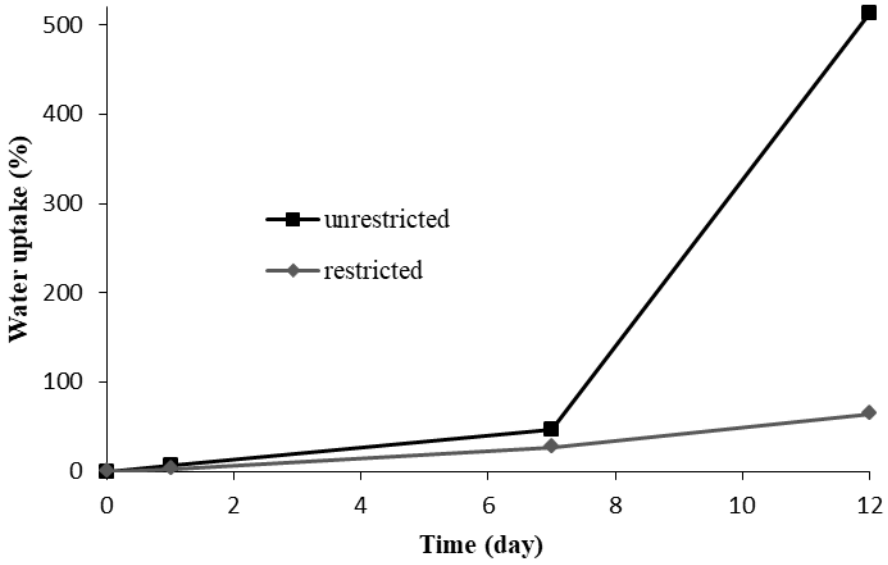


Figure 3.B.13 Theophylline release of restricted and unrestricted implants containing PLGA with a molecular weight of 25 kDa with w/w 10% w/w drug in phosphate buffer pH 7.4 at 37°C (n=3 +/−SD)

The water uptake was approximately two fold higher under unrestricted than under restricted condition at day 7. However, at day 12 the difference became more pronounced, being approximately eight fold higher (**Figure 3.B.14**). This correlated with the results from the mass loss study (**Figure 3.B.14)**. The difference in mass loss was not pronounced until day 7, but it became approximately three fold higher at day12 under restricted condition compared to the unrestricted one. Since the swelling is dependent on the amount of water and free volume available and under restricted swelling condition, the available volume is decreased, a decrease in water uptake can be observed. Due to the decreased water uptake, the solubility and mobility of the polymer degradation products could be decreased impairing their diffusion out of the matrix. This could lead to faster polymer degradation in the erosion phase under restricted condition.



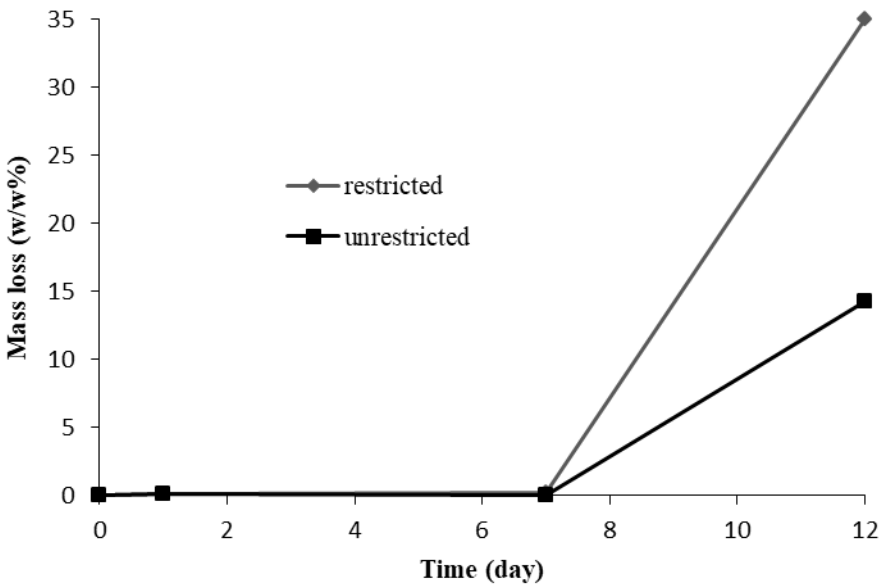


Figure 3.B.14 Comparison of water uptake and mass loss of restricted and unrestricted implants containing 503H in phosphate buffer pH 7.4 at 37°C (n=2)

Since the difference in drug release between unrestricted implants and restricted implants was observed in the erosion phase, the polymer degradation was monitored over this time period in both systems and compared to each other.

The peak of the lognormal molecular weight distribution of PLGA (log M_p) decreased with incubation time for restricted and unrestricted implants. In accordance with the mass loss study **(Figure 3.B.14)**, the change of the molecular weight was similar until day 7 under both restricted and unrestricted conditions. However, at day 12, the polymer molecular weight of the implants in the device was lower than the unrestricted implants and the difference in molecular weight became more pronounced over the time **(Figure 3.B.15)**. This means that the PLGA degradation of implants under restricted condition became faster than under the unrestricted ones, which is in agreement with the degradation of PLGA *in vivo* and *in vitro* described in literature (Grayson et al., 2012; Kamei et al., 1992; Spenlehauer, Vert, Benoit, & Boddaert, 1989; Tracy et al., 1999).

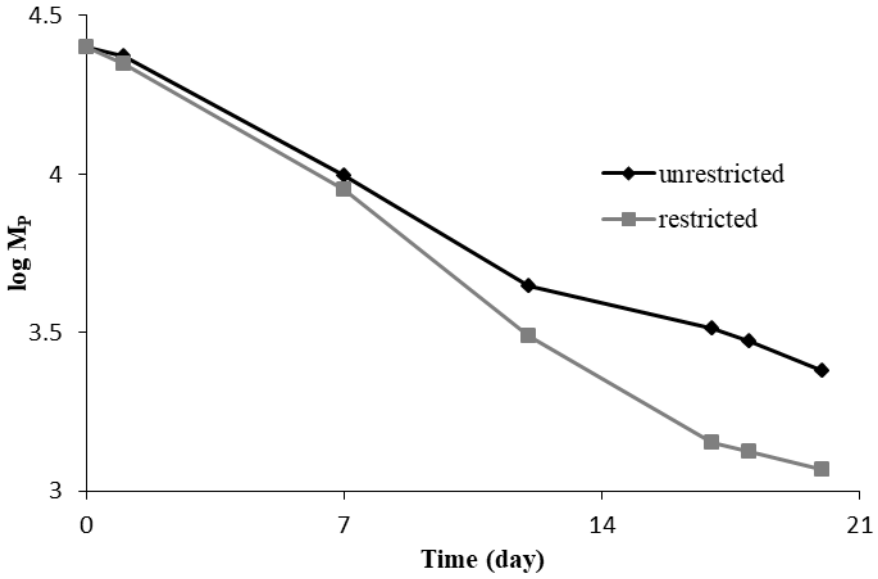


Figure 3.B.15 Changes in molecular weight of restricted and unrestricted implants containing 503H (n=2)

The surface area of the unrestricted implants increased due to the swelling. The implants in the device were not able to take up water and swell, and thus, had a constant surface area. Due to the higher surface area of the unrestricted implants, the degradation products diffused more easily out of the matrix in the erosion phase. Because the water uptake of the implant in the device was lower, the degradation products were less mobile, diffused more slowly out and accumulated within the implants. This caused an acidic pH and accelerated the polymer erosion of the implants in the device due to an autocatalytic effect like reported previously for PLGA 50:50 foams (Lichun Lu et al., 2000).

3.B.3.4 Determination of µpH of restricted and unrestricted implants using EPR

Non-destructive EPR spectroscopy was chosen as an established method to measure the µpH values within the PLGA implants (Capancioni et al., 2003; C. Kroll, Mäder, Stößer, & Borchert, 1995; Christian Kroll & Herrmann, 2001; Siepe et al., 2006).

During the whole experiment, the µpH of restricted and unrestricted implants was acidic. The µpH of unrestricted implants remained constant between 3.5 and 4.5 during the complete experiment (**Figure 3.B.16**). In contrast, the µpH of implant placed in the device was reduced

constantly up to pH 1.5 within 9 days and remained constant during the following 7 days. The lowest accurate pH, which was possible to measure, was 2.2. Although the measured pH values of implants in the device might not be very accurate, the comparative results between the two different systems could be enough to explain their behavior. The degradation of PLGA and production of oligomers reduced the µpH; if the diffusion of water through the matrix is decreased by, e.g., physical barrier around the implant, they can accumulate causing a faster µpH drop, and thus, accelerating the erosion and degradation of PLGA (**Figure 3.B.16)**.

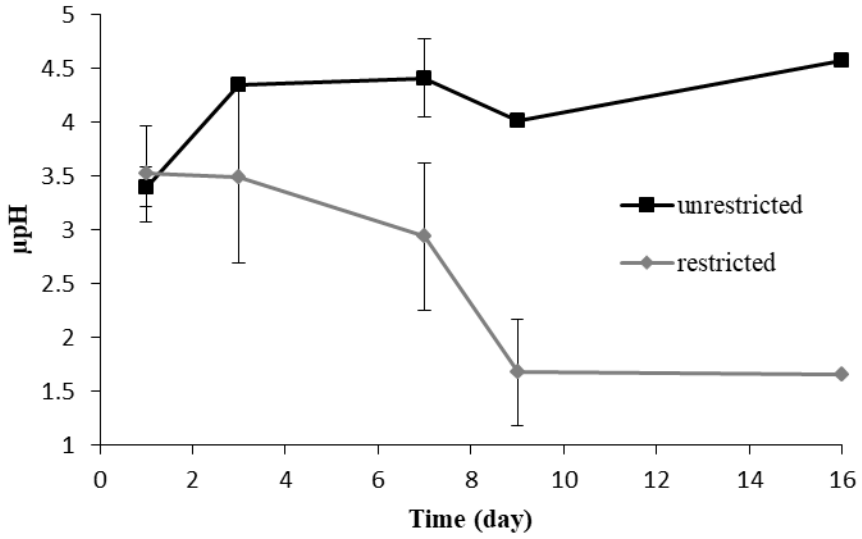


Figure 3.B.16 Determination of µpH in restricted and unrestricted implants containing 503H (n=3 for restricted implants and day 7, n=1 for day 16 restricted and unrestricted implants)

3.B.4 Conclusion

A novel device was designed to simulate the restricted physiological space of the tissue and to restrict the polymer swelling in order to study the effect of restricted swelling of polymer on drug release with three model drugs of different ionization state.

For all three model drugs, basic, acidic and nonionic drugs, the burst release and diffusion controlled drug release were reduced and lag phase was prolonged for implants in the device. This was attributed to the decreased surface area of restricted implant and reduced water uptake and swelling, which lead to a decreased drug release during the diffusion controlled phase. In the

erosion phase, the release rate of drug was faster for the restricted implants due to the accumulation of acidic degradation products from PLGA as a result of reduction of water uptake and oligomers efflux, which caused higher autocatalysis and a faster PLGA degradation. Under restricted condition, drug release was mainly controlled by the polymer erosion independent of the nature of the drug (acidic, basic or neutral)**.**

C

Application of biphasic test model as a new biorelevant model for drug release from PLGA implants and investigate its effect on risperidone release from PLGA-based implants

3.C.1 Background

In order to optimize the drug release media, the effect of pH, buffer capacity, ionic composition, osmolarity and for longer experiments, prevention of microbiological contamination have been studied. Phosphate buffered saline pH 7.4 is the medium most commonly used for non-oral dosage forms (Iyer et al., 2006). Unlike for the gastrointestinal passage standardized biorelevant simulated body fluids have not been generally established for parenteral sites of application (Diakidou, Vertzoni, Dressman, & Reppas, 2009; Jantratid, Janssen, Reppas, & Dressman, 2008). There have been, however, reports of using blood or blood components for dissolution testing of parenteral dosage forms (Blanco-Príeto et al., 1999; Kamberi et al., 2009; Sternberg et al., 2007; G. X. Wang et al., 2010). Rarely, other additives from natural sources have been used for the preparation of dissolution media, such as rat brain homogenate (Chen & Lu, 2008). Some authors report the use of liquids referred to as simulated body fluids or for example simulated synovial fluid, simulated tear fluid or simulated lung fluids (Chen & Lu, 2008; Colombo, Monhemius, & Plant, 2008; Conzone, Brown, Day, & Ehrhardt, 2002; Kokubo & Takadama, 2006; Kortesuo et al., 2000; Nagarwal, Kumar, Dhanawat, & Pandit, 2011; G. X. Wang et al., 2010), but these were mostly intended to evaluate issues other than drug release, such as bone-bonding ability. Also, the use of a modification of Hank's balanced salt solution has been suggested to be biorelevant for subcutaneous implants (Iyer, Barr, & Karnes, 2007). In some studies agar gel was used to simulate the extracellular matrix because of its rheological property and high water content (Delplace et al., 2012; Hoang Thi et al., 2010; Nastruzzi, Esposito, et al., 2008). Shuwisitkul et al. used for the first time a lipophilic liquid (Lipofundin^{TM}) as an alternative release medium to mimic the *in vivo* subcutaneous condition, which resulted in less water uptake, the deformation of the PLGA implant, and faster drug release (Shuwisitkul, 2011).

As mentioned before, in case of intramuscular or subcutaneous administration, the implant is placed in the body in contact with muscle or adipose cells and extracellular fluids. Many studies have been performed to simulate body fluid; however, the role of tissue cells on drug release has not yet been studied. Depending on the lipophilic character of the drug, it may be partitioned into the cells, and be released back into interstitial fluid, as a function of $-k$D, the re-distribution rate. This could lead to formation of a localized depot in the tissue and affect the drug absorption and release.

Furthermore the implant is in contact with cell membranes, which might influence the drug delivery system (Iyer et al., 2006). In previous chapter, the influence of the physiological space of the tissue on drug release from PLGA implants was studied. In this study, a bicompartment system was used to simulate the tissue and body fluid. The oil phase mimics the surrounding tissue and cell membrane and the water phase mimics the body fluid. The biphasic release test was used in oral application for drug with poor solubility, to maintain sink condition. However in this study it is used to mimics the physiological environment/ surrounding tissue and to investigate its effect on risperidone release from PLGA-based implants. Octanol and olive oil were used as the oil phases.

3.C.2 Risperidone release in biphasic system

The release profile of risperidone from PLGA implants containing 5050 DLG 1A and 10% risperidone in biphasic system is shown in **Figure 3.C.1**. The drug concentration was measured in both aqueous and organic phase. The drug released in the aqueous phase over 15 days was less than 2% while it increased in the octanol phase. This attributed mainly to the partitioning of the drug into the oily phase due to higher o/w partition coefficient of risperidone (3.27). This is supposed to mimic the absorption process of lipophilic drugs in the surrounding tissue.

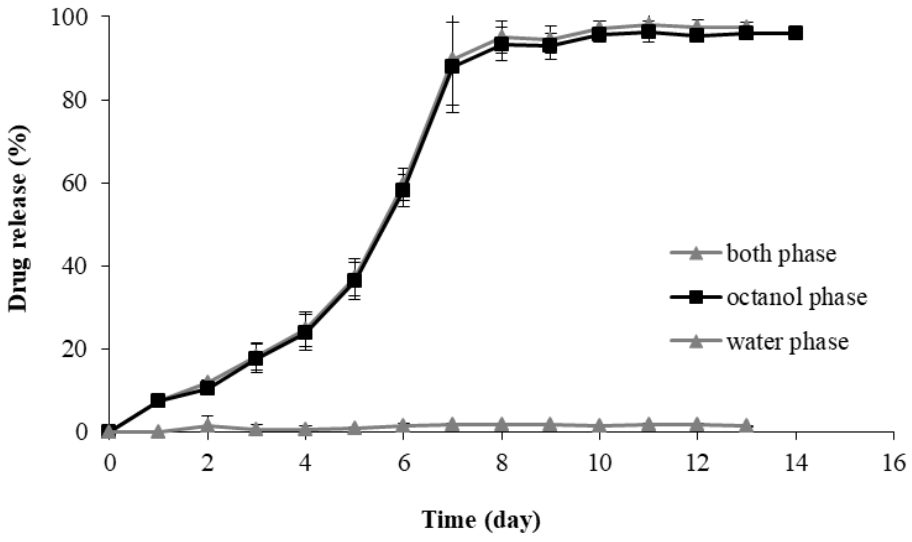


Figure 3.C.1 Risperidone release from 5050 DLG 1A PLGA implant with 10% drug loading in biphasic system at 37°C (n=3 +/−SD)

3.C.3 Risperidone release from 5050 DLG 1A PLGA implant in monophasic system vs. biphasic system

Risperidone release from implants in both systems showed triphasic drug release profile: an initial release, a short diffusion and lag phase followed by an erosion-controlled release thereafter.

In both monophasic and biphasic systems, there was an initial burst release of risperidone (6%) from implants containing polymer 5050 DLG 1A and 10% w/w drug (**Figure 3.C.2a**). Biphasic system resulted in a higher release rate in the erosion phase. Up to day 8 more than 90% drug was released in biphasic system, while in monophasic system only 32% drug was released (**Figure 3.C.2a**). Higher release rate in biphasic system can be explained by higher drug solubility in biphasic system and the affinity of drug to octanol phase.

Since the drug release was governed predominantly by polymer erosion, octanol might influence polymer degradation as well; therefore, water uptake and mass loss study were done. Water uptake by the implants increased with time. At day 1 in both biphasic and monophasic systems no difference was observed in water uptake. However, afterwards, the water uptake by the biphasic system was more than 1.5 folds compared to the monophasic system **(Figure 3.C.2b)**. This indicates faster polymer degradation in biphasic system. Faster degradation leads to faster decrease in molecular weight, polymer with lower molecular weight has higher hydrophilicity and is able to absorb higher amount of water. The mass loss of the implants followed the same trend (**Figure 3.C.2c**). The risperidone release accelerating effect of octanol correlated well with the water uptake and mass loss. These showed a high evidence of faster polymer degradation in biphasic system compared to monophasic system.

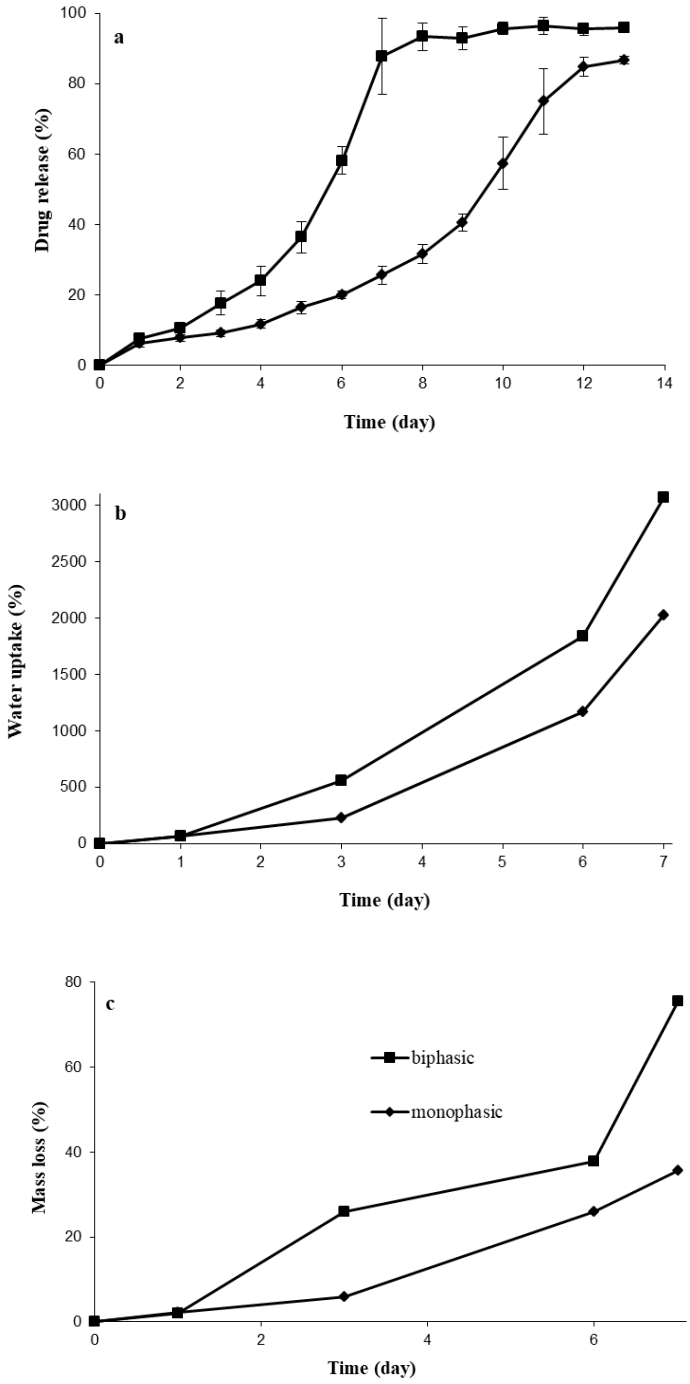


Figure 3.C.2 (a) Release profile (n=3 +/−SD), (b) water uptake (n=1) and (C) mass loss (n=1) of implant containing 5050 DLG 1A and 10% w/w risperidone in biphasic system and monophasic system at 37°C

3.C.4 Risperidone release from 503H implant in monophasic system vs. biphasic system

The release of risperidone from the PLGA 503H implants in both systems was triphasic with an initial burst, followed by a period of negligible risperidone release and a rapid release phase after the first week (**Fig.3.C.5 a and b**). Thus, the release was governed by combination of drug diffusion and polymer erosion mechanisms.

3.C.4.1 Effect of drug loading

In the monophasic system, no difference in the drug release rate between implants loaded with 10% and 30% w/w of the drug during the two initial release phases, burst and lag phases, was observed (**Figure 3.C.4 a**). During the erosion phase the release rate of implants containing 30% drug was slightly higher than implants with 10% drug (**Figure 3.C.4 a**). The reason behind this was, risperidone enhanced polymer degradation due to its basic and nucleophilic character (Selmin, Blasi, & DeLuca, 2012; Souza et al., 2015). In biphasic system, the release rate in initial phase, lag phase and erosion phase was higher for implants containing 30% drug compared to implants containing 10% drug **(Figure 3.C.4 b).** This could attribute to the high affinity of the drug to octanol phase and partitioning of the drug into the oily phase.

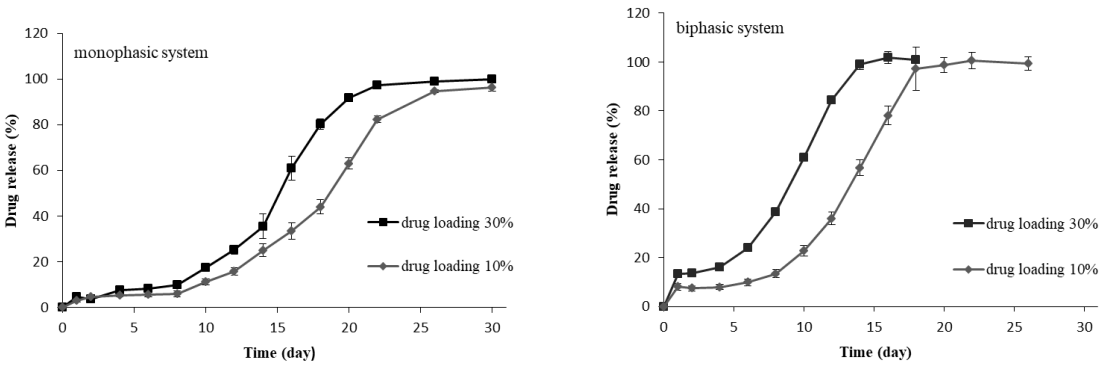


Figure 3.C.4 Release profile of 503H implant containing 10% and 30% w/w risperidone in monophasic system (left) and biphasic system (right) at 37°C (n=3 +/−SD)

3.C.4.2 Effect of dissolution method

In the biphasic system the burst release was higher, the lag phase was shorter and the release rate during erosion phase was higher compared to the monophasic system for both implants containing 10% and 30% drug loading (**Figure 3.C.5 a and b**). For implants containing 30% drug the difference became more pronounced than the one containing 10% drug. Higher burst release of implants with 10% and 30% in biphasic system was attributed to the partition of risperidone into the organic phase as soon as it was released (**Figure 3.C.1**). The biphasic test permits a rapid removal of drug from the aqueous phase by partitioning into the organic phase, so it could be assumed that the amount of drug in the organic phase represents the amount of drug accumulated in the tissue *in vivo*. However, the shorter lag phase and higher release rate in erosion phase for biphasic system indicated faster polymer erosion in biphasic system compared to monophasic system.

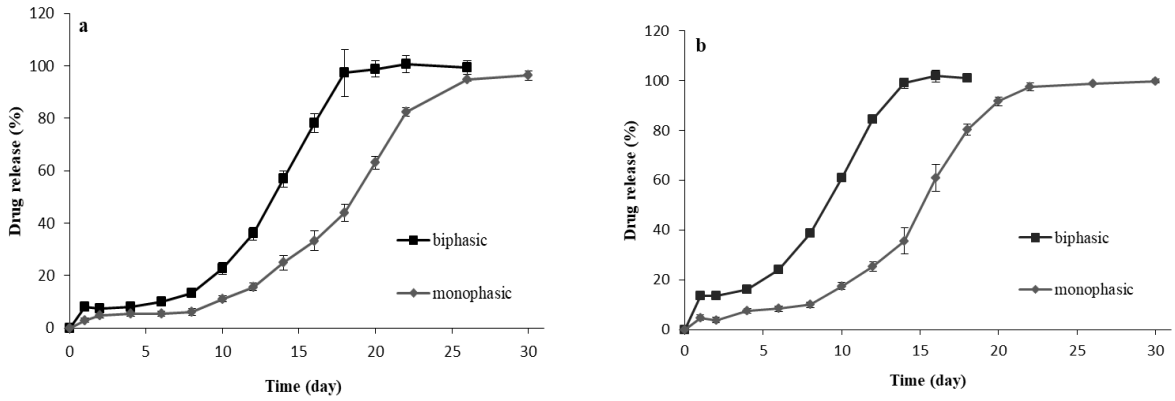


Fig.3.C.5 Release profile of implant containing PLGA 503H and (a) 10% w/w (b) 30% w/w risperidone in biphasic system and monophasic system at 37°C (n=3 +/−SD)

Apart from the polymer type, the environmental conditions such as pH, temperature, and enzymes affect the kinetics of polymer degradation (Yoshioka, Kawazoe, Tateishi, & Chen, 2008). In order to find out the effect of octanol on drug release in erosion phase, the polymer degradation was monitored over time in both systems and compared to each other. The peak of the log-normal molecular weight distribution of PLGA (log Mp) decreased with incubation time for both systems and there was no difference in change in molecular weight **(Figure 3.C.6)**. Higher drug release in erosion phase in biphasic system might therefore be associated with

higher drug solubility in biphasic system, so it could leave the matrix faster after bigger pores have formed as a consequence of polymer degradation. Furthermore, octanol could plasticize PLGA, which leads to faster polymer degradation. Increase in drug release in erosion phase might be also caused by increasing the solubility of PLGA degradation products by octanol rather than its effect on polymer degradation rate.

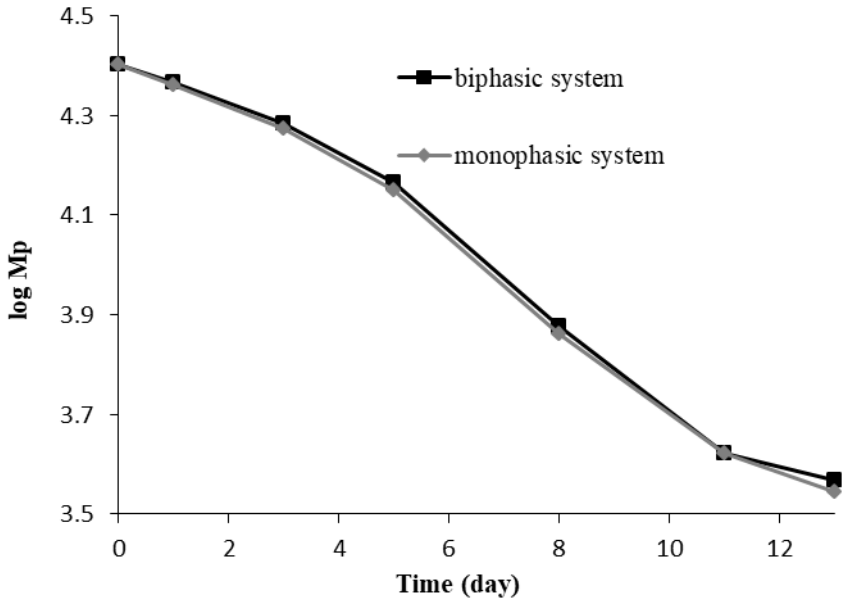


Figure 3.C.6 Changes in MW of implants containing 503H in biphasic system and monophasic system (n=2)

No difference was observed in water uptake study for implants until day 5 in both systems. However, during erosion phase water uptake of implants in biphasic system increased two folds compared to the monophasic system (**Figure 3.C.7**). Similar results were obtained with mass loss study (**Figure 3.C.8**). The water uptake and mass loss studies indicated that octanol affect the drug release in erosion phase.

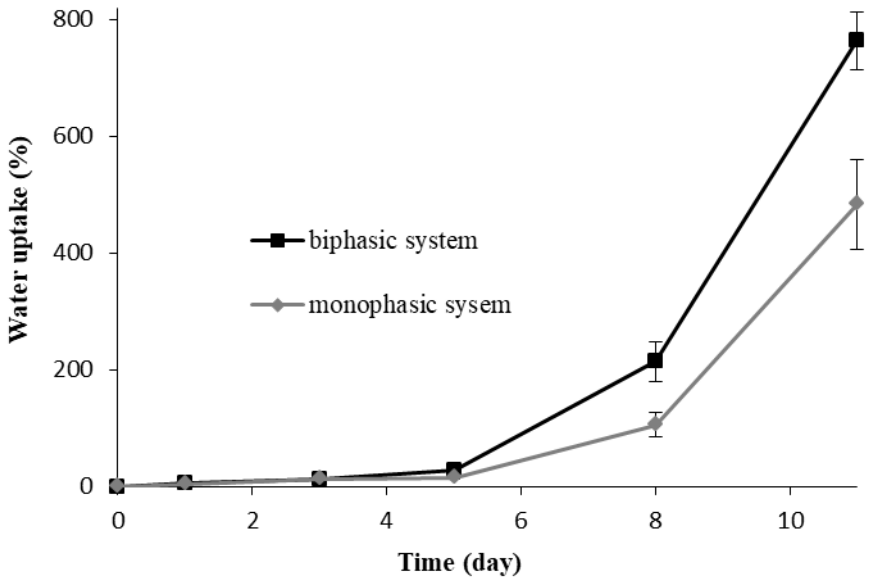


Figure 3.C.7 Water uptake of implants containing 503H in biphasic and monophasic system at 37°C (n=3 +/−SD),

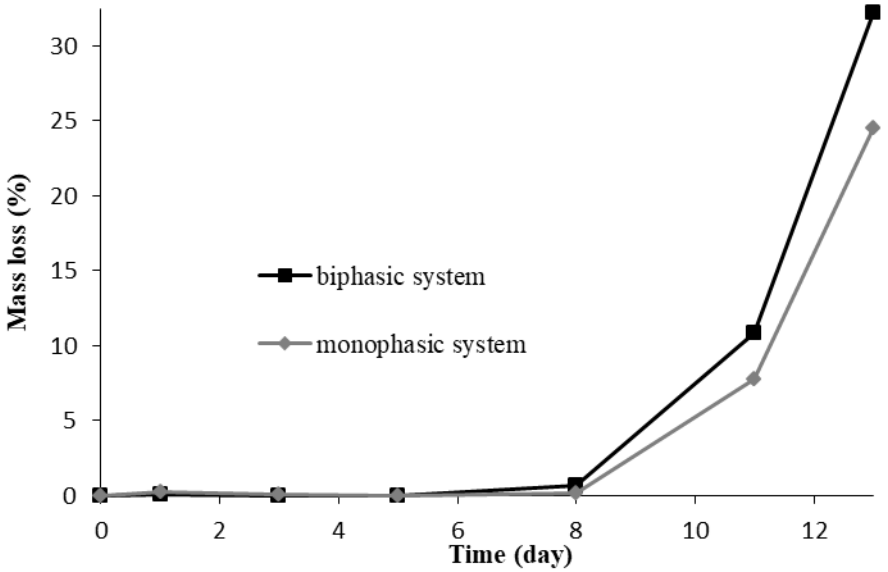


Figure 3.C.8 Mass loss of implant containing 503H in biphasic and monophasic system (n= 1)

The glass transition temperature of risperidone implants decreased from 49°C to 46°C and 36°C after 1 day of incubation in monophasic and biphasic systems, respectively. The decrease in glass transition temperature in monophasic system was probably because of water absorption, which

acts as plasticizer (Omelczuk & McGinity, 1992). The glass transition temperature of the implant in biphasic system was much lower compared to the monophasic system, which might be attributed to the additional plasticizing effect of octanol. The 13°C decrease in glass transition temperature may have increased the molecular mobility and free volume in the polymeric implant and, consequently increased the polymer erosion rate and drug diffusion through the polymeric matrix.

3.C.5 Effect of the amount of octanol and the ratio of octanol/phosphate buffer on drug release

The drug release from implants was independent of the amount of octanol and octanol/phosphate buffer ratio (**Figure 3.C.9 and 10**). The reason for this could be that small amount of octanol was enough to interact with polymer and plasticize it. With increasing the amount of octanol, it formed a heterogeneous system and it cannot improve the plasticizing effect and decrease the glass transition temperature any further.

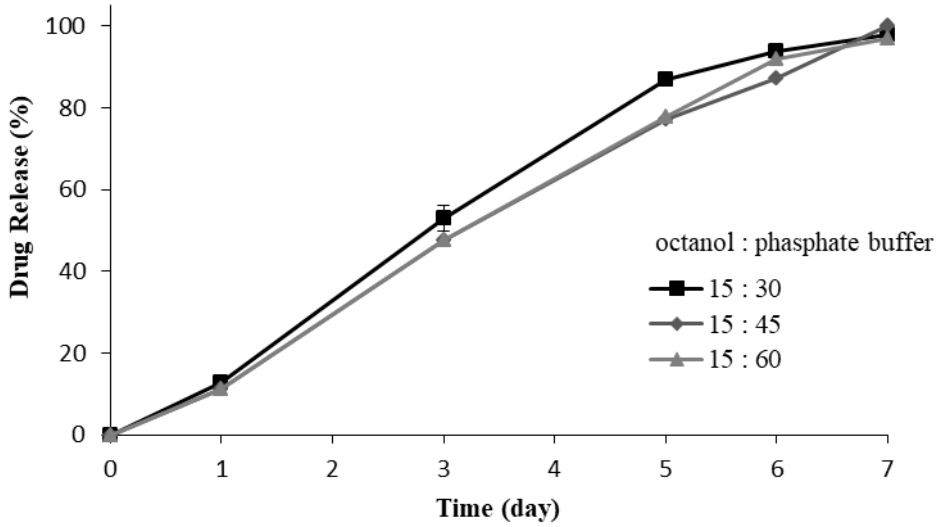


Figure 3.C.9 Effect of different octanol/phosphate buffer pH 7.4 ratio on drug release at 37°C (n=3 +/−SD)

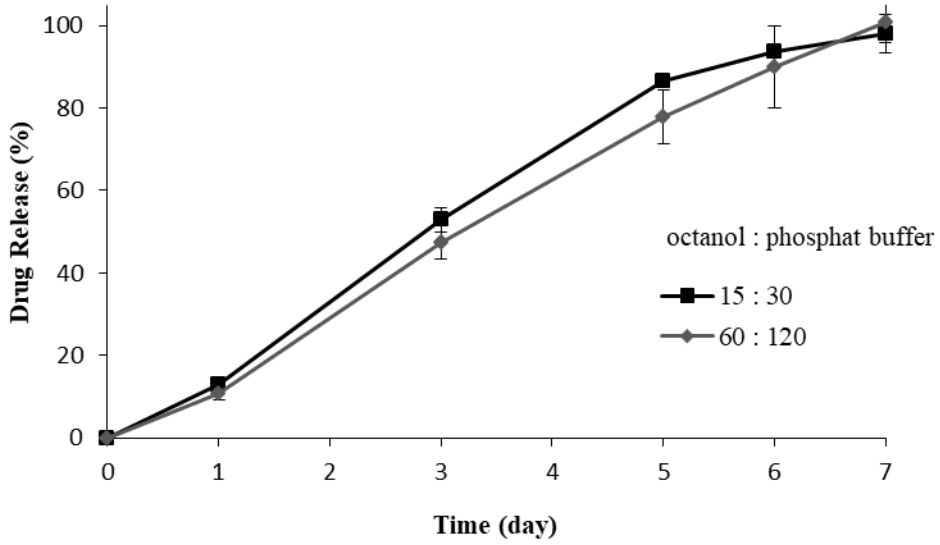


Figure 3.C.10 Effect of different amount of octanol on drug release with constant octanol/ phosphate buffer pH 7.4 ratio at 37°C (n=3 +/−SD)

3.C.6 Drug release from biphasic system containing olive oil

Adipocytes are characterized by the presence of a large triglyceride (TAG) rich lipid droplet bounded by a monolayer of phospholipids. TAG is a neutral lipid consisting of a glycerol moiety esterified with three fatty acids. In humans, the most abundant fatty acids esterified to TAG, in order of decreasing amount, are palmitate (C16:0), stearate (C18:0), oleate (C18:1n9), and linoleate (C18:2n6). These four fatty acids make up around 85% of all TAG (Yew Tan et al., 2015). Olive oil has a similar fatty acid composition and was chosen as oil phase instead of octanol to simulate the subcutaneous tissue better. However, it was not possible to determine the amount of drug in both phases using UV spectrometry, therefore the remaining amount of drug in the implants was extracted and quantified. The study was run for 20 days as after this time point handling of the implants was not possible.

The drug release during diffusion and lag phase was similar in both systems. During the erosion phase the drug release increased in the biphasic system (**Figure 3.C.11**). The water uptake and mass loss were higher in the erosion phase as well (**Figure 3.C.12 and 3.C.13**). The drug release, water uptake, and mass loss study using olive oil indicated faster drug release in erosion

phase. This behavior was similar to the results obtained from biphasic system using octanol. The polymer degradation wasn't influenced by olive oil (**Figure 3.C.14**), which indicate that olive oil, has most likely a plasticizing effect and increases the drug release by accelerating polymer erosion. Furthermore, the drug solubility could also be increased in biphasic system using olive oil due to the high hydrophobicity and high partition coefficient of risperidone. These studies showed that octanol and olive oil have similar effect when used as oily phase.

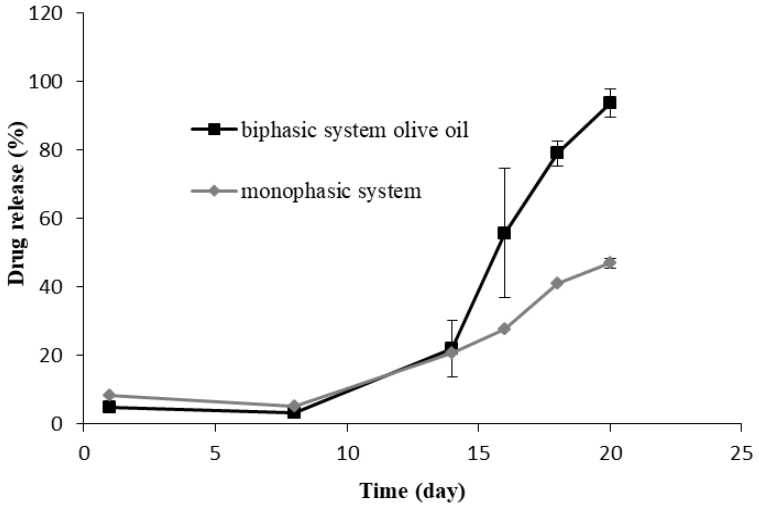


Figure 3.C.11 Release profile of implant containing PLGA 503H and 10% w/w risperidone in biphasic system with "olive oil" and monophasic system at 37°C (n=3 +/−SD),

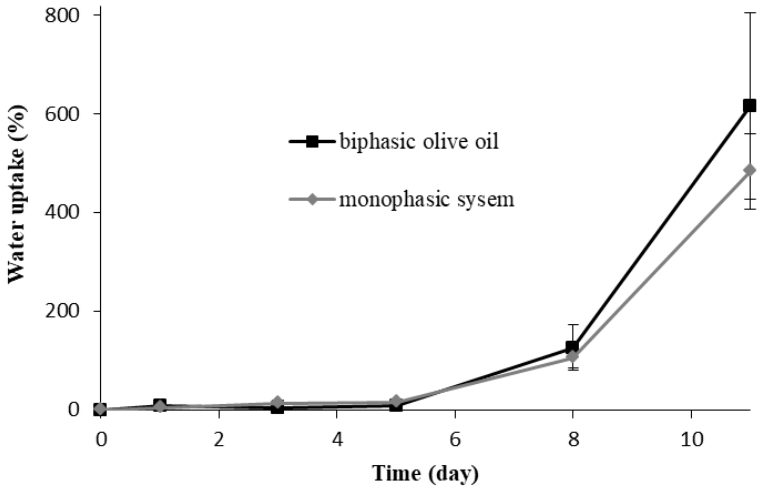


Figure 3.C.12 Water uptake of implants containing 503H in biphasic system with "olive oil" and monophasic system at 37°C (n=3 +/−SD),

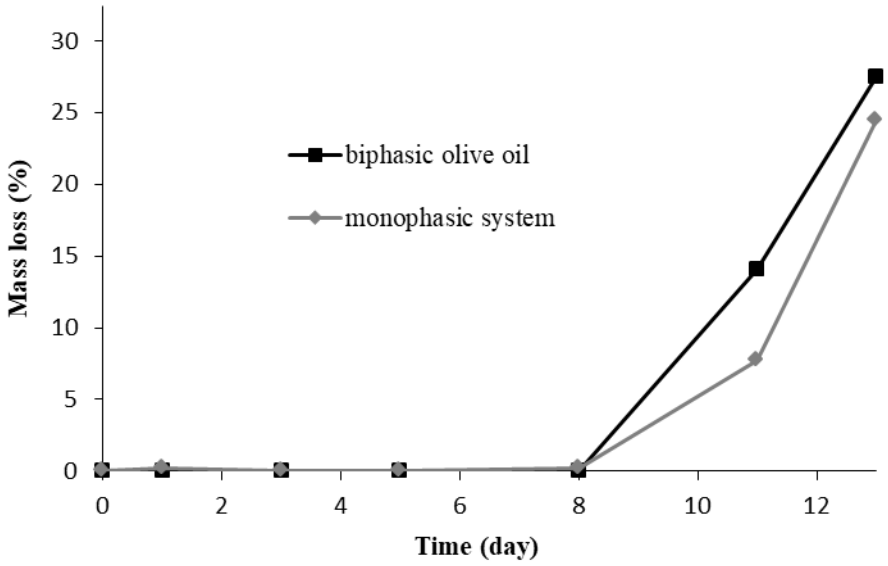


Figure 3.C.13 Mass loss of implants containing 503H in biphasic system with "olive oil" and monophasic system at 37°C (n:1)

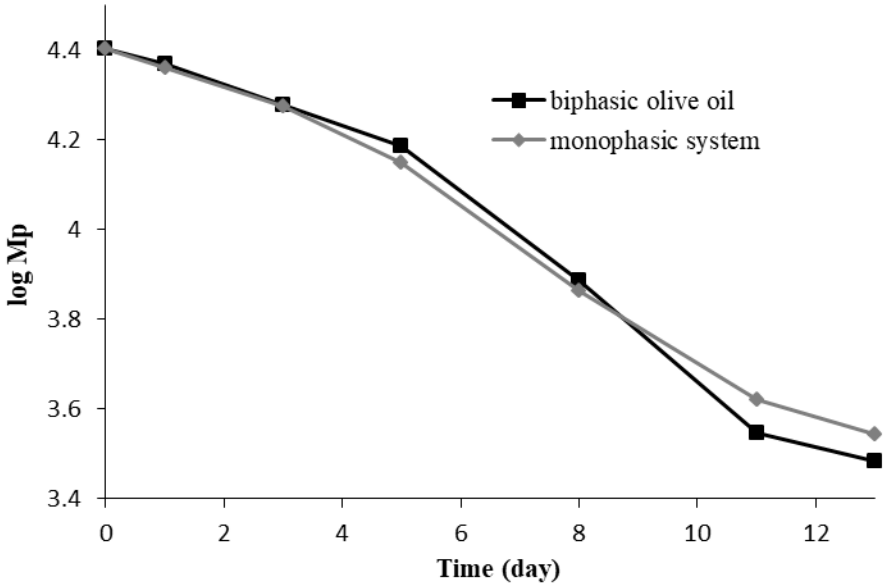


Figure 3.C.14 Changes in MW of implants containing 503H in biphasic system with "olive oil" and monophasic system (n:2)

3.C.7 Effect of incorporation of octanol in implants on drug release

Excipients used to adjust drug release from PLGA-based drug delivery systems are usually low molecular weight salts such as magnesium hydroxide (Kang & Schwendeman, 2002) or hydrophilic polymers such as PEG (Ghalanbor et al., 2010). The problem with these excipients is that they do not stay in the matrix over the complete release time and diffuse out during diffusion phase. Hence these excipients could change the burst release and lag phase, but they usually do not have any influence on drug release in the erosion phase (Ghalanbor et al., 2010). Since risperidone release was mainly erosion controlled and octanol influenced the polymer erosion, octanol was incorporated into risperidone implants in order to adjust risperidone release.

Incorporation of 5% w/w octanol (based on polymer) into 5050 DLG 1A implants containing 10% w/w risperidone did not change the initial burst release, but increased the rate of risperidone release in the erosion phase (**Figure 3.C.15**). The release approximated linear characteristics against time without an uncontrolled burst. However, risperidone release profile did not change with increasing the amount of octanol from 5% to 10% (**Figure 3.C.15**) and the drug release was independent of the amount of octanol in the implant. This can be explained by the glass transition temperature of 5050 DLG 1A. 5% octanol reduced the glass transition temperature, but the glass transition temperature of this polymer was so low that increasing the amount of octanol to 10% did not cause further reduction of the glass transition temperature of 5050 DLG 1A **(Figure3.C.17).**

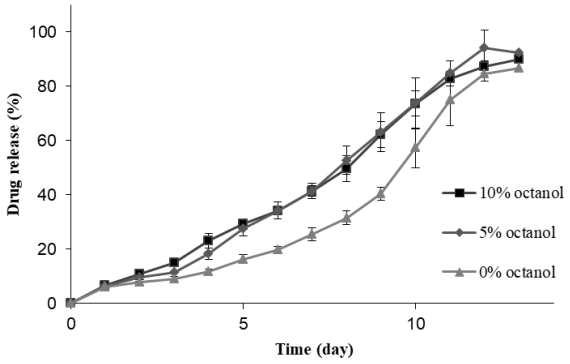


Figure 3.C.15 Effect of octanol level in 5050 DLG 1A implants on risperidone release in phosphate buffer pH 7.4 at 37°C (n=3 +/−SD)

As it was shown in **Figure 3.B.10**, the drug release with polymer 5050 DLG 1A was governed mainly by the polymer erosion mechanism; to better understand the effect of octanol on risperidone release in diffusion phase and lag phase, polymer 5050 DLG 1A was replaced by PLGA 503H.

Incorporation of 5% and 10% w/w octanol (based on polymer) into PLGA 503H implants containing 10% risperidone did not change drug release profile until day 3, which shows that octanol did not influence the initial burst release. But it led to a shorter lag phase and increased the rate of risperidone release in the erosion phase (**Figure 3.C.16a**). The release rate increased with increasing amount of octanol during the erosion phase. These findings suggest that octanol increase the release rate in erosion phase by decreasing the glass transition temperature (**Figure 3.C.17**). The effect of octanol on erosion correlated well with increased water uptake and mass loss. There was no difference in water uptake with all three implants containing 0%, 5% and 10% octanol on day 1, but from day 5 the water uptake increased 2 folds and 4 folds for implants containing 5% and 10% octanol respectively (**Figure 3.C.16b**). The difference became more pronounced over the time. The mass loss of implants without octanol and the implant containing 5% octanol did not start until day 5, the mass loss of implants containing 10% octanol started already at day 3 and the percentage of mass loss increased with increasing the amount of octanol (**Figure 3.C.16c**). It was hypothesized that the degradation and/or erosion rate of the polymer would increase in the presence of octanol due to its plasticizing effect. Furthermore, octanol with a hydrophobic, long alkyl chain and a polar hydroxyl group membrane has an affinity to the PLGA staying longer in the polymer, it does not diffuse out in contrast to hydrophilic excipients like PEG (Ghalanbor et al., 2010).

Incorporation of 10% octanol into PLGA 503H implants resulted in release approximated the linear characteristics without any uncontrolled burst effect (**Figure 3.C.16a).**

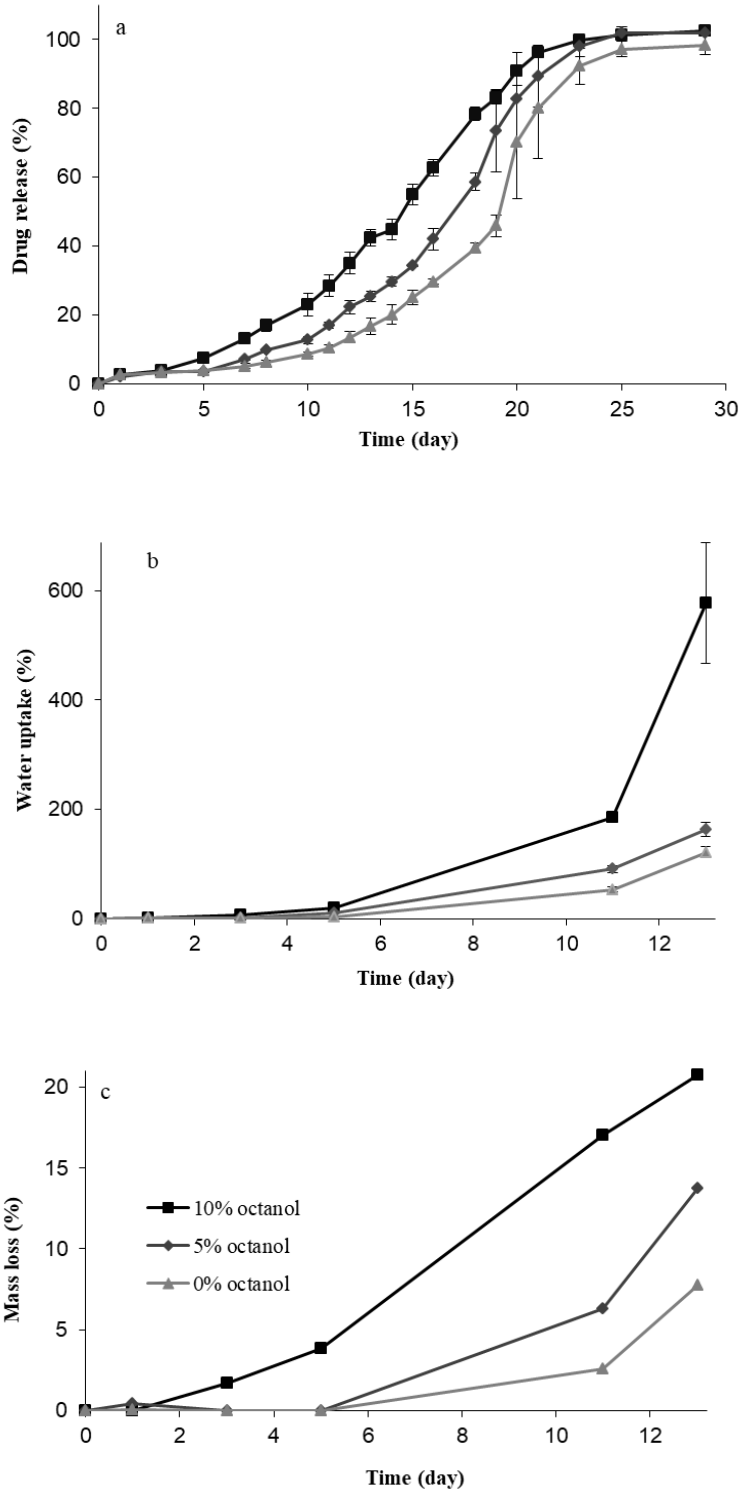


Figure 3.C.16 Effect of percentage of octanol in 503H implants containing 10% risperidone on (a) durg release (b) on water uptake at 37°C (n=3 +/−SD) (c) on mass loss at 37°C (n=2) in phosphate buffer pH 7.4

3.C.8 Effect of octanol on glass transition temperature of two types of PLGA implants containing 10% risperidone

To prove the plasticizing effect of octanol on the PLGA polymer, the glass transition temperature of two types of polymers (503H and 5050 DLG 1A) without octanol, with 5% and 10% octanol was measured. In case of 503H implants 5% and 10% octanol decreased the glass transition temperature by about 6°C and 12°C, respectively (**Figure 3.C.17**), which showed that there was a linear relationship between decrease of glass transition temperature and the amount of octanol incorporated into the implant. In case of 5050 DLG 1A implants, incorporation of 5% octanol decreased the glass transition temperature by 12°C, but in contrast to implants containing PLGA 503H, increasing the amount of octanol to 10% did not cause further reduction of the glass transition temperature of 5050 DLG 1A (**Figure 3.C.17**). An excipient can act as a plasticizer, only when it exists in the same phase as the polymer. At a certain concentration the excipient forms a heterogeneous system and it cannot act as a plasticizer and decrease the glass transition temperature (Blasi et al., 2005). PLGA 503H, with molecular weight of 24 kDa, is a more hydrophobic polymer than 5050 DLG 1A (Molecular weight 7 kDa); higher amount of octanol can be absorbed into this polymer and plasticize it, and due to the higher affinity to the polymer it stays longer in the polymer matrix.

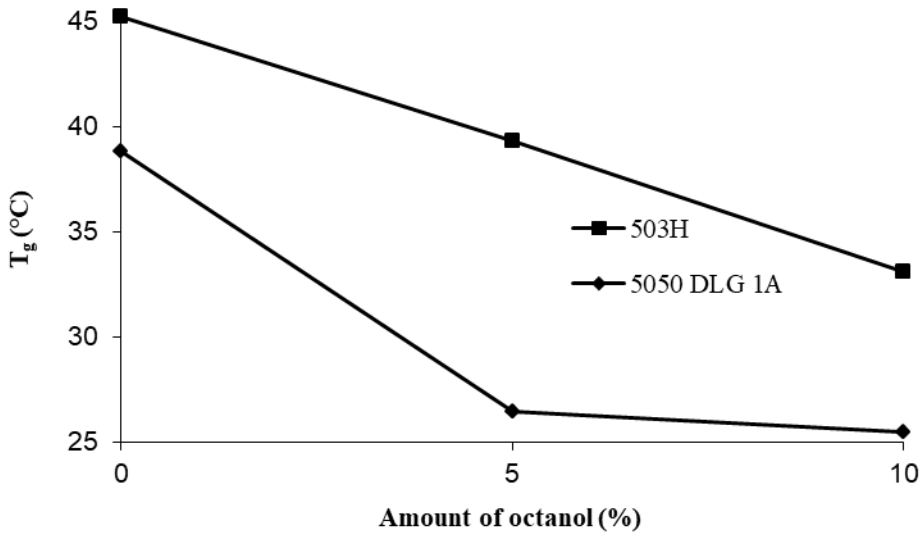


Figure 3.C.17 Effect of percentage of octanol on glass transition temperature of PLGAs (n:2)

3.C.9 Changes in polymer molecular weight of implant containing 503H and octanol

As expected, there were no differences in degree of polymer degradation in implants with 5% octanol or without octanol (**Figure 3.C.18).** However, the degradation of implants containing 10% octanol was slightly faster during the first 5 days (**Figure 3.C.18**). There were no differences in molecular weight in the erosion phase (**Figure 3.C.18**). These results suggested that octanol accelerate polymer erosion due to its plasticizing effect. In addition, it might have an effect on oligomers solubility, which leave the matrix and were not detectable with GPC.

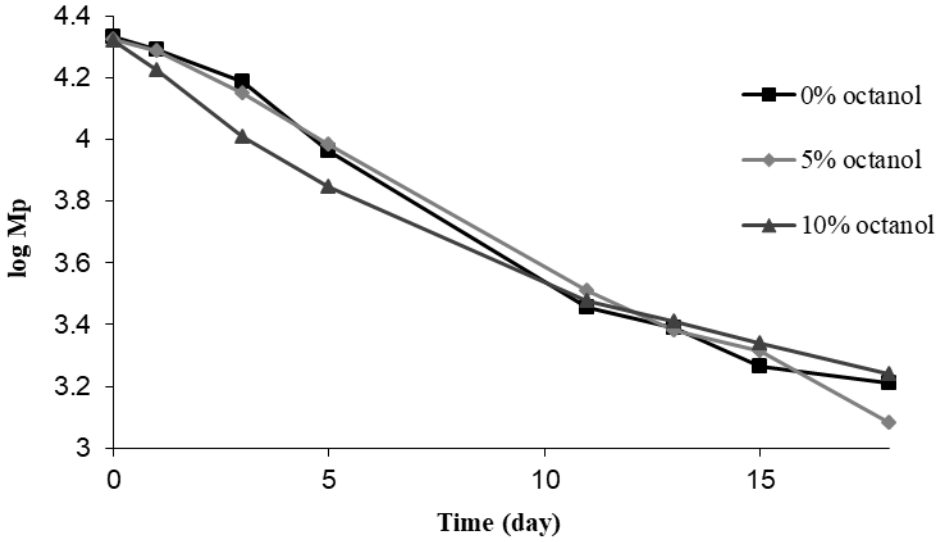


Figure 3.C.18 Effect of octanol on the changes in molecular weight of implants containing 503H

3.C.10 Conclusions

In biphasic system, risperidone was accumulated into the organic phase due to the high affinity of the drug to the octanol phase. Furthermore, the drug release rate was higher in biphasic system compared to monophasic. This was attributed to the plasticizing effect of octanol and increased oligomers solubility, which accelerated polymer erosion without affecting the degradation. Olive oil has the same effect as octanol. Biphasic release model could be potentially appropriate biorelevant media for risperidone PLGA-based implant, since octanol and olive oil can simulate lipid membrane of cells.

Octanol could also be used as an excipient to adjust risperidone release in erosion, since it influences the polymer erosion, without affecting burst release and lag phase. With PLGA 503H implants it was possible to adjust the release rate by varying amount of octanol incorporated into the implant. The release rate increased with increasing amount of octanol in implant during the erosion phase. DSC data showed a linear relationship between depression of glass transition temperature and the amount of octanol incorporated into the implant. Increased release rate was also observed with 5050 DLG 1A implants, however risperidone release profile did not change with increasing the amount of octanol, due to the low glass transition temperature of 5050 DLG 1A polymer. It must be taken into consideration that octanol is not FDA approved and cannot be used as excipient in parenteral formulations, however excipients with similar structure, which are FDA approved, could have the same effect as octanol and could be used to adjust risperidone release in erosion phase.

4. Summary

The biorelevance of the *in vitro* conditions should be considered when designing dissolution or release test methods for controlled release for orals and parenterals. Nevertheless, there are very few reports on such test systems for parenteral dosage forms and most IVIVCs have been achieved without considering physiologically relevant testing conditions in the *in vitro* system. Usually the *in vitro* methods are developed when *in vivo* data is already available and are optimized to match these data. Thus, IVIVC in this case is mainly understood as a tool for quality control purposes, e.g. when dealing with post-approval issues, and the release methods were adapted irrespective of the underlying physiologic conditions to achieve data correlation.

The approach to developing biorelevant test systems is different, even though these tests might also be suitable for establishing IVIVC. Biorelevant test systems may be of outstanding value in the preclinical phase of development providing a sound basis for dosage form optimization and selection of the most promising candidates for clinical studies. As *in vivo* fate of formulation is typically very complex and is furthermore subject to inter- and intra-individual variability, it would be difficult for a biorelevant test system to simulate all *in vivo* conditions accurately.

The major drawback of almost all of the currently used *in vitro* release tests for controlled release parenteral formulations is that the formulations are exposed only to release medium, which does not mimic the physiological environment. When a PLGA-based implant is completely surrounded only by release medium, it can absorb water and swell to its maximal degree, differently to *in vivo*, where less water is available and the formulation is not only in contact with body fluid, but also exposed to the tissue cells.

The purpose of this study was to evaluate the swelling of the implant *ex vivo*, to measure the force applied to the formulation after implantation in the body and to calculate the created pressure in the tissue. To design a new *in vitro* test to mimic mechanical properties of the tissue, which may influence dosage form performance in a standardized and reproducible manner and to study its effect on drug release. To assess biphasic system as biorelevant media and investigate its effect on risperidone release from PLGA-based implants.

The shape, surface area and water uptake of PLGA-implants in *ex vivo* and *in vitro* experiments was determined. Implants were incubated either in phosphate buffer (*in vitro*) or implanted in turkey breast (with different amount of buffer). Surface area and water uptake of implants

increased continuously and they expanded notably and maintained their cylindrical shape under *in vitro* conditions, whereas under *ex vivo* conditions their surface area increased to a lower degree, they expanded only minimal and were deformed, indicating that the swelling was suppressed. The water uptake of both *ex vivo* tests was lower as compared to *in vitro* tests. The water uptake of implant under *ex vivo* test utilizing low amount of buffer increased at a very slow rate. No difference in the water uptake of implants was observed for both *ex vivo* experiments over 3 days, but in the subsequent erosion phase when the implants became soft, the water uptake increased at about the same rate under the *ex vivo* experiment utilizing high amount of phosphate buffer and *in vitro* experiment, as high amount of buffer was available for tissue to be moistened. It can be concluded that the mechanical properties of the tissue and amount of water available *in vivo* both play an important role for the restriction of swelling and the shape change of implants.

A new method - using a texture analyzer and a balloon catheter - was established to quantify the force applied to the formulation *ex vivo*. The force, reached after injecting certain amount of water into the balloon catheter, was determined, when the balloon catheter was in air and under a known weight. A linear relationship was found between the injection force and the force applied to the balloon catheter and an appropriate equation was determined. The measurement was run with different plunger displacements, since this leads to a different degree of expansion of the balloon catheter. Afterwards the balloon catheter was inserted into a turkey breast and injection force was measured. The tissue force applied to the formulation *ex vivo* was calculated with the same plunger displacements using the appropriate equations. This measured force was used to calculate the force applied to the formulation *ex vivo*. The force applied to the balloon catheter *ex vivo* increased with increasing expansion of the balloon catheter. This is caused by the mechanical properties of the tissue, which counteracts the expansion of the balloon catheter. This indicated that the force created in the tissue after implanting an object should be taken into consideration because it might influence the DDSs and therefore also the drug release.

For simulation of limited physiological space, a device was designed to prevent the swelling of formulation. The acidic, basic and nonionic model drugs (e.g.,ibuprofen, risperidone and theophylline) were used for this experiment. All PLGA-based implants showed a typical multiphasic release profile: burst release, diffusion phase followed by lag phase and an erosion

controlled release phase. The risperidone release during diffusion and lag phase was slightly lower for restricted implants compared to unrestricted, due to the lower wettability of implants in the device. This difference in the diffusion and lag phase was more pronounced for ibuprofen and theophylline compared to risperidone due to their higher solubility. From the beginning of erosion phase, drug release rate under restricted conditions was higher than unrestricted implants for all three drug models. Under restricted swelling conditions water uptake was decreased. The decreased water uptake leads to the decreased solubility and mobility of the polymer degradation products so that they could not diffuse out of the matrix. Furthermore, the surface area of the unrestricted implants increased with increase in the swelling hence the degradation products diffuse more easily out of the matrix under these conditions. At the same time the mass loss and polymer degradation was determined and it was found out that the mass loss and polymer degradation were both accelerated under restricted condition. The monitoring of µpH of implants under restricted and non-restricted condition indicated that µpH of the later was between 3.5 and 4.5 during the complete time of follow-up. In contrast, the µpH of implant in device was reduced constantly up to 1.5 within 9 days and remained constant for the following 7 days. As indicated the accumulation of degradation products was higher in the implant in the device due to decrease on surface area and decreased hydration. Oligomers stay in the matrix, causing a faster pH drop and accelerated erosion.

Risperidone release from implants was studied in monophasic and biphasic systems (octanol: water or olive oil: water). A triphasic drug release profile was found for both systems. In biphasic system drug was accumulated into the organic phase due to its high affinity to the octanol phase. In the biphasic system the burst release was higher, the lag phase was shorter and the release rate during erosion phase was higher compared to the monophasic system indicating faster polymer degradation in erosion phase in biphasic system. In order to find out the effect of octanol on drug release in erosion phase, the water uptake, mass loss and polymer degradation was monitored over time in both systems. Results confirmed higher water uptake and mass loss in erosion phase; however, no difference was obtained from degradation study in both systems. Accelerated effect in biphasic system was attributed to the plasticizing effect of octanol. Octanol decreased polymer glass transition temperature, increasing the molecular mobility and free volume in the implant, and consequently, increased the polymer erosion and drug diffusion through the polymeric matrix. Octanol might also increase oligomers solubility, which

accelerated polymer erosion. Olive oil has a similar effect as octanol. Biphasic release model could be potentially the appropriate biorelevant media for risperidone PLGA-based implant, since octanol and olive oil both with a hydrophobic and hydrophilic group can simulate lipid membrane of tissue cells.

The Effect of amount of octanol incorporated in implants was studied on risperidone release from implants. With 503H-based implants it was possible to adjust the release rate with adjusting the amount of octanol incorporated into the implant. The release rate increased with increasing amount of octanol in implant during the erosion phase without affecting burst release and lag phase. DSC data showed a linear relationship between glass transition temperature decrease and the amount of octanol incorporated into the implant. Increased release rate was also observed with 5050 DLG 1A implants. However, risperidone release profile didn't change with increasing the amount of octanol, due to the low glass transition temperature of 5050 DLG 1A polymer.

In conclusion, it may not be possible to simulate all of the biologically relevant reactions at the site of administration through the use of single *in vitro* system, since the biological environment surrounding the formulation is very complex and not static. However, it is still possible to simulate single *in vivo* aspect *in vitro*, in order to investigate its effect on drug release and to develop *in vitro* tests, which are more biorelevant than the current available *in vitro* tests.

5. Zusammenfassung

Die Biorelevanz sollte bei der Entwicklung von In-Vitro-Methoden für orale und parenterale Formulierungen mit kontrollierter Freisetzung berücksichtigt werden. Dennoch gibt es nur wenige Berichte über solche Methoden für parenterale Formulierungen und die meisten IVIVCs wurden ohne Berücksichtigung physiologisch relevanter Testbedingungen in In-vitro-Methoden erreicht. In der Regel werden die In-Vitro-Methoden entwickelt, wenn In-Vivo Daten bereits vorhanden sind und sie werden optimiert, um den In-Vivo Daten zu entsprechen. Somit wird eine IVIVC in diesem Fall hauptsächlich zur Qualitätskontrolle benutzt und die Freisetzungsmethoden wurden unabhängig von den zugrunde liegenden physiologischen Bedingungen angepasst, um eine Datenkorrelation zu erreichen. Der Zweck für die Entwicklung biorelevanter Testsysteme ist aber anders, obwohl diese Tests auch für die Etablierung von IVIVC geeignet sind. Biorelevante Testsysteme können in der präklinischen Entwicklungsphase von herausragender Bedeutung sein um eine solide Grundlage für die Optimierung der Dosierungsform und die Auswahl der aussichtsreichsten Kandidaten für klinische Studien zu liefern. Da die In-Vivo Vorgänge für die Formulierung typischerweise sehr komplex sind und darüber hinaus einer inter- und intraindividuellen Variabilität unterliegen, wäre für ein biorelevantes Testsystem schwierig, alle In-Vivo-Bedingungen genau zu simulieren. Der Hauptnachteil von fast allen derzeit verwendeten In-vitro-Freisetzungstests für parenterale Formulierungen mit kontrollierter Freisetzung besteht darin, dass die Formulierungen nur dem Freisetzungsmedium ausgesetzt werden, was der physiologischen Umgebung nicht entspricht.

Wenn ein PLGA-basiertes Implantat durch das Freisetzungsmedium vollständig umgeben ist, kann es unbegrenzt Wasser aufnehmen und bis zu seinem maximalen Ausmaß quellen, anders als In-Vivo, wo weniger Wasser vorhanden ist und die Formulierung nicht nur in Kontakt mit Körperflüssigkeiten ist, sondern auch zu den Gewebezellen exponiert ist.

Das Ziel dieser Studie war, die Implantatsquellung Ex-Vivo zu bewerten, um die auf die Formulierung ausgeübte Kraft nach der Implantation im Körper zu messen und den erzeugten Druck im Gewebe zu berechnen. Ein neuer standardisierter und reproduzierbarer In-Vitro-Test sollte etabliert werden, der die mechanischen Eigenschaften des Gewebes auf die Arzneisform simuliert und den Effekt auf die Arzneimittelfreisetzung untersucht. Das zweiphasen System sollte als biorelevantes Medium beurteilt und sein Effekt auf die Risperidon-Freisetzung von PLGA-basierten Implantaten untersucht werden.

Die Form, die Oberfläche und die Wasseraufnahme von PLGA-Implantaten wurden in Ex-Vivo und In-Vitro Experimenten untersucht. Die Implantate wurden entweder in Phosphatpuffer (In-Vitro) inkubiert oder in Putenbrust implantiert (mit unterschiedlichen Puffermengen). Unter In-Vitro Bedingungen hat sich die Oberflächenfläche und die Wasseraufnahme von Implantaten kontinuierlich erhöht. Die Implantate expandierten deutlich und haben ihre zylindrische Form beibehalten. Unter Ex-Vivo Bedingungen nahm die Oberfläche nur in einem geringeren Ausmaß zu. Die Implantate expandierten nur minimal und wurden deformiert, was darauf hinweist, dass die Quellung eingeschränkt wurde. Die Wasseraufnahme im Ex-Vivo-Test war im Vergleich zu In-Vitro-Tests geringer.

Die Wasseraufnahme von Implantaten im Ex-Vivo-Test mit einer geringen Menge an Puffer erhöhte sich mit einer sehr langsamen Geschwindigkeit. Es wurde kein Unterschied wurde in der Wasseraufnahme von Implantaten für beide Ex-Vivo-Experimente über 3 Tage beobachtet, jedoch erhöhte sich in der anschließenden Erosionsphase, in der die Implantate weicher wurden, die Wasseraufnahme mit etwa der gleichen Geschwindigkeit im Ex-Vivo-Experiment unter Verwendung einer hohen Menge an Phosphatpuffer wie im In-Vitro Experiment, da eine hohe Menge an Puffer für das zu befeuchtende Gewebe zur Verfügung stand. Es kann gefolgert werden, dass die mechanischen Eigenschaften des Gewebes und die Menge an Wasser, die In-Vivo verfügbar ist, beide eine wichtige Rolle für die eingeschränkte Quellung und die Deformierung von Implantaten spielen.

Eine neue Methode - mit einem Texturanalysator und einem Ballonkatheter - wurde etabliert, um die auf die Formulierung ausgeübte Kraft Ex-Vivo zu quantifizieren. Die Kraft, die nach der Injektion bestimmter Wassermenge in den Ballonkatheter erreicht wurde, wurde in Abhängigkeit einer auf dem Ballonkatheter bekannten Gewicht bestimmt. Es wurde ein linearer Zusammenhang zwischen der Injektionskraft und der Kraft, die auf den Ballonkatheter angewendet wurde, ermittelt und eine Gleichung bestimmt. Die Messung wurde mit unterschiedlichen Ausdehnungsgrad des Ballonkatheters durchgeführt. Danach wurde der Ballonkatheter in eine Putenbrust eingeführt und die Injektionskraft gemessen. Die auf die Formulierung aufgebrachte Gewebekraft Ex-Vivo wurde unter Verwendung der entsprechenden

Gleichungen berechnet. Diese gemessene Kraft wurde verwendet, um die auf die Formulierung aufgebrachte Kraft Ex-Vivo zu berechnen.

Die auf den Ballonkatheter ausgeübte Kraft nahm mit zunehmender Ausdehnung des Ballonkatheters zu. Dies wird durch die mechanischen Eigenschaften des Gewebes verursacht, die der Ausdehnung des Ballonkatheters entgegenwirken.

Dies zeigte, dass nach der Implantation eines Implants bestimmte Kraft vom Gewebe auf die Implantate ausgeuebt wird, die in Betracht gezogen werden sollte, weil er das DDS und damit auch die Arzneimittelfreisetzung beeinflussen könnte.

Um den begrenzten physiologischen Raum zu simulieren wurde eine Vorrichtung entworfen, die die Quellung der Formulierung verhindert. Es wurde festgestellt, dass die Arzneistofffreisetzung von der Vorrichtung nicht kontrolliert wurde. Die drei Arzneistoffe, Risperidone, Ibuprofen und Theophyllin wurden in diesem Experiment verwendet. Alle PLGA-basierten Implantate zeigten ein typisches multiphasisches Freisetzungsprofil: Burst-Freisetzung, Diffusionsphase, gefolgt von einer Verzögerungsphase und einer erosionsgesteuerten Freisetzungsphase. Die Risperidon-Freisetzung während der Diffusions- und Verzögerungsphase war niedriger für Implantate mit eingeschränkter Quellung im Vergleich zu Implantaten mit uneingeschränkter Quellung, aufgrund ihrer geringeren Hydratation in der Vorrichtung. Der Unterschied in der Diffusions- und Verzögerungsphase war für Ibuprofen und Theophyllin, aufgrund ihrer höheren Löslichkeit im Vergleich zu Risperidon, stärker ausgeprägt. Sobald die Erosionsphase erreicht wurde, die Arzneistoff-Freisetzungsrate unter eingeschränkten Quellbedingungen höher als bei uneingeschränkt gequollenen Implantaten für alle drei Arzneistoffmodelle.

Unter eingeschränkten Quellbedingungen wurde die Wasseraufnahme verringert. Die verminderte Wasseraufnahme führt zu einer verminderten Löslichkeit und Diffusion der Polymerabbauprodukte, so dass sie nicht aus der Matrix diffundieren konnten. Weiterhin erhöhte sich die Oberfläche der uneingeschränkt gequollenen Implantaten mit zunehmender Quellung, so dass die Abbauprodukte unter diesen Bedingungen leichter aus der Matrix diffundieren können. Gleichzeitig wurde der Massenverlust und der Polymerabbau bestimmt und es wurde festgestellt, dass der Massenverlust und der Polymerabbau unter eingeschränktem Quellzustand beschleunigt wurden. Bei uneingeschränkter Quellung war der MikropH während der gesamten

Experimentzeit zwischen 3,5 und 4,5. Im Gegensatz dazu reduzierte sich der MikropH der Implantaten in der Vorrichtung innerhalb von 9 Tagen auf 1,5 und blieb für die folgenden 7 Tage konstant. Wie angedeutet, war die Diffusion von Abbauprodukten aus der Implantat in der Vorrichtung, aufgrund der reduzierte Oberfläche und verminderter Hydratation, verringert. Oligomere bleiben in der Matrix, was einen schnellere Mikro-pH-Abnahme und eine beschleunigte Erosion verursacht.

Die Risperidon-Freisetzung aus den Implantaten wurde in monophasischen und biphasischen Systemen (Octanol: Wasser oder Olivenöl: Wasser) untersucht. Beide Systeme zeigten ein triphasisches Freisetzungsprofil, welches vorher schon beschrieben wurde. Im biphasischen System akkumulierte der Arzneistoff, aufgrund seiner hohen Affinität zur Octanolphase, in die organische Phase. In dem biphasischen System war die Burst-Freisetzung höher, die Verzögerungsphase kürzer und die Freisetzungsrate während der Erosionsphase höher im Vergleich zu dem monophasischen System, was auf einen schnelleren Polymerabbau in der Erosionsphase im biphasischen System hinweist. Um die Wirkung von Octanol auf die Arzneistoff-Freisetzung und Erosionsphase zu untersuchen, wurde die Wasseraufnahme, der Massenverlust und der Polymerabbau in Abhängigkeit von der Zeit in beiden Systemen untersucht. Die Ergebnisse bestätigten eine höhere Wasseraufnahme und Massenverlust in der Erosionsphase. Allerdings wurde es kein Unterschied in der Polymerabbaurate in beiden Systemen beobachtet.

Die beschleunigte Wirkung im biphasischen System wurde durch den plastifizierenden Effekt von Octanol verursacht. Octanol verringerte die Polymer-Glasübergangstemperatur, was die molekulare Beweglichkeit und das freie Volumen im Implantat erhöht und folglich die Polymererosion und die Arzneistoffdiffusion durch die Polymermatrix erhöht. Octanol könnte auch die Löslichkeit der Oligomeren erhöht haben, was die Polymererosion zusätzlich beschleunigt haben könnte. Olivenöl hatte eine ähnliche Wirkung wie Octanol. Das biphasische Freisetzungsmodell könnte als biorelevantes Medium für das Risperidon-PLGA-basierte Implantat geeignet sein, da Octanol und Olivenöl beide sowohl eine hydrophobe als auch eine hydrophile Gruppe besitzen und somit die Lipidmembran der Gewebezellen simulieren können.

Es wurde der Effekt der Menge an Octanol im Implantat auf die Arzneistoff-Freisetzung aus dem Implantat untersucht. Mit 503H-basierten Implantaten war es möglich, die Freisetzungsrate mit der Menge an Octanol, die im Implantat vorhandeln war, einzustellen. Die Freisetzungsrate nahm mit zunehmender Menge an Octanol im Implantat während der Erosionsphase zu ohne die Burst-Freisetzung und die Lag-Phase zu beeinflussen. DSC-Daten zeigten einen linearen Zusammenhang zwischen der Abnahme der Glasübergangstemperatur und der Zunahme von Octanol, das in das Implantat zugesetzt worden war. Eine erhöhte Freisetzungsrate wurde auch bei 5050 DLG 1A Implantaten beobachtet. Das Risperidon-Freisetzungsprofil von 5050 DLG 1A-Polymer änderte sich im Gegensatz zum 503H-basierten Implantat nicht proportional mit der Menge an Octanol aufgrund der niedrigen Glasübergangstemperatur.

Abschließend lässt sich festhalten, dass es wahrscheinlich nicht möglich ist, durch einen einzelnen In-Vitro Test alle biologisch relevanten Reaktionen am Applikationsort zu simulieren, da die biologische Umgebung, die die Formulierung umgibt, sehr komplex und nicht statisch ist. Es ist jedoch möglich, einzelne In-Vivo-Aspekte, In-Vitro zu simulieren, um deren Effekte auf die Arzneistofffreisetzung zu untersuchen und In-Vitro-Tests zu entwickeln, die biorelevanter als die derzeitig verfügbaren In-Vitro-Tests sind.

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