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C. Bode, Heiko Kranz, Florence Siepmann, Juergen Siepmann

Research article

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C. Bode,[1] H. Kranz,[2] F. Siepmann,[1] J. Siepmann,[1,*]

[1]*Univ. Lille, Inserm, CHU Lille, U1008, 59000 Lille, France*

[2]*Bayer AG, Muellerstraße 178, 13353 Berlin, Germany*

*correspondence:

Professor Juergen Siepmann, Ph.D.

Univ. Lille, College of Pharmacy,

INSERM U1008 Controlled Drug Delivery Systems and Biomaterials

3, rue du Professeur Laguesse

59006 Lille, France

Phone: +33-3-20964708

juergen.siepmann@univ-lille2.fr

Abstract

Different types of *in-situ* forming implants based on poly(lactic-co-glycolic acid) (PLGA) and N-methyl-pyrrolidone (NMP) were prepared for controlled ocular delivery of dexamethasone. The impact of the volume of the release medium, initial drug content, polymer molecular weight and PLGA concentration on the resulting drug release kinetics were studied and explained based on a thorough physico-chemical characterization of the systems. This included for instance the monitoring of dynamic changes in the implants' wet and dry mass, morphology, PLGA polymer molecular weight, pH of the surrounding bulk fluid and water/NMP contents upon exposure to phosphate buffer pH 7.4. Importantly, the systems can be expected to be rather robust with respect to variations in the vitreous humor volumes encountered in vivo. Interestingly, limited drug solubility effects *within* the implants as well as in the surrounding aqueous medium play an important role for the control of drug release at a drug loading of only 7.5 %. Furthermore, the polymer molecular weight and PLGA concentration in the liquid formulations are decisive for *how* the polymer precipitates during solvent exchange and for the swelling behavior of the systems. These features determine the resulting inner system structure and the conditions for mass transport. Consequently, they affect the degradation and drug release of the *in-situ* formed implants.

Key words: PLGA; *in-situ* forming implant; dexamethasone; autocatalysis; swelling

1. Introduction

Age-related macular degeneration (AMD) and diabetic retinopathy are two of the leading causes for irreversible blindness and vision impairment (Hughes et al., 2005; Edelhauser et al., 2010). Late AMD exists in two forms: the atrophic (or "dry") AMD and the neovascular (or "wet") AMD. Yet, up to now, wet AMD is the only treatable form. It is triggered by vascular endothelial growth factor (VEGF), causing the blood vessels in the retina to grow erratically, eventually breaking through the Bruch's membrane (the innermost layer of the choroid). This leads to blood and protein leakage in the macula, resulting in a blurry vision or sudden vision loss (Chiou, 2011; Bonilha et al., 2013). In the case of diabetic retinopathy, microvascular complications are the result of poorly adjusted diabetes. Sustained hyperglycaemia ultimately causes microaneurysms and a breakdown of endothelial tight junctions in the blood-retinal barrier (BRB), allowing proteins to leak into the vitreous. At later stages, choroidal neovascularization of the retina occurs (Kowluru and Mishra, 2015; Wan et al., 2015; Zaki et al., 2016). Both diseases (wet AMD and diabetic retinopathy) are commonly treated by intravitreal injections of anti-VEGF agents and corticosteroids. Anti-VEGF agents inhibit the growth of the blood vessels, while corticosteroids reduce inflammation by minimizing the expression of inflammatory cytokines and of VEGF. Hence, the choroidal neovascularization is stabilized, decreasing the breakdown rate of the blood-retinal barrier (Kurz et al., 2008; Wykoff et al., 2015; Rodríguez Villanueva et al., 2017).

For an effective treatment, the drugs have to reach the retina in the back of the eye. However, the anatomy and physiology of the eye hamper this: For instance, when using eye drops, less than 5% of the administered drug is generally absorbed through the cornea to reach the anterior chamber (Urtti, 2006). This is due to the low permeability of the cornea (with its different layers and polarities), dilution with tear fluid, rapid lacrimal drainage and other factors. Most importantly, only a very small fraction of the drug is finally found inside the vitreous: the

site of action (approximately 0.001 – 0.0004 % of the administered drug) (Urtti, 2006; Wilson et al., 2011; Kaur and Kakkar, 2014). It has to be pointed out that systemic drug administration also encounters a crucial hurdle: The blood-retinal barrier, preventing most drugs from reaching the vitreous. The attempt to overcome this hurdle with very high systemically administered drug amounts to achieve therapeutic levels in the eye is limited by severe side effects (Edelhauser et al., 2010; Kaur and Kakkar, 2014).

For these reasons intravitreal drug injections are currently considered as the most appropriate way to assure that the drug reaches its site of action. However, every injection bears a risk of infections and other serious side effects, like retinal detachment, retinal haemorrhage, endophthalmitis, increased intraocular pressure, cataract or vitreous haemorrhage (Edelhauser et al., 2010; Giudice and Galan, 2012; Ying et al., 2013; Kaur and Kakkar, 2014; Bisht et al., 2017). Apart from these risks, the discomfort of receiving a needle in the eye leads to limited compliance (Droege et al., 2013; Ghazala et al., 2013).

To assure treatment efficacy, therapeutic drug concentrations must be provided over prolonged periods of time at the site of action. Since dexamethasone has a half-life of approximately 5.5 h in the vitreous, frequent injections are, thus, necessary to remain within the therapeutic range (Chan et al., 2011). Local controlled drug delivery systems can help overcoming all these hurdles: The risk of side effects can be reduced, patient compliance improved and the therapeutic efficacy increased. Nowadays, non-biodegradable implants are approved by the FDA for intraocular administration, releasing dexamethasone over prolonged periods of time [e.g., Retisert (retisert.com), Iluvien (iluvien.com)]. However, these implants have to be removed surgically upon drug exhaust, which is associated with similar risks as the initial insertion, or remain in the vitreous where they accumulate over time (Yasin et al., 2014). To avoid the second surgery for device removal, *biodegradable* implants offer an interesting potential. For example, Allergan developed Ozurdex, a biodegradable implant containing 0.7

mg dexamethasone in a poly(lactic-co-glycolic acid) (PLGA) matrix, which is injected through a 22G needle (Chan et al., 2011). But large needles can be problematic in practice. The injection of a *liquid* solution, that precipitates *in-situ* in the eye and sustains drug release, could effectively reduce the required needle size. Importantly, smaller needles are associated with less pain experienced by the patients (Rodrigues et al., 2011).

Different types of *in-situ* forming implants have been described in the literature, for various types of applications (Kranz and Bodmeier, 2007, 2008; Schoenhammer et al., 2009, 2010; Kempe and Mäder, 2012; Parent et al., 2013, 2017). The transformation from the liquid state (allowing for facilitated administration) to the solid state (allowing for controlled drug release over prolonged periods of time) can be induced by different phenomena, such as solvent exchange, changes in the pH or temperature, or *in-situ* cross-linking (Kempe and Mäder, 2012). In the case of solvent exchange, generally a water-insoluble polymeric matrix former is dissolved in a biocompatible, water-miscible organic solvent. The drug is dissolved and/or dispersed in this polymer solution. Upon injection into aqueous body fluids, the organic solvent diffuses into the surrounding environment (being miscible with water), while water diffuses into the formulation. Since the polymer is water-insoluble, it precipitates and forms the solid implant. The drug molecules or particles are trapped within the implant and slowly released over time. Different formulation parameters can be used to alter implant formation and performance. For instance, the addition of hydrophilic polymers [such as hydroxypropyl methylcellulose (HPMC)] has been proposed to increase the bioadhesion of *in-situ* forming implants releasing antimicrobial drugs in periodontal pockets for the treatment of periodontitis (Do et al., 2014, 2015b, 2015a; Agossa et al., 2017). Poly(lactic-co-glycolic acid) (PLGA) is a well-known biodegradable and biocompatible matrix former in parenteral controlled release formulations (Kranz et al., 2000; Luan et al., 2006; Desai et al., 2008; Kempe et al., 2010; Fredenberg et al., 2011; Ghalanbor et al., 2013; Schwendeman et al., 2014; Gasmi et al., 2015a,

2015b; Huang et al., 2015; Gasmi et al., 2016; Hirota et al., 2016; Hamoudi-Ben Yelles et al., 2017). In the case of *in-situ* forming implants based on PLGA, often N-methyl-pyrrolidone (NMP) is used as a water-miscible organic solvent, for example in the following commercially available drug products: Atridox (for injection into periodontal pockets) (Thakur et al., 2014); Eligard (for subcutaneous injection) (eligard.com); Nuflor (for intramuscular or subcutaneous injection in beef) (merck-animal-health-usa.com/product/cattle/Nuflor-Injectable-Solution/1); Doxirobe gel (for injection into periodontal pockets in dogs) (zoetisus.com/products/dogs/doxirobe-gel.aspx). Furthermore, the group of AG Mikos (Ueda et al., 2007) reported on NMP-based in-situ forming ocular drug delivery systems for luocinolone acetonide, which are based on poly(propylene fumarate) as polymeric matrix former. It has to be pointed out that the toxicity of NMP upon intraocular injection should be investigated in the future.

The aim of this study was to prepare different types of *in-situ* forming implants based on PLGA for intraocular dexamethasone delivery. The systems were thoroughly characterized physico-chemically, including for instance dynamic changes in the wet mass, dry mass, water/NMP content, morphology, polymer molecular weight, potential changes in the pH of the release medium, and drug release kinetics.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (50:50, -COOH end groups; PLGA, Resomer RG 502 H and Resomer RG 504 H; Evonik, Darmstadt, Germany); dexamethasone (Discovery Fine Chemicals, Dorset, UK); N-methyl-pyrrolidone, acetonitrile and tetrahydrofuran (Fisher Scientific, Illkirch, France); ethanol 96% (VWR, Fontenay-sous-Bois, France).

2.2. Preparation of the liquid formulations

Appropriate amounts of PLGA and dexamethasone were dissolved in NMP in glass vials under stirring at 500 rpm (Multipoint Stirrer, Thermo Scientific, Loughborough, UK) at room temperature for 60 min. Afterwards, the vials were kept without stirring for 1 h at room temperature in order to remove air bubbles. The formulations were stored at 2-8 °C, and allowed to reach room temperature prior to use.

2.3. *In-situ* formation of implants

Eppendorf vials were filled with 2.25 or 4.5 mL phosphate buffer pH 7.4 (USP 40) and kept at 37 °C overnight. One hundred µl of the liquid PLGA/dexamethasone/NMP formulations (prepared as described in *section 2.2.*) were injected into the vials using a syringe pump (2 mL/min; PHD 2000; Harvard Apparatus, Holliston, USA). Solvent exchange initiated polymer precipitation and *in-situ* implant formation. The Eppendorf vials were placed into a horizontal shaker (80 rpm, 37 °C; GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany).

2.4. Characterization of *in-situ* formed implants

In vitro drug release: At determined time points, the phosphate buffer pH 7.4 was completely renewed. The amount of dexamethasone in the withdrawn bulk fluid was determined by HPLC-UV analysis, using a Thermo Fisher Scientific Ultimate 3000 Series HPLC, equipped with a LPG 3400 SD/RS pump, an auto sampler (WPS-3000 SL) and a UV-Vis detector (VWD-3400RS) (Thermo Fisher Scientific, Waltham, USA). Samples were centrifuged for 2.5 min at 10,000 rpm (Centrifuge Universal 320; Hettich, Tuttlingen, Germany), and filtered with a 0.45 µm PVDF syringe filter (Millex-HV, Merck Millipore,

Tullagreen, Ireland). Fifty µL samples were injected into an A C18 RP column (Gemini 3 µm C18 110 Å, 100 mm x 4.6 mm; Phenomenex, Le Pecq, France). The mobile phase consisted of acetonitrile and water (33:67 v/v), the flow rate was 1.5 mL/min. Dexamethasone had a retention time of approximately 3.8 min, the detection wavelength was λ = 254 nm. The calibration curve was linear (R > 0.999) within the range of 0.06 to 0.00003 mg/mL. To determine the amount of dexamethasone potentially remaining in the implants after 35 d exposure to phosphate buffer pH 7.4, the remnants were freeze-dried for 3 d (Christ Epsilon 2–4 LSC; Martin Christ, Osterode, Germany) and the lyophilisates were dissolved in a mixture of acetonitrile and ethanol (2:1 v/v). The solutions were filtered using 0.45 µm PVDF filter syringes, and analyzed for their drug contents by HPLC-UV (as described above). In case of incomplete drug release at the end of the observation period, the "missing" amounts were experimentally recovered in the implant remnants. All experiments were conducted in triplicate. In addition, the pH of the release medium was measured at pre-determined time points using a pH meter (InoLab pH Level 1; WTW, Weilheim, Germany) (n = 3).

Implant swelling and erosion: At pre-determined time points, implant samples were withdrawn, excess water carefully removed using Kimtech precision wipes (Kimberly-Clark, Rouen, France) and weighed [*wet mass (t)*]. The samples were lyophilized for 3 d (Christ Epsilon 2–4 LSC) and weighed again [*dry mass (t)*]. The *wet mass (%) (t)*, *water/NMP content (%) (t)*, and *dry mass loss (%) (t)* were calculated as follows:

$$wet\ mass\ (\%)(t) = \frac{wet\ mass\ (t)}{formulation\ mass} \times 100\ \% \quad (1)$$

$$water/NMP\ content\ (\%)(t) = \frac{wet\ mass\ (t) - dry\ mass(t)}{wet\ mass\ (t)} \times 100\ \% \quad (2)$$

$$dry\ mass\ loss\ (\%)(t) = \frac{dry\ mass\ (0) - dry\ mass\ (t)}{dry\ mass\ (0)} \times 100\ \% \quad (3)$$

where *formulation mass* is the initial total mass of the liquid formulation (PLGA + dexamethasone + NMP), and *dry mass (0)* is the dry mass of the liquid formulation prior to exposure to the release medium (PLGA + dexamethasone). All experiments were conducted in triplicate.

Polymer degradation: At pre-determined time points, implants were withdrawn, freeze-dried and the lyophilisates were dissolved in tetrahydrofuran (at a concentration of 3 mg/mL). The average polymer molecular weight (Mw) of the PLGA was determined by Gel Permeation Chromatography (GPC, Separation Modules e2695 and e2695D, 2419 RI Detector, Empower GPC software; Waters, Guyancourt, France) using a PLGel 5 µm MIXED-D column, 7.5 x 300 mm (Agilent Technologies, Interchim, Montluçon, France). The injection volume was 50 µL. Tetrahydrofuran was the mobile phase (flow rate: 1 mL/min). Polystyrene standards with molecular weights between 1,090 and 70,950 Da (Polymer Labaratories, Varian, Les Ulis, France) were used to prepare the calibration curve. All experiments were conducted in triplicate.

Implant morphology: At pre-determined time points, implants were withdrawn and optionally freeze-dried. Cross-sections were obtained by manual breaking. Pictures were taken with an optical image analysis system (Nikon SMZ-U; Nikon, Tokyo, Japan), equipped with a Zeiss camera (AxioCam ICc1; Zeiss, Jena, Germany).

2.5. Determination of the drug solubility

The solubility of dexamethasone (as received) in phosphate buffer pH 7.4 at 37 °C was determined in agitated glass flasks. An excess amount of dexamethasone powder (approximately 30 mg) was exposed to 80 mL bulk fluid, kept at 37 °C under horizontal shaking (80 rpm; GFL 3033). Samples were withdrawn, filtered (0.45 µm PVDF syringe filter), diluted

and analyzed for their drug content by HPLC-UV (as described above, using an injection volume of 20 µL) until equilibrium was reached. Each experiment was conducted in triplicate.

3. Results and Discussion

3.1. Importance of the volume of the release medium

Since the investigated implants are formed *in-situ* following solvent exchange, it was important to evaluate the impact of the volume of the release medium into which the PLGA/drug/NMP solutions were injected. Potentially, the volume of this aqueous phase can affect the diffusion rate of NMP into the surrounding aqueous phase and/or the diffusion rate of water into the (initially) liquid formulation. Such changes might affect the resulting implant size and inner structure and, hence, the drug release kinetics.

The volume of vitreous humor in humans has been reported to be about 4 to 5 mL (Bennett, 2016). To monitor potential effects of variations in the bulk fluid volume in this order of magnitude on the key properties of the *in-situ* formed implants, 2.25 and 4.5 mL have been investigated in this study. Furthermore, most drugs are eliminated via the anterior pathway (Toris et al., 1999; Urtti, 2006). To simulate drug elimination and fluid renewal, the release medium was completely exchanged every day during the first week (which is most decisive for implant formation) in this study.

Figure 1 shows macroscopic pictures of PLGA-based implants formed upon injection of 100 µL of a PLGA/dexamethasone/NMP solution into 2.25 or 4.5 mL phosphate buffer pH 7.4 (37 °C). The liquid formulations contained 30 % Resomer RG 502H and 0.75 % dexamethasone. The photos were taken after 3 d. At the top, implants in Eppendorf tubes (filled with the release medium) are shown. Below, higher magnifications of implants, which had been

carefully withdrawn from the release medium are illustrated (surfaces). At the bottom, surfaces and cross-sections of implant samples after freeze-drying are shown. The cross-sections were obtained by manual breaking. The dashed regions highlight the hollow cores of the implants. As it can be seen, there was no remarkable impact of the volume of the aqueous bulk fluid (2.25 vs. 4.5 mL) on the resulting implant morphology: left vs. right hand side in Figure 1. Please note that caution must be paid when drawing conclusions from the pictures of lyophilized implants, because of artifact creation during freeze-drying. Importantly, the implants were hollow also in the wet state (data not shown). This can be explained as follows: Upon contact with water, NMP diffuses into the outer bulk fluid and water diffuses into the liquid NMP formulation. Since PLGA is soluble in NMP, but not in water, at a certain time point the polymer precipitates (once the solubility of the polymer in the water/NMP mixture is reached). This process likely starts at the "formulation – aqueous bulk fluid" interface, because the water concentration is highest and the NMP concentration lowest at this location. The continuous decrease in PLGA solubility in the NMP/water mixture (the NMP content decreases, whereas the water content increases) leads to continued polymer precipitation. Thus, the PLGA "shell" becomes thicker and thicker, growing "inwards". Once all PLGA has precipitated, potentially remaining inner volumes (here the centers of the implants) cannot be filled with polymer and become water-filled cavities. Please note that complete solvent exchange took up to several days in this study: Thus, the implant cores remained liquid for a significant period of time. Importantly, no noteworthy impact of the bulk fluid volume on this cavity formation was observed.

Figures 2a and b show the resulting dexamethasone release kinetics and the dynamic changes of the systems' water/NMP contents over time. The water/NMP contents of the implants were determined gravimetrically as the difference between the wet and dry mass of the withdrawn samples (before and after freeze-drying). As it can be seen, the drug release

curves were virtually overlapping for the investigated bulk fluid volumes (2.25 vs. 4.5 mL). Also, the resulting water/NMP contents were very similar. This can probably be attributed to the fact that NMP and water are freely miscible: So, there are no saturation effects, resulting in potentially reduced NMP diffusion rates into smaller (eventually more saturated) outer aqueous phases (and vice versa).

Importantly, limited drug solubility effects in the surrounding release medium are unlikely to affect dexamethasone release from the *in-situ* forming implants at an initial drug loading of 0.75 %: The solubility of dexamethasone in phosphate buffer pH 7.4 at 37 °C was determined to be 77 ± 4 µg/mL. In NMP, the drug is freely soluble. Thus, at early time points (when the surrounding bulk fluid contains considerable amounts of NMP) saturation effects in the surrounding bulk fluid are unlikely. Furthermore, even if assuming the absence of any NMP in the surrounding bulk fluid from day 3 on (this is a "worst case scenario" for the drug solubility), sink conditions were also provided for the remaining observation period (considering the drug solubility determined in pure phosphate buffer pH 7.4 at 37 °C).

These findings are important, since they demonstrate that variations in the volume of the bulk fluid into which the PLGA/drug/NMP solutions are injected, are not substantially affecting the key properties of the resulting implants. In other words: The proposed *in-situ* forming implant formulations can be expected to be rather robust with respect to variations in the vitreous humor volumes encountered in vivo.

3.2. Impact of the drug loading

Figure 3 shows the impact of the initial drug loading of the *in-situ* forming implant formulations on the resulting dexamethasone release kinetics and the dynamic changes in the implants' wet mass as well as water/NMP contents. The initial drug content was varied from 0.25 to 7.5 %, as indicated. Please note that 100 µL of the formulation with the intermediate

drug loading (0.75 %) contain a similar drug dose as the commercially available drug product Ozurdex (0.7 mg) (Chan et al., 2011). The release medium was 2.25 mL phosphate buffer pH 7.4. Resomer RG 502H (30 %) was the polymer. Clearly, the relative drug release rates were similar for formulations loaded with 0.25 and 0.75 % dexamethasone (filled and open circles in Figure 2a), whereas the relative drug release rate was substantially lower at 7.5 % drug loading (filled triangles). This cannot be attributed to differences in the dynamic changes in the systems' wet mass, as illustrated in Figure 3b (which were rather similar for all drug loadings).

Given the limited solubility of dexamethasone in the release medium (77 ± 4 µg/mL in phosphate buffer pH 7.4 at 37 °C), one hypothesis can be that the substantially reduced drug release rate at 7.5 % initial dexamethasone loading is due to saturation effects. To evaluate the validity of this hypothesis, the renewal rate of the release medium was altered: Figures 4a and b show the resulting drug release kinetics and degrees of bulk fluid saturation (with respect to the drug) observed at a higher and lower sampling frequency (at each sampling time point, the release medium was completely renewed). The degrees of saturation of the release medium were calculated based on the solubility of dexamethasone in phosphate buffer pH 7.4 at 37 °C. Since the surrounding bulk fluid contained important amounts of NMP at early time points, and since dexamethasone is soluble in NMP, no values are indicated in the first week (Figure 4b). Clearly, the higher sampling frequency lead to faster drug release after about 1 week, corresponding to lower degrees of bulk fluid saturation with the drug. Furthermore, after about 3 weeks, the degree of bulk fluid saturation substantially decreased (to about 10 % = sink conditions) in the case of the higher sampling frequency, while the release rate *de*creased. These observations indicate that saturation effects likely refer to both: dexamethasone saturation in the *surrounding* bulk fluid as well as drug saturation effects *within* the implants: At an initial drug loading of 7.5 %, important parts of the dexamethasone can be expected to precipitate

within the *in-situ* forming PLGA implants upon water penetration into and NMP leaching out of the system. Consequently, dissolved and non-dissolved dexamethasone co-exist within the implant. It has to be pointed out that only dissolved drug is available for diffusion and can be released into the surrounding bulk fluid (Siepmann and Siepmann, 2012, 2008). Hence, drug release is also likely to be limited by saturation effects *within* the implants.

Please note that during the first week, the observed dexamethasone release rates were very similar for the lower and higher sampling frequency (filled and open circles in Figure 4a). This might be explained by the fact that during this time period noteworthy amounts of NMP were still present *within* the implants and the surrounding bulk fluid (limiting the importance of drug saturation effects).

Furthermore, the initial drug loading had no major impact on the resulting dynamic changes in the implants' wet mass over time (Figure 3b). The latter increased during the first 2.5 weeks, and then decreased again. The initial increase can be attributed to the progressing PLGA degradation and subsequent water penetration into the more and more hydrophilic polymer matrices. The subsequent decrease is likely attributable to the dissolution/disappearance of the remnants (more hydrated regions dissolving faster than less hydrated regions). The water/NMP contents were very high during the observation period, irrespective of the initial drug loading (Figure 3c).

3.3. Impact of the PLGA polymer molecular weight

The effects of the polymer molecular weight of the PLGA on drug release and the dynamic changes in the implants' wet mass as well as water/NMP contents upon exposure to phosphate buffer pH 7.4 are illustrated in Figure 5: Resomer RG 502H (Mw about 15 k Da) and Resomer RG 504H (Mw about 45 k Da) are compared. The initial dexamethasone loading was 0.25 %, the polymer concentration in the liquid formulation was 30 %, and the volume of the release

346 medium was 2.25 mL. As it can be seen, the polymer molecular weight substantially impacted
347 the dynamic changes in the systems' wet mass and water/NMP content: Implants based on
348 longer chain PLGA took up fundamentally less water than systems based on shorter chain
349 PLGA. This can be attributed to the facts that: (i) longer chain PLGA is more hydrophobic than
350 shorter chain PLGA, and (ii) longer chain PLGA is likely to precipitate earlier than shorter
351 chain PLGA upon water penetration into the system and NMP diffusion out of the formulation.
352 The observed differences in the wet mass of the implants based on shorter and longer chain
353 PLGA (Figure 5b) are consistent with the different water/NMP contents of systems (Figure 5c).
354 Whereas the implants based on the more hydrophilic Resomer RG 502H show high water
355 contents right from the beginning, the water contents of Resomer RG 504H-based systems was
356 initially substantially lower, but significantly increased during the observation period (due to
357 the progressive polymer chain cleavage). From a practical point of view, substantial implant
358 swelling should be avoided to minimize any related side effects in vivo. This might for instance
359 be achieved via the selection of appropriate PLGA molecular weights or monomer (lactic acid:
360 glycolic acid) ratios, or specific additives (Do et al., 2014, 2015a,b).

361 Interestingly, these substantial differences in the implants' compositions and water uptake
362 behaviors are "not fully" reflected in the observed release kinetics (Figure 5a). This is because
363 drug release was almost complete within the first few days: the time period of implant
364 formation. For instance, after 4 d only 3.8 ± 0.8 and 10.5 ± 1.0 % dexamethasone remained
365 trapped within the implants based on Resomer RG 502H and Resomer RG 504H, respectively.
366 These amounts were slowly released during the subsequent 3 weeks. The observed slower drug
367 release from Resomer RG 504H-based implants compared to Resomer RG 502H-based
368 implants can at least partially be attributed to the lower water contents of the systems (and, thus,
369 denser polymer networks). Please note that with other drugs, which are not almost completely
370 released within the first few days during implant formation, substantial differences in the

resulting release kinetics can be expected from Resomer RG 502H- and Resomer RG 504H-based implants, due to the fundamentally different conditions for drug release in these systems (Figures 5b and c).

3.4. Impact of the polymer concentration

Figure 6 shows the observed dexamethasone release kinetics from *in-situ* formed implants prepared with drug-polymer solutions in NMP containing 30 vs. 45% Resomer RG 502H, or 15 vs. 30 % Resomer RG 504H. Please note that in the latter case, higher polymer concentrations lead to considerable viscosities, rendering injection difficult. The volume of the release medium was 2.25 mL, the initial drug content 0.25 %. As it can be seen, the polymer concentration in the liquid formulations affected the resulting drug release kinetics, irrespective of the PLGA polymer molecular weight: With increasing polymer concentration the dexamethasone release rate decreased. This can at least partially be attributed to differences in the implants' inner structure, as shown in Figure 7: At the top, cross-sections of freeze-dried implants based on Resomer RG 502H are illustrated, at the bottom cross-sections of implants based on Resomer RG 504H. The implants were lyophilized after 3 d exposure to phosphate buffer pH 7.4. Again, please note that caution should be paid because of potential artifact creation during freeze-drying. The dashed regions indicate the hollow central implant cavities. Clearly, higher polymer concentrations in the liquid formulations lead to smaller cavities. This can be attributed to the fact that PLGA precipitation started at the "liquid formulation – aqueous bulk fluid" interface. Subsequent PLGA precipitation "filled" the *in-situ* forming implants. In the case of higher polymer concentrations, more polymer was available to fill the interior of the systems, resulting in smaller cavities. The thicker the polymer shells, the longer are the diffusion pathways through the PLGA matrices to be overcome by the trapped drug. Thus,

higher polymer concentrations in the formulations lead to thicker polymer shells/barriers and, hence, slower drug release (irrespective of the polymer molecular weight).

Furthermore, the smaller central implant cavities at higher initial PLGA concentrations resulted in lower increases in the systems' wet mass and lower water contents, irrespective of the PLGA polymer molecular weight (Figures 8 and 9). Figures 10 to 12 illustrate the dynamic changes in the polymer molecular weight (Mw) of the PLGA, the pH of the surrounding bulk fluid and the dry mass loss kinetics of the systems. Importantly, the smaller central implant cavities observed at higher initial polymer concentrations lead to accelerated ester chain cleavage (Figure 10: open symbols always below filled symbols). This can be attributed to an increase in the importance of autocatalytic effects in these systems: Water is present throughout the implants, thus, polymer chain cleavage occurs in the entire polymer matrices. The generated (water-soluble) shorter chain acids slowly diffuse into the surrounding bulk fluid, where they are (at least partially) neutralized. In addition, bases from the surrounding phosphate buffer diffuse into the implants and neutralize (at least partially) the generated acids. However, the rate at which the acids are generated within the implants can be higher than the rate at which they are neutralized. Consequently, the micro-pH can locally drop (Brunner et al., 1999; Ding and Schwendeman, 2004; Li and Schwendeman, 2005; Ding and Schwendeman, 2008; Schädlich et al., 2014), resulting in pH gradients within the implants. Since hydrolytic ester bond cleavage is catalyzed by protons, PLGA degradation is accelerated at locations with low pH values (Grizzi et al., 1995; Lu et al., 1999). The importance of such autocatalytic effects strongly depends on the systems' dimensions and porosity (Siepmann et al., 2005; Klose et al., 2006). With increasing polymer concentration in the liquid formulation the thickness of the polymer "shells" increases (Figure 7), hence, autocatalysis is likely more pronounced. The experimentally measured PLGA degradation kinetics shown in Figure 10 clearly confirm this hypothesis: The polymer backbone is more rapidly cleaved at higher PLGA concentrations

(open vs. filled symbols). Interestingly, this faster PLGA degradation at higher polymer concentrations is not reflected in the drug release kinetics (Figure 6), demonstrating the dominance of the thickness of the PLGA shells (the lengths of the diffusion pathways through the polymeric matrices) in this case.

Furthermore, the diffusion of the short chain acids out of the implants into the surrounding bulk fluid can lead to a decrease in pH of the latter. As it can be seen in Figure 11, decreasing pH values of the release medium were indeed observed in all cases. At higher polymer concentrations the "pH drops" were much more pronounced than in the case of lower PLGA concentrations, irrespective of the polymer molecular weight. This can probably be attributed to the fact that thicker polymer "shells" are created at high PLGA concentrations, resulting in more pronounced autocatalytic effects (since the generated short chain acids more slowly diffuse out and bases from the release medium more slowly diffuse in, due to the longer diffusion pathways to be overcome). The potential consequences of (slight) acidifications of the surrounding environment in vivo should be addressed in future studies. The fact that dexamethasone is an anti-inflammatory drug might help minimizing tissue irritation, but caution should be taken when speculating on these aspects based on in vitro data.

Comparing the dynamic changes in the pH values of the surrounding bulk fluids in the case of implants based on Resomer RG 502H and Resomer RG 504H (Figure 11 a vs. 11b), it can be seen that the "pH drops" occur at later time points in the case of the longer chain PLGA. This can at least partially be attributed to the fact that the initial polymer molecular weight was higher, thus, more time is needed to generate short chain, water-soluble acids, which can diffuse out. Interestingly, the "*clear* pH drops" in the bulk fluid observed at higher polymer concentrations (open symbols in Figures 11a,b) are followed by distinct increases in the systems' dry mass loss (open symbols in Figures 12a,b): The dry mass loss nicely reflects the leaching of the shorter chain (water-soluble) acids out of the implants into the release medium.

4. Conclusion

In-situ forming PLGA-based implants offer an interesting potential for ocular dexamethasone delivery. Importantly, the systems can be expected to be rather robust with respect to variations in the vitreous humor volumes encountered in vivo. Depending on the initial drug loading, drug saturation effects *within* the implants and in the surrounding aqueous medium can play an important role for the control of dexamethasone release. The polymer molecular weight as well as the PLGA concentration in the liquid formulations determine *how* the macromolecules precipitate as well as the extent and rate of system swelling. These are key features, being decisive for the mobility of water, drug, polymer degradation products and bases within the system. For example, they affect the thickness of the polymer shell, water content of the system and importance of local drops in the micro-pH (and, thus, autocatalysis). The inner implant structure and conditions for mass transport within the *in-situ* forming implants determine polymer degradation and drug release.

In the future, the toxicity of the solvent NMP for the ocular tissue as well as the potential consequences of local drops in pH due to leaching of PLGA degradation products should be studied in vivo. It would also be interesting to investigate the effects of the monomer ratio (lactic acid to glycolic acid) of the PLGA as well as the impact of potential additives, altering the formation of the implants and the conditions for mass transport. Such formulation changes might be used to adjust desired release kinetics for given drugs and drug doses during specific target release periods.

References

Agossa, K., Lizambard, M., Rongthong, T., Delcourt-Debruyne, E., Siepmann, J., Siepmann, F., 2017. Physical key properties of antibiotic-free, PLGA/HPMC-based in-situ forming implants for local periodontitis treatment. Int. J. Pharm. 521, 282–293. https://doi.org/10.1016/j.ijpharm.2017.02.039

Bennett, L., 2016. Other Advances in Ocular Drug Delivery, in: Addo, R.T. (Ed.), Ocular Drug Delivery: Advances, Challenges and Applications. Springer International Publishing, pp. 165–185. https://doi.org/10.1007/978-3-319-47691-9_10

Bisht, R., Jaiswal, J.K., Oliver, V.F., Eurtivong, C., Reynisson, J., Rupenthal, I.D., 2017. Preparation and evaluation of PLGA nanoparticle-loaded biodegradable light-responsive injectable implants as a promising platform for intravitreal drug delivery. J. Drug Deliv. Sci. Technol. 40, 142–156. https://doi.org/10.1016/j.jddst.2017.06.006

Bonilha, V.L., Shadrach, K.G., Rayborn, M.E., Li, Y., Pauer, G.J.T., Hagstrom, S.A., Bhattacharya, S.K., Hollyfield, J.G., 2013. Retinal deimination and PAD2 levels in retinas from donors with age-related macular degeneration (AMD). Exp. Eye Res. 111, 71–78. https://doi.org/10.1016/j.exer.2013.03.017

Brunner, A., Mäder, K., Göpferich, A., 1999. pH and Osmotic Pressure Inside Biodegradable Microspheres During Erosion1. Pharm. Res. 16, 847–853. https://doi.org/10.1023/A:1018822002353

Chan, A., Leung, L.-S., Blumenkranz, M.S., 2011. Critical appraisal of the clinical utility of the dexamethasone intravitreal implant (Ozurdex®) for the treatment of macular edema related to branch retinal vein occlusion or central retinal vein occlusion. Clin. Ophthalmol. Auckl. NZ 5, 1043–1049. https://doi.org/10.2147/OPTH.S13775

Chiou, G.C.Y., 2011. Pharmacological treatment of dry age-related macular degeneration (AMD). Taiwan J. Ophthalmol. 1, 2–5. https://doi.org/10.1016/j.tjo.2011.08.001

Desai, K.G.H., Mallery, S.R., Schwendeman, S.P., 2008. Effect of formulation parameters on 2-methoxyestradiol release from injectable cylindrical poly(dl-lactide-co-glycolide) implants. Eur. J. Pharm. Biopharm. 70, 187–198. https://doi.org/10.1016/j.ejpb.2008.03.007

Ding, A.G., Schwendeman, S.P., 2008. Acidic Microclimate pH Distribution in PLGA Microspheres Monitored by Confocal Laser Scanning Microscopy. Pharm. Res. 25, 2041–2052. https://doi.org/10.1007/s11095-008-9594-3

Ding, A.G., Schwendeman, S.P., 2004. Determination of water-soluble acid distribution in poly(lactide-co-glycolide). J. Pharm. Sci. 93, 322–331. https://doi.org/10.1002/jps.10524

Do, M.P., Neut, C., Delcourt, E., Seixas Certo, T., Siepmann, J., Siepmann, F., 2014. In situ forming implants for periodontitis treatment with improved adhesive properties. Eur. J. Pharm. Biopharm. 88, 342–350. https://doi.org/10.1016/j.ejpb.2014.05.006

Do, M.P., Neut, C., Metz, H., Delcourt, E., Mäder, K., Siepmann, J., Siepmann, F., 2015a. In-situ forming composite implants for periodontitis treatment: How the formulation determines system performance. Int. J. Pharm. 486, 38–51. https://doi.org/10.1016/j.ijpharm.2015.03.026

Do, M.P., Neut, C., Metz, H., Delcourt, E., Siepmann, J., Mäder, K., Siepmann, F., 2015b. Mechanistic analysis of PLGA/HPMC-based in-situ forming implants for periodontitis treatment. Eur. J. Pharm. Biopharm. 94, 273–283. https://doi.org/10.1016/j.ejpb.2015.05.018

Droege, K.M., Muether, P.S., Hermann, M.M., Caramoy, A., Viebahn, U., Kirchhof, B., Fauser, S., 2013. Adherence to ranibizumab treatment for neovascular age-related macular degeneration in real life. Graefes Arch. Clin. Exp. Ophthalmol. 251, 1281–1284. https://doi.org/10.1007/s00417-012-2177-3

Edelhauser, H.F., Rowe-Rendleman, C.L., Robinson, M.R., Dawson, D.G., Chader, G.J., Grossniklaus, H.E., Rittenhouse, K.D., Wilson, C.G., Weber, D.A., Kuppermann, B.D., Csaky, K.G., Olsen, T.W., Kompella, U.B., Holers, V.M., Hageman, G.S., Gilger, B.C., Campochiaro, P.A., Whitcup, S.M., Wong, W.T., 2010. Ophthalmic Drug Delivery Systems for the Treatment of Retinal Diseases: Basic Research to Clinical Applications. Investig. Opthalmology Vis. Sci. 51, 5403. https://doi.org/10.1167/iovs.10-5392

Fredenberg, S., Wahlgren, M., Reslow, M., Axelsson, A., 2011. The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems—A review. Int. J. Pharm. 415, 34–52. https://doi.org/10.1016/j.ijpharm.2011.05.049

Gasmi, H., Danede, F., Siepmann, J., Siepmann, F., 2015a. Does PLGA microparticle swelling control drug release? New insight based on single particle swelling studies. J. Controlled Release 213, 120–127. https://doi.org/10.1016/j.jconrel.2015.06.039

Gasmi, H., Siepmann, F., Hamoudi, M.C., Danede, F., Verin, J., Willart, J.-F., Siepmann, J., 2016. Towards a better understanding of the different release phases from PLGA microparticles: Dexamethasone-loaded systems. Int. J. Pharm., In Honour of Professor Alexander T. FlorenceIn Honour of Professor Alexander T. Florence 514, 189–199. https://doi.org/10.1016/j.ijpharm.2016.08.032

Gasmi, H., Willart, J.-F., Danede, F., Hamoudi, M.C., Siepmann, J., Siepmann, F., 2015b. Importance of PLGA microparticle swelling for the control of prilocaine release. J. Drug Deliv. Sci. Technol. 30, 123–132. https://doi.org/10.1016/j.jddst.2015.10.009

Ghalanbor, Z., Körber, M., Bodmeier, R., 2013. Interdependency of protein-release completeness and polymer degradation in PLGA-based implants. Eur. J. Pharm. Biopharm. 85, 624–630. https://doi.org/10.1016/j.ejpb.2013.03.031

539 Ghazala, F., Hovan, M., Mahmood, S., 2013. Improving treatment provision of Wet AMD with
540 intravitreal ranibizumab. BMJ Qual. Improv. Rep. 2, u201733.w993.
541 https://doi.org/10.1136/bmjquality.u201733.w993
542 Giudice, G.L., Galan, A., 2012. Basic Research and Clinical Application of Drug Delivery
543 Systems for the Treatment of Age-Related Macular Degeneration.
544 https://doi.org/10.5772/31524
545 Grizzi, I., Garreau, H., Li, S., Vert, M., 1995. Hydrolytic degradation of devices based on
546 poly(dl-lactic acid) size-dependence. Biomaterials 16, 305–311.
547 https://doi.org/10.1016/0142-9612(95)93258-F
548 Hamoudi-Ben Yelles, M.C., Tran Tan, V., Danede, F., Willart, J.F., Siepmann, J., 2017. PLGA
549 implants: How Poloxamer/PEO addition slows down or accelerates polymer
550 degradation and drug release. J. Controlled Release 253, 19–29.
551 https://doi.org/10.1016/j.jconrel.2017.03.009
552 Hirota, K., Doty, A.C., Ackermann, R., Zhou, J., Olsen, K.F., Feng, M.R., Wang, Y., Choi, S.,
553 Qu, W., Schwendeman, A.S., Schwendeman, S.P., 2016. Characterizing release
554 mechanisms of leuprolide acetate-loaded PLGA microspheres for IVIVC development
555 I: In vitro evaluation. J. Controlled Release, The 14th edition of the European
556 Symposium on Controlled Drug Delivery, Egmond aan Zee, The Netherlands on April
557 13-15, 2016 244, 302–313. https://doi.org/10.1016/j.jconrel.2016.08.023
558 Huang, J., Mazzara, J.M., Schwendeman, S.P., Thouless, M.D., 2015. Self-healing of pores in
559 PLGAs. J. Controlled Release 206, 20–29.
560 https://doi.org/10.1016/j.jconrel.2015.02.025
561 Hughes, P.M., Olejnik, O., Chang-Lin, J.-E., Wilson, C.G., 2005. Topical and systemic drug
562 delivery to the posterior segments. Adv. Drug Deliv. Rev., Drug Delivery Strategies to

563 Treat Age-Related Macular Degeneration 57, 2010–2032.
564 https://doi.org/10.1016/j.addr.2005.09.004
565 Kaur, I.P., Kakkar, S., 2014. Nanotherapy for posterior eye diseases. J. Controlled Release 193,
566 100–112. https://doi.org/10.1016/j.jconrel.2014.05.031
567 Kempe, S., Mäder, K., 2012. In situ forming implants — an attractive formulation principle for
568 parenteral depot formulations. J. Controlled Release, Drug Delivery Research in Europe
569 161, 668–679. https://doi.org/10.1016/j.jconrel.2012.04.016
570 Kempe, S., Metz, H., Pereira, P.G.C., Mäder, K., 2010. Non-invasive in vivo evaluation of in
571 situ forming PLGA implants by benchtop magnetic resonance imaging (BT-MRI) and
572 EPR spectroscopy. Eur. J. Pharm. Biopharm., Imaging Techniques in Drug
573 Development 74, 102–108. https://doi.org/10.1016/j.ejpb.2009.06.008
574 Klose, D., Siepmann, F., Elkharraz, K., Krenzlin, S., Siepmann, J., 2006. How porosity and size
575 affect the drug release mechanisms from PLGA-based microparticles. Int. J. Pharm.,
576 Local Controlled Drug Delivery to the Brain 314, 198–206.
577 https://doi.org/10.1016/j.ijpharm.2005.07.031
578 Kowluru, R.A., Mishra, M., 2015. Oxidative stress, mitochondrial damage and diabetic
579 retinopathy. Biochim. Biophys. Acta BBA - Mol. Basis Dis. 1852, 2474–2483.
580 https://doi.org/10.1016/j.bbadis.2015.08.001
581 Kranz, H., Bodmeier, R., 2008. Structure formation and characterization of injectable drug
582 loaded biodegradable devices: In situ implants versus in situ microparticles. Eur. J.
583 Pharm. Sci. 34, 164–172. https://doi.org/10.1016/j.ejps.2008.03.004
584 Kranz, H., Bodmeier, R., 2007. A novel in situ forming drug delivery system for controlled
585 parenteral drug delivery. Int. J. Pharm. 332, 107–114.
586 https://doi.org/10.1016/j.ijpharm.2006.09.033

587 Kranz, H., Ubrich, N., Maincent, P., Bodmeier, R., 2000. Physicomechanical Properties of
588 Biodegradable Poly(D,L-lactide) and Poly(D,L-lactide-co-glycolide) Films in the Dry
589 and Wet States. J. Pharm. Sci. 89, 1558–1566. https://doi.org/10.1002/1520-
590 6017(200012)89:12<1558::AID-JPS6>3.0.CO;2-8
591 Kurz, P.A., Suhler, E.B., Flaxel, C.J., Rosenbaum, J.T., 2008. Injectable Intraocular
592 Corticosteroids, in: Becker, M., Davis, J. (Eds.), Surgical Management of Inflammatory
593 Eye Disease. Springer Berlin Heidelberg, pp. 5–16. https://doi.org/10.1007/978-3-540-
594 33862-8_1
595 Li, L., Schwendeman, S.P., 2005. Mapping neutral microclimate pH in PLGA microspheres. J.
596 Controlled Release, Proceedings of the Eight European Symposium on Controlled Drug
597 Delivery 101, 163–173. https://doi.org/10.1016/j.jconrel.2004.07.029
598 Lu, L., Garcia, C.A., Mikos, A.G., 1999. In vitro degradation of thin poly(DL-lactic-co-glycolic
599 acid) films. J. Biomed. Mater. Res. 46, 236–244.
600 Luan, X., Skupin, M., Siepmann, J., Bodmeier, R., 2006. Key parameters affecting the initial
601 release (burst) and encapsulation efficiency of peptide-containing poly(lactide-co-
602 glycolide) microparticles. Int. J. Pharm. 324, 168–175.
603 https://doi.org/10.1016/j.ijpharm.2006.06.004
604 Parent, M., Clarot, I., Gibot, S., Derive, M., Maincent, P., Leroy, P., Boudier, A., 2017. One-
605 week in vivo sustained release of a peptide formulated into in situ forming implants. Int.
606 J. Pharm. 521, 357–360. https://doi.org/10.1016/j.ijpharm.2017.02.046
607 Parent, M., Nouvel, C., Koerber, M., Sapin, A., Maincent, P., Boudier, A., 2013. PLGA in situ
608 implants formed by phase inversion: Critical physicochemical parameters to modulate
609 drug release. J. Controlled Release 172, 292–304.
610 https://doi.org/10.1016/j.jconrel.2013.08.024

611 Rodrigues, E.B., Grumann, A., Penha, F.M., Shiroma, H., Rossi, E., Meyer, C.H., Stefano, V.,
612 Maia, M., Magalhaes, O., Farah, M.E., 2011. Effect of needle type and injection
613 technique on pain level and vitreal reflux in intravitreal injection. J. Ocul. Pharmacol.
614 Ther. Off. J. Assoc. Ocul. Pharmacol. Ther. 27, 197–203.
615 https://doi.org/10.1089/jop.2010.0082
616 Rodríguez Villanueva, J., Rodríguez Villanueva, L., Guzmán Navarro, M., 2017.
617 Pharmaceutical technology can turn a traditional drug, dexamethasone into a first-line
618 ocular medicine. A global perspective and future trends. Int. J. Pharm. 516, 342–351.
619 https://doi.org/10.1016/j.ijpharm.2016.11.053
620 Schädlich, A., Kempe, S., Mäder, K., 2014. Non-invasive in vivo characterization of
621 microclimate pH inside in situ forming PLGA implants using multispectral fluorescence
622 imaging. J. Controlled Release 179, 52–62.
623 https://doi.org/10.1016/j.jconrel.2014.01.024
624 Schoenhammer, K., Boisclair, J., Schuetz, H., Petersen, H., Goepferich, A., 2010.
625 Biocompatibility of an injectable in situ forming depot for peptide delivery. J. Pharm.
626 Sci. 99, 4390–4399. https://doi.org/10.1002/jps.22149
627 Schoenhammer, K., Petersen, H., Guethlein, F., Goepferich, A., 2009. Injectable in situ forming
628 depot systems: PEG-DAE as novel solvent for improved PLGA storage stability. Int. J.
629 Pharm. 371, 33–39. https://doi.org/10.1016/j.ijpharm.2008.12.019
630 Schwendeman, S.P., Shah, R.B., Bailey, B.A., Schwendeman, A.S., 2014. Injectable controlled
631 release depots for large molecules. J. Controlled Release, 30th Anniversary Special
632 Issue 190, 240–253. https://doi.org/10.1016/j.jconrel.2014.05.057
633 Siepmann, J., Elkharraz, K., Siepmann, F., Klose, D., 2005. How Autocatalysis Accelerates
634 Drug Release from PLGA-Based Microparticles: A Quantitative Treatment.
635 Biomacromolecules 6, 2312–2319. https://doi.org/10.1021/bm050228k

636 Siepmann, J., Siepmann, F., 2012. Modeling of diffusion controlled drug delivery. J. Controlled
637 Release, Drug Delivery Research in Europe 161, 351–362.
638 https://doi.org/10.1016/j.jconrel.2011.10.006
639 Siepmann, J., Siepmann, F., 2008. Mathematical modeling of drug delivery. Int. J. Pharm.,
640 Future Perspectives in Pharmaceutics Contributions from Younger Scientists 364, 328–
641 343. https://doi.org/10.1016/j.ijpharm.2008.09.004
642 Thakur, R.R.S., McMillan, H.L., Jones, D.S., 2014. Solvent induced phase inversion-based in
643 situ forming controlled release drug delivery implants. J. Controlled Release 176, 8–23.
644 https://doi.org/10.1016/j.jconrel.2013.12.020
645 Toris, C.B., Yablonski, M.E., Wang, Y.-L., Camras, C.B., 1999. Aqueous humor dynamics in
646 the aging human eye. Am. J. Ophthalmol. 127, 407–412. https://doi.org/10.1016/S0002-
647 9394(98)00436-X
648 Ueda, H., Hacker, M. c., Haesslein, A., Jo, S., Ammon, D. m., Borazjani, R. n., Kunzler, J. f.,
649 Salamone, J. c., Mikos, A. g., 2007. Injectable, in situ forming poly(propylene
650 fumarate)-based ocular drug delivery systems. J. Biomed. Mater. Res. A 83A, 656–666.
651 https://doi.org/10.1002/jbm.a.31226
652 Urtti, A., 2006. Challenges and obstacles of ocular pharmacokinetics and drug delivery. Adv.
653 Drug Deliv. Rev., Ocular Drug Delivery 58, 1131–1135.
654 https://doi.org/10.1016/j.addr.2006.07.027
655 Wan, T.-T., Li, X.-F., Sun, Y.-M., Li, Y.-B., Su, Y., 2015. Recent advances in understanding
656 the biochemical and molecular mechanism of diabetic retinopathy. Biomed.
657 Pharmacother. 74, 145–147. https://doi.org/10.1016/j.biopha.2015.08.002
658 Wilson, C.G., Tan, L.E., Mains, J., 2011. Principles of Retinal Drug Delivery from Within the
659 Vitreous, in: Kompella, U.B., Edelhauser, H.F. (Eds.), Drug Product Development for

the Back of the Eye, AAPS Advances in the Pharmaceutical Sciences Series. Springer US, pp. 125–158.

Wykoff, C.C., Croft, D.E., Brown, D.M., Wang, R., Payne, J.F., Clark, L., Abdelfattah, N.S., Sadda, S.R., 2015. Prospective Trial of Treat-and-Extend versus Monthly Dosing for Neovascular Age-Related Macular Degeneration: TREX-AMD 1-Year Results. Ophthalmology 122, 2514–2522. https://doi.org/10.1016/j.ophtha.2015.08.009

Yasin, M.N., Svirskis, D., Seyfoddin, A., Rupenthal, I.D., 2014. Implants for drug delivery to the posterior segment of the eye: A focus on stimuli-responsive and tunable release systems. J. Controlled Release 196, 208–221. https://doi.org/10.1016/j.jconrel.2014.09.030

Ying, L., Tahara, K., Takeuchi, H., 2013. Drug delivery to the ocular posterior segment using lipid emulsion via eye drop administration: Effect of emulsion formulations and surface modification. Int. J. Pharm. 453, 329–335. https://doi.org/10.1016/j.ijpharm.2013.06.024

Zaki, W.M.D.W., Zulkifley, M.A., Hussain, A., Halim, W.H.W.A., Mustafa, N.B.A., Ting, L.S., 2016. Diabetic retinopathy assessment: Towards an automated system. Biomed. Signal Process. Control 24, 72–82. https://doi.org/10.1016/j.bspc.2015.09.011

Web references

http://eligard.com, accessed on 30 June 2018

https://iluvien.com, accessed on January 30 2018

https://www.merck-animal-health-usa.com/product/cattle/Nuflor-Injectable-Solution/1, accessed on 30 June 2018

http://www.retisert.com, accessed on January 30 2018

https://www.zoetisus.com/products/dogs/doxirobe-gel.aspx, accessed on 30 June 2018

685 **Figure legends**

686

687 Fig. 1. Macroscopic pictures of implants formed *in-situ* upon exposure to phosphate buffer
688 pH 7.4 before and after freeze drying (surfaces and cross-sections). The formulations
689 contained 0.75 % dexamethasone and 30 % PLGA 502H. The volume of the release
690 medium was 2.25 mL (left column) or 4.5 mL (right column). The pictures were taken
691 after 3 d. The dashed regions highlight the hollow cores of the implants.

692 Fig 2. Impact of the volume of the release medium (phosphate buffer pH 7.4) on: a) drug
693 release, and b) the dynamic changes in the water/NMP content of *in-situ* forming
694 implants. The formulations contained 0.75 % dexamethasone and 30 % PLGA 502H.
695 Mean values +/- standard deviation are indicated (n=3).

696 Fig. 3. Impact of the initial drug loading (indicated in the diagrams) of *in-situ* forming
697 implants on the resulting: a) drug release kinetics, b) dynamic changes in the wet mass
698 and c) dynamic changes in the water/NMP content of the systems after exposure to
699 phosphate buffer pH 7.4. The formulations contained 30 % PLGA 502H. The volume
700 of the release medium was 2.25 mL. Mean values +/- standard deviation are indicated
701 (n=3).

702 Fig. 4. Impact of the sampling frequency during the drug release measurements on: a) the
703 cumulative relative amount of drug released, and b) the degree of saturation of the
704 withdrawn samples. The formulations contained 7.5 % dexamethasone and 30 %
705 PLGA 502H. The volume of the release medium was 4.5 mL. Mean values +/-
706 standard deviation are indicated (n=3).

707 Fig. 5. Importance of the polymer molecular weight of the PLGA (Resomer 502H vs. 504H)
708 for: a) drug release, b) the dynamic changes in the wet mass, and c) the dynamic
709 changes in the water/NMP content from/of implants formed *in-situ* upon exposure to
710 phosphate buffer pH 7.4. The formulations contained 0.25 % dexamethasone and

30 % PLGA. The volume of the release medium was 2.25 mL. Mean values +/- standard deviation are indicated (n=3).

Fig. 6. Impact of the PLGA concentration in the formulation on the resulting dexamethasone release kinetics from *in-situ* formed implants upon exposure to 2.25 mL phosphate buffer pH 7.4: a) PLGA 502H and b) PLGA 504H. The drug content was 0.25 %. Mean values +/- standard deviation are indicated (n=3).

Fig. 7. Macroscopic pictures of cross-sections of freeze-dried *in-situ* formed implants after 3 d exposure to 2.25 mL phosphate buffer 7.4. The formulations contained 0.25 % dexamethasone and 30 % or 45 % PLGA 502H or 15 % or 30 % PLGA 504H. The cross-sections were obtained by manual breaking. All implants were hollow, the cavities are highlighted by the dashed areas.

Fig. 8. Impact of the PLGA concentration in the formulation on the dynamic changes in the wet mass of implants formed *in-situ* upon exposure to 2.25 mL phosphate buffer pH 7.4: a) PLGA 502H and b) PLGA 504H. The formulations contained 0.25 % dexamethasone. Mean values +/- standard deviation are indicated (n=3).

Fig. 9. Effects of the PLGA concentration in the formulation on the dynamic changes in the water/NMP content of implants formed *in-situ* upon exposure to 2.25 mL phosphate buffer pH 7.4: a) PLGA 502H and b) PLGA 504H. The formulations contained 0.25 % dexamethasone. Mean values +/- standard deviation are indicated (n=3).

Fig. 10. Impact of the PLGA concentration in the formulation on PLGA degradation in implants formed *in-situ* upon exposure to 2.25 mL phosphate buffer pH 7.4: a) PLGA 502H and b) PLGA 504H. The formulations contained 0.25 % dexamethasone. Mean values +/- standard deviation are indicated (n=3).

Fig. 11. Effects of the PLGA concentration in the formulation on the dynamic changes in the pH of the release medium surrounding implants formed *in-situ* upon exposure to

2.25 mL phosphate buffer pH 7.4: a) PLGA 502H and b) PLGA 504H. The formulations contained 0.25 % dexamethasone. Mean values +/- standard deviation are indicated (n=3).

Fig. 12. Impact of the PLGA concentration in the formulation on the dry mass loss of implants formed *in-situ* upon exposure to 2.25 mL phosphate buffer pH 7.4: a) PLGA 502H and b) PLGA 504H. The formulations contained 0.25 % dexamethasone. Mean values +/- standard deviation are indicated (n=3).

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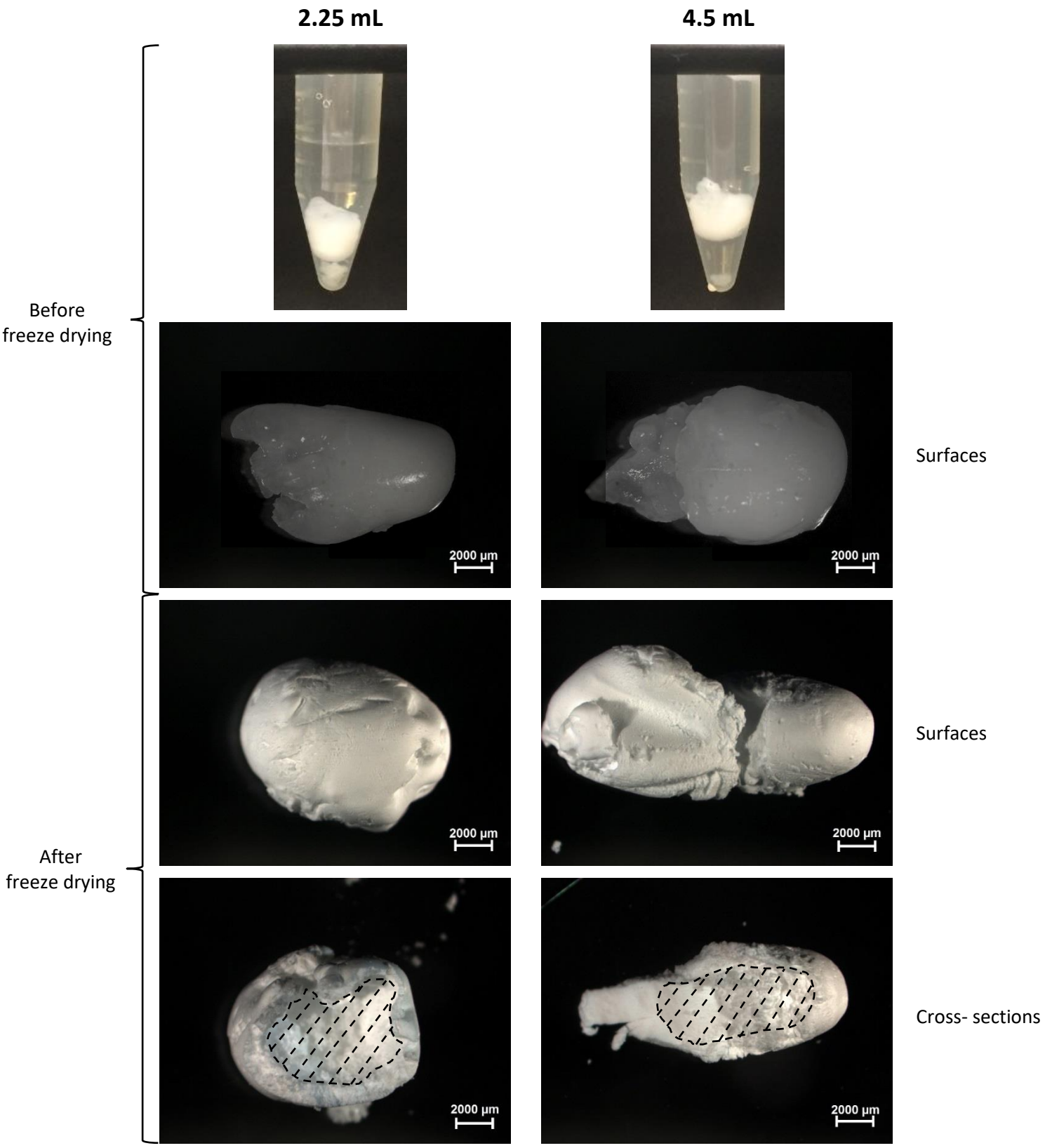

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Figure 1

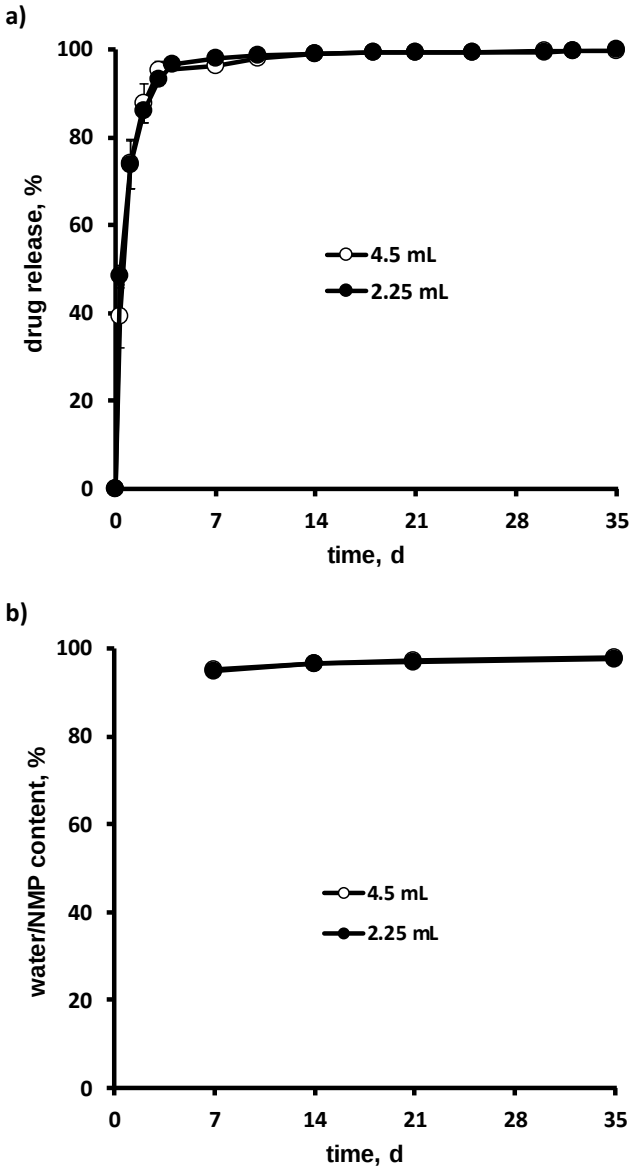

a)
drug release, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
4.5 mL
2.25 mL
b)
water/NMP content, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
4.5 mL
2.25 mL

Figure 2

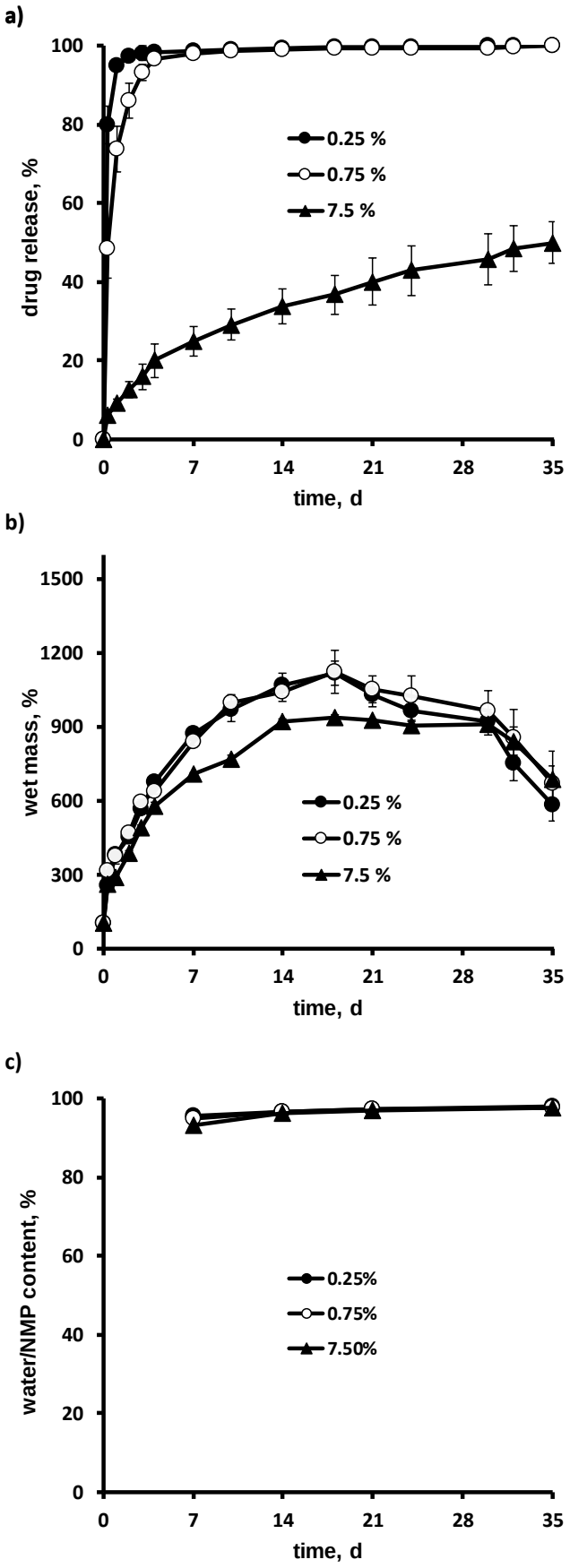

a)
drug release, %
time, d
0.25 %
0.75 %
7.5 %
b)
wet mass, %
time, d
0.25 %
0.75 %
7.5 %
c)
water/NMP content, %
time, d
0.25%
0.75%
7.50%

Figure 3

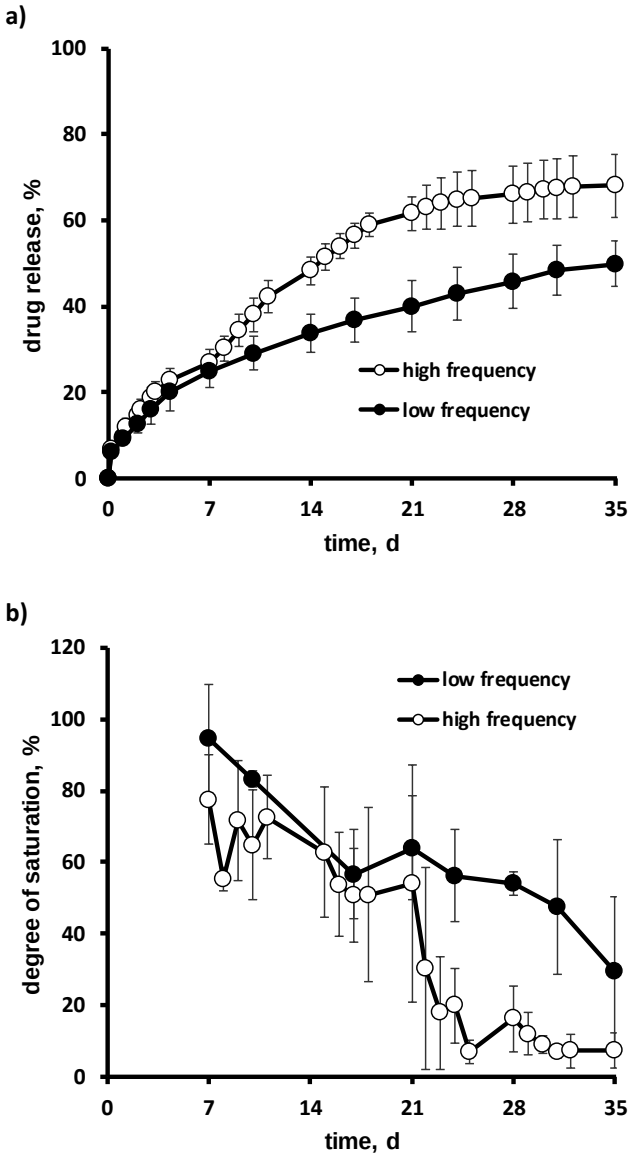

a)
drug release, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
high frequency
low frequency
b)
degree of saturation, %
120
100
80
60
40
20
0
0
7
14
21
28
35
time, d
low frequency
high frequency

Figure 4

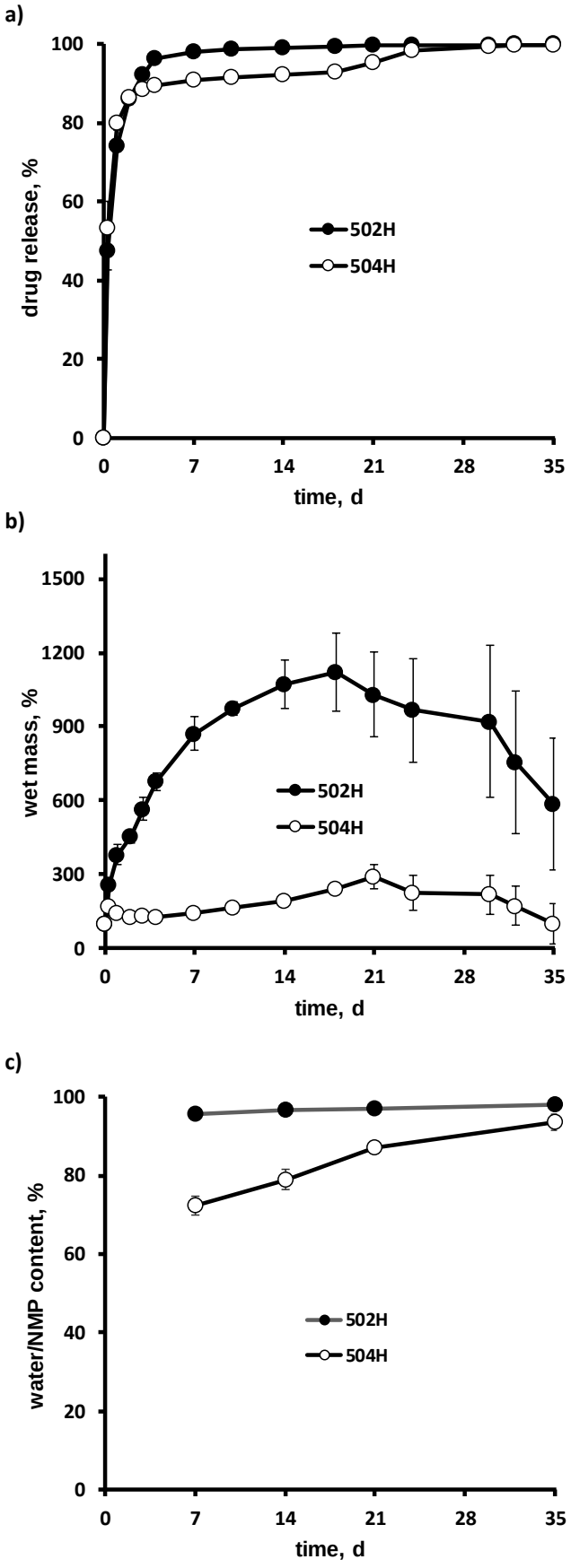

a)
drug release, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
502H
504H
b)
wet mass, %
1500
1200
900
600
300
0
0
7
14
21
28
35
time, d
502H
504H
c)
water/NMP content, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
502H
504H

Figure 5

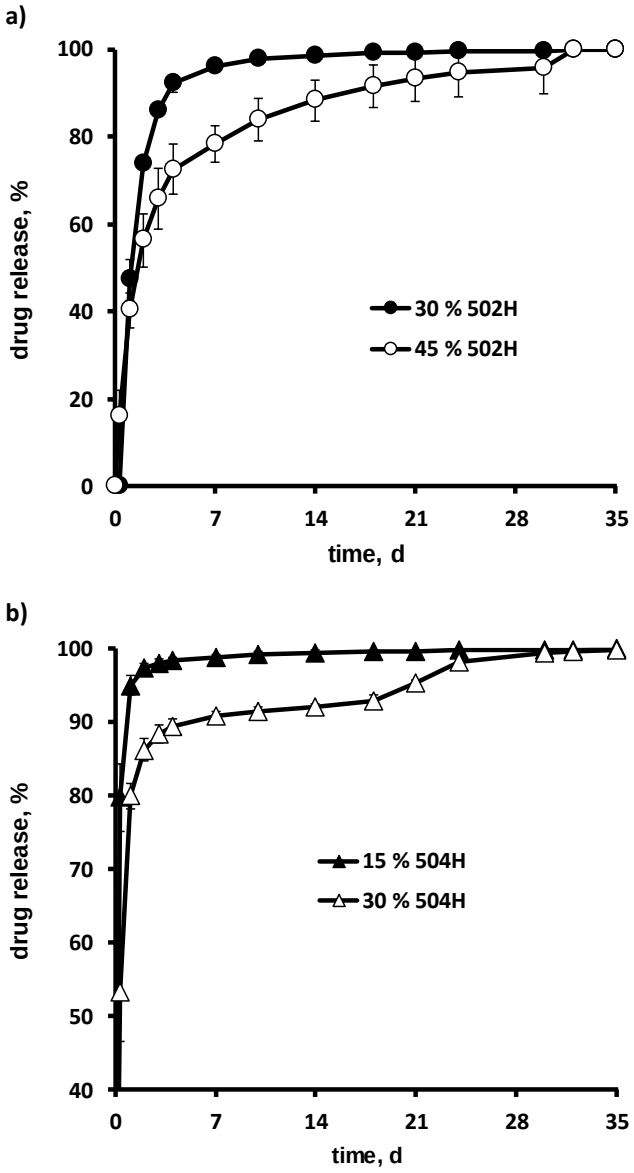
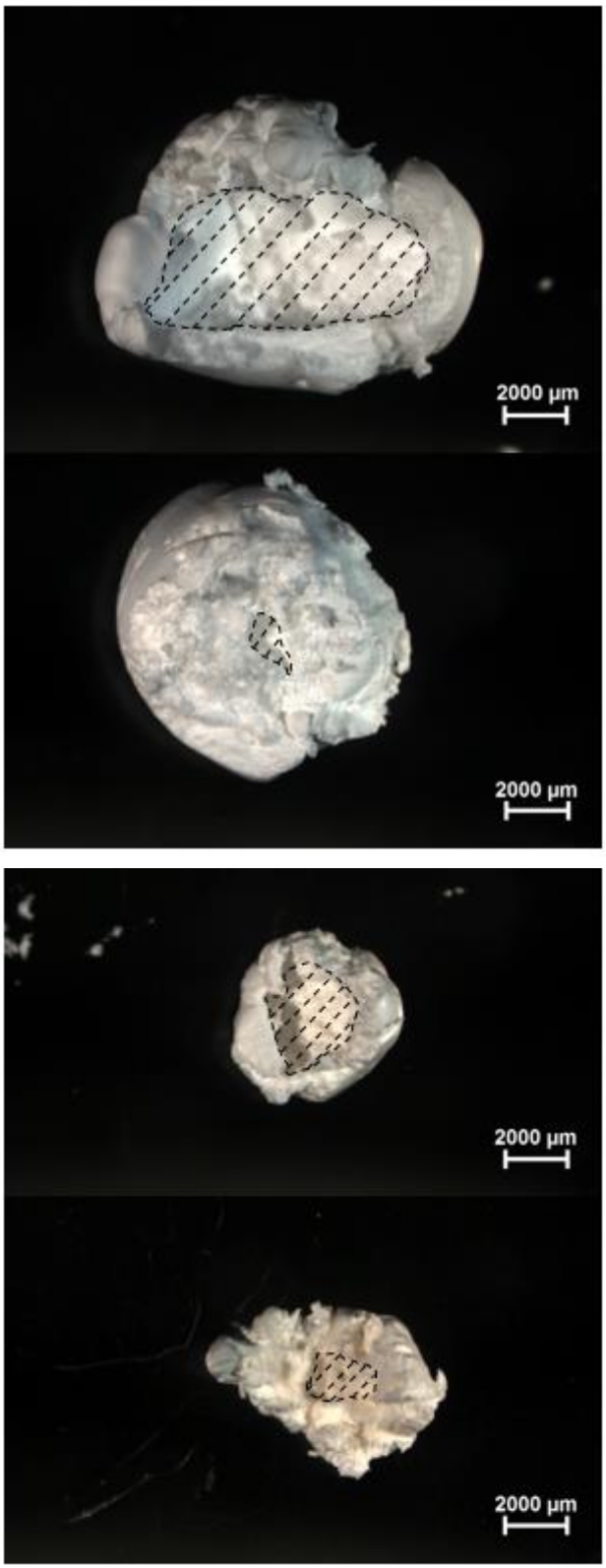

a)
drug release, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
30 % 502H
45 % 502H
b)
drug release, %
100
90
80
70
60
50
40
0
7
14
21
28
35
time, d
15 % 504H
30 % 504H

Figure 6



30 % 502H

45 % 502H

15 % 504H

30 % 504H

Figure 7

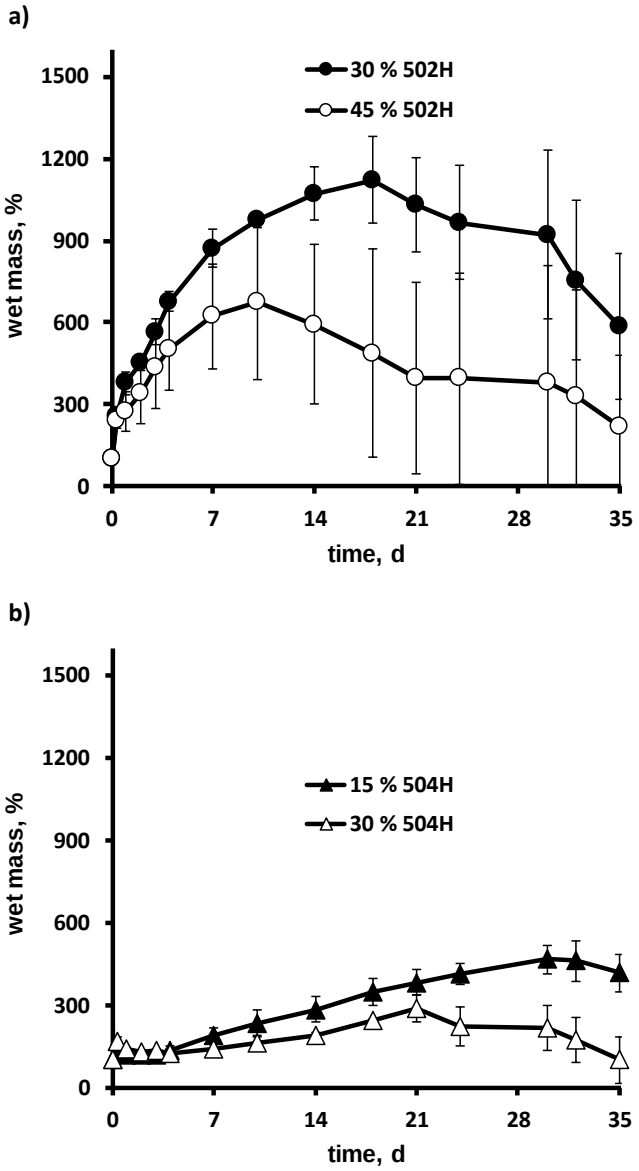

a)
30 % 502H
45 % 502H
wet mass, %
time, d
b)
15 % 504H
30 % 504H
wet mass, %
time, d

Figure 8

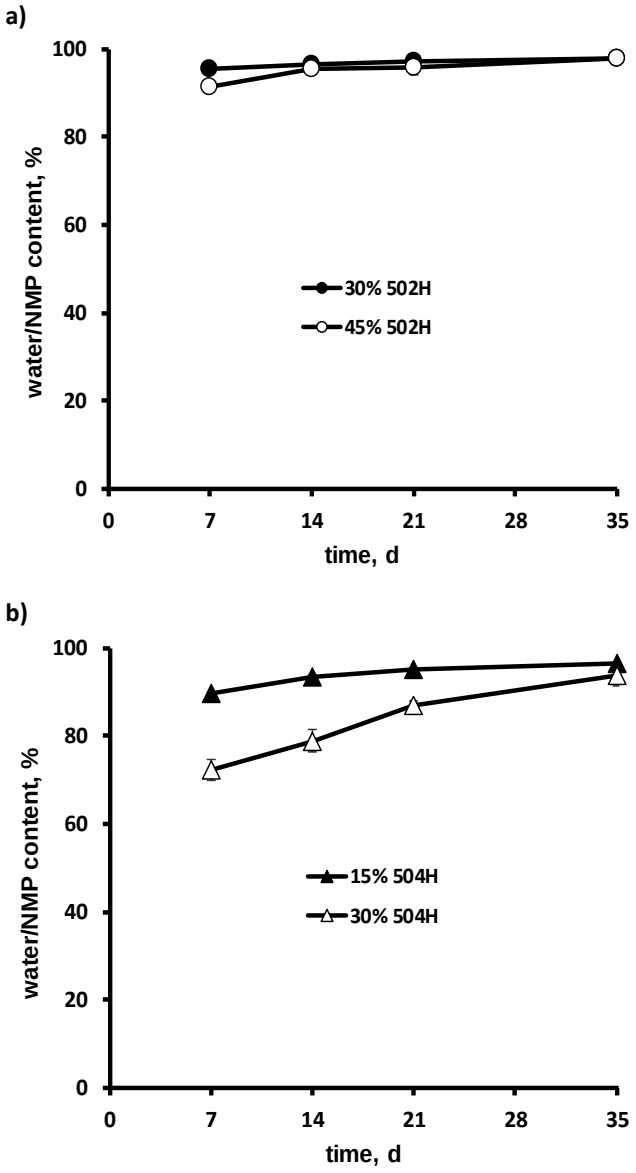

a)
water/NMP content, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
30% 502H
45% 502H
b)
water/NMP content, %
100
80
60
40
20
0
0
7
14
21
28
35
time, d
15% 504H
30% 504H

Figure 9

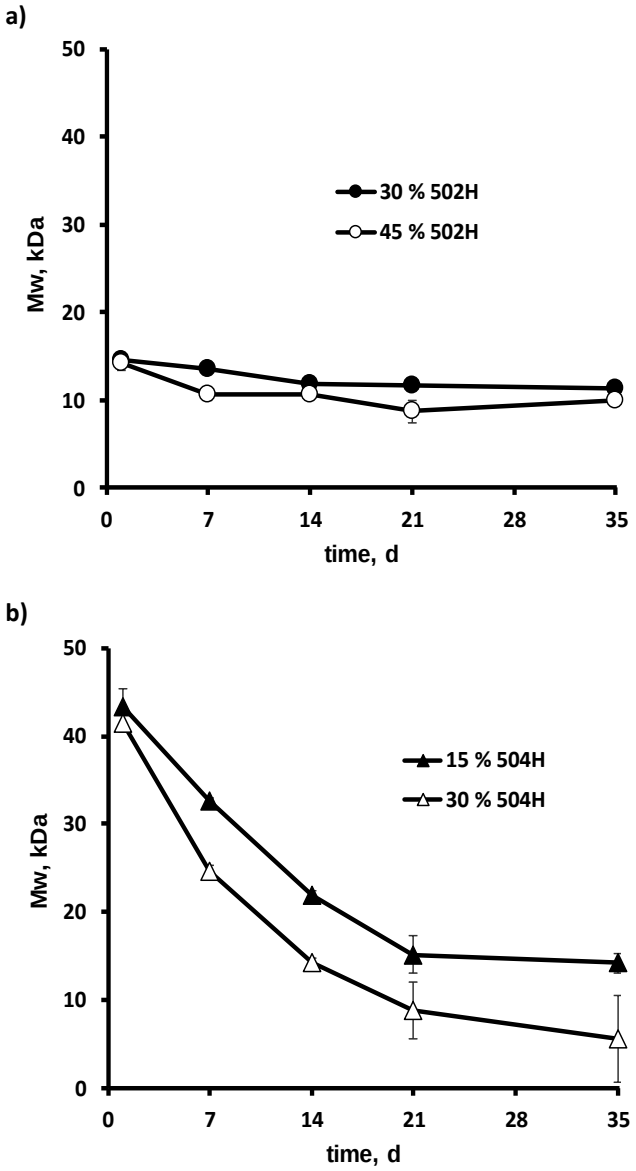

a)
50
40
30
20
10
0
Mw, kDa
30 % 502H
45 % 502H
0
7
14
21
28
35
time, d
b)
50
40
30
20
10
0
Mw, kDa
15 % 504H
30 % 504H
0
7
14
21
28
35
time, d

Figure 10

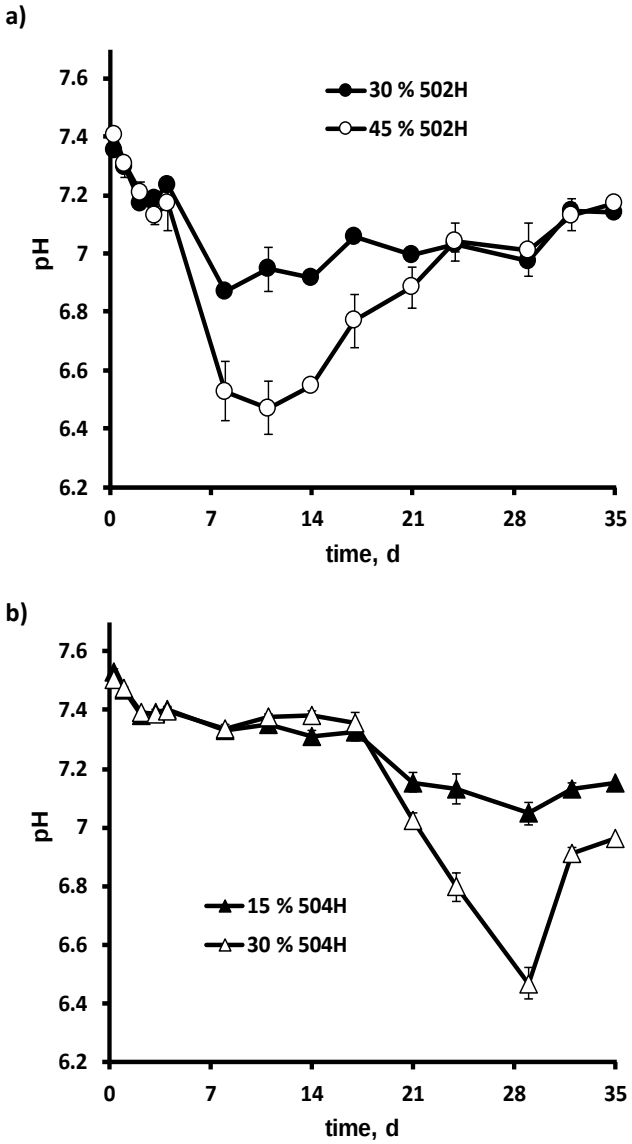

a)
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
pH
30 % 502H
45 % 502H
0
7
14
21
28
35
time, d
b)
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
pH
15 % 504H
30 % 504H
0
7
14
21
28
35
time, d

Figure 11

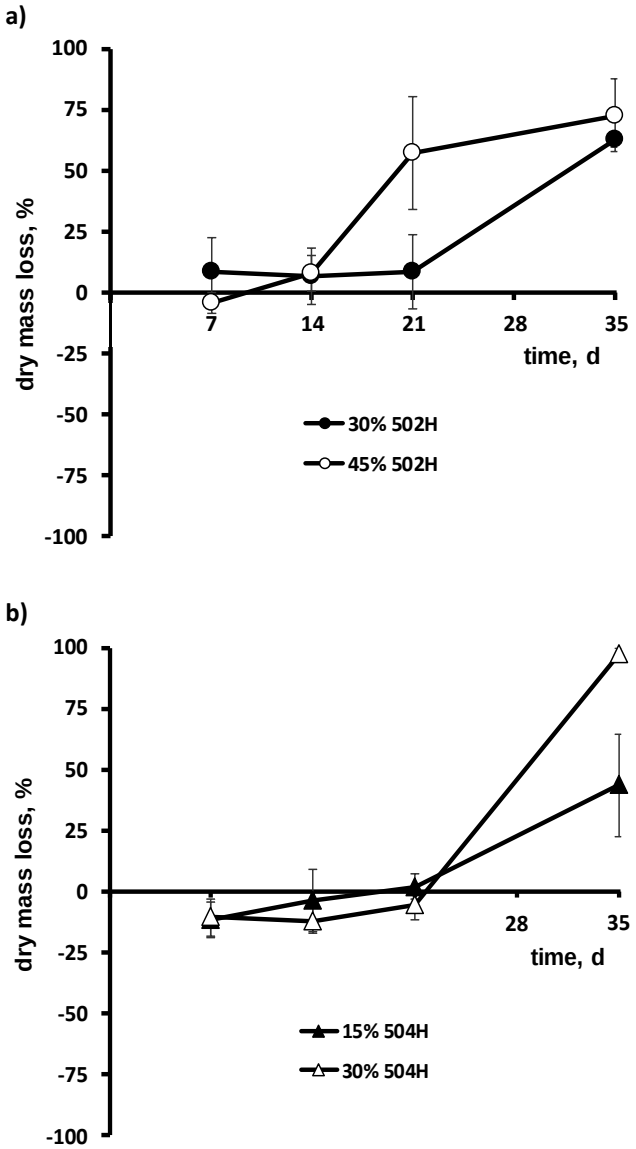

a)
100
75
50
25
0
-25
-50
-75
-100
dry mass loss, %
7
14
21
28
35
time, d
30% 502H
45% 502H
b)
100
75
50
25
0
-25
-50
-75
-100
dry mass loss, %
28
35
time, d
15% 504H
30% 504H

Figure 12