

Research article

***In-situ* forming PLGA implants for intraocular dexamethasone delivery**

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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

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

76 For these reasons intravitreal drug injections are currently considered as the most
77 appropriate way to assure that the drug reaches its site of action. However, every injection bears
78 a risk of infections and other serious side effects, like retinal detachment, retinal haemorrhage,
79 endophthalmitis, increased intraocular pressure, cataract or vitreous haemorrhage (Edelhauser
80 et al., 2010; Giudice and Galan, 2012; Ying et al., 2013; Kaur and Kakkar, 2014; Bisht et al.,
81 2017). Apart from these risks, the discomfort of receiving a needle in the eye leads to limited
82 compliance (Droege et al., 2013; Ghazala et al., 2013).

83 To assure treatment efficacy, therapeutic drug concentrations must be provided over
84 prolonged periods of time at the site of action. Since dexamethasone has a half-life of
85 approximately 5.5 h in the vitreous, frequent injections are, thus, necessary to remain within
86 the therapeutic range (Chan et al., 2011). Local controlled drug delivery systems can help
87 overcoming all these hurdles: The risk of side effects can be reduced, patient compliance
88 improved and the therapeutic efficacy increased. Nowadays, non-biodegradable implants are
89 approved by the FDA for intraocular administration, releasing dexamethasone over prolonged
90 periods of time [e.g., Retisert (retisert.com), Iluvien (iluvien.com)]. However, these implants
91 have to be removed surgically upon drug exhaust, which is associated with similar risks as the
92 initial insertion, or remain in the vitreous where they accumulate over time (Yasin et al., 2014).
93 To avoid the second surgery for device removal, *biodegradable* implants offer an interesting
94 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 $\lambda = 254$ 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:

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

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

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

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 (M_w) 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 μ m MIXED-D column, 7.5 x 300 mm (Agilent Technologies, Interchim, Montluçon, France). The injection volume was 50 μ L. Tetrahydrofuran was the mobile phase (flow rate: 1 mL/min). Polystyrene standards with molecular weights between 1,090 and 70,950 Da (Polymer Laboratories, 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 μ m PVDF syringe filter), diluted

and analyzed for their drug content by HPLC-UV (as described above, using an injection volume of 20 μ L) 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 μ L 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 ($77 \pm 4 \mu\text{g/mL}$ 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 *decreased*. 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

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

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

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

340 3.3. Impact of the PLGA polymer molecular weight

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

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

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

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Figure legends

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

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

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

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

Fig. 5. Importance of the polymer molecular weight of the PLGA (Resomer 502H vs. 504H) for: a) drug release, b) the dynamic changes in the wet mass, and c) the dynamic changes in the water/NMP content from/of implants formed *in-situ* upon exposure to 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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).

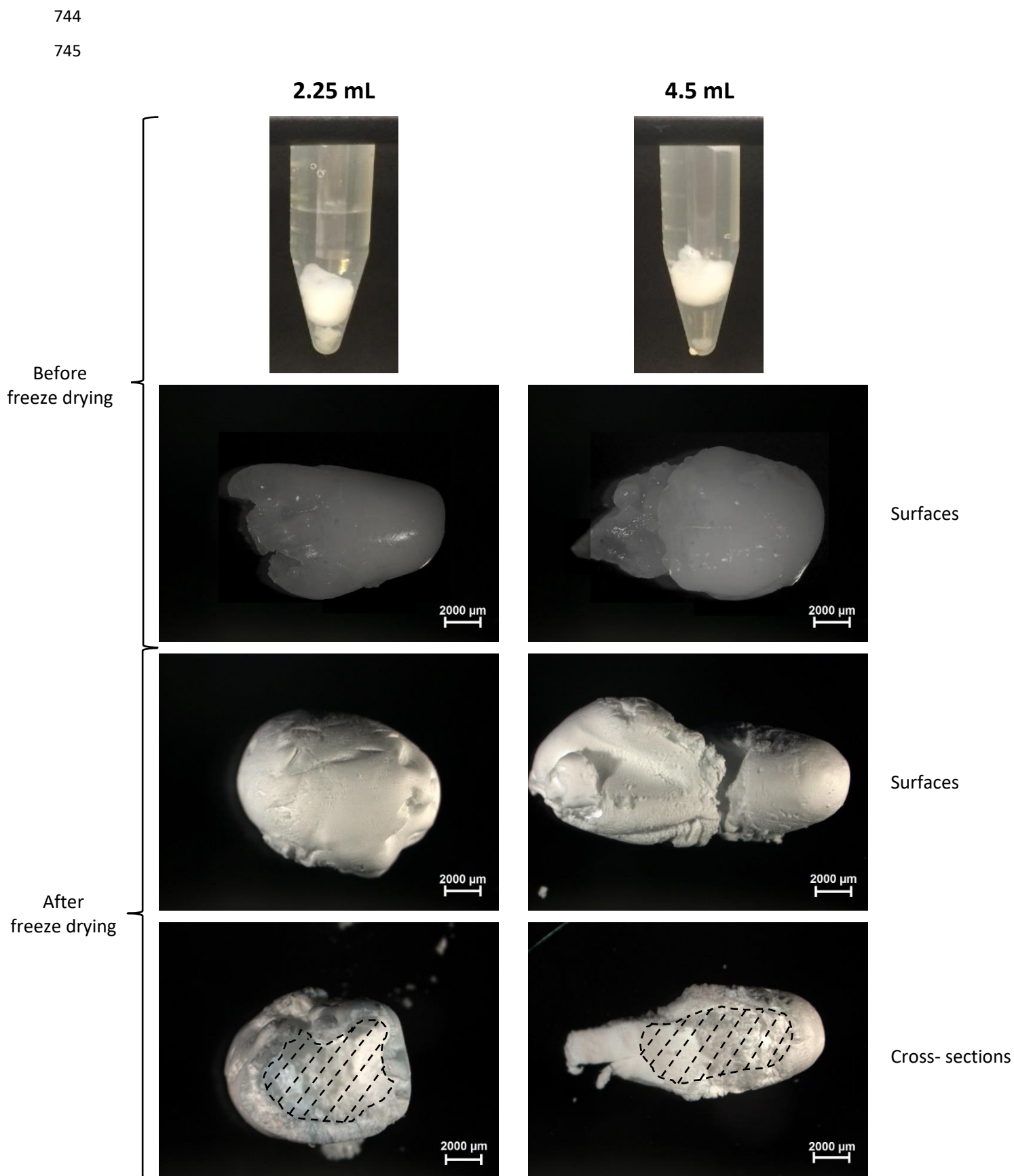


Figure 1

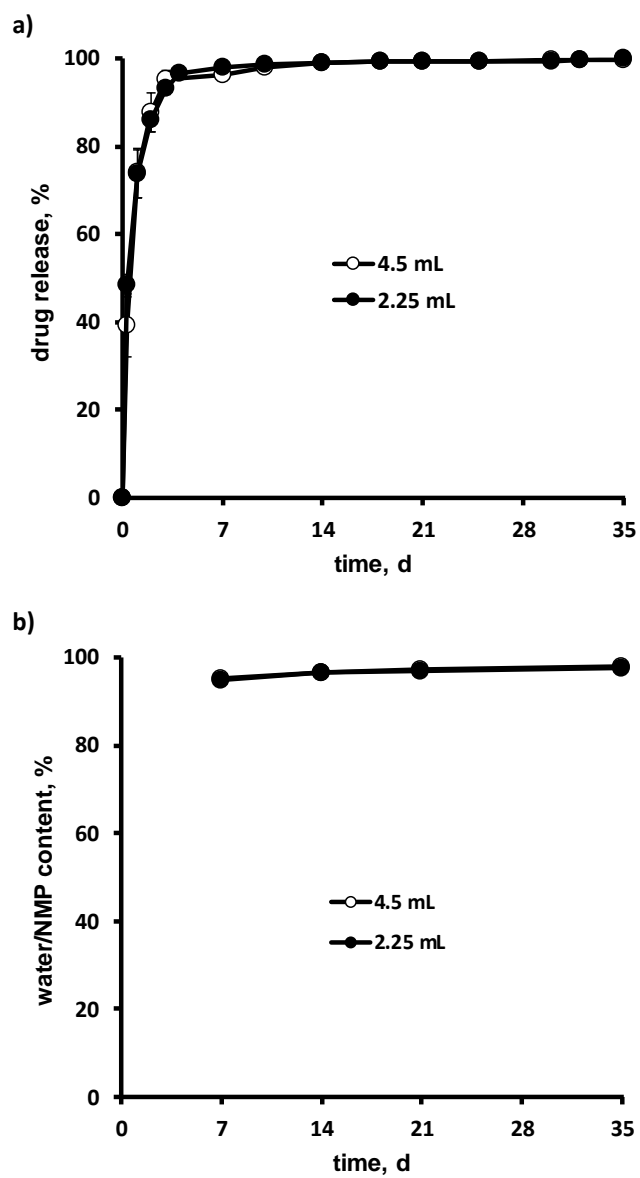


Figure 2

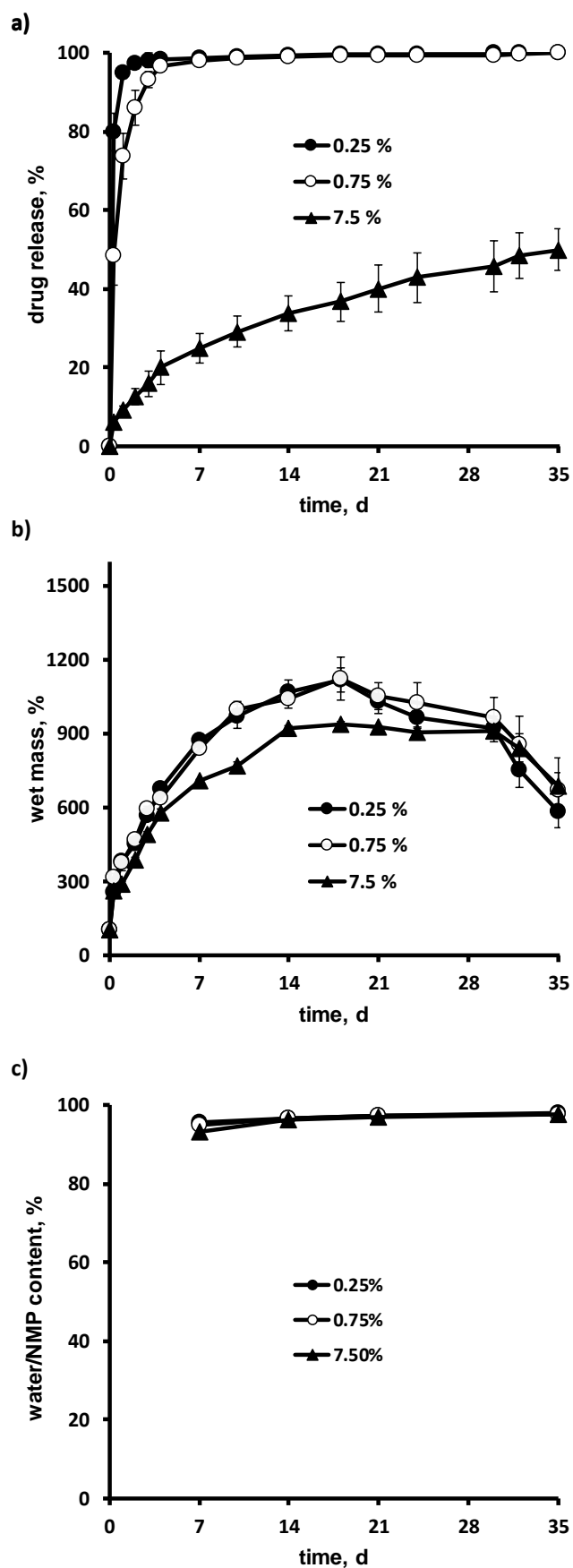


Figure 3

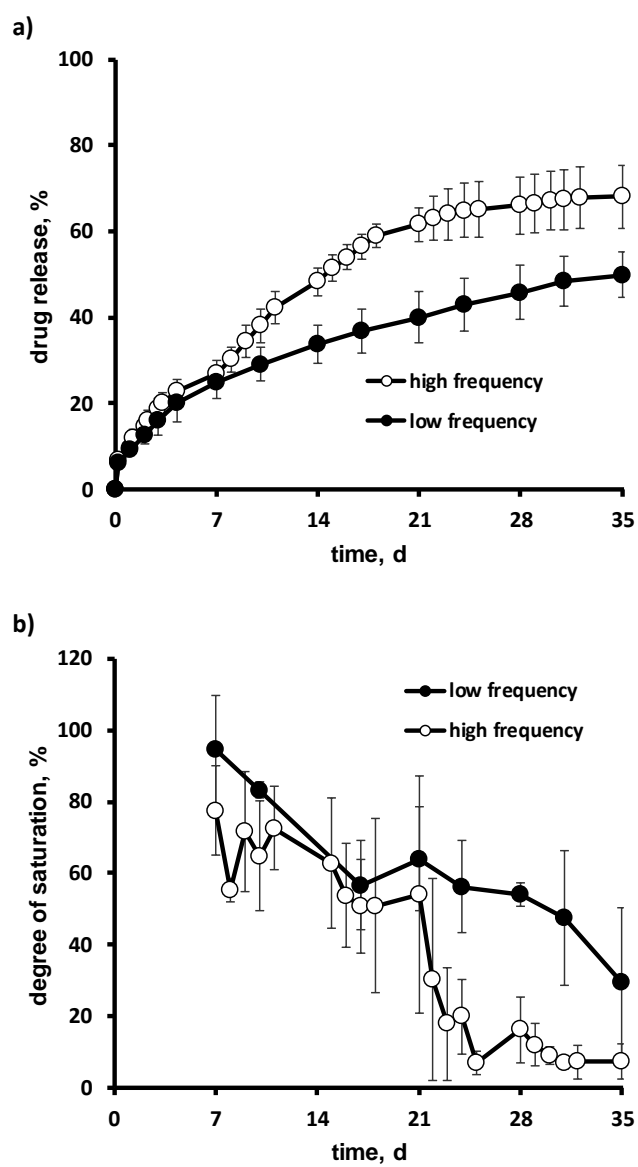


Figure 4

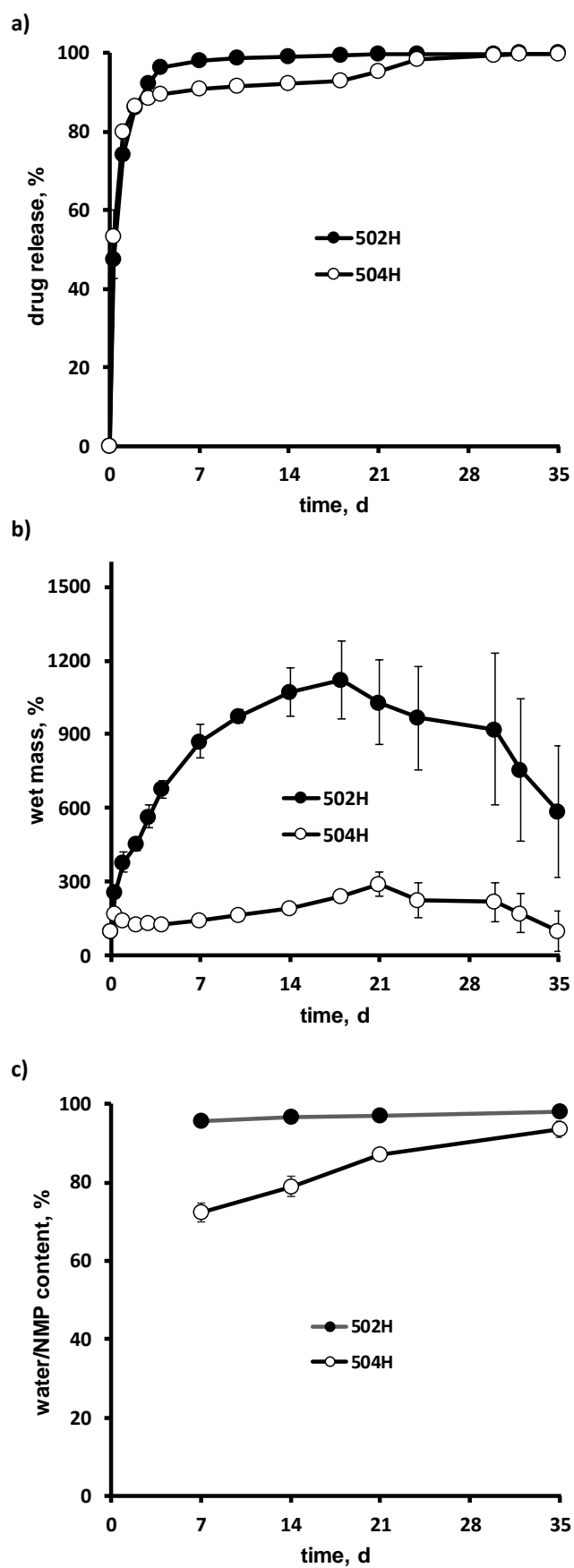


Figure 5

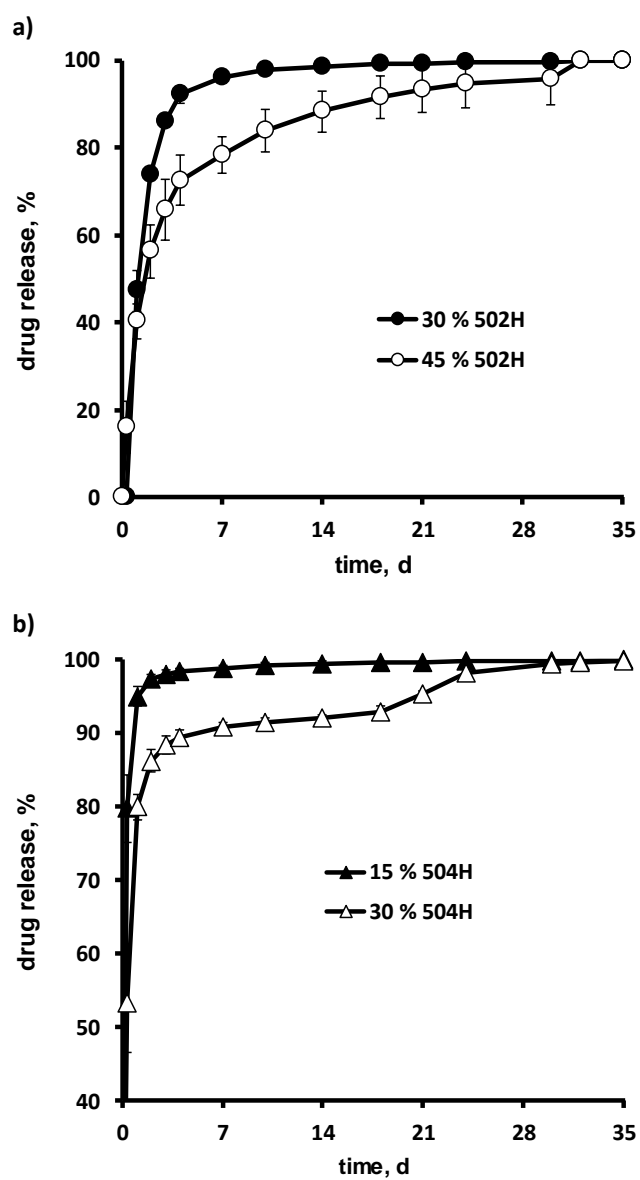
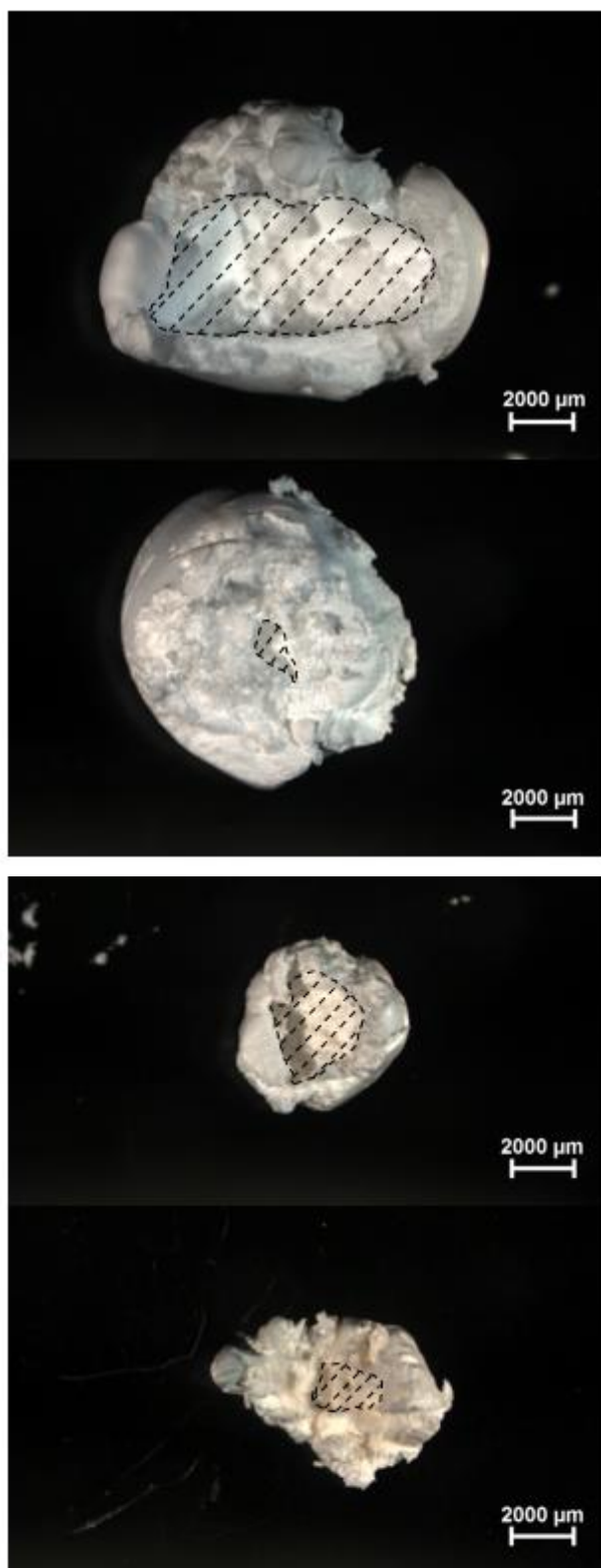


Figure 6



30 % 502H

45 % 502H

15 % 504H

30 % 504H

Figure 7

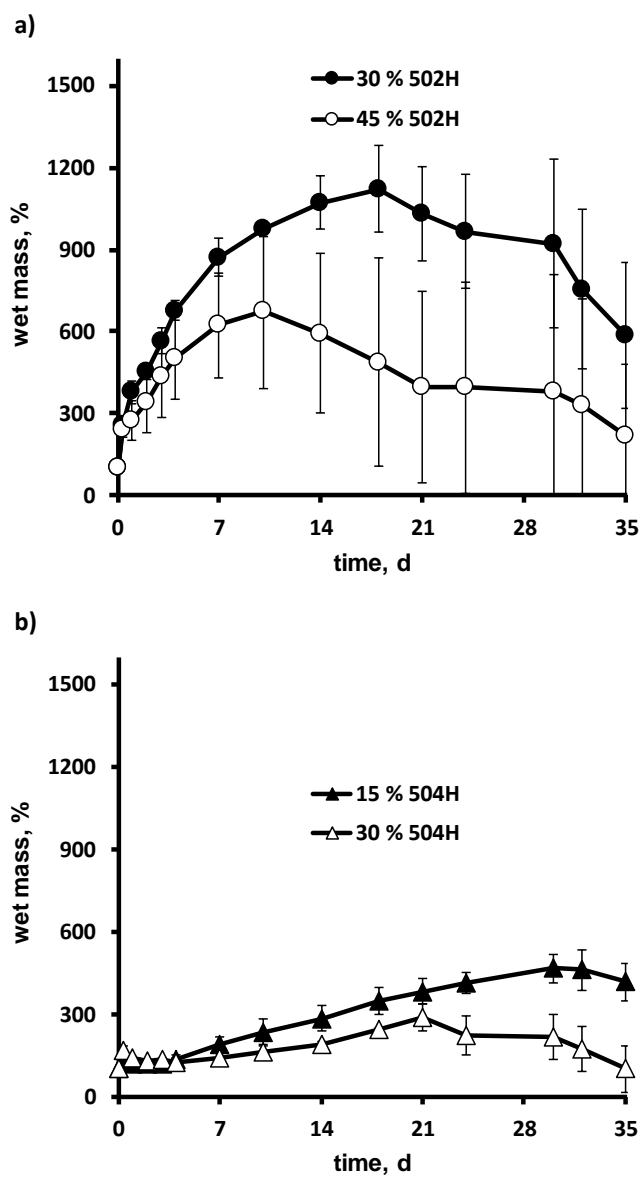


Figure 8

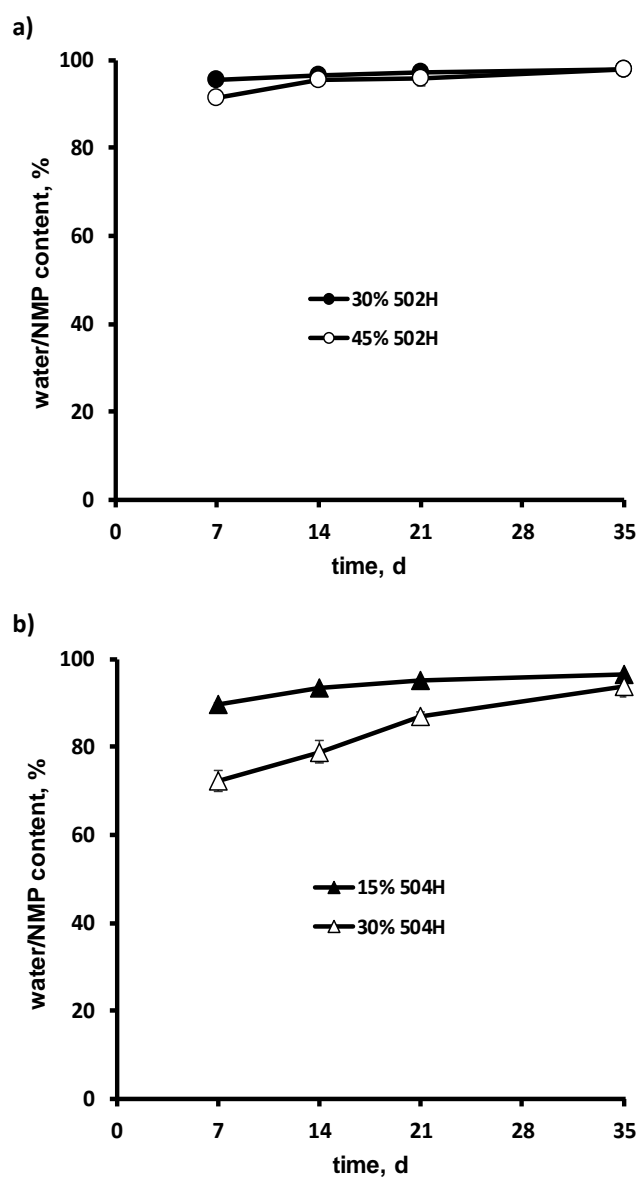


Figure 9

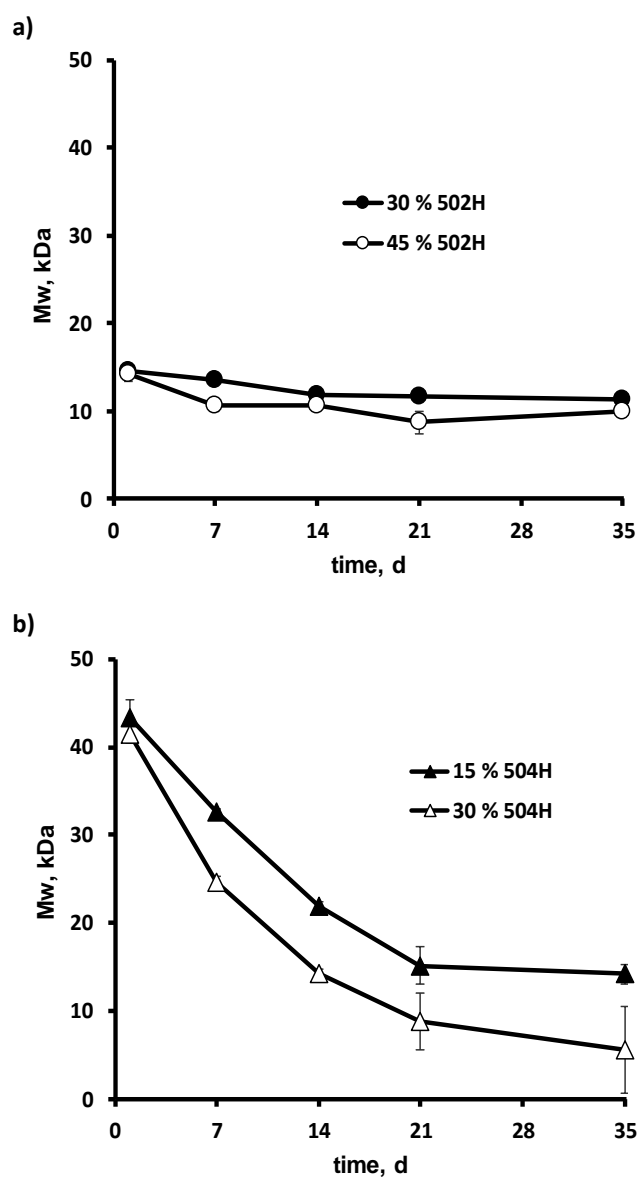


Figure 10

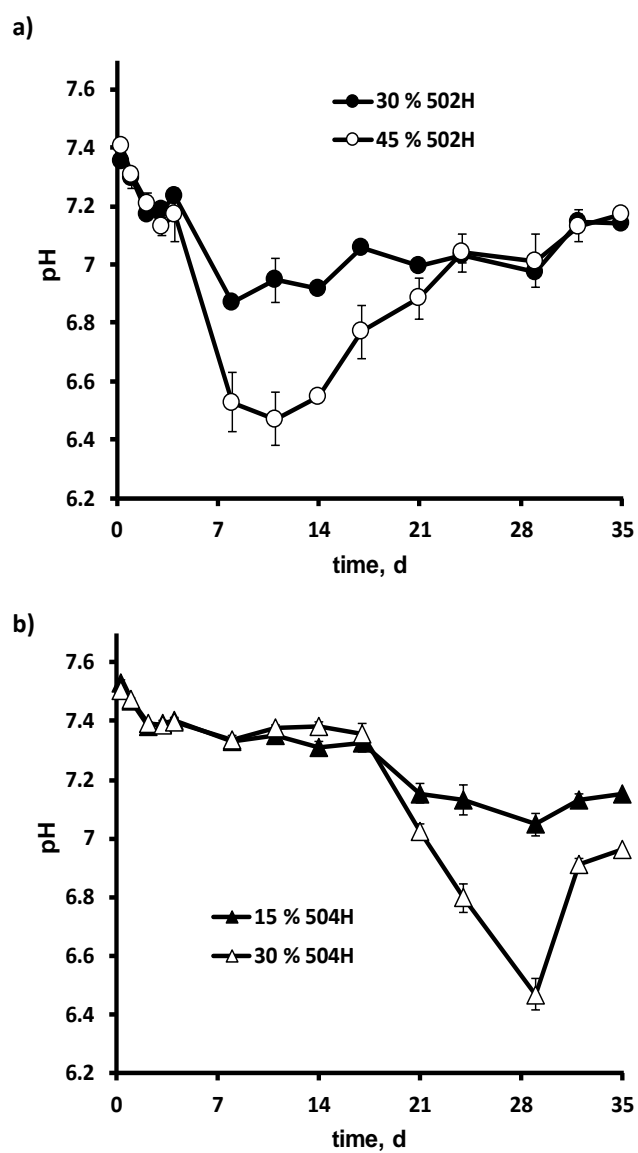


Figure 11

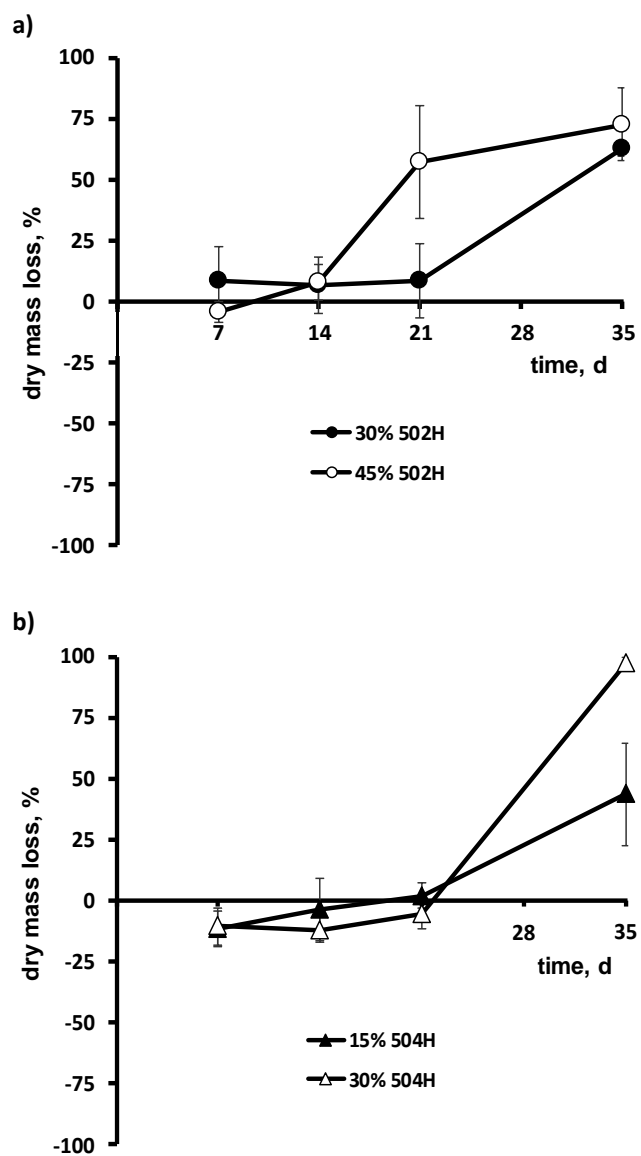


Figure 12