



Towards a better understanding of the different release phases from PLGA microparticles: Dexamethasone-loaded systems



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ABSTRACT

Dexamethasone-loaded, poly(lactic-co-glycolic acid) (PLGA) microparticles were prepared using an oil-in-water solvent extraction/evaporation method. The drug loading was varied from 2.4 to 61.9%, keeping the mean particle size in the range of 52–61 μm . In vitro drug release was characterized by up to 3 phases: (1) an (optional) initial burst release, (2) a phase with an about constant drug release rate, and (3) a final, again rapid, drug release phase. The importance and durations of these phases strongly depended on the initial drug loading. To better understand the underlying mass transport mechanisms, the microparticles were thoroughly characterized before and after exposure to the release medium. The initial burst release seems to be mainly due to the dissolution of drug particles with direct access to the microparticles' surface. The extent of the burst was negligible at low drug loadings, whereas it exceeded 60% at high drug loadings. The second release phase seems to be controlled by limited drug solubility effects and drug diffusion through the polymeric systems. The third drug release phase is likely to be a consequence of substantial microparticle swelling, leading to a considerable increase in the systems' water content and, thus, fundamentally increased drug mobility.

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1. Introduction

Poly(lactic-co-glycolic acid) (PLGA)-based microparticles offer an interesting potential as advanced drug delivery systems, since they: (i) are biodegradable (avoiding the removal of empty remnants), (ii) are generally biocompatible (Fournier et al., 2003), (iii) allow for a precise control of the resulting drug release kinetics during time periods ranging from a few hours up to several weeks or months, (iv) can rather easily be injected using standard syringes, and (v) can be used for a variety of drugs and medical treatments. Since many years a number of drug products based on PLGA microparticles are commercially available.

However, despite the considerable practical success of PLGA microparticles as advanced drug delivery systems, the underlying mass transport mechanisms controlling drug release are often not fully understood (Faisant et al., 2002; Siepmann et al., 2002; Siepmann and Siepmann, 2008). This can at least partially be

explained by the potential complexity of the involved physical and chemical phenomena (Siepmann and Goepferich, 2001; Fredenberg et al., 2011). For instance, the following processes might play a role in the control of drug release from PLGA-based microparticles: Water penetration into the systems upon contact with aqueous body fluids, drug dissolution (Siepmann and Siepmann, 2013), drug diffusion through the intact PLGA matrix and/or drug diffusion through water-filled pores, drug partitioning between an aqueous phase located in pores and a continuous PLGA matrix, limited drug solubility effects, pore closing effects (Kang and Schwendeman, 2007; Huang et al., 2015), plasticization of PLGA by water (Blasi et al., 2005) or drugs (Albertini et al., 2015; Gasmi et al., 2015a,b), polymer chain cleavage and the diffusion of water-soluble degradation products through the eroding microparticles into the surrounding environment, osmotically driven water-influx into the system, diffusion of bases from the surrounding bulk fluid into the microparticles, as well as microparticle swelling (note that this list is not exhaustive). In addition, autocatalytic effects might play a major role (Fu et al., 2000; von Burkensroda et al., 2002; Dunne et al., 2000; Siepmann et al., 2005; Li and Schwendeman, 2005; Gu et al., 2016) as explained in the following: The rate at which water penetrates into the system is much higher than the

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rate of ester bond cleavage. Consequently, the polymer chains are cleaved throughout the system and short chain acids are generated everywhere in the microparticles. Often, the rate at which these acids are generated is higher than the rate at which they are neutralized (since the diffusion of acids and bases in the systems is often slower compared to the generation of the acids). Thus, the micro-pH within the PLGA particles might locally substantially drop (Brunner et al., 1999; Li and Schwendeman, 2005). This is particularly true for the center of the microparticles (since the length of the diffusion pathways is the longest). Since protons catalyze the ester bond cleavage in PLGA, this results in autocatalytic effects (this means that one of the products of the chemical reaction catalyzes the reaction itself).

Interestingly, often mono-, bi-, or tri-phasic drug release is reported from PLGA microparticles (Siepmann et al., 2005; Klose et al., 2006; Fredenberg et al., 2011). Generally, an initial burst release is followed by a second phase with an about constant release rate, and a final (again rapid) drug release phase. This can serve as an indication that the conditions for drug release are likely to substantially change with time. The fact that drug release from PLGA microparticles is not always tri-phasic, might be explained as follows: In certain systems the drug is already completely released once the conditions for the second or third drug release phase are provided (Siepmann et al., 2005; Klose et al., 2006). Note that the relative importance of the different, potentially involved mass transport phenomena might substantially vary between different types of PLGA-based microparticles. For instance, the type of drug might substantially affect its release: Certain drugs act as plasticizers for PLGA (Albertini et al., 2015; Gasmi et al., 2015a, b). Also, the drug can be more or less soluble in the polymer matrix, and the physical state of the drug in the system might affect the release mechanism (Siepmann and Siepmann, 2008, 2012, 2013). Furthermore, if the drug is an acid or a base, its presence might affect PLGA degradation (ester bond cleavage being catalyzed by protons and hydroxide ions). In addition, the drug loading and preparation method of the microparticles might impact the systems' inner and outer morphology and, thus, the relative importance of the involved mass transport phenomena (Wang et al., 2015; Klose et al., 2006). Furthermore, the PLGA microparticle size and porosity might impact the importance of potentially involved autocatalytic effects, determining the pathway lengths to be overcome and altering the mobility of acids leaving and of bases entering the systems (Siepmann et al., 2005; Klose et al., 2006). Moreover, the surrounding environment might substantially impact the observed drug release kinetics, for example via limited drug solubility effects and/or alterations in the pH values (potentially accelerating PLGA degradation) (Klose et al., 2009, 2011; Zolnik and Burgess, 2008).

In order to better understand which physical and chemical processes are of importance for the control of drug release from a particular type of PLGA particles, the latter should ideally be thoroughly characterized before and after exposure to the release medium. This includes for instance thermal analyses (e.g., DSC), X-ray diffraction measurements, SEM, optical microscopy, GPC and the monitoring of single microparticle swelling (Gasmi et al., 2015a, b). It has recently been shown with an acidic and a basic drug (ketoprofen and prilocaine free base, both acting as efficient plasticizers for PLGA) that the third drug release phase seems to be initiated by substantial microparticle swelling (Gasmi et al., 2015a, b). However, as yet it is unknown whether this phenomenon also plays a role for neutral drugs, and/or drugs which do not plasticize PLGA.

The aim of this study was to prepare different types of dexamethasone-loaded PLGA microparticles using an oil-in-water solvent extraction/evaporation method. The initial drug loading was varied from 2.4 to 61.9%, while the mean microparticle size

was intentionally kept in the range of 52–61 μm , in order to avoid particle size effects (Siepmann et al., 2004). One of the reasons for selecting dexamethasone-loaded PLGA microparticles was their interesting therapeutic potential, for instance for the reduction of foreign body reactions to glucose sensors (prolonging the latter's lifetime) (Gu et al., 2015; Gu and Burgess, 2015). The different types of systems were thoroughly characterized before and after exposure to phosphate buffer pH 7.4. The idea was to better understand the underlying mass transport mechanisms controlling the different release phases from PLGA-based microparticles based on the obtained results.

2. Materials and methods

2.1. Materials

Poly(D,L lactic-co-glycolic acid) (PLGA; Resomer RG 504H; 50:50 lactic acid:glycolic acid; Evonik; Darmstadt; Germany), dexamethasone (99.0% purity; Discovery Fine Chemicals, Dorset, United Kingdom), dichloromethane (VWR, Fontenoy-sous-Bois, France); dimethylsulfoxide (DMSO) and tetrahydrofuran (HPLC Grade; Fisher Scientific, Illkirch, France); poly(vinyl alcohol) (Mowiol 4-88; Sigma-Aldrich, Steinheim, Germany).

2.2. Microparticle preparation

Dexamethasone-loaded, PLGA-based microparticles were prepared using an oil-in-water (O/W) emulsion solvent extraction/evaporation technique. Depending on the theoretical drug loading (which was varied between 3.9% to 63.2% w/w), 41.3–722.1 mg drug and 420.1–1016.1 mg PLGA were dissolved in a mixture of dimethylsulfoxide (DMSO) and dichloromethane (CH_2Cl_2) (Table 1). The composition and volume of the organic phase was adjusted to provide similar viscosities and, most importantly, a similar mean microparticle diameter (in the range of about 50–60 μm). This organic phase was emulsified within 400 mL of an outer aqueous poly(vinyl alcohol) solution (0.25% w/w; previously cooled to +4 °C) using a three-blade propeller (Eurostar power-b; Ika-Werke, Staufen, Germany; 2000 rpm), inducing microparticle formation. Stirring was continued for 30 min. The particles were hardened by adding 1 L of the same outer aqueous poly(vinyl alcohol) solution (cooled to +4 °C) and further stirring at 700 rpm during 4 h. The particles were then separated by filtration (Nylon, 0.45 μm , 13 mm; GE Healthcare Life Sciences Whatman, Kent, UK, vacuum pump) and subsequently freeze-dried (Christ Epsilon 2-4 LSC; Martin Christ, Osterode, Germany; freezing at –45 °C for 2 h, primary drying at –9 °C/0.014 mbar for 10 h, secondary drying at +20 °C/0.0014 mbar for 10 h).

2.3. Microparticle characterization

Particle sizes were determined by optical microscopy (diameters of surface equivalent circles). Pictures were taken using an Axiovision Zeiss Scope-A1 microscope, AxioCam ICc1 camera and Axiovision Zeiss Software (Carl Zeiss, Jena, Germany). Each measurement included 200 microparticles.

Table 1

Composition of the inner organic phase used for microparticle preparation.

Theoretical drug loading (%)	3.9	9.8	14.9	22.4	33.7	63.2
DMSO (mL)	2.0	2.0	2.0	2.0	2.0	2.5
CH_2Cl_2 (mL)	5.0	5.0	4.6	4.3	4.3	3.8
PLGA (mg)	1016.1	1014.6	910.2	835.2	623.3	420.1
Drug (mg)	41.3	110.6	159.6	240.6	317.2	722.1

The practical drug loading of the microparticles was determined by dissolving accurately weighed amounts of samples in DMSO, followed by filtration (PVDF syringe filters, 0.45 μm ; GE Healthcare) and drug content analysis by HPLC (Thermo Fisher Scientific Ultimate 3000 Series, equipped with a pump: LPG 3400 SD/RS, an autosampler: WPS-3000 SL, a column compartment: TCC 3000 D/RS and a UV-vis detector: VWD-3400RS; Thermo Fisher Scientific, Waltham, USA). A reversed phase column C18 (Gemini 5 μm , 110 Å; 150 mm \times 4.6 mm, Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of acetonitrile:water (33:67 v:v), the flow rate was 1.5 mL/min. The detection wavelength was 254 nm. The lower quantification limit was 6×10^{-7} mg/L, the linear range was 0.0001–50 mg/L. Fifty microliter samples were injected. Each experiment was conducted in triplicate.

In vitro drug release was measured as follows: Depending on the drug loading, 5–10 mg dexamethasone-loaded microparticles were placed in amber glass flasks filled with 15–100 mL phosphate buffer pH 7.4 (USP 35) in order to provide sink conditions (except for high drug loadings at very late time points, at which 40–50% drug saturation was observed). Note that the differences in microparticle mass:bulk fluid volume did not affect the pH of the surrounding bulk fluid to a noteworthy extent (otherwise, drug release might have been influenced by this ratio, e.g. [Klose et al., 2010](#)): The pH of the release medium stayed in the range of 7.3–7.4 throughout all experiments. The flasks were horizontally shaken at 80 rpm at 37 °C (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, samples were withdrawn (one fifth of the release medium was replaced with fresh phosphate buffer pH 7.4), filtered (PVDF syringe filters 0.45 μm , GE Healthcare) and analyzed for their drug content by HPLC (as described above, injecting 100 μL samples). Each experiment was conducted in triplicate.

The decrease in polymer molecular weight (Mw) of the PLGA during drug release was measured by gel permeation chromatography (GPC, Separation Modules e2695 and e2695D, 2419 RI Detector; Waters, Guyancourt, France) column: PLgel 5 μm MIXED-D, 7.5 \times 300 mm (Polymer Laboratories, Varian, Les Ulis, France). Tetrahydrofuran was used as mobile phase at a flow rate of 1 mL/min. Microparticles were treated as described for the in vitro drug release measurements. At predetermined time points, the contents of the glass flasks were filtered and the microparticles freeze-dried. Three milligram samples were dissolved in 1 mL tetrahydrofuran. Fifty microliter samples were injected. Molecular weights were calculated using the Empower GPC software and polystyrene standards (Polymer Laboratories).

The glass transition temperature (T_g) of the polymer was measured using differential scanning calorimetry (DSC 1 Star System; Mettler Toledo, Greifensee, Switzerland). Approximately 3 mg samples were placed in sealed aluminum pans, which were heated to 110 °C, cooled to –10 °C and reheated to 110 °C at a rate of 10 °C/min. The glass transition temperature was determined during second heating cycle. For reasons of comparison, PLGA powder (as received) was also studied.

X-ray powder diffraction: A X-ray wide angle diffractometer Inel CSP 120 (λ Cu, $K\alpha_1 = 1.54$ Å; Inel, Artenay, France) was used to characterize the microparticles, as well as pure dexamethasone and PLGA powders (as received) for reasons of comparison. Powder samples were placed in Lindemann glass capillaries (diameter 0.7 mm).

Scanning electron microscopy (SEM): The external and internal morphology of the microparticles was studied using a Hitachi S-4000 scanning electron microscope (Hitachi High-Technologies Europe, Krefeld, Germany). Samples were fixed with a ribbon carbon double-sided adhesive and covered with a fine carbon layer. Cross-sections were obtained after inclusion of the microparticles

into water-based glue (UHU twist & glue, Buehl, Germany) and cutting with a Leica UM EC7 ultra-microtome using a 45° diamond cutter. Microparticles were observed before and after exposure to the release medium. In the latter case, the samples were treated as for the in vitro release measurements (described above). At predetermined time points, samples were withdrawn, filtered (Nylon, 0.45 μm , 13 mm; GE healthcare) and freeze-dried.

The swelling of individual microparticles was monitored in 96-well standard microplates (Carl Roth, Karlsruhe, Germany) as follows: Approximately 70 microparticles were introduced into each well, which was filled with 130 μL phosphate buffer pH 7.4 (USP 35). The well plates were kept at 37 °C in a horizontal shaker (80 rpm, GFL 3033). To minimize water evaporation, the well plates were closed and surrounded with Parafilm (Pechiney Plastic Packaging, Chicago, USA). However, partial evaporation of the medium could not completely be avoided and once a week fresh phosphate buffer pH 7.4 was added to assure about 130 μL liquid in each well during the entire observation period. At pre-determined time points, pictures were taken using a Nikon Eclipse E600 microscope, equipped with a Nikon Digital camera DXM1200C and NIS-Element Basic Research Imaging software (Nikon, Tokyo, Japan).

3. Results and discussion

3.1. Key physicochemical properties of the microparticles

[Fig. 1](#) shows the experimentally measured dexamethasone release kinetics from the investigated PLGA-based microparticles. The practical drug loading was varied from 2.4 to 61.9%, as indicated. The encapsulation efficiency increased with increasing theoretical drug loading, probably because of drug saturation effects of the outer aqueous phase during microparticle preparation. Importantly, the mean particle size was similar in all cases (in the range of “52–61 μm ”, [Table 2](#)). The release medium was phosphate buffer pH 7.4, and in no case the pH of the bulk fluid decreased below 7.3 during the observation periods (this is to be highlighted, because the release of acidic degradation products might eventually lead to a noteworthy drop in the pH of the release medium, resulting in accelerated PLGA degradation). As it can be seen, the drug release patterns were bi- or tri-phasic, depending on

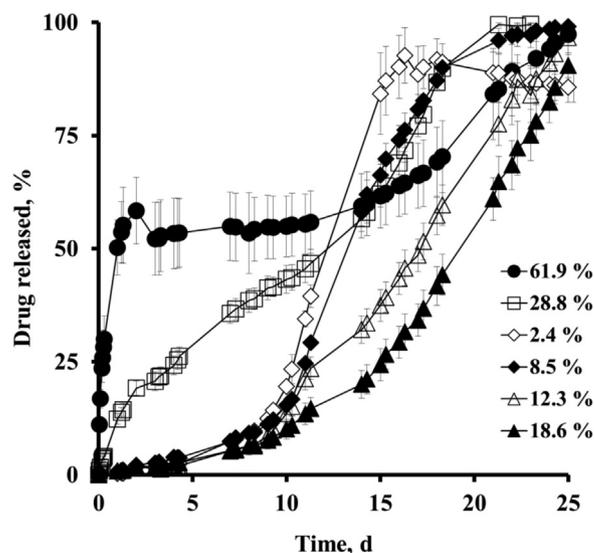


Fig. 1. Effects of the practical drug loading (indicated in the diagram) on dexamethasone release from PLGA-based microparticles in phosphate buffer pH 7.4.

Table 2Impact of theoretical drug loading on the practical drug loading, encapsulation efficiency and mean size of the investigated microparticles (mean values \pm SD).

Theoretical drug loading (%)	3.9	9.8	14.9	22.3	33.7	63.2
Practical drug loading (%)	2.4 \pm 0.1	8.5 \pm 0.1	12.3 \pm 0.2	18.6 \pm 0.1	28.8 \pm 0.7	61.9 \pm 0.4
Encapsulation efficiency (%)	61.0 \pm 0.0	87.0 \pm 0.9	82.6 \pm 1.1	83.6 \pm 0.2	85.5 \pm 2.1	98.1 \pm 0.5
Size (μ m)	61 \pm 20	58 \pm 27	53 \pm 21	60 \pm 28	52 \pm 27	61 \pm 23

the practical dexamethasone loading: At loadings equal to or higher than 28.8%, an initial burst release was followed by a phase with an about constant dexamethasone release rate and a final, again rapid, drug release phase. In contrast, the burst release was negligible at drug loadings up to 18.6%. Interestingly, the practical dexamethasone loading also affected the duration of respective release phase as well as their onset time points. For example, the onset of the final release phase was delayed at high initial drug loadings. To better understand why these different release phases were observed and how the initial drug loading mechanistically affects them, the microparticles were thoroughly characterized physico-chemically before and after exposure to the release medium.

The DSC thermograms of dry microparticles (before exposure to the release medium) are illustrated in Fig. 2a. The practical drug loading is indicated on the left hand side. For reasons of comparison, also PLGA powder (as received) was studied. Clearly, a glass transition (T_g) was observed in all cases at about 48 °C. Importantly, this T_g was virtually unaffected by the initial drug loading (Fig. 2b). This might serve as an indication for the fact that dexamethasone is not acting as a plasticizer for the PLGA, in contrast to other drugs (Gasmi et al., 2015a,b). Fig. 3 shows the X-ray diffraction patterns of these microparticles: for the different initial drug loadings, before exposure to the release medium. For reasons of comparison, also PLGA and drug powder (as received) were studied. Clearly, the dexamethasone raw material was highly crystalline, whereas the polymer was X-ray amorphous. At drug loadings equal to and above 28.8%, crystalline drug was visible in the microparticles. At dexamethasone loadings equal to and below 18.6%, the microparticles were X-ray amorphous. This might be due to the presence of amorphous drug particles, extremely small drug crystals (too small to be detectable by X-ray diffraction) and/or drug being dissolved in the polymeric matrix (in the form of individual molecules; but not acting as a plasticizer, Fig. 2). It was beyond the scope of this study to distinguish between these different possible states.

Fig. 4 shows SEM pictures of surfaces and cross-sections of PLGA microparticles loaded with 61.9% dexamethasone. The upper row shows pictures obtained before exposure to the release medium, the bottom row shows pictures obtained after 5 d exposure to phosphate buffer pH 7.4 at 37 °C (and subsequent filtering and freeze-drying, so artifact creation cannot be excluded). As it can be seen, the microparticles initially exhibited a smooth surface and numerous drug particles were homogeneously distributed throughout the system. After 5 d exposure to the release medium, the surface became much more rough and porous, while numerous drug particles were still clearly visible inside the system. Note that some of the particles seemed to be partially collapsed. These deformations might have occurred during sample preparation, indicating that the microparticles had become partially highly porous (and fragile). Thus, certain microparticle regions seem to have become almost devoid of drug, whereas other regions still contained high amounts of drug.

The decrease in the polymer molecular weight of the PLGA as a function of the exposure time to the release medium and initial drug loading is illustrated in Fig. 5. Clearly, polymer degradation

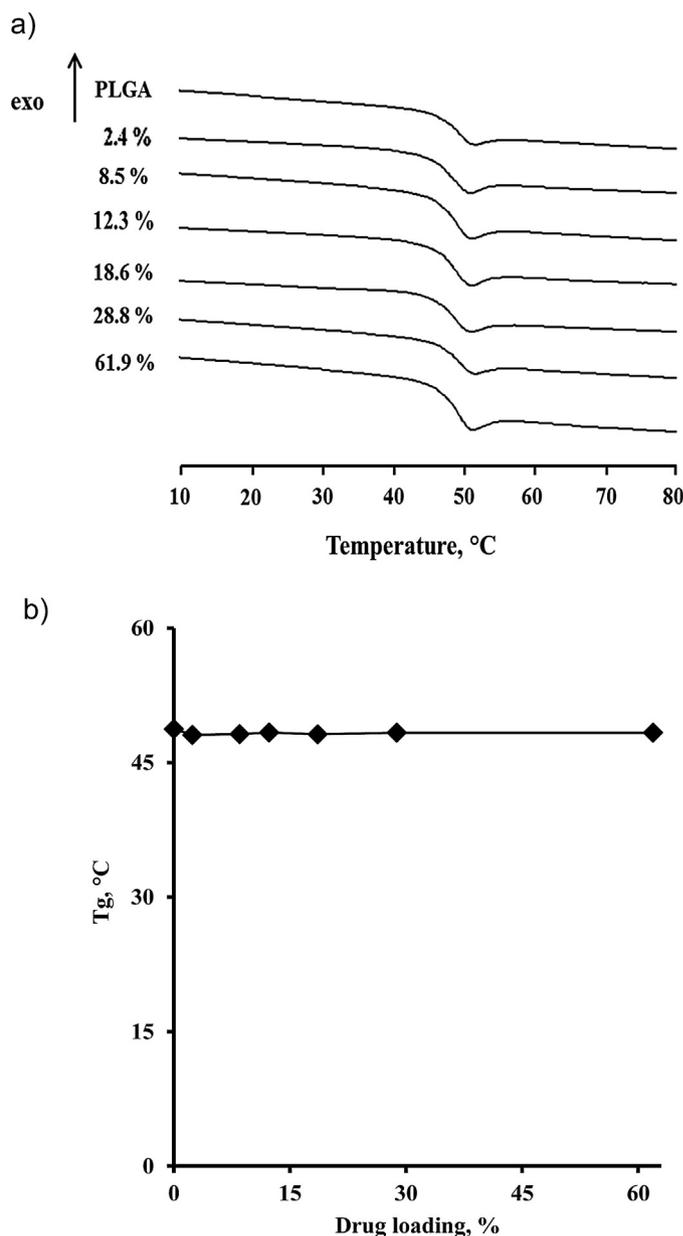


Fig. 2. (a) DSC thermograms of the investigated PLGA microparticles, loaded with different amounts of dexamethasone (indicated in the diagram). The systems were measured in the dry state (before exposure to the release medium). For reasons of comparison, also the DSC thermogram of PLGA powder (as received) is shown. (b) Dependence of the glass transition temperature (T_g) of the PLGA in the dexamethasone-loaded microparticles (measured in the dry state) on the practical drug loading of the systems.

slowed down with increasing drug loading. This can probably be attributed to the occurrence of autocatalytic effects and the substantial difference in microparticle porosity upon drug exhaust. The cartoon at the bottom of Fig. 5 illustrates the involved phenomena. The black circles represent the drug, the white circles

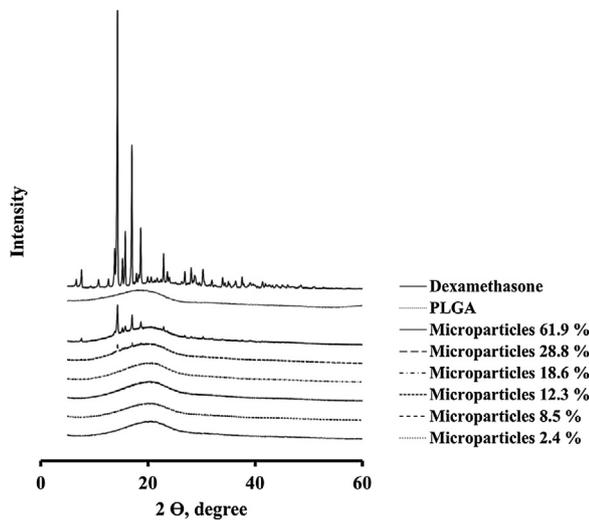


Fig. 3. X-ray diffraction patterns of the investigated dexamethasone-loaded microparticles (before exposure to the release medium.). The practical drug loading is indicated in the diagram. For reasons of comparison, also the X-ray diffraction patterns of the drug and polymer powders (as received) are shown.

pores and the red color indicates local drops in the micro-pH (the darker the color, the more pronounced is the drop). As illustrated, upon drug release microparticles with high initial dexamethasone loadings become highly porous. Consequently, short chain acids (which are generated in the microparticles due to PLGA degradation) and bases coming from the surrounding bulk fluid, are relatively mobile. Thus, the acidic PLGA degradation products within the microparticles can rather rapidly diffuse out, or be neutralized in the system. Hence, the pH within the microparticles drops only to a limited extent. In contrast, at low initial dexamethasone loadings, the resulting system porosity upon drug exhaust is low and short chain acids generated inside the system can cumulate, resulting in substantial drops in micro-pH (especially at the center, due to the longer diffusion pathways).

This leads to accelerated PLGA degradation (Brunner et al., 1999; Klose et al., 2006), since the hydrolysis of ester bonds is catalyzed by protons. This phenomenon is also called “auto-catalysis” (because one of the degradation products catalyzes the chemical reaction).

Furthermore, Fig. 6 shows the swelling kinetics of individual, dexamethasone-loaded, PLGA microparticles upon exposure to the release medium. Exemplarily, a small (19 μm) and a medium-sized (58 μm) microparticle are shown. As it can be seen, the microparticles did not swell during the first 8 d, but then their diameter substantially increased. Importantly, these swelling kinetics did not depend on the microparticle size (this was also true for larger particles, data not shown). It has previously been reported that such substantial microparticle swelling coincided (or preceded) the onset of the final (rapid) drug release phase from prilocaine- and ketoprofen-loaded PLGA microparticles (Gasmi et al., 2015a,b). For this reason, the swelling kinetics of the single dexamethasone-loaded microparticles were plotted together with the drug release kinetics from the respective ensembles of microparticles in Figs. 7–9 (for the different drug loadings). The filled diamonds correspond to the left y-axes (showing the percentages of drug release), whereas the open triangles correspond to the right y-axes (showing the microparticle diameter). The pictures at the bottom of Figs. 7 and 8 show photomicrographs of dexamethasone-loaded PLGA microparticles obtained after different exposure times to the release medium (as indicated). Note that in contrast to previously reported prilocaine- and ketoprofen-loaded systems (Gasmi et al., 2015a,b), the microparticles were much more mobile in the well plates. Hence, individual microparticles more easily changed their position in the well plate. This might at least in part be explained by the absence of any plasticizing effect of dexamethasone for the PLGA (Fig. 2), in contrast to prilocaine and ketoprofen, which effectively decreased the glass transition temperature of this polymer (and, thus, likely increased microparticle sticking).

Importantly, the microparticles did not swell during a specific time period, but then the diameter of the systems started to substantially increase. The onset of this considerable system swelling coincided with (or preceded) the onset of the final rapid

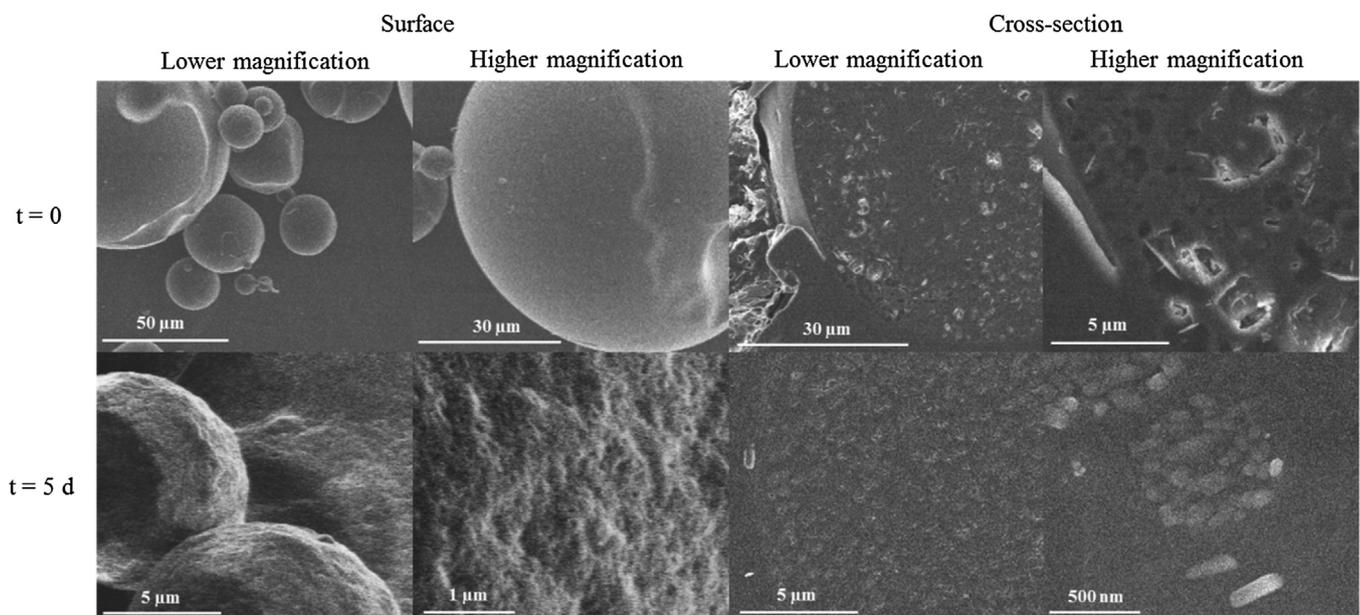


Fig. 4. SEM pictures of surfaces and cross-sections (lower and higher magnification) of dexamethasone-loaded microparticles before and after 5 d exposure to phosphate buffer pH 7.4. The initial drug loading was 61.9%. Note the different scale bars.

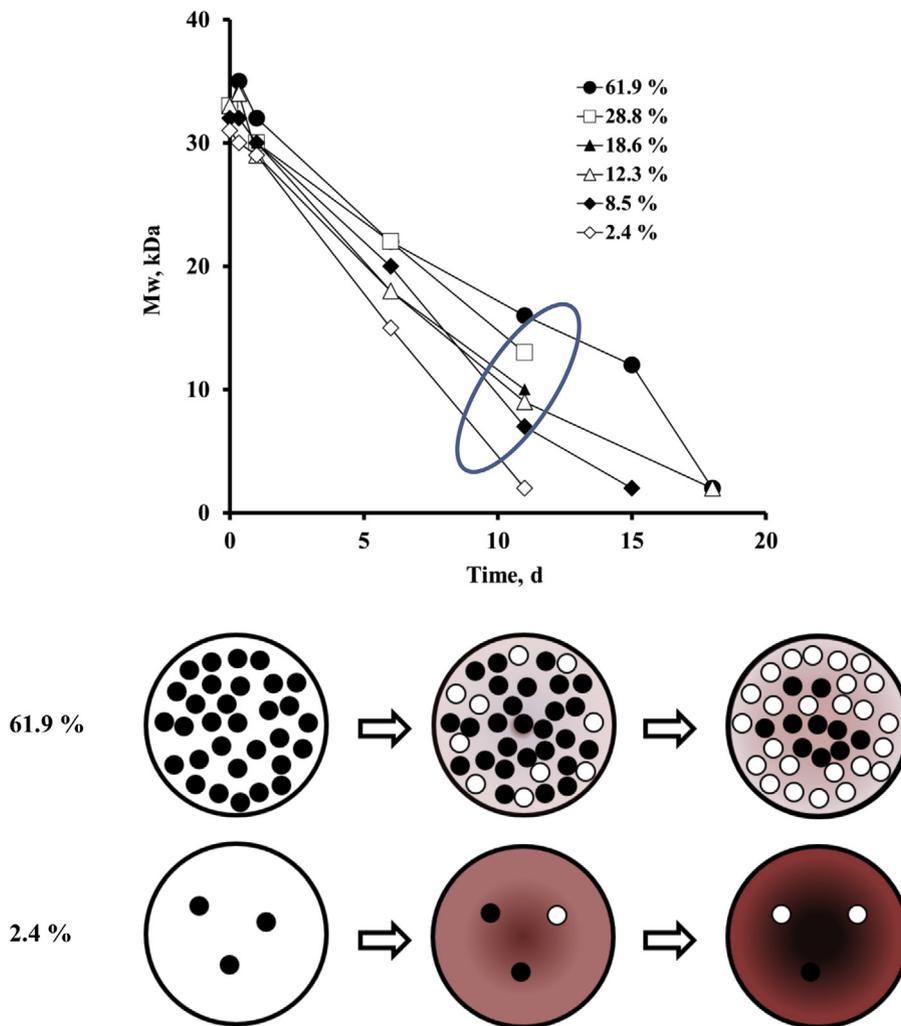


Fig. 5. Effects of the initial (practical) dexamethasone loading of the investigated microparticles (indicated in the diagram) on PLGA degradation upon exposure to phosphate buffer pH 7.4 at 37 °C. The polymer molecular weight was determined by GPC analysis. The blue ellipse indicates the onset regions for substantial microparticle swelling. The cartoon at the bottom shows schematically the changes in microparticle porosity with time (for low and high initial dexamethasone loadings), as well as the drop in micro-pH within the systems. Black circles represent drug particles, white circles pores, and the red color indicates local drops in the micro-pH (the darker the color, the more pronounced is the drop). Details are discussed in the text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

drug release phase. This was true for all the investigated drug loadings (Figs. 7–9). Interestingly, and in contrast to the previously reported prilocaine- and ketoprofen-loaded PLGA microparticles (Gasmi et al., 2015a,b), there was no specific “critical molecular weight of the PLGA” at which substantial microparticle swelling set on, that was common for all initial drug loadings. Instead, system swelling started at higher PLGA molecular weights in the case of high initial dexamethasone loadings (blue ellipse in Fig. 5). This difference might be explained as follows: In addition to the polymer molecular weight, also the porosity of the microparticles is likely to be of crucial importance for the onset of substantial microparticle swelling (induced for instance by the osmotic pressure within the systems, which is resulting from the generation of short chain degradation products). Once the mechanical stability of the polymeric structure becomes insufficient (the degree of polymer chain entanglement decreases due to polymer degradation, and system porosity increases due to drug release), substantial amounts of water can penetrate into the microparticles. At higher initial dexamethasone loadings, highly porous structures remain upon drug exhaust, thus, the critical

polymer molecular weight allowing for substantial microparticle swelling is higher compared to low dexamethasone loadings (Note that eventually also the drug particle size and shape might affect these phenomena.).

3.2. Drug release mechanisms

Based on the above described physico-chemical key properties of the investigated PLGA microparticles, the following drug release mechanisms, explaining the observed bi- and tri-phasic dexamethasone release patterns can be hypothesized:

The initial burst release phase is likely due to the dissolution of drug particles, which are directly in contact with the microparticles' surface (and, thus, in direct contact with the release medium). In addition, at high initial drug loadings, also drug particles, which are in direct contact with such drug particles having “direct access” to the microparticles' surface can rapidly dissolve and be released (e.g. by diffusion through large pores created by released drug particles). This mechanism can also explain the substantial increase in the relative importance of the

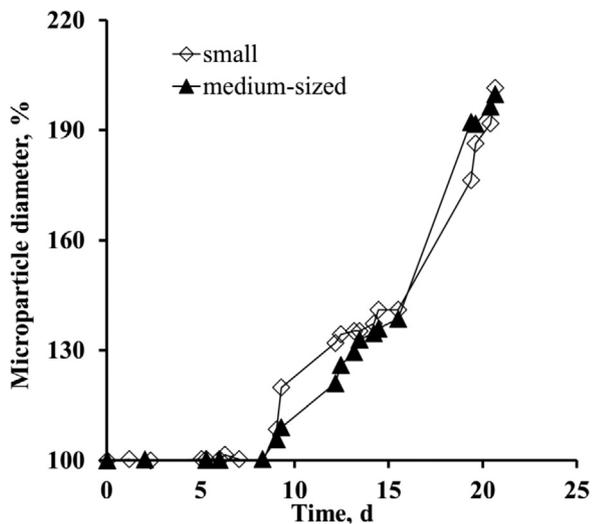


Fig. 6. Dynamic changes in the diameter of *single* PLGA-microparticles, loaded with 2.4% dexamethasone, upon exposure to phosphate buffer pH 7.4: Exemplarily, a small (initially 19 μm) and a medium-sized (initially 58 μm) microparticle are shown.

burst release from the investigated dexamethasone-loaded PLGA microparticles with increasing initial drug loading (Fig. 10a). The SEM pictures of the microparticles with an initial dexamethasone

loading of 61.9% after 5 d exposure to the release medium (Fig. 4) are also consistent with this hypothesis: Certain microparticle regions seem to still contain numerous drug particles, whereas others seem to be collapsed (indicating virtually complete drug exhaust in these regions). Note that the collapse probably only occurred during sample preparation, since during drug release the pores are likely to be filled with water. In contrast, at low initial dexamethasone loadings the burst release is negligible, because only very few drug particles have direct access to the microparticles' surface. In Fig. 10a, "non-trapped drug" means "drug, which is not completely trapped and has direct and rapid access to the microparticles' surface" (it does not mean "non-encapsulated drug").

The second drug release phase is characterized by an approximately constant drug release rate (Fig. 10b). Interestingly, this release rate (the slope of the straight lines) substantially depends on the initial dexamethasone loading: the release rate was low at low and very high drug loadings, whereas it was high at the intermediate drug loading of 28.8%. This might be explained as follows: At very high drug loadings (here 61.9%), a substantial portion of the drug has direct access to the microparticles' surface and is rapidly released during the "burst phase". But parts of the drug (in this case around 40%) does not have this direct access and is effectively trapped by the PLGA. This drug is likely released by diffusion through the polymeric barrier in the second drug release phase. Importantly, a saturated dexamethasone solution is most probably provided within the system: the solubility of this drug in

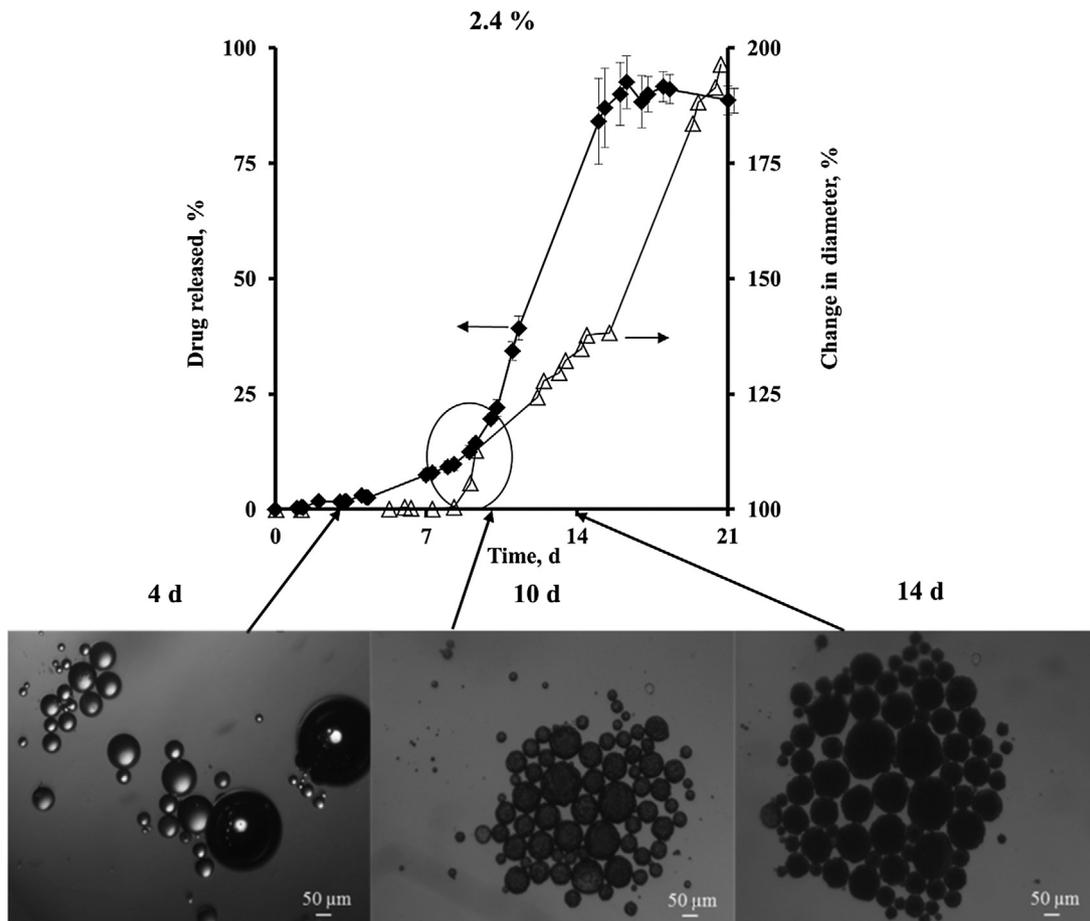


Fig. 7. Dexamethasone release from *ensembles* of microparticles (filled diamonds, left y-axis) and dynamic changes in the diameter of *single* PLGA-microparticles (open triangles, right y-axis). The release medium was phosphate buffer pH 7.4, the drug loading was 2.4%. The ellipse indicates the onset of substantial microparticle swelling, coinciding with the onset of the final rapid drug release phase. At the bottom, optical microscopy pictures of the microparticles are shown after different exposure times (as indicated).

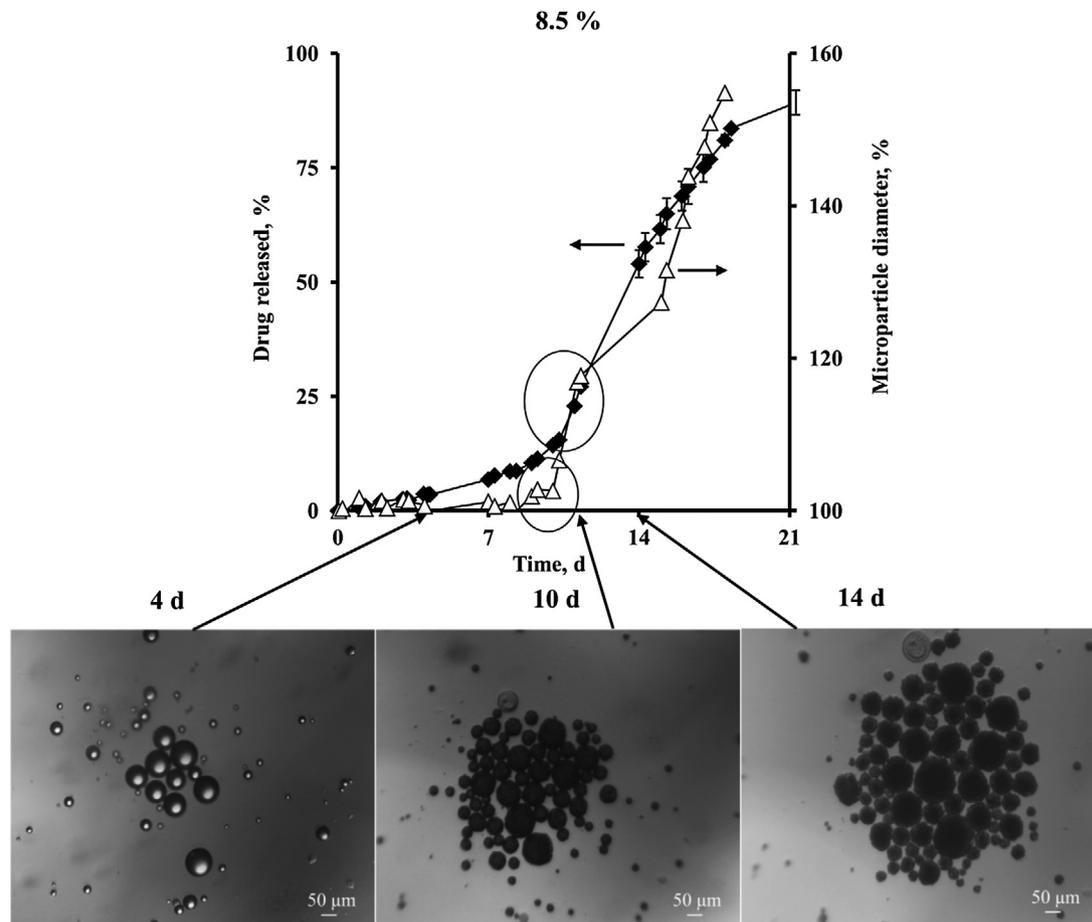


Fig. 8. Dexamethasone release from *ensembles* of microparticles (filled diamonds, left y-axis) and dynamic changes in the diameter of *single* PLGA-microparticles (open triangles, right y-axis). The release medium was phosphate buffer pH 7.4, the drug loading was 8.5%. The ellipses indicate the onset of substantial microparticle swelling and the onset of the final rapid drug release phase. At the bottom, optical microscopy pictures of the microparticles are shown after different exposure times (as indicated).

phosphate buffer pH 7.4 at 37 °C is 73.9 ± 0.4 mg/L (Delplace et al., 2012), and the amount of water available for drug dissolution inside the microparticles is limited. In contrast, in the surrounding bulk fluid, sink conditions were provided during this second phase of drug release in all cases. Thus, about constant drug concentration gradients exist during these time periods (saturated solutions inside versus negligible concentrations outside), leading to about constant drug release rates (filled circles in Fig. 10b). Also at low initial drug loadings, the dexamethasone particles are likely to be effectively trapped, resulting in low drug release rates (filled and open triangles and diamonds in Fig. 10b). Since again, saturated solutions are provided inside the systems and sink conditions outside, the resulting drug release rates are about constant. In contrast, at the intermediate drug loading of 28.8%, the initial burst release was about 20%, and the remaining (not yet released) drug particles have a relatively high probability to be in direct contact with each other. This can be expected to lead to the formation of a porous network upon drug exhaust. However, these pores are much less numerous and much smaller than those created upon drug exhaust at high drug loadings. Once all drug with direct rapid access to the microparticles' surface has been released during the burst release phase, the remaining drug is released through a network of small pores. Since again, saturated solutions are provided inside the microparticles and sink conditions outside, an about constant drug release results (Fig. 10b open squares).

The final rapid drug release phase sets on as soon as (or shortly after) substantial microparticle swelling occurs. As discussed

above, this swelling might result from the osmotic pressure built up within the systems (more and more shorter chain degradation products are generated and dissolve in the available water). The swelling starts as soon as the polymeric structure becomes sufficiently weak (the decreasing polymer molecular weight results in a decreasing degree of macromolecular entanglement, and drug release leads to an increase in system porosity). The penetration of substantial amounts of water into the system leads to a considerable increase in drug permeability (outweighing the increase in the lengths of the diffusion pathways), and hence, increased release rates. Interestingly, in the case of dexamethasone, even the presence of these considerable amounts of water in the microparticles during this final stage of drug release is not sufficient to dissolve all drug: The slope of the relative drug release curves decreases with increasing initial drug loading (Fig. 10c). Thus, again saturated drug solutions are provided within the microparticles, explaining the observed about constant drug release rates in the final drug release phase.

Importantly, the type of drug and manufacturing procedure can significantly impact the relative importance of the mass transport phenomena involved in the control of drug release from PLGA microparticles (e.g., Klose et al., 2008). For example, it has previously been shown that the relative release rate of the acidic drug ketoprofen and of the free base prilocaine steadily increases with increasing initial drug loading, also in the final rapid release phase (Gasmi et al., 2015a,b). Furthermore, the onset of the final rapid drug release phase was shifted to earlier time points with

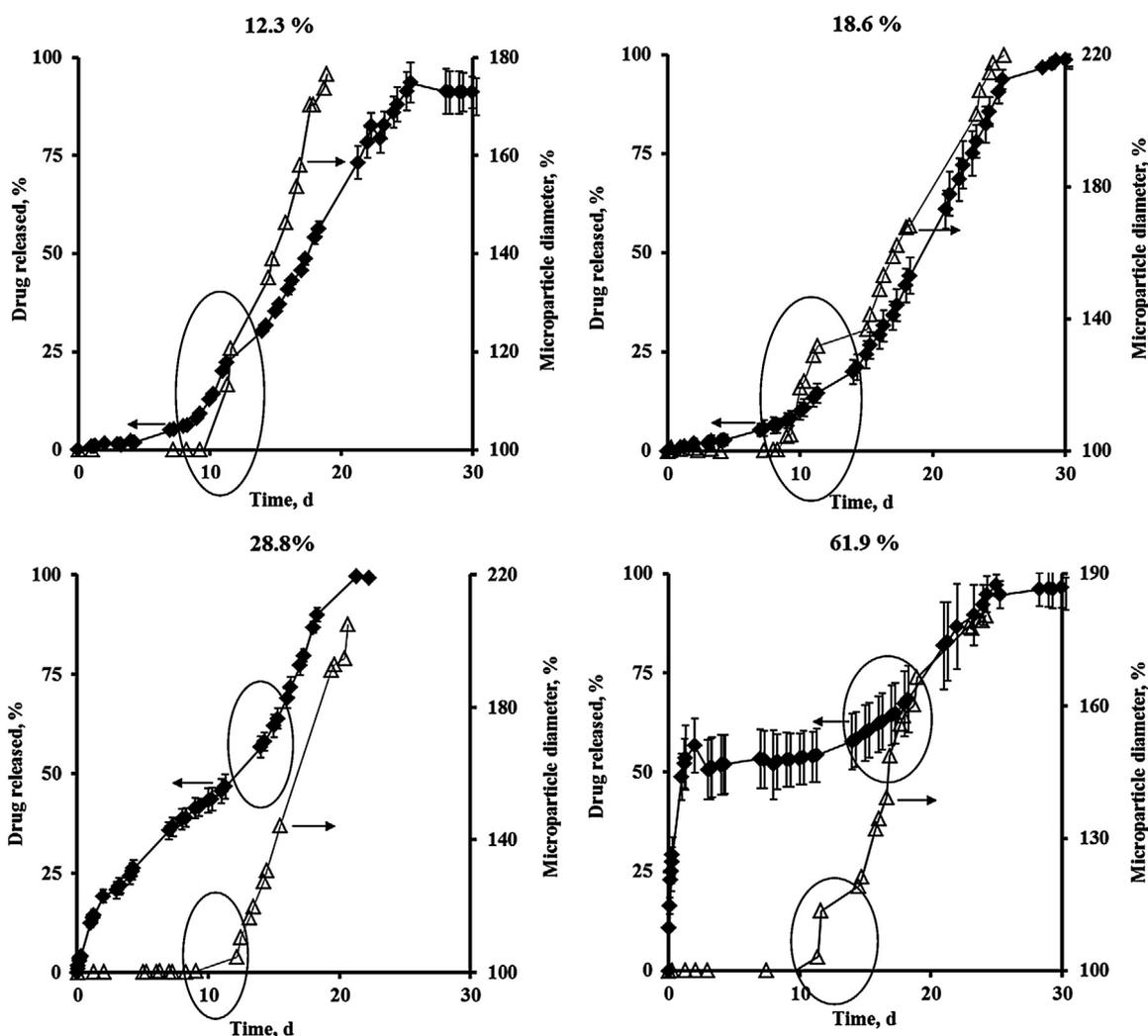


Fig. 9. Dexamethasone release from *ensembles* of microparticles (filled diamonds, left y-axis) and dynamic changes in the diameter of *single* PLGA-microparticles (open triangles, right y-axis). The release medium was phosphate buffer pH 7.4, the drug loading was 12.3, 18.6, 28.8 and 61.9%, as indicated. The ellipses indicate the onset of substantial microparticle swelling and the onset of the final rapid drug release phase.

increasing initial loadings for these two drugs. This is in contrast to dexamethasone, where the relative release rate decreases in the final release phase with increasing drug loading, and where the onset of the final drug release phase is shifted to later time points with increasing initial drug loading. The reasons for these differences probably include the facts that ketoprofen and prilocaine (free base) accelerate PLGA degradation (ester bond cleavage being catalyzed by protons and hydroxide ions), and that both drugs act as efficient plasticizers for PLGA. Furthermore, the preparation method (e.g. using oil-in-water emulsions and solvent extraction/evaporation techniques *versus* spray-drying) can significantly affect the inner and outer morphology of the systems. This can remarkably impact the mobility of acids, bases and the drug, determining for instance the importance of autocatalytic effects (Klose et al., 2006), but also of other phenomena. Thus, the exact mechanisms governing drug release from a specific type of PLGA microparticles can vary from one system to the other. However, the following phenomena are likely to occur in virtually all PLGA microparticles:

- If drug particles have direct access to the microparticles' surface, they can be expected to rapidly dissolve upon contact with aqueous media, leading to a "burst release".
- Once all drug with direct and rapid access to the microparticles' surface has been released, the remaining ("trapped") drug is slowly released. Importantly, saturated drug solutions can be expected to exist within the systems, even in the case of freely water-soluble drugs. This is because the amounts of water available for drug dissolution in the PLGA microparticles at this stage are generally very limited. These saturated drug solutions combined with sink conditions provided outside the microparticles (in the surrounding environment) lead to about constant drug concentration gradients. The latter are the driving forces for diffusion, which is likely to play a major role in this phase of drug release (together with the limited drug solubility effects). Hence, about constant drug release results ("zero order phase") (Siepmann and Siepmann, 2012). The slope of the respective straight lines essentially depends on the permeability of the polymeric system for the drug.
- At a certain time point, the PLGA microparticles become sufficiently labile to allow for substantial microparticle swelling. This is due to the degradation of the polymer and drug release (resulting in a decreasing degree of macromolecular entanglement and increasing system porosity). The water influx is at least partially driven by the osmotic pressure, which is built up within the system due to the PLGA degradation products and drug

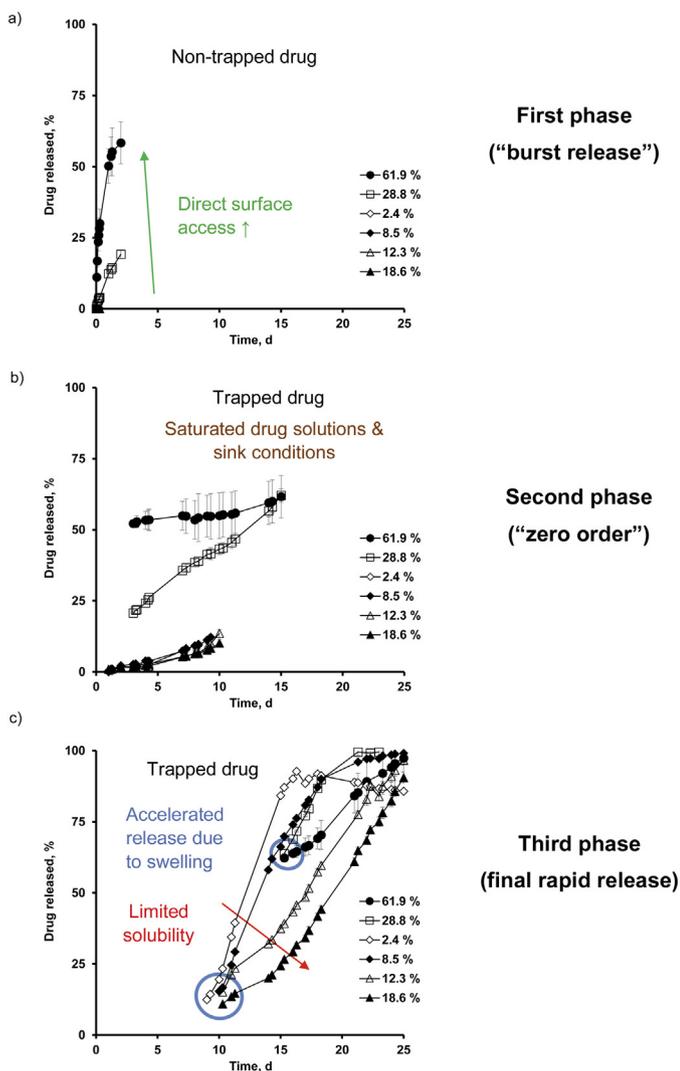


Fig. 10. The different phases of drug release from the investigated dexamethasone-loaded PLGA microparticles: (a) burst release phase, (b) "zero order release phase" and, (c) final rapid drug release phase. The drug loading is indicated in the diagrams. Details on the underlying drug release mechanisms are given in the text. Here, "non-trapped drug" means "drug, which is not completely trapped and has direct and rapid access to the microparticles' surface".

dissolution. In addition, the hydrophilicity of the system increases with time (shorter chain PLGA containing more COOH- and OH-groups is more hydrophilic than longer chain PLGA). This facilitates water imbibition. The resulting substantial microparticle swelling leads to a significantly increased drug mobility (outweighing the increase in the length of the diffusion pathways) and, thus, accelerated drug release.

Please note that in certain systems, only *mono-* or *bi-*phasic drug release is observed. This might at least partially be attributable to the fact that the drug is already completely released before the conditions for the subsequent release phases are provided. For example, if 100% of the drug has rapid and direct access to the microparticles' surface, only a burst release is observed. Also, if all drug is already released during the first and second release phases (burst release and zero order release phase), once substantial microparticle swelling sets on (leading to significantly increased system permeability), obviously no further drug can be released.

4. Conclusion

The obtained new insight into the underlying mass transport mechanisms determining the different release phases from PLGA microparticles can be very helpful to better understand the *in vitro* (and probably also the *in vivo*) behavior of these systems. In practice, this knowledge can for instance be used to facilitate product optimization and/or troubleshooting during production (e.g., via root cause analyses). In particular, the effects of formulation and processing parameters (e.g., of the initial drug loading) on the performance of the systems are likely to be more easily understood. Thus, the drug products do not need to be treated as "black boxes". Ideally, mechanistically realistic mathematical models could be used in the future to quantitatively predict the impact of the design of PLGA microparticles on the resulting drug release kinetics.

However, caution should be paid when trying to generalize the findings of this study: The presented results were obtained with dexamethasone-loaded PLGA microparticles, which were prepared using an oil-in-water solvent extraction/evaporation method. The importance of the physico-chemical processes contributing to the control of drug release in PLGA microparticles might strongly depend on the systems' composition and manufacturing procedure (the latter can for instance affect the inner and outer particles' structure). It would be interesting to conduct similar and even more comprehensive studies in the future with other types of PLGA-microparticles (e.g. loaded with different drugs).

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