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Research paper

Importance of PLGA microparticle swelling for the control of prilocaine release

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ABSTRACT

It has recently been reported that system swelling is likely to control the onset of the third release phase from *ketoprofen*-loaded PLGA microparticles: However, yet it is unclear whether this type of release mechanism is also valid for other types of drugs. In this study, PLGA microparticles were loaded with different amounts of the free base prilocaine, keeping the microparticle size constant. The systems were characterized using GPC, DSC, SEM, X-ray powder diffraction, drug release measurements and the monitoring of *single* microparticle swelling. At lower drug loadings, tri-phasic release patterns were observed: An initial burst was followed by a period with an about constant release rate, which was followed by a third, again rapid release phase. Interestingly, the beginning of this final rapid drug release phase coincided with the onset of substantial microparticle swelling. GPC analysis revealed that the PLGA molecular weight was about 18–19 k Da at these "onset time points". Thus, it seems that as soon as a critical polymer molecular weight is reached, important amounts of water penetrate into the system, leading to significantly increased polymer and drug mobility. Hence, microparticle swelling seems to cause the onset of the final release phase of different types of drugs.

1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) offers major advantages as matrix former for parenteral controlled drug delivery systems, because: (i) It is biodegradable [1]. (ii) It is biocompatible [2]. (iii) Drug release can be controlled over broad ranges of time periods [3–8]. Often, PLGA *microparticles* are used as delivery systems, since they can be rather easily administered (e.g., s.c. or i.m.). Since many years a variety of controlled release drug products based on PLGA microparticles is available on the market, in particular for cancer treatments. It has to be pointed out that the observed drug release kinetics might depend on the surrounding environment [9,10]. Generally, drug release from PLGA microparticles is mono-, bi-, or tri-phasic [9,11,12]. In the latter case, an initial burst release is followed by a period with an about constant release rate and a final (again rapid) drug release phase.

Interestingly, yet relatively little knowledge is available on the exact underlying mass transport mechanisms controlling drug release from PLGA microparticles. This can be attributed to the complexity of the involved physico-chemical phenomena [13–17]: Upon contact with aqueous body fluids, water penetrates into the system, leading to drug dissolution (if the latter is not already dissolved in the polymer) and PLGA degradation (ester bond cleavage). Once dissolved, the drug can diffuse out of the system (through water-filled channels and/or intact polymeric networks). In addition, shorter chain PLGA degradation products can diffuse out of the system, while molecules and ions of the surrounding bulk fluid can penetrate into the microparticles. It has been shown that water penetration into PLGA microparticles is generally much more rapid than the subsequent ester hydrolysis [18]. Consequently, the systems are soon completely wetted and polymer degradation occurs throughout the microparticles (leading to "bulk erosion"). Depending on various factors (including for instance the system size and porosity), the generation of shorter chain acids can be faster than the diffusion of these acidic degradation products out of the microparticles (and the diffusion of bases from the environment into the system). Consequently, acids can accumulate and the

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micro-pH within the particles might significantly drop (especially in large and non-porous systems) [19,20]. Since ester hydrolysis is catalyzed by protons, this can lead to autocatalytic effects and accelerated polymer degradation and drug release [21,22]. Furthermore, the initial presence of tiny pores at the microparticles' surface during the first hours might explain the often observed "burst release" from these systems. Upon exposure to the release medium, these pores might be closed, resulting in decreased drug mobility [23–25].

Concerning the exact reasons for the onset of the *third* (and again rapid) drug release phase from PLGA microparticles, very little is known up to now. Recently, it has been reported that in the case of PLGA microparticles loaded with the acidic drug ketoprofen, significant particle swelling coincided with the onset of this third release phase [26]: The monitoring of *single* particle swelling (by optical microscopy) allowed correlating swelling and drug release kinetics of different types of particles. However, yet, it is unclear whether this correlation between particle swelling and drug release was eventually only a coincidence by hazard, or whether it is only observed in the case of acidic drugs, or whether PLGA microparticle swelling is generally the cause for the onset of the final rapid drug release phase from this type of advanced drug delivery systems.

The aim of the present study was to prepare different types of PLGA microparticles loaded with the free base prilocaine: The initial drug loading was varied from 2 to 35% (w:w). Importantly, the mean particle size was kept about constant in order to minimize microparticle size effects [21,22]. Gel permeation chromatography (GPC), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), X-ray powder diffraction, drug release measurements and the monitoring of *single* microparticle swelling (by optical microscopy) were used to characterize the systems before and after exposure to phosphate buffer pH 7.4.

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); prilocaine (free base) and polyvinyl alcohol (Mowiol 4–88) (Sigma–Aldrich, Steinheim, Germany); acetonitrile and dichloromethane (VWR, Fontenoy-sous-Bois, France); tetrahydrofurane (HPLC Grade; Fisher Scientific, Illkirch, France).

2.2. Preparation of PLGA microparticles

Prilocaine (free base)-loaded PLGA microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: Depending on the theoretical drug loading [which was varied from 3 to 50% (w:w)], 31.5–527.2 mg drug and 518.8–1015.1 mg PLGA were dissolved in 4.1–8.0 mL dichloromethane (Table 1) (the volume of the organic solvent was adapted to keep the mean microparticle diameter in the range of 80–90 μm in all cases). This organic phase was emulsified within 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25%, w/w) for 30 min under stirring with a three-blade propeller (2000 rpm), inducing microparticle formation. The particles were hardened by adding 2.5 L of the same outer aqueous polyvinyl alcohol solution and further stirring at 700 rpm during 4 h. The microparticles were subsequently separated by filtration and freeze-dried (Christ Epsilon 2–4 LSC, Martin Christ, Osterode, Germany).

2.3. Microparticle characterization

2.3.1. Microparticle size

Particles sizes were determined by optical microscopy: Pictures were taken using an Axiovision Zeiss Scope-A1 microscope (Carl Zeiss Microimaging, Goettingen, Germany), equipped with an AxioCam ICc1 camera and Axiovision Zeiss Software (Carl Zeiss, Jena, Germany). Each measurement included 200 microparticles.

2.3.2. Practical drug loading

Accurately weighed amounts of microparticles (approximately 20 mg) were dissolved in 10 mL acetonitrile, followed by filtering (PTFE syringe filters, 0.45 μm) and determination of the prilocaine content by HPLC analysis (Prostar 210 pump, 410 autosampler, 335 Photodiode array Detector, Galaxy Software; Varian, Les Ulis, France). A reversed phase column C18 (Gemini 5 μm, 110 A; 150 mm × 4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of acetonitrile:phosphate buffer pH 8 (Ph. Eur. 7) (40:60 v:v). The flow rate was 1 mL/min, the detection wavelength 260 nm (linear concentration range from 1 to 200 μg/mL). Twenty microliter samples were injected. Each experiment was conducted in triplicate.

2.3.3. In vitro drug release

Fifty milligrams of prilocaine-loaded microparticles were placed in 12 mL glass tubes, filled with 10 mL phosphate buffer pH 7.4 (USP 35). The tubes were horizontally shaken at 80 rpm at 37 °C (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, 2 mL samples were withdrawn and replaced with fresh medium. The samples were filtered using PTFE syringe filters (0.45 μm, VWR, Fontenoy-sous-Bois, France) and their drug content was analyzed by HPLC analysis, as described above (but injecting 50 instead of 20 μL samples). Each experiment was conducted in triplicate. Sink conditions were provided throughout the experiments.

2.3.4. Gel permeation chromatography (GPC)

The decrease in polymer molecular weight (Mw) of PLGA in the microparticles during drug release was measured by GPC analysis

Table 1
Composition of the organic phases used for the preparation of PLGA microparticles and thin films, loaded with the free base prilocaine (using an O/W solvent extraction/evaporation technique and film casting method, respectively).

Theoretical loading, % (w:w)	3.0	6.7	9.3	15	23	33	40	50
Microparticles								
CH_2Cl_2, mL	8.0	7.7	7.6	7.0	6.4	5.5	5.0	4.1
PLGA, mg	1015.1	978.5	951.6	877.1	804.6	698.9	625.5	518.8
drug, mg	31.5	70.1	97.3	155.3	242.5	351.6	420.5	527.2
Films								
CH_2Cl_2, mL	8.4	8.4	8.2	7.6	7.0	6.0	5.5	5.0
PLGA, mg	3926.2	3820.8	3945.6	3439.1	3237.2	2832.6	2427.6	2023.0
Drug, mg	123.1	273.2	404.6	606.9	970.5	1420.8	1630.4	2057.2

(Prostar 230 pump, 410 autosampler, 356-LC RI Detector; Varian, Les Ulis, France). A PLgel 5 μm Mixed-D column (7.5 × 300 mm, kept at 35 °C; Polymer Laboratories, Varian, Les Ulis, France) was used. The mobile phase was tetrahydrofurane, the flow rate was 1 mL/min. Prilocaine-loaded microparticles were treated as described above for the in vitro drug release studies. At predetermined time points, samples were withdrawn, filtered (Nylon, 0.45 μm, 13 mm, GE Healthcare, Buckinghamshire, UK) and freeze-dried. Three milligrams of microparticles were dissolved in 1 mL tetrahydrofurane. Fifty microliter samples were injected. The molecular weights (Mw) were calculated using the Cirrus GPC software (Polymer Laboratories). Polystyrene standards (Polymer Laboratories) were used for calibration.

2.3.5. Differential scanning calorimetry (DSC)

In order to determine the glass transition temperature (Tg) of PLGA in the microparticles, DSC thermograms were recorded using a DSC 1 Star System (Mettler Toledo, Greinfensee, Switzerland). Approximately 3 mg microparticle samples (for reasons of comparison also pure PLGA and drug) were heated in sealed aluminum pans from room temperature to 100 °C, cooled down to −70 °C and reheated to 100 °C (rate of all heating and cooling steps = 10 °C/min). Dry and wet microparticles were studied. In the latter case, microparticles were treated as for the in vitro drug release studies described above. After 48 h exposure to the release medium, samples were withdrawn and filtered (Nylon, 0.45 μm, 13 mm; GE healthcare, Buckinghamshire, UK). The aluminum pans were pierced in the case of dry microparticles, closed pans were used for wet microparticles.

2.3.6. X-ray powder diffraction

A X-ray wide angle diffractometer Inel CSP 120 (λ Cu, Kα1 = 1.54 A; Inel, Artenay, France) was used to characterize the microparticles (as well as pure prilocaine and PLGA, for reasons of comparison). Powder samples were placed in Lindemann glass capillaries (diameter 0.7 mm). Samples were studied right after microparticle preparation as well as after one year storage at 4 °C in a refrigerator in closed glass vials.

2.3.7. Scanning electron microscopy (SEM)

The external and internal morphology of the microparticles before and after exposure to the release medium 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 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 microparticles were treated as for the in vitro release studies (described above). At predetermined time points, samples were withdrawn, filtered (Nylon, 0.45 μm, 13 mm; GE healthcare) and freeze-dried.

2.3.8. Swelling of individual microparticles

Approximately 50 microparticles were introduced into each well of a 96-well standard microplate (Carl Roth, Karlsruhe, Germany), filled with 100 μL phosphate buffer pH 7.4 (USP 35). The microplates were placed into a horizontal shaker (80 rpm, 37 °C; 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 100 μL liquid in each well during the entire observation period. At predetermined time points, pictures were taken using an Axiovision Zeiss Scope-A1 microscope and the microparticle sizes determined as described above.

2.4. Preparation of thin PLGA films

Thin, PLGA-based films, loaded with different amounts of prilocaine (free base) were prepared by solvent casting: Appropriate amounts of drug and polymer were dissolved in 5.0–8.4 mL dichloromethane (Table 1). The volume of the organic solvent was adapted to the amount of PLGA in order to provide similar viscosities. The solutions were cast into Teflon molds and dried at room temperature for 5 d. The thickness of the films was between 80 and 90 μm in all cases, determined with a Minitest (Electro Physik, Cologne, Germany) at 9 positions on each film sample.

2.5. Film characterization

The elongation at break (%) of the films was measured using the puncture test and a texture analyzer (TAXT Plus, TAXT Plus, Surrey, Godalming, UK) at room temperature. Samples were mounted on a film holder. The puncture probe (spherical end: 5 mm diameter) was fixed on the load cell (5 kg) and driven downward with a cross-head speed of 0.1 mm/s to the center of the film holder's hole (diameter: 10 mm). Load versus displacement curves were recorded until rupture of the film and used to calculate the elongation at break (%) as follows:

$$\text{elongation at break } (\%) = \frac{\sqrt{R^2 + d^2} - R}{R} \cdot 100\%$$

Here, R denotes the radius of the film exposed in the cylindrical hole of the holder and d the displacement to puncture.

3. Results and discussion

3.1. In vitro drug release

Irrespective of the theoretical drug loading, the encapsulation efficiency for prilocaine in the microparticles was about 70% (Table 2). Thus, the practical drug loadings varied between 2.1 and 34.8%. Fig. 1 shows the impact of this initial drug loading on the resulting drug release kinetics from the PLGA microparticles in phosphate buffer pH 7.4. Throughout the experiments, perfect sink conditions were provided (drug solubility in the release medium at 37 °C: 8.2 ± 0.1 mg/mL [27]). As it can be seen, the relative drug release rate increased with increasing initial prilocaine loading. This might be attributable to the facts that: (i) With increasing initial drug content the microparticle porosity increases upon drug

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

Theoretical drug loading, % (w:w)	3.0	6.7	9.3	15	23	33	40	50
Practical drug loading, % (w:w)	2.1 ± 0.0	4.6 ± 0.0	6.5 ± 0.1	10.9 ± 0.1	16.0 ± 0.0	23.3 ± 0.0	28.6 ± 0.1	34.8 ± 0.0
Encapsulation efficiency, %	68.9 ± 0.0	69.3 ± 0.0	70.6 ± 0.8	72.9 ± 0.1	69.1 ± 0.1	69.7 ± 0.1	71.2 ± 0.3	69.0 ± 0.0
Microparticle diameter, μm	89 ± 30	89 ± 34	89 ± 25	85 ± 34	89 ± 27	81 ± 27	82 ± 24	82 ± 31

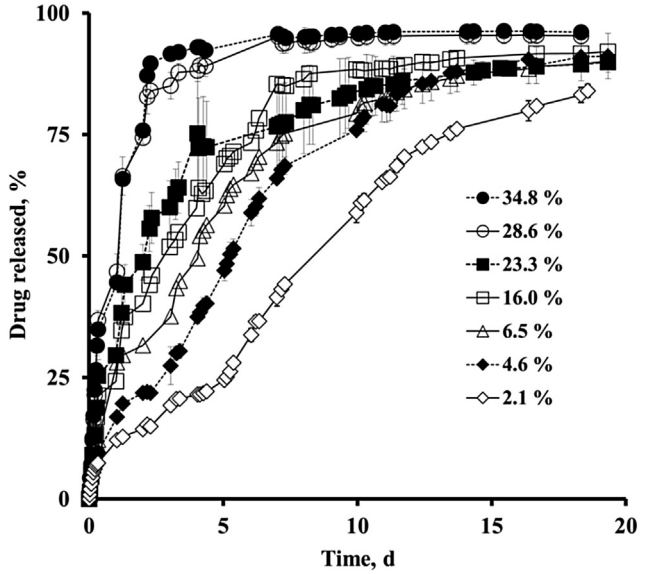

Fig. 1. Effects of the initial drug loading (indicated in the diagram, w:w) on prilocaine release from PLGA-based microparticles in phosphate buffer pH 7.4.

exhaust. Thus, more and more porous polymeric structures result, in which the remaining drug becomes more and more mobile. (ii) Prilocaine is a basic drug and PLGA degradation is catalyzed by bases. Thus, polymer degradation is likely to be accelerated and drug mobility to be increased with increasing initial drug content [11]. (iii) Prilocaine might act as a plasticizer for PLGA, increasing polymer molecular mobility and, thus, also drug mobility [28].

Importantly, not only the slope, but also the shape of the release profiles was strongly affected by the initial prilocaine loading (Fig. 1): At relatively low loadings [2.1, 4.6 and 6.5% (w:w)], *triphasic* drug release patterns were observed: An initial burst release phase was followed by a time period with an about constant drug release rate and finally an again rapid drug release phase. In contrast, at the investigated higher initial drug loadings, different release phases could hardly be distinguished: The profiles were more or less mono-phasic. Furthermore, at low initial drug loadings, the onset of the final rapid drug release phases was shifted to earlier time points with increasing prilocaine loading.

It has to be pointed that the observed differences in the drug release kinetics as a function of the initial drug loading cannot be attributed to differences in the microparticle sizes, since all types of microparticles exhibited a mean diameter of 81–89 μm (Table 2). To better understand underlying mass transport mechanisms, the different types of microparticles were thoroughly characterized physico-chemically before and after exposure to the release medium. In addition, thin films of identical composition were prepared and their mechanical properties measured.

3.2. Physico-chemical characterization of the microparticles and films

The degradation kinetics of PLGA in the investigated microparticles upon exposure to the release medium are illustrated in Fig. 2. The upper diagram shows the decrease in the polymer molecular weight (Mw) as a function of time, the lower diagram as a function of the initial drug loading. In the latter case, the curves represent specific time periods of exposure to phosphate buffer pH 7.4. Clearly, the PLGA degradation rate increased with increasing drug loading. This confirms the hypothesis that the observed substantial increase in the relative drug release rate from the microparticles

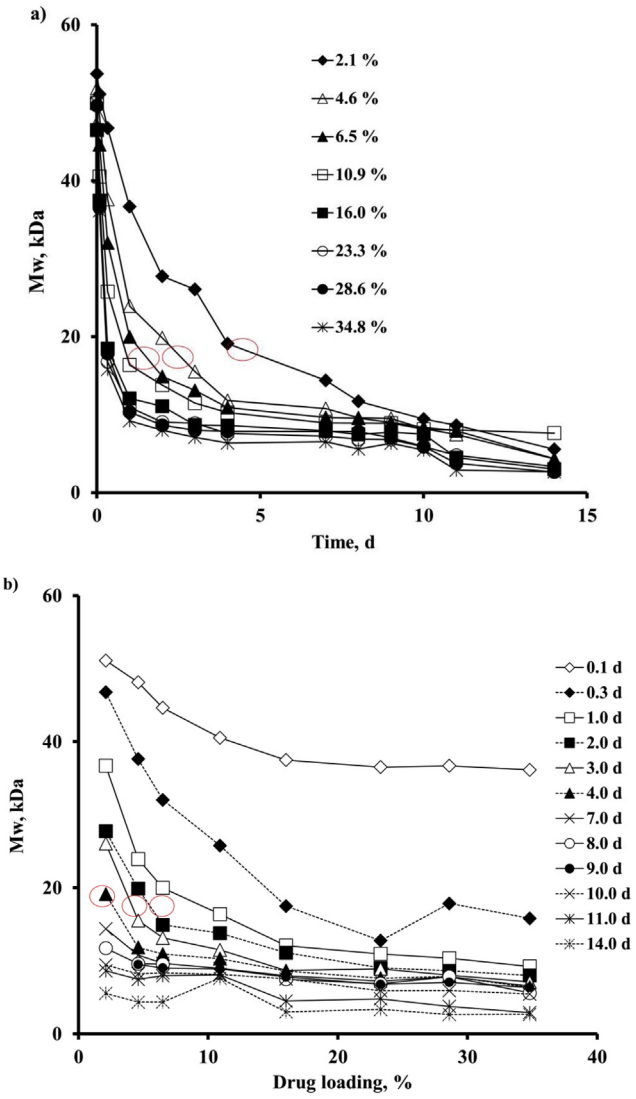

Fig. 2. Decrease in polymer molecular weight (Mw) of PLGA in the investigated prilocaine-loaded microparticles upon exposure to phosphate buffer pH 7.4: a) Mw decrease as a function of time; the drug loading (w:w) is indicated in the diagram; b) Mw decrease as a function of the initial drug loading; the curves correspond to different exposure times to the release medium (indicated in the diagram). The red ellipses indicate the time periods for the onset of substantial microparticles swelling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with increasing initial prilocaine content (Fig. 1) can at least partially be attributed to the fact that the basic nature of the drug leads to catalyzed polyester chain cleavage. Shorter chain PLGA is less entangled and, thus, more mobile, resulting in increased drug mobility. In addition, shorter chain PLGA is more hydrophilic, attracting more water into the system, water being mandatory for drug dissolution (a pre-requisite for drug diffusion) and acting as a plasticizer for PLGA [29,30].

The DSC thermograms of the investigated prilocaine-loaded microparticles are shown in Fig. 3a. For reasons of comparison, also the thermograms obtained with the raw materials PLGA and prilocaine (free base) are shown. The arrows mark glass transition temperatures (Tgs). All microparticles in this diagram were measured in the dry state. As it can be seen, the drug reference powder shows an endothermic event, starting at about 38 °C, corresponding to the melting peak of crystalline prilocaine free base.

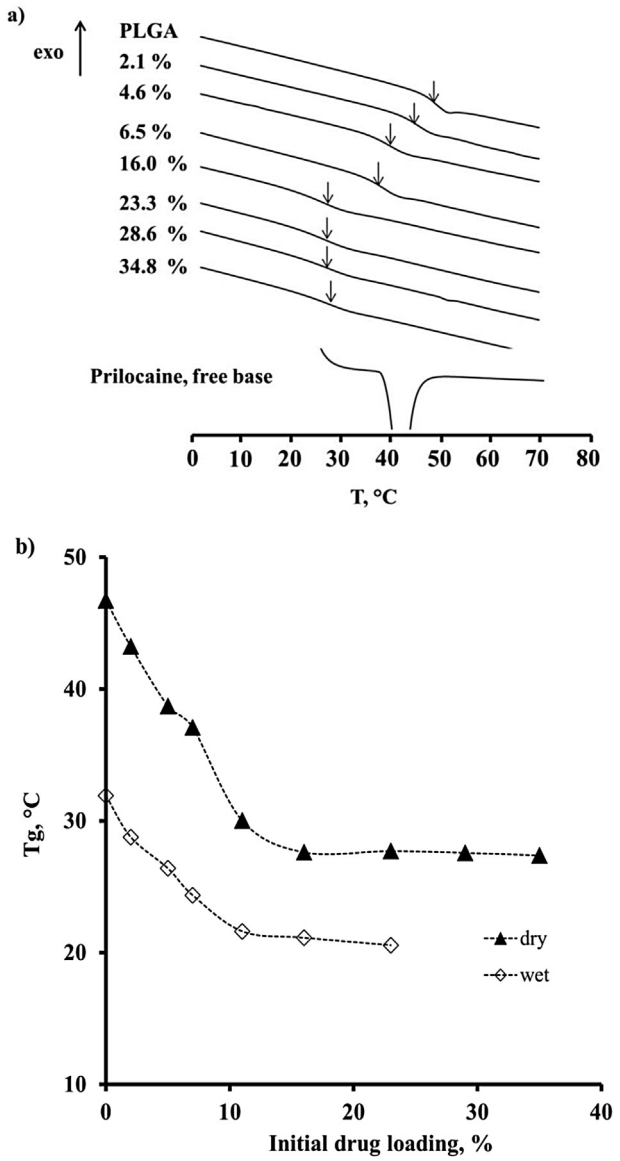

Fig. 3. a) DSC thermograms of PLGA (powder as received), drug-loaded microparticles (measured in the dry state; the prilocaine loading is indicated in the diagram) and prilocaine free base (powder as received). The arrows mark glass transition temperatures. b) Dependence of the glass transition temperature (Tg) of PLGA in the investigated microparticles on the initial drug loading (w:w). The values were determined from DSC scans of microparticles in the dry or wet state (upon 48 h exposure to the release medium).

The PLGA powder exhibits a glass transition at about 47 °C. Importantly, this Tg significantly decreased with increasing initial drug content until a plateau value was reached. The black triangles in Fig. 3b illustrate this decrease in the Tg of the PLGA in the dry microparticles with increasing prilocaine content. Thus, the drug acts as a plasticizer for the polymer. So, also the hypothesis that plasticizing effects of prilocaine are likely to contribute to the increase in the relative drug release rate from the microparticles with increasing initial prilocaine content (Fig. 1) is confirmed.

The leveling off of the Tg values (at around 27 °C) above an initial drug content of 10–15% (Fig. 3b) probably indicates that the polymer phase becomes saturated with the drug: Excess amounts of prilocaine do not dissolve in the polymer, but form a separate phase. Since no drug melting peaks were observed at around 38 °C, the drug is likely to be in an amorphous state. Note that water is known to act as a plasticizer for PLGA, and upon exposure to the release medium the Tg decreases approximately by 10 °C [26]. For the control of drug release from the PLGA microparticles, the conditions in the wet state are more relevant than those in the dry state, since water is known to rather rapidly penetrate into the systems upon exposure to the release medium [18]. The open diamonds in Fig. 3b indicate the glass transition temperatures of PLGA in microparticles, which had been exposed to phosphate buffer pH 7.4 for 48 h. Clearly, the Tg values were shifted by about 10 °C to lower values, due to the plasticizing effects of water. Thus, in all cases the PLGA is likely to be in the rubbery state during most parts of the release periods. Interestingly, again the Tg significantly decreased with increasing prilocaine content and leveled off at around 10–15% drug content.

The pronounced plasticizing effect of the free base prilocaine for PLGA was further confirmed by mechanical analysis of thin, free films: Prilocaine and PLGA were dissolved in dichloromethane and the solutions cast into Teflon molds. Films of virtually the same composition as the microparticles formed upon solvent evaporation, and were studied using a texture analyzer and the puncture test (at room temperature). Fig. 4 shows the observed dependence of the percent elongation at break of the films on the initial drug content. For reasons of comparison, also drug-free films were investigated. As it can be seen, the films became more flexible with increasing prilocaine content, clearly demonstrating the plasticizing effect of this drug for this polymer. Interestingly, the values leveled off at 10–15% drug loading, thus, in the same range as the glass transition temperatures measured by DSC. This confirms a likely (at least apparent) solubility limit of about 10–15% (w/w) of prilocaine (free base) in the investigated PLGA.

Fig. 5a shows the X-ray diffraction patterns of the investigated prilocaine-loaded PLGA microparticles (right after preparation). For reasons of comparison, also the X-ray patterns of pure PLGA and pure drug powder are shown. Clearly, the prilocaine (free base) raw material was crystalline, whereas neither the PLGA powder, nor any of the drug-loaded microparticles showed X-ray diffraction peaks. This is consistent with the DSC measurements, indicating that the drug is partly dissolved in the polymer (up to 10–15%) and – if excess amounts are present – these excess amounts are in an

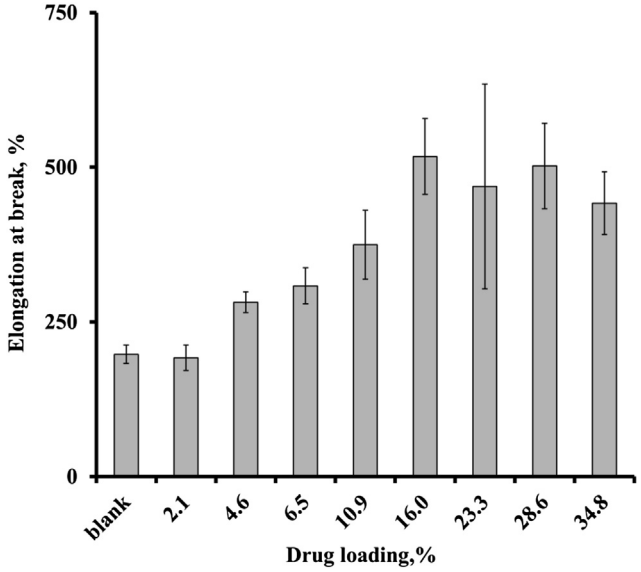

Fig. 4. Elongation at break (%) of thin PLGA-based films, loaded with different amounts of prilocaine (free base). The measurements were performed with a texture analyzer at room temperature in the dry state.

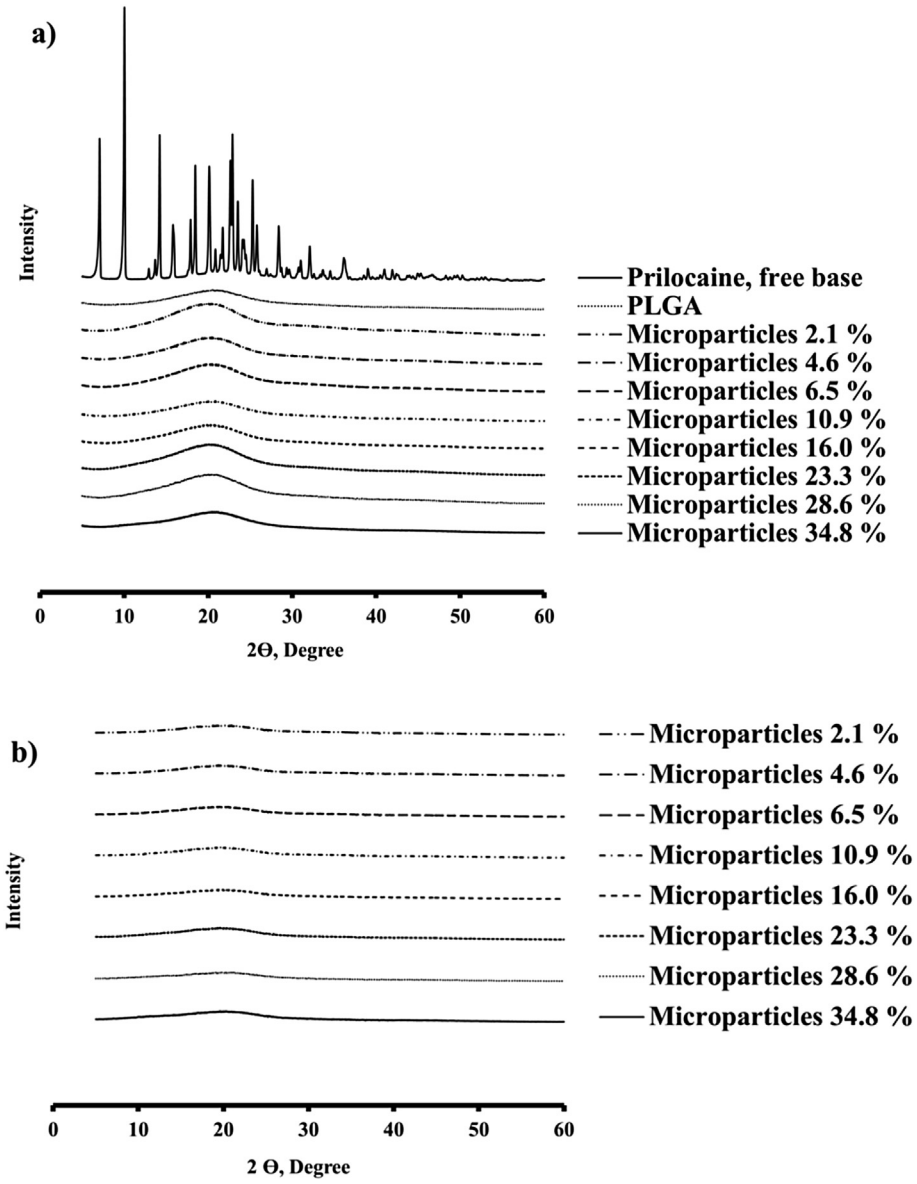

Fig. 5. a) X-ray diffraction patterns of prilocaine free base (powder, as received), PLGA (powder, as received) and drug-loaded microparticles after manufacturing. The drug loading is indicated in the diagram (w:w). b) X-ray diffraction patterns of the drug-loaded microparticles shown in a) after 1 year storage at 4 °C.

amorphous form. Fig. 5b shows that the physical states of the drug and polymer within the microparticles seem to be long-term stable under the given conditions: X-ray diffraction patterns of the different systems after 1 year storage at 4 °C are shown, and no significant differences compared to Fig. 5a are visible.

Fig. 6 shows SEM pictures of surfaces and cross-sections of microparticles loaded with 2.1, 16.0 or 34.8% prilocaine (free base). The upper two rows show microparticles *before* exposure to the release medium, the lower two rows *after 48 h* exposure to phosphate buffer pH 7.4. Note that in the latter cases, the microparticles were freeze-dried prior to the measurements. Thus, artifact creation cannot be excluded. As it can be seen, the microparticles were spherical in shape and initially non-porous (at the surface and internally). Upon 48 h exposure to the release medium pores became visible, especially at higher drug loadings. They can be attributed to drug release and matrix erosion.

Based on these findings it can be concluded that prilocaine seems to be dissolved within the PLGA at initial drug loadings below 10–15% (w:w), whereas it is partly dissolved and partly dispersed in the form of tiny amorphous drug particles at drug loadings above 10–15% (w:w). Furthermore, prilocaine acts as a plasticizer for PLGA and the glass transition temperature of the latter is well below 37 °C during drug release, hence, the microparticles are in the rubbery state. In addition, the basic drug accelerates polymer degradation. However, it still remains unclear why at 2.1, 4.6 and 6.5% prilocaine contents *tri-phasic* drug release kinetics were observed (the onset of the final rapid drug release phase being shifted to earlier time points with increasing drug content), whereas at higher drug loadings more or less *mono-phasic* drug release was observed.

3.3. Individual microparticle swelling

Fig. 7 shows optical microscopy pictures of PLGA microparticles loaded with 2.1, 16.0 and 34.8% prilocaine. The photos were taken after 1 h, 3 d or 7 d exposure to phosphate buffer pH 7.4 at 37 °C in

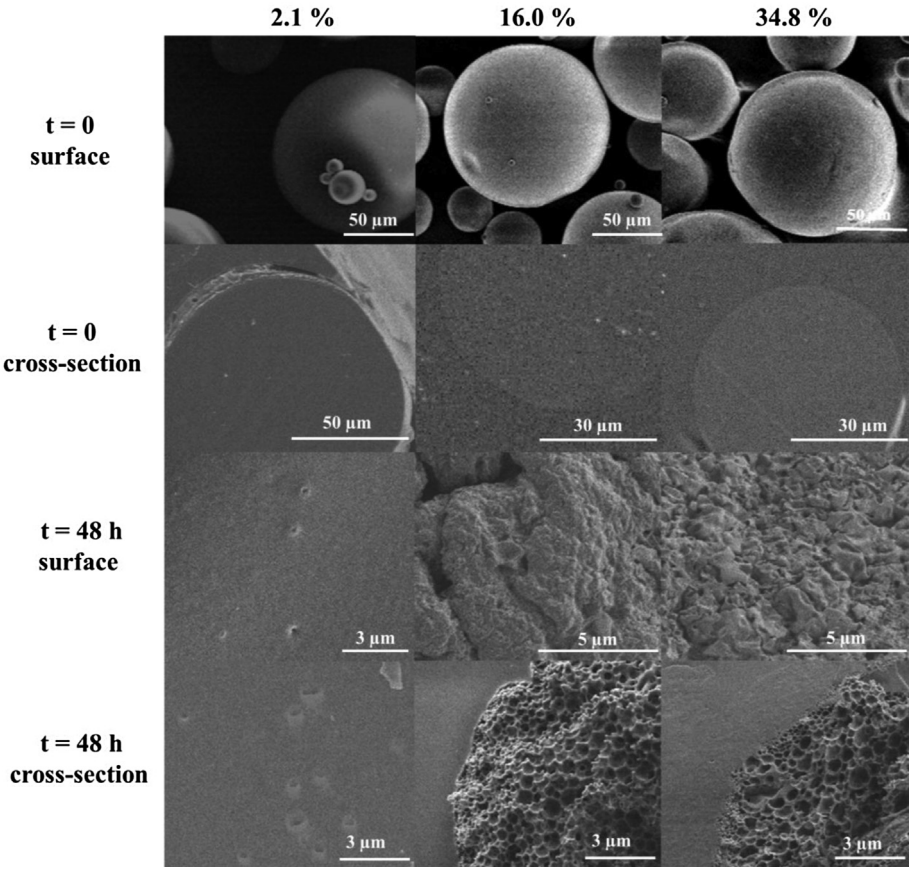

Fig. 6. SEM pictures of surfaces and cross-sections of prilocaine-loaded microparticles before and after exposure to phosphate buffer pH 7.4 for 48 h (as indicated on the left hand side). The initial drug loading (w:w) is given at the top.

96-well standard microplates. Importantly, the microparticles could be followed individually: In each column, the arrows highlight the same microparticle, observed at different time points. This is decisive, since this allows *single* microparticle swelling measurements.

As it can be seen in Fig. 7, significant size changes occurred upon exposure to the release medium. The relative changes in the microparticles' diameter (loaded with 2.1% drug) are plotted as a function of time in Fig. 8. The swelling kinetics of 3 differently sized

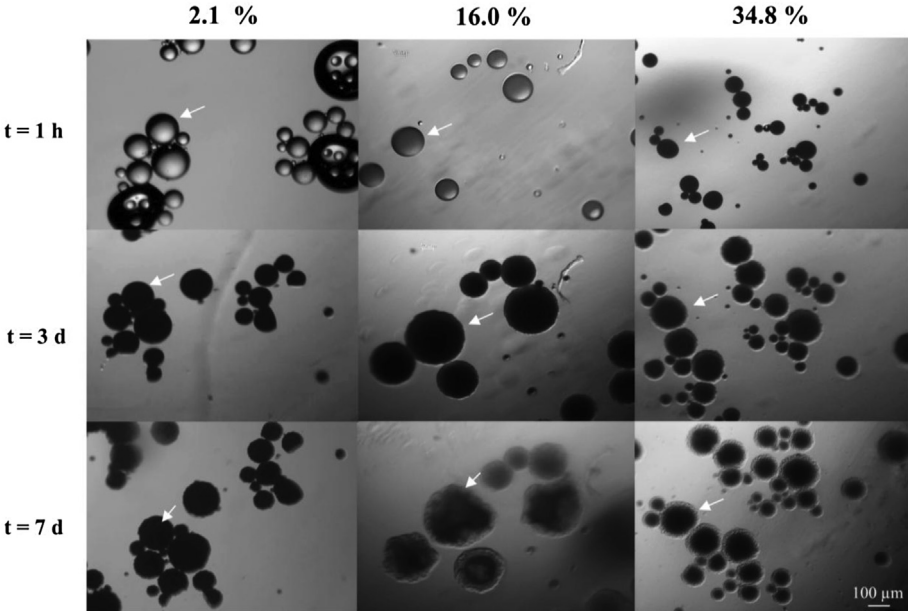

Fig. 7. Optical microscopy pictures of the investigated PLGA-based microparticles after 1 h, 3 d and 7 d exposure to phosphate buffer pH 7.4 (as indicated). The prilocaine loading (w:w) is given at the top. In each column, the arrows mark the same microparticle (observed at different time points).

microparticles are shown: A small (initially 50 μm), a medium-sized (initially 82 μm) and a large (initially 188 μm) microparticle. In addition, the mean values are indicated. Clearly, the microparticle size remained about constant until about 4.5 d. Then, significant swelling set on. Importantly, the onset time point of this remarkable microparticle swelling as well as the shape of the "swelling curves" did not depend on the microparticle size (at least in the investigated range). This is very interesting, since significant system swelling strongly alters the conditions for drug transport in the microparticles: The water content dramatically increases: For instance, a 50% increase in diameter corresponds to a 237.5% increase in microparticle volume. Such dramatic changes in the water contents of the systems can be expected to strongly affect drug mobility: Prilocaine is likely to become much more mobile. In addition, eventually non-dissolved drug (due to limited amounts of water in the microparticles prior to the onset of substantial microparticle swelling) can dissolve and becomes available for diffusion.

In order to evaluate the potential impact of the observed onset of substantial microparticle swelling on drug release, the swelling kinetics were plotted in the same diagrams as the prilocaine release kinetics: Figs. 9–11 show drug release and the dynamic changes in the size of PLGA microparticles loaded with 2.1, 4.6 and 6.5% prilocaine upon exposure to phosphate buffer pH 7.4. The filled diamonds (corresponding to the left y-axes) illustrate the drug release kinetics, whereas the open triangles (corresponding to the right y-axes) show the changes in the microparticles' diameter. Below each diagram two optical microscopy pictures are shown, which were taken after different exposure periods to the release medium (as indicated). Interestingly, in all cases the onset of substantial microparticle swelling is followed by the onset of the third (again rapid) drug release phase. This can be explained by the substantial increase in the water content of the microparticles, resulting in increased drug mobility (and eventually additional drug dissolution). Comparing Figs. 9–11, it becomes evident that the onset of substantial microparticle swelling is shifted towards earlier time points with increasing initial drug content. This can at least partially be attributed to the catalyzing effect of the basic drug prilocaine for PLGA degradation: As it can be seen in Fig. 2, the

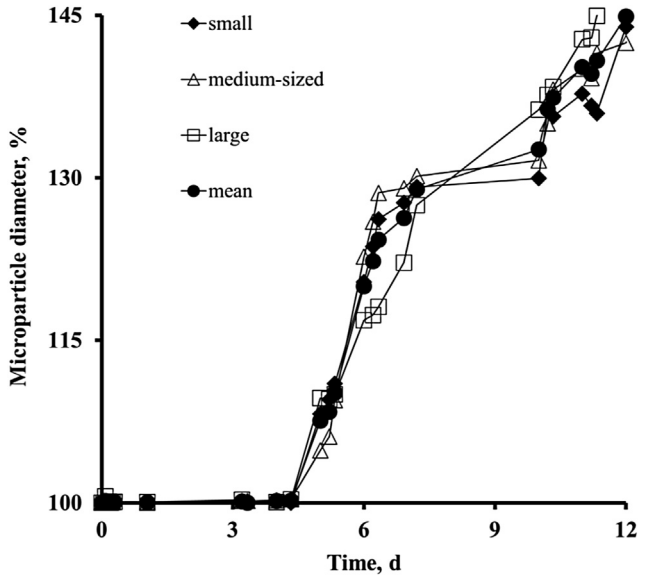

Fig. 8. Dynamic changes in the diameter of individual PLGA-based microparticles (measured by optical microscopy) upon exposure to phosphate buffer pH 7.4: A small (initially 50 μm), a medium-sized (initially 82 μm) and a large (initially 188 μm) microparticle were studied. Also the mean values are indicated. The prilocaine (free base) loading was 2.1% (w:w).

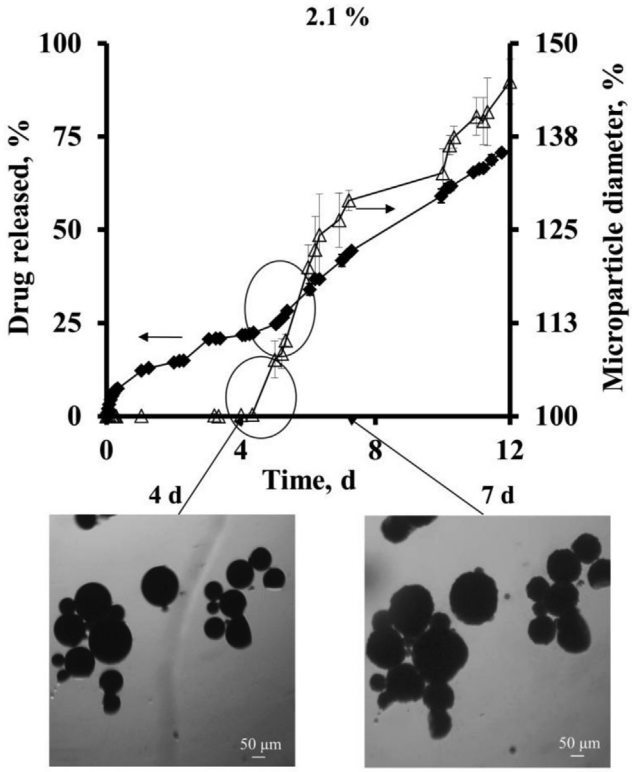

Fig. 9. Drug release from and swelling of PLGA microparticles loaded with 2.1% (w:w) prilocaine (free base) upon exposure to phosphate buffer pH 7.4 (upper diagram). Optical microscopy pictures of microparticles after 4 and 7 d exposure to the release medium (pictures at the bottom).

decrease in polymer molecular weight is more rapid at higher drug loadings. Interestingly, there seems to be some kind of critical Mw threshold value, at which substantial PLGA microparticle swelling starts: around 18–19 k Da: The red ellipses in Fig. 2 mark the time points at which microparticle swelling sets on. This threshold value is consistent with the one recently observed with ketoprofen-loaded PLGA-based microparticles [23]. Thus, this threshold value does not seem to depend on the basic or acidic nature of the drug. It seems that as soon as this critical polymer molecular weight is reached, the system becomes sufficiently hydrophilic to allow for the penetration of substantial amounts of water (longer PLGA chains are less hydrophilic than shorter PLGA chains, since the −COOH end groups are hydrophilic, whereas the polymer backbone is more hydrophobic). Also, the degree of polymer chain entanglement decreases with decreasing polymer molecular weight, resulting in weakened polymeric networks. Fig. 12 shows the drug release kinetics and swelling behavior of PLGA microparticles loaded with 23.3 or 34.8% prilocaine (free base). As it can be seen, at these initial drug loadings, substantial microparticle swelling occurs right from the beginning and different drug release phases are difficult to distinguish.

Thus, these results suggest that the mechanistic reason for the onset of the third drug release phase, which is often observed with PLGA-based microparticles, is likely to be substantial microparticle swelling. The latter starts as soon as a critical polymer molecular weight threshold value is reached.

4. Conclusion

This study provides further evidence that the onset of the often observed, third (and again rapid) drug release phase from PLGA

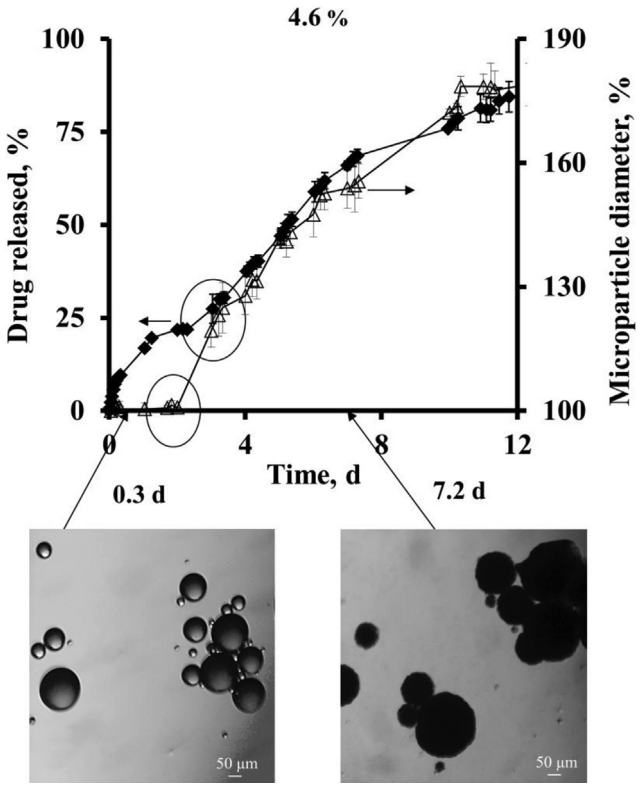

Fig. 10. Drug release from and swelling of PLGA microparticles loaded with 4.6% (w:w) prilocaine (free base) upon exposure to phosphate buffer pH 7.4 (upper diagram). Optical microscopy pictures of microparticles after 0.3 and 7.2 d exposure to the release medium (pictures at the bottom).

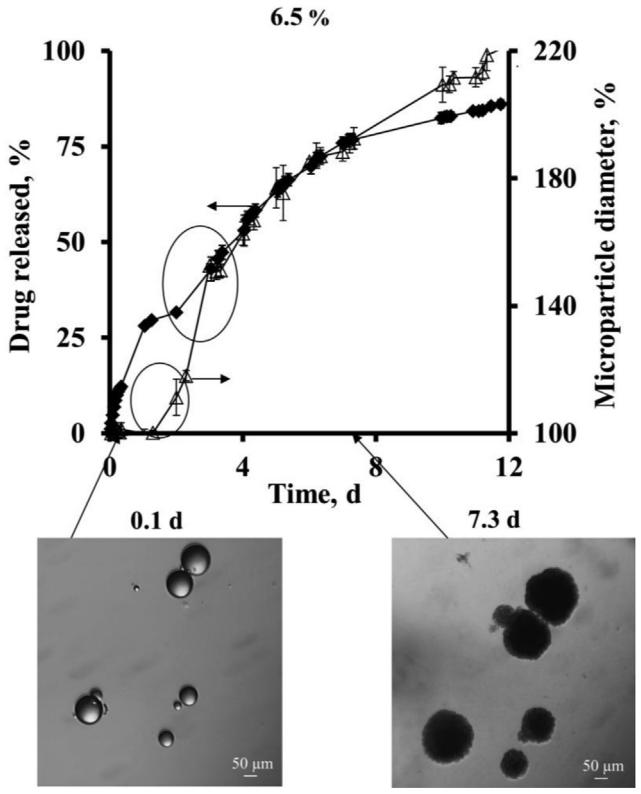

Fig. 11. Drug release from and swelling of PLGA microparticles loaded with 6.5% (w:w) prilocaine (free base) upon exposure to phosphate buffer pH 7.4 (upper diagram). Optical microscopy pictures of microparticles after 0.1 and 7.3 d exposure to the release medium (pictures at the bottom).

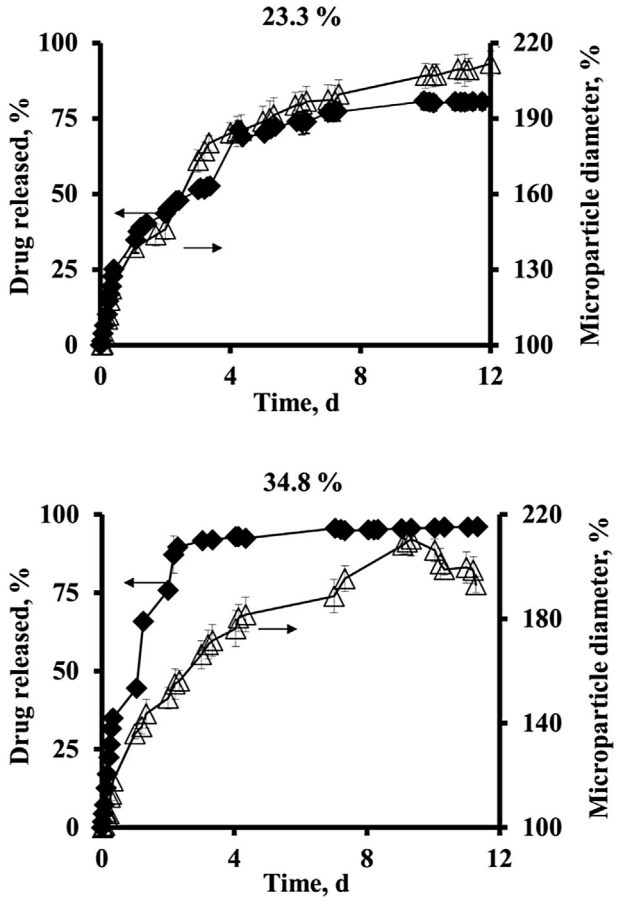

Fig. 12. Drug release from and swelling of PLGA microparticles loaded with 23.3 or 34.8% (w:w) prilocaine (as indicated) upon exposure to phosphate buffer pH 7.4.

microparticles is caused by system swelling: As soon as the polymer chains are sufficiently short (and, thus, sufficiently hydrophilic) and the polymer network sufficiently weak, important amounts of water penetrate into the system. This leads to strongly increased drug mobility (and potentially further drug dissolution). Recently, this type of behavior has been reported for the acidic drug ketoprofen. The present study shows that this release mechanism is likely to be valid also for the basic drug prilocaine. Note that both drugs are acting as plasticizers for PLGA. Thus, in the future it will be interesting to investigate drugs, which are neither acidic/basic, nor a plasticizer for PLGA.

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