



Unintended potential impact of perfect sink conditions on PLGA degradation in microparticles

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ABSTRACT

Yet, no standardized test method for drug release measurements from PLGA-based microparticles has been generally agreed on, or described by the regulatory authorities. Often, perfect sink conditions are provided *in vitro* to avoid artificial drug saturation effects. However, the maintenance of such conditions might strongly affect PLGA degradation. The involved physicochemical processes are complex and the potential impact of perfect sink conditions is not yet well understood. Differently sized, highly porous, carbamazepine- and ibuprofen-loaded PLGA microparticles were prepared by a W/O/W emulsion solvent extraction/evaporation technique. The initial drug loading was intentionally low (3–4%) so that the two drugs were molecularly dispersed within the polymeric matrices (monolithic solutions). This was important to be able to exclude potential limited drug solubility effects on the resulting release kinetics. Drug release into phosphate buffer pH 7.4 was measured under perfect sink conditions. SEC, DSC and SEM were used to characterize polymer degradation. The decrease in the average polymer molecular weight, glass transition temperature as well as changes in the inner and outer morphology of the PLGA microparticles were strongly affected by the bulk fluid's volume. In the case of the poorly water-soluble drug carbamazepine, much lower "microparticle mass:phosphate buffer volume" ratios were required to maintain perfect sink conditions, resulting in stable pH values within the bulk fluid, slower PLGA degradation and, thus, lower drug release rates. Thus, great care has to be taken when defining the conditions for *in vitro* drug release measurements from PLGA-based microparticles, avoiding potentially artificial conditions for polymer degradation.

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1. Introduction

Poly(lactic-co-glycolic acid) (PLGA)-based microparticles have received great interest in the last 2 decades, because they offer various advantages: (i) drug release can be controlled from days to months (Freiberg and Zhu, 2004); (ii) PLGA is biodegradable (Anderson and Shive, 1997), avoiding the removal of empty remnants; (iii) PLGA shows good biocompatibility (Middleton and Tipton, 2000), even with brain tissue (Fournier et al., 2003; Menei et al., 2004; Wu and Ding, 2004); and (iv) PLGA-based drug products have been approved by the FDA for human use. However, the design of this type of advanced drug delivery systems is not straightforward, because many factors influence the resulting drug release rate from PLGA microparticles, including the: preparation method (Sansdrap and Moës, 1993; O'Donnell and McGinity, 1997;

Mandal, 1998; Thote et al., 2005), particle size (Dunne et al., 2000; Berkland et al., 2003; Siepmann et al., 2005), system's porosity (Kang and Schwendeman, 2007; Klose et al., 2006), polymer molecular weight (Park, 1994; Ravivarapu et al., 2000), monomer ratio (lactic acid:glycolic acid) (Ravivarapu et al., 2000), and type of drug (Frank et al., 2005). Furthermore, the experimental method used to monitor drug release can be of importance (D'Souza and DeLuca, 2006), such as the type and concentrations of ions in the release medium (Faisant et al., 2006), degree of agitation and temperature of the bulk fluid the microparticles are exposed to (Bain et al., 1999; Shameem et al., 1999; D'Souza and DeLuca, 2005; Zolnik et al., 2006), type of apparatus used (Conti et al., 1995; Kostanski and DeLuca, 2000; Aubert-Pouëssel et al., 2002) and "microparticle mass:bulk fluid volume" ratio (Klose et al., 2010).

For conventional pharmaceutical dosage forms, the type of experimental setup to be used for *in vitro* drug release studies is generally specified in the Pharmacopoeia, e.g. USP or Ph.Eur. But this is not yet the case for controlled release microparticles (Burgess et al., 2002; Siewert et al., 2003; Burgess et al., 2004; Martinez et al., 2008, 2010). Generally, perfect sink conditions are provided during drug release measurements in order to avoid

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artificial saturation effects. The latter do often not occur *in vivo* due to drug distribution within the neighboring tissue and/or uptake into the systemic circulation. In this study, the term “perfect sink conditions” is understood as follows: the drug concentration in the release medium does not exceed 10% of the solubility of the drug in this medium under the given conditions at any time point. If the drug is poorly water-soluble, this means that low amounts of PLGA microparticles must be exposed to high amounts of release medium. Importantly, more and more new chemical entities exhibit poor aqueous solubility (Tyrchan et al., 2009). Thus, the number of poorly water-soluble drugs to be incorporated into PLGA-based microparticles, offering the benefits of controlled parenteral delivery, can be expected to increase.

PLGA microparticles are known to undergo bulk erosion: water penetration into the systems upon contact with aqueous body fluids is rapid compared to polymer hydrolysis. Thus, the entire particles become wetted and PLGA degradation occurs throughout the systems. Due to concentration gradients, the degradation products, e.g. shorter chain acids, subsequently diffuse out of the microparticles into the surrounding bulk fluid. In addition, bases from the release medium diffuse into the microparticles. However, diffusional mass transport is relatively slow and the rate at which the acids are generated by PLGA hydrolysis might be significantly higher than the rate at which they are neutralized. This can lead to pronounced drops in the micro-pH within the systems (Brunner et al., 1999; Fu et al., 2000; Li and Schwendeman, 2005). Furthermore, the pH of the bulk fluid can significantly decrease (Grizzi et al., 1995; Sah and Chien, 1995; Fu et al., 2000; Klose et al., 2010). As the hydrolysis of an ester is catalyzed by acids, the subsequent degradation of PLGA is accelerated. For these reasons it is possible that the maintenance of perfect sink conditions potentially strongly affects the resulting polymer degradation and, thus, drug release patterns from PLGA microparticles. However, yet this phenomenon has not been studied in detail and its practical relevance is poorly understood.

The aim of the present study was to prepare PLGA microparticles containing the poorly water-soluble drug carbamazepine and – for reasons of comparison – ibuprofen (solubility in phosphate buffer pH 7.4:0.2 versus 7.5 mg/mL, respectively) (Zhou et al., 2008; Klose et al., 2010). Both drugs exhibit similar molecular weights: carbamazepine 236 Da and ibuprofen 206 Da. Drug release was measured under perfect sink conditions and the systems were characterized using differential scanning calorimetry, size exclusion chromatography and scanning electron microscopy in order to get deeper insight into the underlying mass transport mechanisms.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA, Resomer RG 504H, PLGA 50:50; Boehringer Ingelheim, Ingelheim, Germany), carbamazepine (BASF, Ludwigshafen, Germany), ibuprofen (free acid; Novartis, Barleben, Germany), acetonitrile, chloroform and dichloromethane (Rotisol HPLC; Carl Roth, Karlsruhe, Germany), and polyvinyl alcohol (Mowiol 4-88; Kuraray, Frankfurt, Germany).

2.2. Microparticle preparation

Carbamazepine- and ibuprofen-loaded, PLGA-based microparticles were prepared using a water-in-oil-in-water (W/O/W) emulsion solvent extraction/evaporation technique: 46 mg of drug was dissolved in 9 g of dichloromethane. 1 g of PLGA was added to this solution, which was shaken at room temperature to allow for complete polymer dissolution. Then, 0.5 mL of demineralized

water was emulsified within this solution using an Ultra-Thurax (60 s, 20,000 rpm, T25 basic; IKA, Staufen, Germany). This primary water-in-oil (W/O) emulsion was dispersed into 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25% w/w) under stirring with a three-blade propeller for 30 min (2000 rpm). Upon solvent extraction/evaporation the microparticles formed. They were hardened by subsequent addition of 2.5 L of outer aqueous phase and 4 h gentle stirring (700 rpm). The particles were separated by filtration and subsequently freeze-dried to minimize the residual solvents' content. Different size fractions were obtained by wet sieving prior to freeze-drying (average pore size of the sieves: 200, 125, 100, 80, 63, 50 and 40 μm ; Retsch, Haan, Germany).

2.3. Particle size analysis

Mean particle diameters were determined with a microscope (Axioskop; Carl Zeiss, Jena, Germany), equipped with an optical imaging system (EasyMeasure; INTEQ, Berlin, Germany). Each measurement included 200 particles.

2.4. Determination of the practical drug loading

The initial, practical drug loading was determined by dissolving accurately weighed amounts of microparticles (approximately 25 mg) in 5/50 mL (in the case of ibuprofen/carbamazepine) acetonitrile and subsequent UV drug detection ($\lambda_{\text{ibuprofen}} = 264 \text{ nm}$, $\lambda_{\text{carbamazepine}} = 286 \text{ nm}$, Anthelie Advanced; Secomam, Domont, France). Each experiment was conducted in triplicate.

2.5. In vitro drug release studies

To provide perfect sink-conditions in all cases, 400 mg of ibuprofen-loaded microparticles were placed in 40 mL glass bottles filled with 40 mL phosphate buffer pH 7.4 (USP 32), whereas 50 mg of carbamazepine-loaded microparticles were placed in 100 mL glass bottles filled with 100 mL phosphate buffer pH 7.4. The bottles were horizontally shaken at 37 °C (80 rpm, GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At pre-determined time points, 1 mL samples were withdrawn (replaced with fresh medium) and analyzed UV-spectrophotometrically ($\lambda_{\text{ibuprofen}} = 264 \text{ nm}$, $\lambda_{\text{carbamazepine}} = 286 \text{ nm}$, Anthelie Advanced). Each experiment was conducted in triplicate. The pH of the bulk fluids was measured using a pH meter (InoLab pH Level 1, WTW, Weilheim, Germany; $n = 1$).

2.6. Monitoring of changes in the physicochemical properties of the microparticles upon exposure to the release medium

Microparticles were treated as described in Section 2.5. At pre-determined time points, microparticles were separated by filtration (0.45 μm) from the release medium, freeze-dried and stored at 4 °C for further analysis.

The average polymer molecular weight of PLGA was determined by size exclusion chromatography (SEC). Microparticles were dissolved in chloroform (2% w/w). Fifty μL of this solution were injected into a SEC apparatus (SCL-10A; Shimadzu, Tokyo, Japan) [column: PLgel 5 μm MIXED-D, 7.5 mm \times 300 mm (Polymer Laboratories, Church Stretton, UK); mobile phase: chloroform containing 0.1% (w/w) triethanolamin; flow rate: 1 mL/min; column temperature: 40 °C; detector: refractometer]. All indicated molecular weights are weight-average molecular weights (M_w), calculated using the Cirrus GPC software (Polymer Laboratories) and polystyrene standards (580–299,400 Da; Polymer Laboratories; $n = 1$).

The glass transition temperature of the polymer (T_g) was determined by differential scanning calorimetry (DSC, DSC821e; Mettler

Toledo, Giessen, Germany; $n = 1$). Approximately 10 mg samples were heated in sealed aluminum pans (investigated temperature range: -10 to $+80$ °C, heating rate: 5 °C/min).

Scanning electron microscopy (SEM) was used to characterize the *internal and external morphology* of the microparticles (S-4000; Hitachi High-Technologies Europe, Krefeld, Germany). Samples were covered under an argon atmosphere with a fine gold layer (10 nm; SCD 040; Bal-tec, Witten, Germany). Cross-sections of the microparticles were obtained after inclusion into water-based glue and cutting with a razor blade.

3. Results and discussion

3.1. Microparticle morphology, drug loading and size

Highly porous, spherical microparticles of different size loaded with carbamazepine or ibuprofen were obtained. Figs. 1 and 2 show examples of SEM pictures of surfaces and cross-sections of small, medium-sized and large microparticles. The high initial porosity can be attributed to the preparation method (W/O/W solvent evaporation technique). Importantly, autocatalytic effects can, thus, be expected to be less pronounced compared to initially non-porous, PLGA-based microparticles (Klose et al., 2006). Despite the different solubility of carbamazepine and ibuprofen, both drugs were successfully incorporated in the microparticles using the same preparation technique. The encapsulation efficiency was 65% for carbamazepine-loaded systems and 87% for ibuprofen-loaded microparticles, respectively. The practical drug loading was intentionally low ($3.0 \pm 0.1\%$ for carbamazepine and $4.0 \pm 0.1\%$ for ibuprofen), assuring that both drugs were molecularly dispersed within the polymeric matrices. Such systems are also called “monolithic solutions”. Consequently, limited drug solubility effects are highly unlikely to play a major role for the control

of drug release from the investigated microparticles. No drug melting peaks were visible in the DSC diagrams (data not shown), and no evidence for drug crystals or amorphous aggregates could be seen on the SEM pictures (e.g., Figs. 1 and 2). The mean diameters of the microparticles were 27, 64, 89, 107, 141 μm in the case of carbamazepine-loaded systems, and 24, 52, 79, 113, 143 μm in the case of ibuprofen-loaded devices, respectively.

3.2. In vitro drug release

The solubility of carbamazepine and ibuprofen in phosphate buffer pH 7.4 (37 °C) is equal to 0.2 and 7.5 mg/mL, respectively (Zhou et al., 2008; Klose et al., 2010). To provide perfect sink conditions during drug release in both cases, 400 mg of ibuprofen-loaded microparticles were exposed to 40 mL release medium ($\rightarrow 10$ mg/mL), whereas 50 mg of carbamazepine-loaded microparticles were exposed to 100 mL of this bulk fluid ($\rightarrow 0.5$ mg/mL). Fig. 3 shows the experimentally measured release kinetics of carbamazepine and ibuprofen from the differently sized microparticles. Clearly, drug release was much faster in the case of ibuprofen (Fig. 3b) compared to carbamazepine (Fig. 3a) (note the different scaling of the x-axes), irrespective of the microparticle diameter. Importantly, this phenomenon cannot be attributed to the different water-solubility of the two compounds, since both are dissolved in the polymeric matrix (as discussed above). Thus, no carbamazepine or ibuprofen dissolution step is involved in the control of drug release. Furthermore, the drug release rate monotonically decreased with increasing microparticle size, irrespective of the type of drug. This is in good agreement with data reported in the literature on initially highly porous, PLGA microparticles (Klose et al., 2006). The trend indicates that potential autocatalytic effects do not (completely) compensate the increase in the length of the diffusion pathways with increasing microparticle size, as it can be

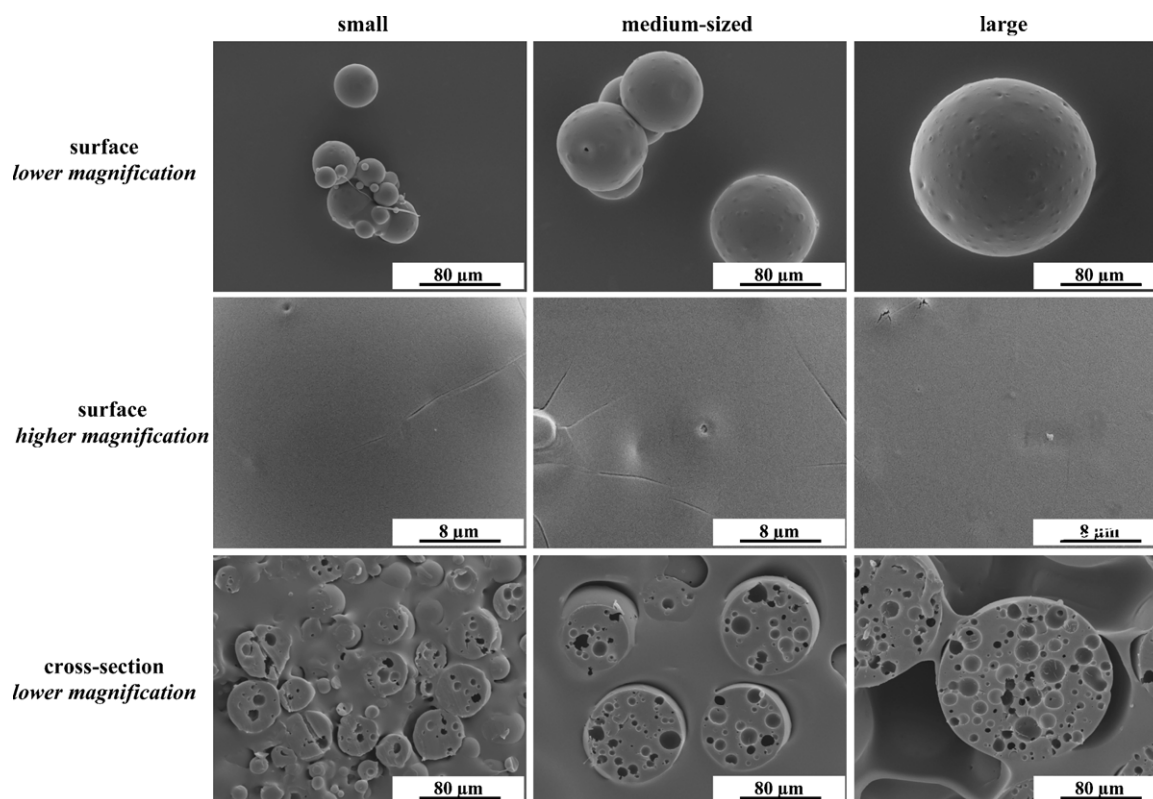


Fig. 1. Morphology of small, medium-sized and large carbamazepine-loaded, PLGA-based microparticles before exposure to phosphate buffer pH 7.4 ($t = 0$ d): SEM pictures of surfaces and cross-sections (note the different magnifications/scale bars).

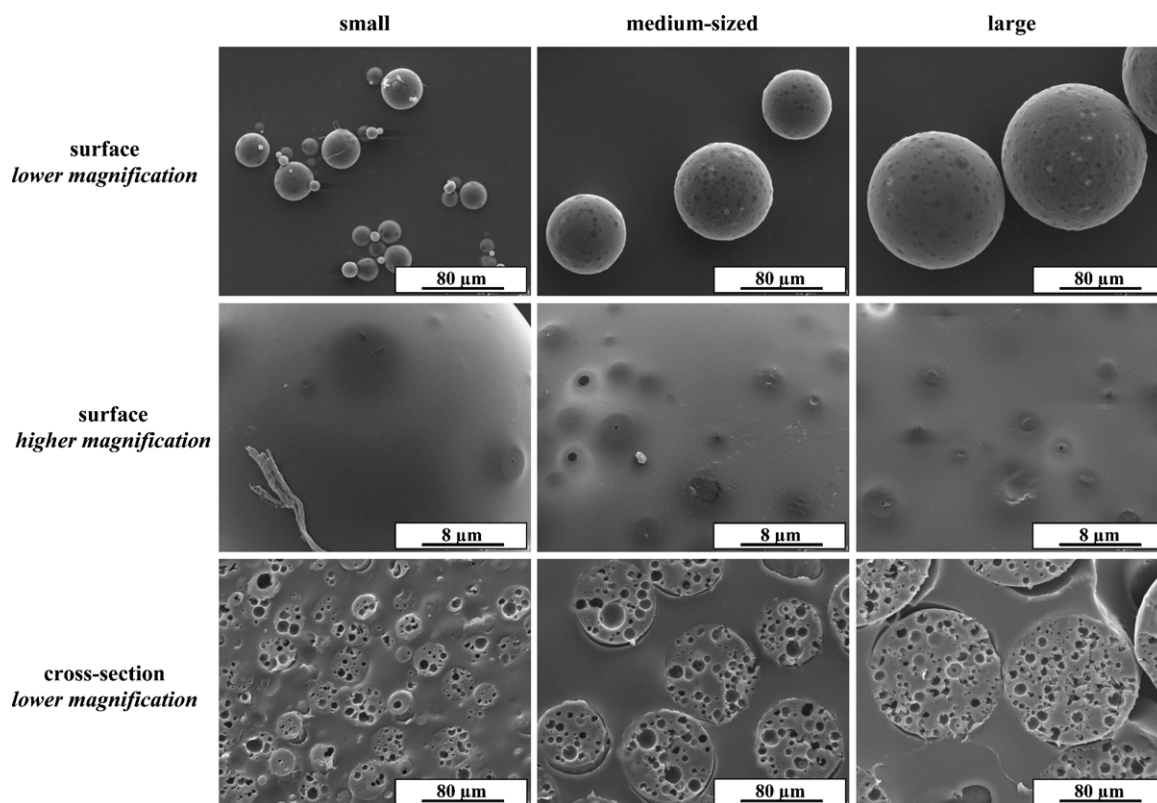


Fig. 2. Morphology of small, medium-sized and large ibuprofen-loaded, PLGA-based microparticles before exposure to phosphate buffer pH 7.4 ($t=0$ d): SEM pictures of surfaces and cross-sections (note the different magnifications/scale bars).

the case with initially non-porous, PLGA microparticles (Siepmann et al., 2005). The fact that ibuprofen release was much faster than carbamazepine release might at least partially be attributed to: (i) differences in drug-polymer interactions (Klose et al., 2008), (ii) differences in the molecular weight of the drugs. However, this effect is likely to be limited: carbamazepine: 236 Da, ibuprofen: 206 Da; and (iii) the fact that ibuprofen is an acid, whereas carbamazepine is neutral: it is well known that PLGA degradation is catalyzed by protons. However, as the drug loading is very low, the importance of this effect can be expected to be limited. It has recently been shown that: (1) PLGA degradation in initially non-porous microparticles loaded with 4% ibuprofen was similar to that in initially non-porous microparticles loaded with 4% lidocaine (Klose et al., 2008), and that (2) polymer degradation in initially porous PLGA microparticles loaded with 4% lidocaine was not much faster than in initially porous, drug-free microparticles (Klose et al., 2006). Thus, the “drug acidity effect” is unlikely to fully explain the observed differences in the drug release rates shown in Fig. 3 (carbamazepine versus ibuprofen). In addition to these three potential reasons, also the fact that carbamazepine-loaded microparticles were exposed to higher bulk fluid volumes than ibuprofen-loaded systems (or more precisely that the “microparticle mass:release medium volume” ratio was much lower in the case of carbamazepine-loaded microparticles) may play a role. In practice, the volume of the bulk fluid is often increased or the microparticle mass decreased, if the water-solubility of the drug is low, in order to provide perfect sink conditions. However, this environmental parameter might potentially affect the physicochemical phenomena involved in the control of drug release from this type of dosage forms to a significant extent. This is generally ignored. In order to better understand the importance of such “perfect sink conditions” effects, the degradation behavior of the PLGA microparticles was studied using SEC, DSC and SEM.

3.3. Microparticle degradation

Fig. 4 shows the experimentally measured decrease in the average molecular weight of PLGA (symbols) in the investigated microparticles upon exposure to phosphate buffer pH 7.4. Clearly, the hydrolysis of the polyester was more rapid in ibuprofen-loaded microparticles (Fig. 4b) compared to carbamazepine-loaded systems (Fig. 4a). This might at least partially be attributed to the acidic nature of ibuprofen: Ester hydrolysis is known to be catalyzed by protons. In contrast, carbamazepine is neutral and its presence is unlikely to accelerate PLGA degradation. However, as discussed above, the initial ibuprofen loading is very low (4%) and the “acidity effect” is likely to be limited.

To quantitatively describe the polymer degradation kinetics measured by SEC, the following (pseudo-) first order equation was fitted to the experimental results (Kenley et al., 1987):

$$M_w(t) = M_{w0} \cdot \exp(-k_{\text{degr}} \cdot t) \quad (1)$$

where $M_w(t)$ and M_{w0} are the average polymer molecular weight at time t and $t=0$ (before exposure to the release medium), respectively; k_{degr} denotes the apparent degradation rate constant of the polymer. As it can be seen in Fig. 4, good agreement between theory (curves) and experiments (symbols) was obtained in all cases, irrespective of the type of drug and microparticle size. Based on these fittings, the apparent degradation rate constant of PLGA in the investigated microparticles upon exposure to phosphate buffer pH 7.4 could be determined (Table 1). As it can be seen, for both types of drugs the degradation rate constant moderately increased with increasing microparticle size. This indicates that autocatalytic effects are of importance, despite the high initial porosity of the systems: With increasing microparticle dimension, the length of the diffusion pathways for short chain acids (generated upon PLGA degradation and to be released into the surrounding bulk fluid)

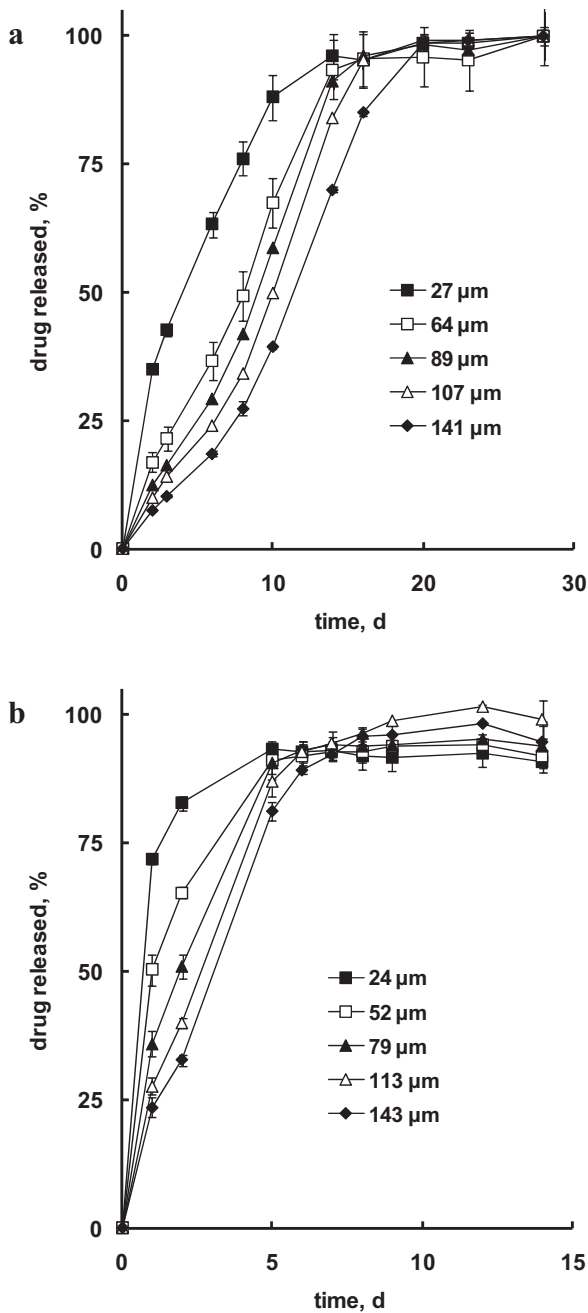


Fig. 3. Drug release from differently sized PLGA-based microparticles (diameters are indicated in the diagram) in phosphate buffer pH 7.4: (a) carbamazepine-loaded systems and (b) ibuprofen-loaded microparticles (note the different scaling of the x-axes).

Table 1

Apparent degradation rate constant of PLGA (k , week⁻¹) in initially porous microparticles containing 4% ibuprofen or 3% carbamazepine upon exposure to phosphate buffer pH 7.4 as a function of the particle size.

Carbamazepine-loaded microparticles					
Mean size, μm	27	–	89	–	141
k , week ⁻¹	0.35	–	0.40	–	0.44
Ibuprofen-loaded microparticles					
Mean size, μm	24	52	79	113	143
k , week ⁻¹	0.74	0.78	0.79	0.82	0.83

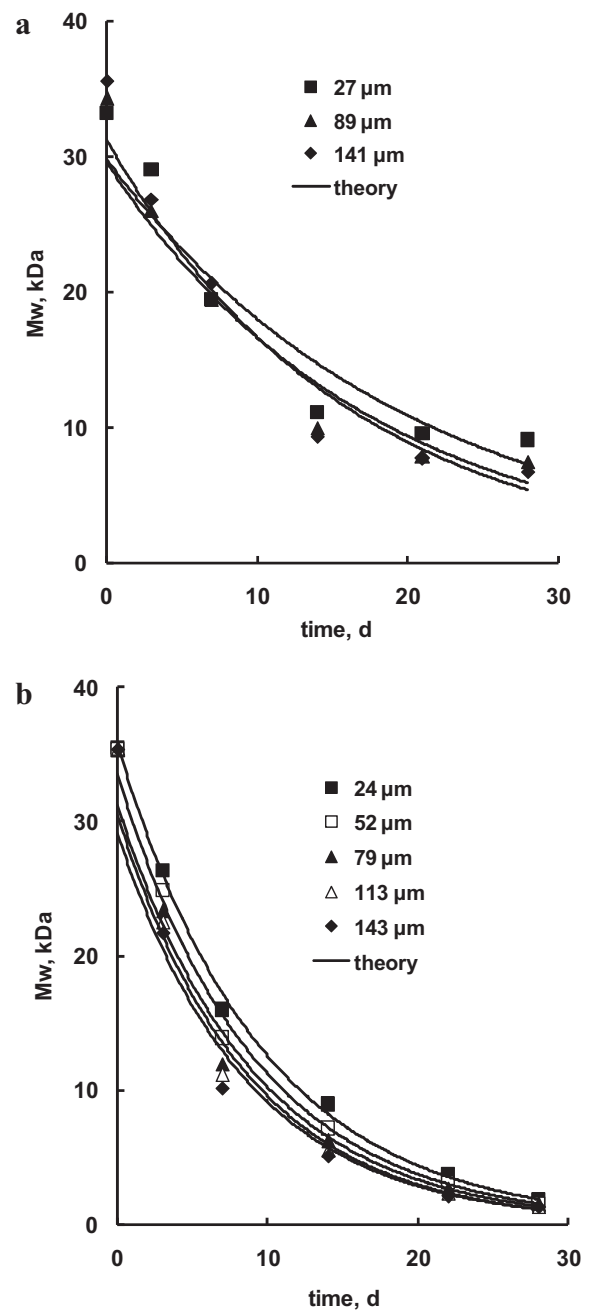


Fig. 4. Decrease in the polymer molecular weight (M_w , measured by SEC) of PLGA in microparticles of different size (diameters are indicated in the diagrams) upon exposure to phosphate buffer pH 7.4: (a) carbamazepine-loaded devices, and (b) ibuprofen-loaded systems. The symbols represent the experimentally determined values, the curves the fitted theory (Eq. (1)).

increases. In addition, the length of the diffusion pathways for bases penetrating from the release medium into the microparticles increases. Consequently, the diffusion rates of these acids and bases decrease, resulting in decreased acid neutralization rates and, thus, more pronounced drops in the micro pH within the dosage forms (Siepmann et al., 2005). As polyester hydrolysis is catalyzed by protons, this leads to accelerated PLGA degradation. Comparing the apparent degradation rate constants of PLGA in carbamazepine- and ibuprofen-loaded microparticles (Table 1), it becomes obvious that polymer degradation is about twice as rapid in ibuprofen-loaded systems. As discussed above, this can partially be attributed to the acidic nature of ibuprofen. However, as the drug loading was very low and for the above-explained reasons, it is unlikely that

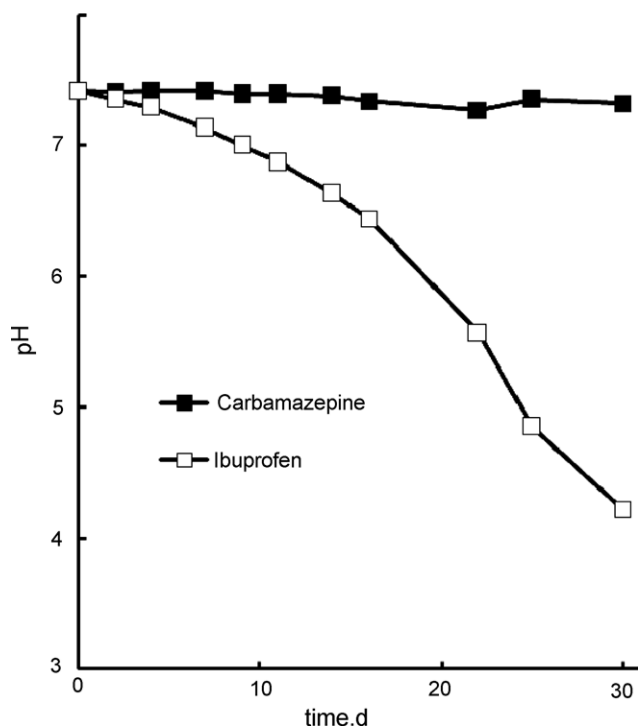


Fig. 5. Changes in the pH of the release medium during drug release measurements from carbamazepine-loaded (filled circles) and ibuprofen-loaded (open circles), medium-sized microparticles. In both cases, perfect sink conditions were provided throughout the experiments.

this phenomenon can fully explain the difference in the polymer degradation rate.

Another parameter is likely to be of significance: the volume of the release medium the microparticles are exposed to, or more precisely the “microparticle mass:bulk fluid volume” ratio. In the case of carbamazepine this ratio is much lower than in the case of ibuprofen. Thus, the ability of the phosphate buffer to neutralize the shorter chain acids generated upon PLGA degradation and released into the bulk fluid is much higher. Hence, it can be expected that the pH of the bulk fluid surrounding the microparticles is higher in the case of carbamazepine-loaded microparticles compared to ibuprofen-loaded microparticles (and this not only due to the acidity of the latter drug). Fig. 5 shows that this phenomenon was indeed observed experimentally: the pH of the bulk fluid significantly decreased in the case of ibuprofen-loaded microparticles, whereas it remained about constant in the case of carbamazepine-loaded systems.

These observations were further confirmed by DSC measurements: the experimentally determined changes in the glass transition temperature of PLGA (T_g) in the differently sized microparticles loaded with carbamazepine or ibuprofen upon exposure to phosphate buffer pH 7.4 are shown in Fig. 6. Clearly, the T_g remained about constant during the observation period in the case of carbamazepine-loaded systems, because this drug is neutral and the bulk fluid volume considerable with respect to the microparticle mass. In contrast, the glass transition temperature significantly decreased in the case of ibuprofen-loaded microparticles. This leads to significantly increased polymer chain mobility and, thus, increased drug mobility. Interestingly, the T_g decreased more rapidly in larger microparticles than in smaller ones, which is in good agreement with the above discussed (moderate) impact of autocatalytic effects in the investigated systems.

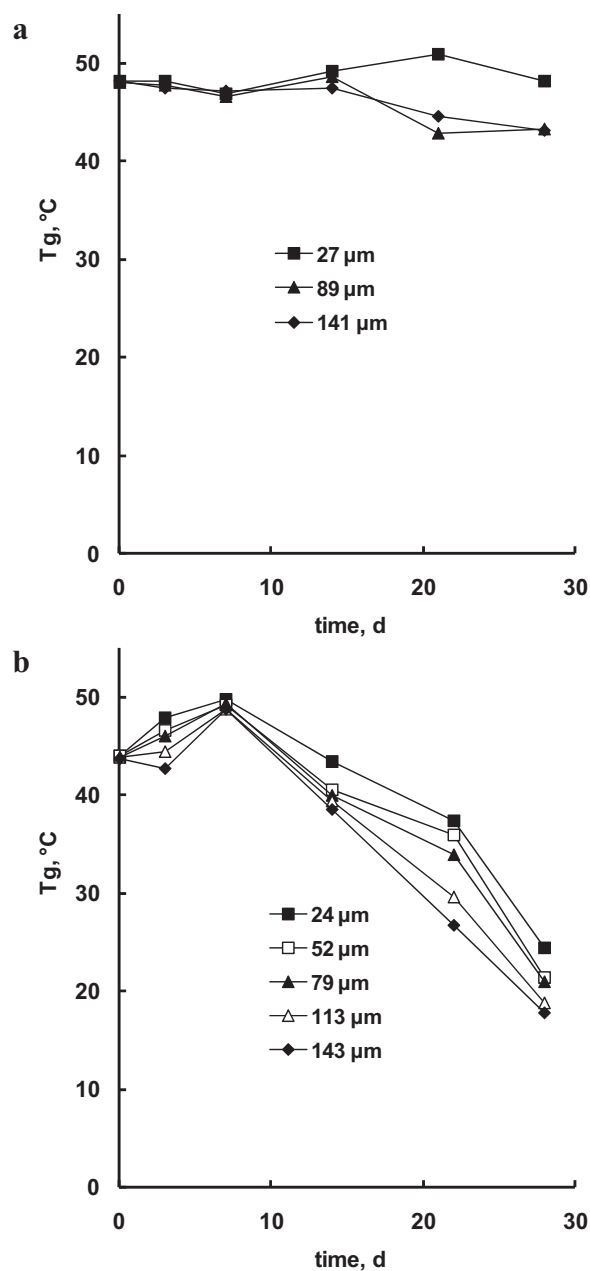


Fig. 6. Changes in the glass transition temperature (T_g) of PLGA in microparticles of different size (diameters are indicated in the diagrams), loaded with: (a) carbamazepine, or (b) ibuprofen upon exposure to phosphate buffer pH 7.4.

The importance of the bulk fluid volume (or more precisely “microparticle mass:release medium volume” ratio) and of the type of drug on the microparticle degradation was further confirmed by scanning electron microscopy: Fig. 7 shows for example surfaces and cross-sections of small, medium-sized and large carbamazepine-loaded microparticles after 14 d exposure to phosphate buffer pH 7.4 at different magnifications. Clearly, the microparticles became much more porous compared to their initial state (before exposure to the release medium, Fig. 1): internally as well as externally. This is due to the hydrolytic degradation of the polymer (and to a minor extent to drug leaching). Importantly, it was not possible to obtain such SEM pictures of cross sections of ibuprofen-loaded microparticles, because polymer degradation was too advanced at this time point.

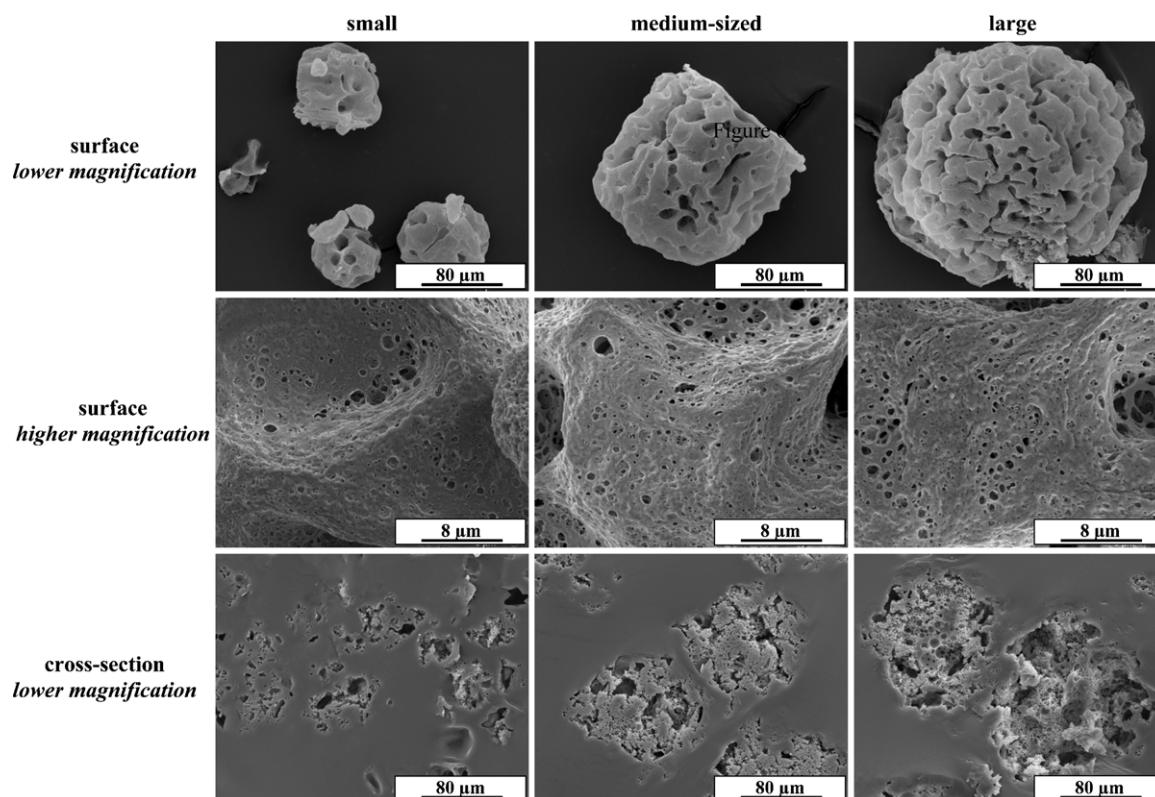


Fig. 7. Morphology of small, medium-sized and large carbamazepine-loaded, PLGA-based microparticles after 14 d exposure to phosphate buffer pH 7.4: SEM pictures of surfaces (lower and higher magnifications) and cross-sections (lower magnifications) (note the different magnifications/scale bars).

4. Conclusions

When measuring drug release from a controlled delivery system, generally perfect sink conditions are provided in order to avoid that drug accumulated in the bulk fluid slows down further release. This is generally justified, because drug released *in vivo* is often rapidly eliminated from the direct vicinity of the dosage form. However, in the case of PLGA-based drug delivery systems the use of high amounts of bulk fluid required to maintain perfect sink conditions for poorly water-soluble drugs can have a significant, unintended impact: The degradation of the release rate controlling matrix former can be altered, resulting in eventually unrealistic conditions for drug release and artificial release patterns. Thus, great care must be taken when defining the experimental conditions for the measurement of *in vitro* drug release from PLGA-based dosage forms. Comprehensive *in vivo* results monitoring PLGA degradation at the injection site are highly desirable in the near future to allow for the establishment of reasonable conditions for drug release measurements *in vitro*.

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