International Journal of Pharmaceutics 383 (2010) 123–131

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Drug release from PLGA-based microparticles: Effects of the "microparticle:bulk fluid" ratio

D. Klose[a,b], F. Siepmann[a], J.F. Willart[c], M. Descamps[c], J. Siepmann[a,*]

[a] College of Pharmacy, JE 2491, Univ. Lille Nord de France, 3 Rue du Prof. Laguesse, 59006 Lille, France
[b] College of Pharmacy, Freie Universitaet Berlin, Kelchstr. 31, 12169 Berlin, Germany
[c] Laboratoire de Dynamique et Structure des Matériaux Moléculaires, UMR CNRS 8024, Univ. Lille Nord de France, 59655 Villeneuve d'Ascq, France

ARTICLE INFO

Article history:
Received 30 June 2009
Received in revised form 2 September 2009
Accepted 7 September 2009
Available online 11 September 2009

Keywords:
PLGA
Microparticle
Controlled release
In vitro release
Release conditions

ABSTRACT

The aim of this study was to better understand the importance of the "microparticle mass:bulk fluid volume" ratio during *in vitro* drug release measurements from PLGA microparticles. Initially porous/non-porous, ibuprofen/lidocaine/propranolol HCl-loaded systems were exposed to phosphate buffer pH 7.4 in agitated test tubes, varying the microparticle concentration from 5:1 to 20:1 mg:mL. Interestingly, drug release was virtually unaffected by the "microparticle mass:bulk fluid volume" ratio in the case of initially porous, ibuprofen-loaded microparticles, exhibiting complete drug release within about 1 week. Optical microscopy, SEM, DSC and pH measurements of the bulk fluid revealed no major impact of the microparticle concentration on the systems' properties within the first couple of days. However, a more rapid and pronounced decrease in the pH of the release medium occurred after 10–14 d at elevated "microparticle mass:bulk fluid volume" ratios. This resulted in an accelerated: (i) decrease in the glass transition temperature, (ii) microparticle agglomeration, and (iii) increase in the internal and external microparticle porosity. Importantly, this phenomenon did not significantly affect drug release from initially porous, lidocaine-loaded microparticles, exhibiting complete release within about 18 d. In contrast, drug release became significantly faster at higher "microparticle mass:bulk fluid volume" ratios in the case of initially non-porous, lidocaine-loaded microparticles and initially porous, propranolol HCl-loaded systems, exhibiting complete release after 1 and 2 months, respectively. Thus, depending on the type of system, the "microparticle mass:bulk fluid volume" ratio may or may not affect the observed release kinetics *in vitro*. This should be carefully taken into account when defining the experimental conditions for drug release measurements from this type of advanced drug delivery systems.

1. Introduction

In the last decades, poly(lactic-co-glycolic acid) (PLGA) has received great interest in the medical and pharmaceutical field because of its biodegradability, toxicological safety and good biocompatibility (Ignatius and Cleas, 1996; Anderson and Shive, 1997; Fournier et al., 2003; Menei et al., 2004). It is one of the few synthetic polymers, which have been approved for human clinical use. Nowadays, PLGA is widely applied in parenteral controlled drug delivery systems, including nano- and microparticles (Giteau et al., 2008) as well as implants (Guse et al., 2006). Several products are available on the market based on PLGA microparticles, such as "Lupron Depot", containing the anticancer drug leuprolide acetate. The great success of PLGA-based microparticles as controlled drug delivery systems can be attributed to the various advantages this type of dosage forms offer, including: (i) the possibility to control the release rate during periods, which can range from a few days up to several months, (ii) relatively easy administration using standard needles and syringes (compared to the surgical insertion of implants), (iii) complete biodegradability, and (iv) good biocompatibility, even with brain tissue (Menei et al., 1993). Several formulation parameters can be varied in order to adjust desired drug release patterns from PLGA-based microparticles, including the polymer molecular weight (Ravivarapu et al., 2000a), microparticle size (Berkland et al., 2003; Siepmann et al., 2005), microparticle porosity (Kang and Schwendeman, 2007; Klose et al., 2006), and drug loading (Ravivarapu et al., 2000b).

However, despite of the steadily increasing practical importance of PLGA-based microparticles as parenteral controlled drug delivery systems, yet little is known about the importance of the experimental conditions used for *in vitro* drug release measurements and the underlying mass transport mechanisms controlling drug release from this type of dosage forms (Shameem et al., 1999; Siepmann and Goepferich, 2001; D'Souza and DeLuca, 2006;

* Corresponding author. Tel.: +33 3 20964708; fax: +33 3 20964942.
E-mail address: juergen.siepmann@univ-lille2.fr (J. Siepmann).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.09.012

Siepmann and Siepmann, 2008). So far, no regulatory guidelines are available defining experimental conditions, although the need for such standards has been pointed out in workshops initiated by the FDA and USP (Burgess et al., 2002) as well as by the EUFEPS (Burgess et al., 2004). In practice, very different experimental conditions are used (Conti et al., 1995; Bain et al., 1999; Aubert-Pouëssel et al., 2002; D'Souza and DeLuca, 2005a) and great caution has to be paid when comparing the obtained results. In particular, the temperature and pH of the release medium have been reported to strongly affect the resulting drug release kinetics (D'Souza et al., 2005b; Faisant et al., 2006). The use of elevated temperatures might be useful for quality controls and to a certain extent during the development phase. However, care needs to be taken, because the underlying drug release mechanisms might be altered.

Interestingly, potential effects of the ratio "microparticle mass exposed to the release medium:volume of the release medium" are generally neglected. This is surprising, because PLGA is degraded into shorter chain acids, which are known to diffuse into the surrounding bulk fluid. Depending on the relative amount of microparticles and volume of the surrounding buffer, it can be expected that the pH of the release medium more or less rapidly drops. Such a drop in the pH of the bulk fluid can be expected to alter subsequent polymer degradation, because PLGA hydrolysis is known to be catalyzed by protons. As the average polymer molecular weight affects drug mobility within the microparticles, the kinetics observed *in vitro* might significantly depend on the chosen "microparticle mass:bulk fluid volume" ratio. It was the aim of this study to better understand the importance of such potential effects, using several types of microparticles releasing different drugs during a large range of release periods. Ibuprofen, lidocaine and propranolol HCl were chosen as model drugs and incorporated into initially porous as well as in initially non-porous PLGA-based microparticles. Three different "microparticle mass:bulk fluid volume" ratios were investigated and changes in the physicochemical properties of the microparticles during drug release measured using optical and scanning electron microscopy, DSC analysis, particle size, drug release and drug loading measurements. If possible, appropriate mathematical theories were used to elucidate the underlying drug release 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), lidocaine (free base; Sigma–Aldrich, Steinheim, Germany), ibuprofen and propranolol HCl (Salutas, Barleben, Germany), acetonitrile and dichloromethane (VWR, Fontenoy-sous-Bois, France), and polyvinyl alcohol (Mowiol 4-88; Kuraray, Frankfurt, Germany).

2.2. Microparticle preparation

Porous, ibuprofen-loaded and lidocaine-loaded, PLGA-based microparticles were prepared using a water-in-oil-in-water (W/O/W) solvent extraction/evaporation technique: 2 g of PLGA were dissolved within 18 g of dichloromethane. Either 90 mg of ibuprofen or lidocaine were added to this solution, which was shaken at room temperature to allow for complete dissolution. Two mL of demineralized water were emulsified into this solution using an Ultra-Thurrax (90 s, 20,000 rpm, T25 basic; IKA-Werke, 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.

Non-porous, lidocaine-loaded, PLGA-based microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: 2 g of PLGA were dissolved within 18 g of dichloromethane. Ninety mg of lidocaine were added to this solution, which was shaken at room temperature to allow for complete dissolution. This organic solution 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.

Porous, propranolol HCl-loaded, PLGA-based microparticles were prepared using a water-in-oil-in-water (W/O/W) solvent extraction/evaporation technique: 2 g of PLGA were dissolved within 18 g of dichloromethane. Two mL of aqueous propranolol HCl solution (25%, w/v) were emulsified into the organic phase using an Ultra-Thurrax (90 s, 20,000 rpm, T25 basic). 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.

In all cases, the microparticles were hardened by the subsequent addition of 2.5 L of further outer aqueous phase and 4 h gentle stirring (700 rpm). The particles were then separated by filtration and subsequently freeze-dried to minimize the residual solvents' content. Very small (<50 μm) and large particles (>160 μm) were excluded by sieving (average pore size of the sieves: 50 and 160 μm; Retsch, Haan, Germany).

2.3. Particle size analysis

Particle size distributions and mean diameters of the complete batch were determined by laser diffractometry (Malvern Mastersizer S, Malvern, Orsay, France) using aqueous microparticle dispersions.

2.4. Determination of the initial drug loading

The initial, practical drug loading was determined by dissolving accurately weighed amounts of microparticles (approximately 20 mg) in 7 mL of acetonitrile and subsequent UV drug detection ($\lambda_{lidocaine}$ = 263 nm, $\lambda_{ibuprofen}$ = 264 nm, $\lambda_{propranolol\,HCl}$ = 290 nm, Anthelie Advanced; Secomam, Domont, France).

2.5. In vitro drug release studies

Drug-loaded microparticles (50, 100 and 200 mg, respectively) were placed within 10 mL phosphate buffer pH 7.4 (USP 32) in 10 mL glass tubes (Fig. 1). The tubes were horizontally shaken at 37 °C (80 rpm, GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At pre-determined time intervals, 1 mL samples were withdrawn (replaced with fresh medium) and analyzed UV-spectrophotometrically ($\lambda_{lidocaine}$ = 263 nm, $\lambda_{ibuprofen}$ = 264 nm, $\lambda_{propranolol\,HCl}$ = 290 nm, Anthelie Advanced). Each experiment was conducted in triplicate.

2.6. Monitoring of changes upon exposure to the release medium

To monitor changes in the physicochemical properties of the bulk fluid and of the microparticles occurring during drug release, microparticles were treated as described in Section 2.5. At pre-determined time intervals:

- Samples were withdrawn and analyzed using an optical imaging system (Nikon SMZ-U; Nikon, Tokyo, Japan), equipped with a

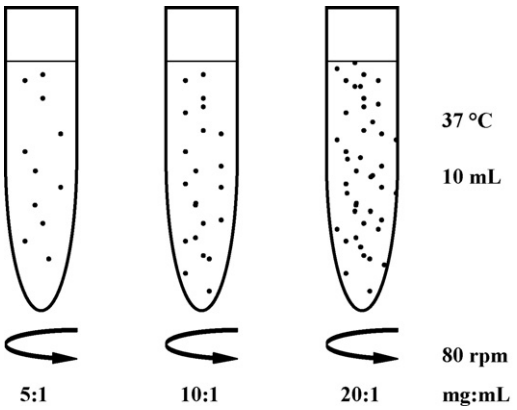

Fig. 1. Schematic presentation of the experimental setup used for *in vitro* drug release measurements.

Sony camera (Hyper HAD model SSC-DC38DP; Elvetec, Templemars, France) and the Optimas 6.0 software (Media Cybernetics, Silver Spring, USA).

- Samples were withdrawn, filtered (syringe with filter needle; 5 μm) and the pH measured (inoLab pH Level 1; WTW, Weilheim, Germany).
- The entire contents of the glass tubes were filtered (5 μm), rinsed with water and the obtained microparticles freeze-dried and stored at 4 °C for further analysis. 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. Furthermore, the glass transition temperature (Tg) of the polymer was determined by differential scanning calorimetry (DSC; Q1000; TA Instruments, Paris, France). Approximately 3–4 mg samples were heated in sealed aluminum pans (investigated temperature range: −10 to +80 °C, heating rate: 5 °C/min). The samples were flushed with nitrogen. Temperature and enthalpy readings were calibrated using indium.

2.7. Solubility measurements

Excess amounts of drug were added to phosphate buffer pH 7.4 (USP 32) in a horizontal shaker (GFL 3033; 37 °C, 80 rpm). Every 24 h, samples were withdrawn and analyzed for their drug content UV-spectrophotometrically as described in Section 2.5 until equilibrium was reached.

2.8. Mathematical modeling of drug release

Drug release from the investigated microparticles was quantitatively described based on Fick's second law of diffusion and taking into account the following initial and boundary conditions:

(i) At $t = 0$ (before exposure to the release medium), the drug is homogeneously distributed throughout the system.
(ii) The initial drug concentration is below the solubility of the drug within the device (molecular dispersion, monolithic solution).
(iii) The diffusional resistance for drug release within the unstirred liquid boundary layers surrounding the microparticles is negligible compared to the diffusional resistance within the spheres under the given experimental conditions.
(iv) Perfect sink conditions are maintained throughout the experiments.
(v) The microparticles are spherical in shape.

Under these conditions the following analytical solution of Fick's second law can be derived (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{1}{n^2} \cdot \exp\left(-\frac{n^2 \cdot \pi^2}{R^2} \cdot D \cdot t\right) \quad (1)$$

where M_t and M_∞ are the absolute, cumulative amounts of drug released at time t and infinity, respectively; R denotes the radius of the microparticles; D is the apparent diffusion coefficient of the drug in the microparticles.

3. Results and discussion

In order to better understand the importance of the "microparticle mass:bulk fluid volume" ratio on the resulting drug release kinetics from PLGA-based microparticles, four types of systems were studied: (i) initially porous, ibuprofen-loaded microparticles, (ii) initially porous, lidocaine-loaded microparticles, (iii) initially non-porous, lidocaine-loaded microparticles, and (iv) initially porous, propranolol HCl-loaded microparticles. Importantly, the release periods from these microparticles were very different, ranging from a couple of days to 2 months (Fig. 2(a)). Perfect sink conditions were provided in all cases [solubility at 37 °C in phosphate buffer pH 7.4: ibuprofen – 7.5 mg/mL; lidocaine – 5 mg/mL; propranolol HCl – 254 mg/mL (Bodmeier and Chen, 1989)].

3.1. Initial microparticle shape, morphology, size and drug loading

All particles were spherical in shape. Those prepared by a W/O/W extraction/evaporation method were initially highly porous. Fig. 3 shows SEM pictures of surfaces and cross-sections of two examples: ibuprofen-loaded and lidocaine-loaded microparticles. Irrespective of the type of drug, numerous pores are distributed throughout the spheres. The morphology of propranolol HCl-loaded microparticles prepared by the above-described W/O/W extraction/evaporation method was very similar (data not shown). In contrast, microparticles loaded with lidocaine and prepared by the O/W extraction/evaporation method detailed in Section 2.2 did not show any internal or external porosity before exposure to the release medium (data not shown).

The average microparticle size was in the range of 79–142 μm. The initial, practical drug loading of all systems varied between 4 and 6%, assuring a molecular distribution of the drug within the polymer (monolithic solution). No drug crystals were visible on the SEM pictures and no drug melting peaks were observed in the DSC measurements (data not shown). The respective average particle sizes and practical loadings were as follows: initially porous, ibuprofen-loaded microparticles: 79 μm and 4%, initially porous, lidocaine-loaded microparticles: 92 μm and 4%, initially non-porous, lidocaine-loaded microparticles: 115 μm and 4%, and initially porous, propranolol HCl-loaded microparticles: 142 μm and 6%.

3.2. In vitro drug release

Fig. 2(a) illustrates the experimentally measured drug release kinetics (symbols) of the different types of microparticles in phosphate buffer pH 7.4. The "microparticle mass:bulk fluid volume" ratio was varied from 5:1 to 20:1 mg:mL, as indicated in the diagrams. Importantly, the observed time periods for complete drug release were substantially different, ranging from approximately 1

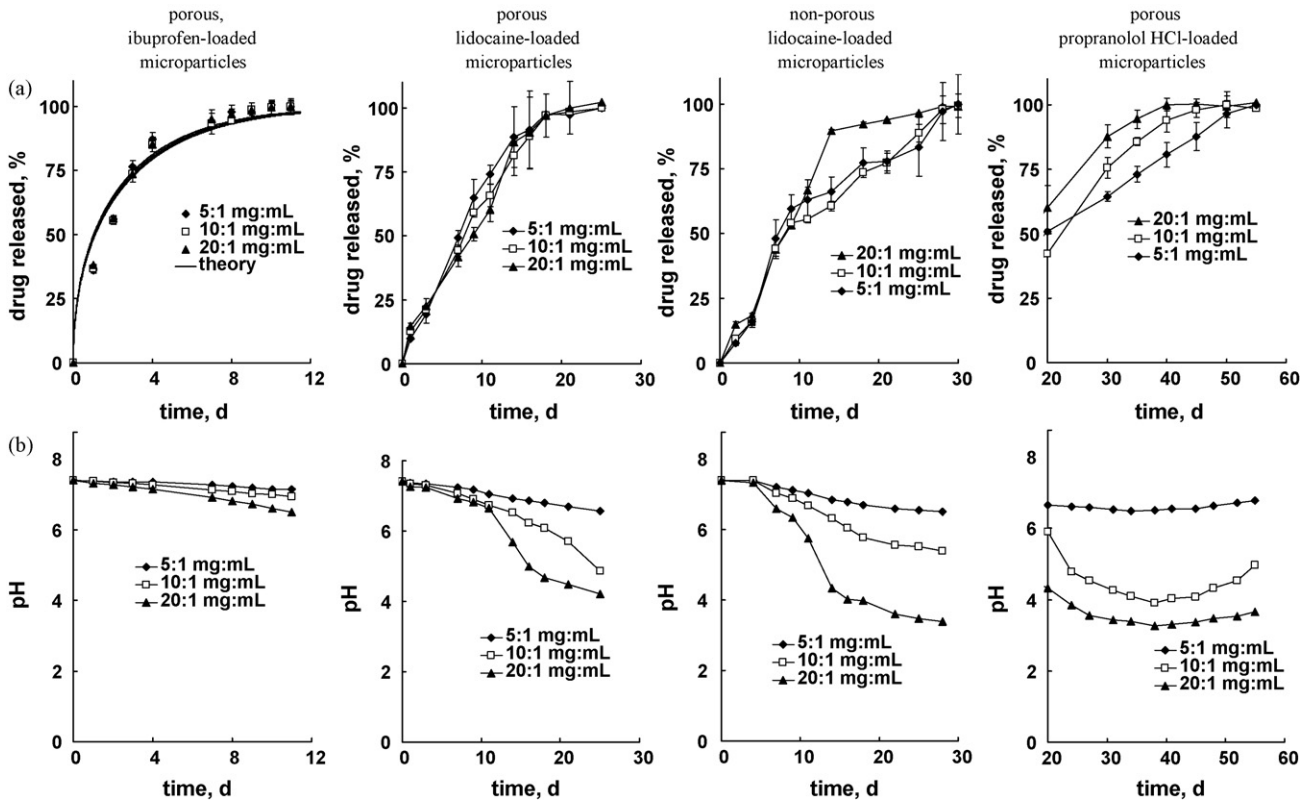

Fig. 2. Effects of the "microparticle mass:bulk fluid volume" ratio (indicated in the diagrams) on: (a) drug release from PLGA-based microparticles in phosphate buffer pH 7.4 [diagram on the left hand side: symbols = experiments; curves = theory (Eq. (1))], and (b) changes in the pH of the bulk fluid during drug release. Note the different scaling of the *x*-axes. The microparticle type is indicated at the top.

week (in the case of initially porous, ibuprofen-loaded systems) to about 2 months (in the case of initially porous, propranolol HCl-loaded microparticles). This can at least partially be explained by the differences in microparticle porosity, drug–polymer interaction (pK_a of ibuprofen = 4.4; lidocaine = 7.9; propranolol HCl = 9.7) and drug mobility within the polymeric systems (Siepmann et al., 2005; Klose et al., 2006, 2008).

Interestingly, in the case of complete drug release within about 1 week, the observed drug release kinetics could be quantitatively described using an appropriate analytical solution of Fick's second law of diffusion, taking into account that: (i) the drug is initially homogeneously and molecularly distributed throughout the system, (ii) the microparticles are spherical in shape, (iii) perfect sink conditions are maintained throughout the experiments, and (iv) drug release is pre-dominantly governed by diffusion with constant diffusivities. Under these conditions, Eq. (1) can be derived. Fitting this equation to the experimentally determined drug release rates from initially porous, ibuprofen-loaded microparticles led

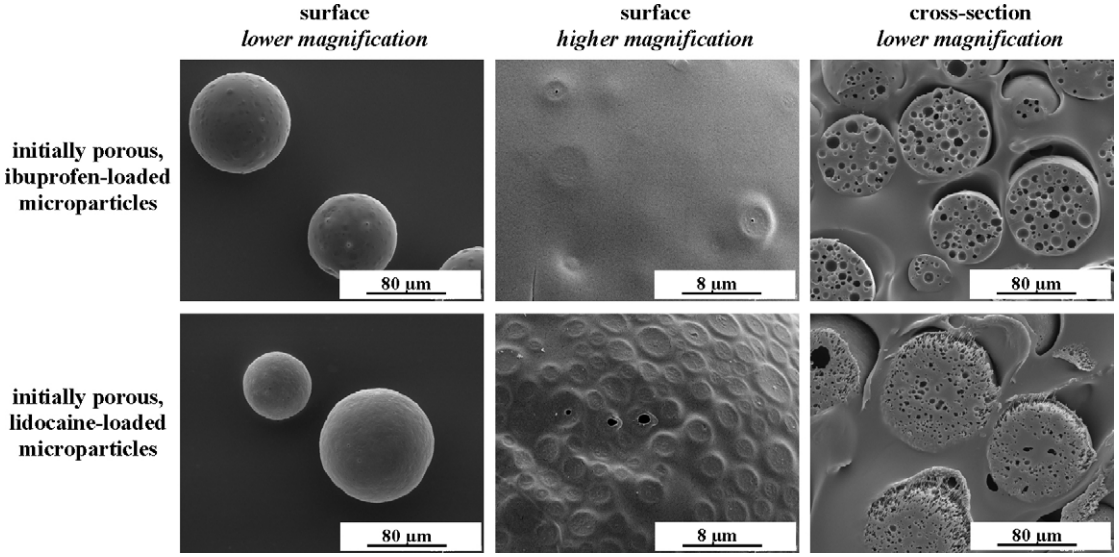

Fig. 3. Morphology of initially porous, ibuprofen- and lidocaine-loaded, PLGA-based microparticles before exposure to phosphate buffer pH 7.4 ($t = 0$): SEM pictures of surfaces (lower and higher magnification) and cross-sections (lower magnification) (note the different scale bars).

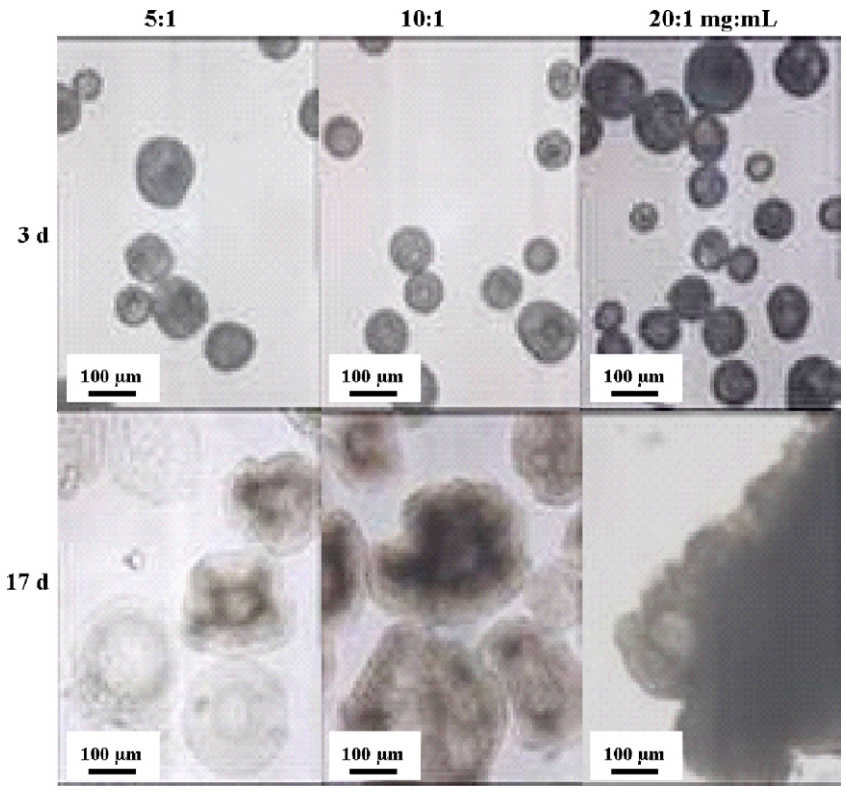

Fig. 4. Importance of the "microparticle mass:bulk fluid volume" ratio (indicated at the top) on the aggregation behavior of initially porous, propranolol HCl-loaded, PLGA-based microparticles upon exposure to phosphate buffer pH 7.4: optical microscopy pictures taken after 3 and 17 d, respectively.

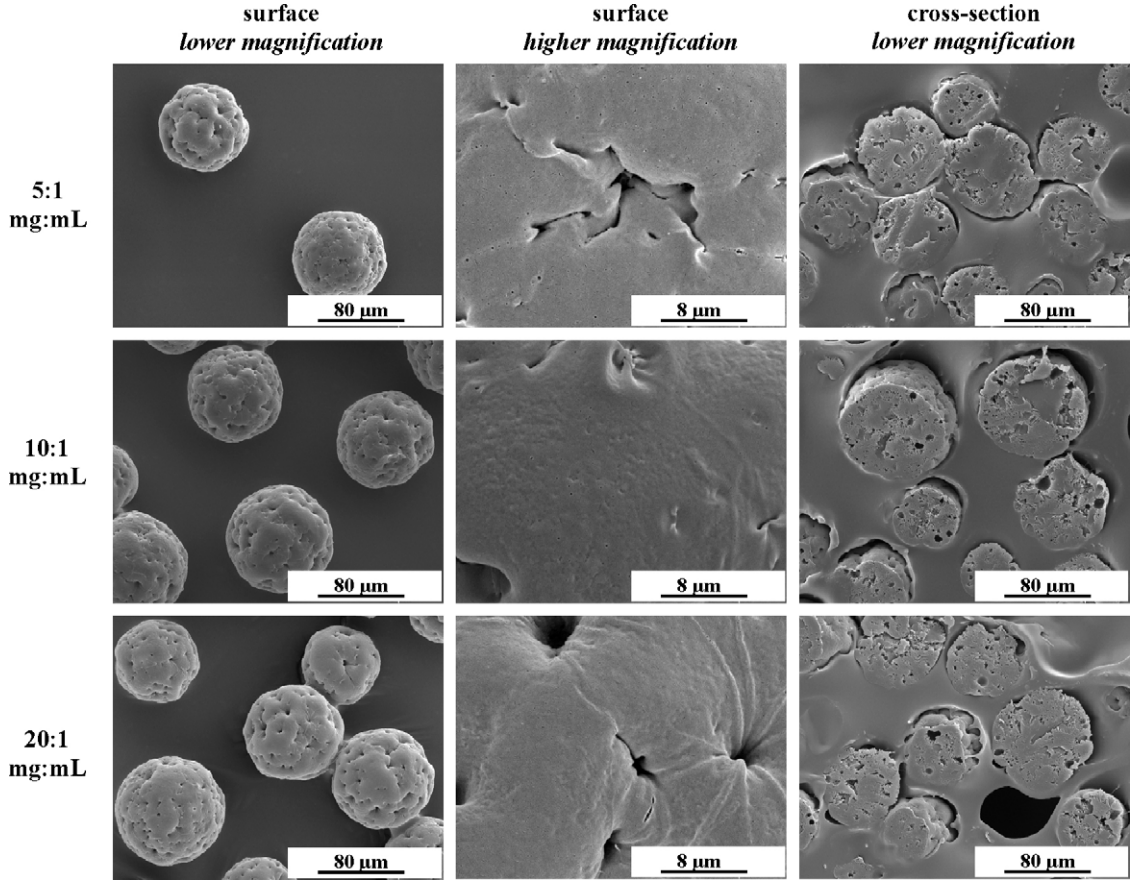

Fig. 5. Inner and outer morphology of initially porous, ibuprofen-loaded, PLGA-based microparticles upon 3 d exposure to phosphate buffer pH 7.4: effects of the "microparticle mass:bulk fluid volume" ratio (indicated on the left hand side). SEM pictures of surfaces (lower and higher magnification) and cross-sections (lower magnification), note the different scale bars.

to good agreement between theory and experiment, irrespective of the "microparticle mass:bulk fluid volume ratio" (symbols and curves in the diagram on the left hand side of Fig. 2(a)). Thus, ibuprofen release from this type of PLGA-based microparticles is pre-dominantly controlled by drug diffusion with time- and position-independent diffusion coefficients. Based on these fittings, the apparent diffusivity of the drug in the microparticles could be determined, being equal to 4.7 (±0.3), 4.8 (±0.1) and 5.0 (±0.2) × 10^{-12} cm^2/s at the "microparticle mass:bulk fluid volume ratios" 5:1, 10:1 and 20:1 mg:mL, respectively. In contrast, fitting Eq. (1) to the experimentally determined drug release kinetics from the other types of microparticles resulted in significant deviations between theory and experiment (data not shown). Thus, in these cases also other phenomena are of importance. This probably includes polymer degradation, which is not taken into account in Eq. (1). It has to be pointed out that throughout the entire release periods, various physicochemical processes can be involved in the control of drug release, for example water penetration into the system, polymer chain cleavage, drug diffusion, creation of acidic microenvironments and microparticle disintegration (Brunner et al., 1999; Siepmann and Goepferich, 2001; Siepmann et al., 2002, 2006; Siepmann and Siepmann, 2008). Importantly, the relative importance of these phenomena can strongly depend on the time period and on the type of system.

Interestingly, the experimentally determined (and theoretically calculated) drug release patterns of ibuprofen from initially porous microparticles were virtually overlapping for all investigated "microparticle mass:bulk fluid volume" ratios (Fig. 2(a), diagram on the left hand side). This is in good agreement with the very similar apparent diffusion coefficients and rather similar changes in the pH of the release medium during drug release (Fig. 2(b), diagram on the left hand side). As it can be seen, the pH slightly decreased in all cases. This can be explained by the fact that PLGA is degraded into shorter chain acids upon contact with water, which diffuse into the bulk fluid.

In contrast, significant differences were observed in the "pH–time" profiles when initially porous, lidocaine-loaded microparticles were exposed to the release medium after approximately 10 d (Fig. 2(b)): the drop in pH was much more pronounced at higher "microparticle mass:bulk fluid volume" ratios. This can be attributed to the fact that higher amounts of shorter chain acids are created per volume release medium at higher "microparticle mass:bulk fluid volume" ratios. As the buffer capacity is identical in all cases, the pH more rapidly decreases at higher microparticle concentrations. It has to be pointed out that the degradation of PLGA is catalyzed by protons (Grizzi et al., 1995; Lu et al., 1999). Thus, a drop in pH can be expected to result in accelerated polymer chain cleavage and, thus, a more rapid decrease in the average polymer molecular weight, leading to an accelerated increase in polymer chain and drug mobility and consequently to accelerated drug release. Interestingly, the impact of the significant differences in the pH changes of the bulk fluid observed in the case of initially porous, lidocaine-loaded microparticles after approximately 10 d did not very much affect the resulting drug release rates (Fig. 2). This can at least partially be explained by the fact that the majority of the drug is already released at the time point when the pH differences become pronounced.

In contrast, drug release was far from being complete when the differences in the changes in the pH of the bulk fluid became significant in the case of initially non-porous microparticles containing lidocaine (Fig. 2). This resulted in a major impact of the "microparticle mass:bulk fluid volume" ratio on drug release: the majority of the lidocaine was released from these microparticles after only 2 weeks, when 200 mg microparticles were exposed to 10 mL phosphate buffer pH 7.4. In contrast, complete drug release

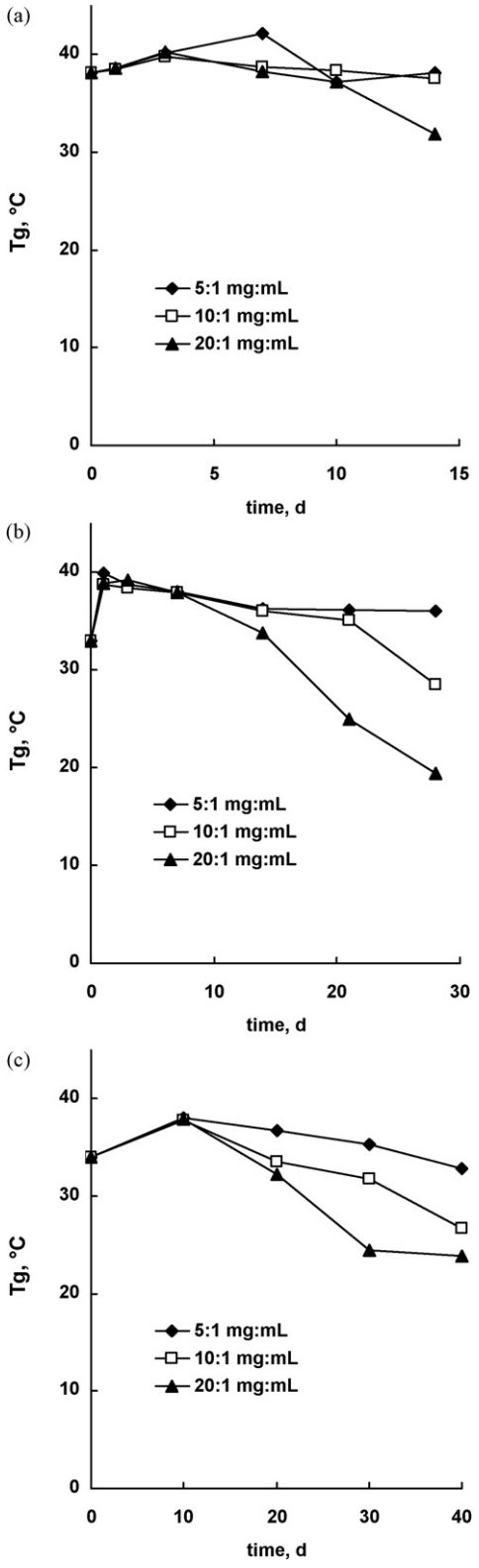

Fig. 6. Effects of the "microparticle mass:bulk fluid volume" ratio (indicated in the diagrams) on changes in the glass transition temperature (Tg) of PLGA in initially porous microparticles, loaded with: (a) ibuprofen, (b) lidocaine, and (c) propranolol HCl upon exposure to phosphate buffer pH 7.4. Note the different scaling of the *x*-axes.

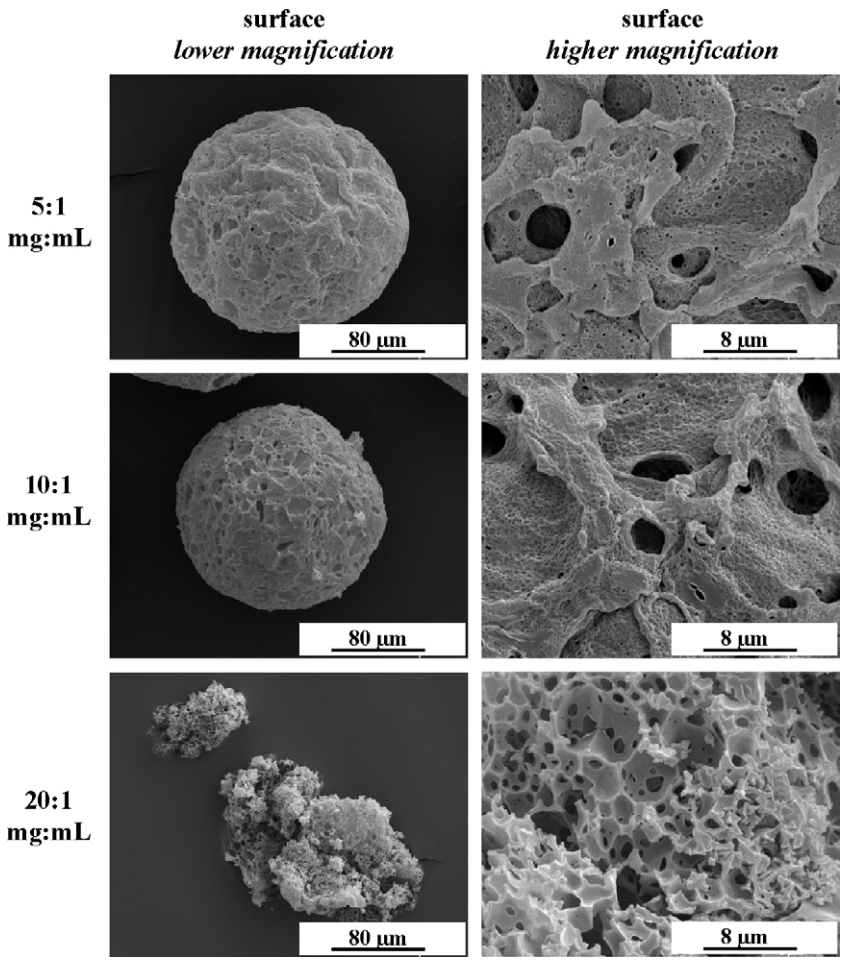

Fig. 7. Outer morphology of initially porous, lidocaine-loaded, PLGA-based microparticles upon 14 d exposure to phosphate buffer pH 7.4: effects of the "microparticle mass:bulk fluid volume" ratio (indicated on the left hand side). SEM pictures of surfaces (lower and higher magnification), note the different scale bars.

was observed after approximately 4 weeks, when 50 mg microparticles were exposed to 10 mL release medium. The much faster drug release at higher "microparticle mass:bulk fluid volume" ratios can be explained by the more rapid decrease in the pH of the bulk fluid, resulting in accelerated polymer degradation and an accelerated increase in drug mobility (as discussed above). The observed differences in the pH changes and drug release kinetics when varying the "microparticle mass:bulk fluid volume" ratio were most pronounced in the case of initially porous, propranolol HCl-loaded systems (Fig. 2, diagrams on the right hand side). This can be explained by the longer time periods during which drug was released from these microparticles. For instance, propranolol HCl release was complete after approximately 40 d when 200 mg microparticles were exposed to 10 mL phosphate buffer pH 7.4, whereas about 55 d were required for complete release when only 50 mg were exposed to the same volume of release medium (Fig. 2).

3.3. Physicochemical changes during drug release

The explanation for the effects of the "microparticle mass:bulk fluid volume" ratio on the resulting drug release kinetics was further confirmed by monitoring dynamic changes in the physicochemical properties of the systems upon exposure to the release medium. Optical and scanning electron microscopy as well as DSC analysis were used for this purpose.

The aggregation behavior of the microparticles was observed using optical microscopy. Fig. 4 shows as an example pictures of initially porous, propranolol HCl-loaded microparticles after 3 and 17 d exposure to phosphate buffer pH 7.4 at different "microparticle mass:bulk fluid volume" ratios (indicated at the top). Clearly, microparticle aggregation was very limited after only 3 d exposure to the release medium, irrespective of the "microparticle mass:bulk fluid volume" ratio (Fig. 4, top row). This is in good agreement with the observed absence of significant differences in the pH changes of the bulk fluid and drug release patterns. Also the alterations of the inner and outer morphology of the microparticles during the first days upon exposure to the release medium did not significantly depend on the "microparticle mass:bulk fluid volume" ratio: Fig. 5 exemplarily shows SEM pictures of surfaces and cross-sections of initially porous, ibuprofen-loaded microparticles after 3 d exposure to phosphate buffer pH 7.4. The external and internal porosity of all particles was similar. Furthermore, no major differences were observed with respect to the changes in the glass transition temperature (Tg) of the polymer upon exposure to the release medium during the first week, irrespective of the type of microparticles and "microparticle mass:bulk fluid volume" ratio (Fig. 6). The glass transition temperature of a polymeric drug delivery system is a very crucial parameter, because it determines whether the macromolecular network is in the glassy or in the rubbery state. In the rubbery state, polymer chain mobility is much higher and, thus, also the mobility of incorporated drugs is increased. It has to be pointed out that the Tgs shown in Fig. 6 were obtained with *dry* microparticles and that water acts as a plasticizer for PLGA (Blasi et al., 2005). Faisant et al. (2002) have shown that the Tg of PLGA microparticles can drop by 10 °C or more upon water penetration into the system. Thus, the polymer is likely to be in the rubbery state in the

investigated microparticles during drug release, allowing for significant drug diffusion through the polymeric network (Siepmann and Siepmann, 2008).

In contrast, after about 10–14 d, significant effects of the "microparticle mass:bulk fluid volume" ratio became visible on the: (i) microparticle aggregation behavior (Fig. 4), (ii) changes in the glass transition temperature of the systems (Fig. 6), and (iii) changes in the microparticle morphology (Fig. 7). This agrees well with the observed effects of the "microparticle mass:bulk fluid volume" on drug release, which started to become important after this time period (except for initially porous, lidocaine-loaded microparticles, from which major parts of the drug were already released at this time point; drug release from initially porous, ibuprofen-loaded microparticles was already complete at this time point) (Fig. 2(a)). Fig. 4 (bottom row) shows for example the aggregation behavior of initially porous, propranolol HCl-loaded microparticles after 17 d exposure to phosphate buffer pH 7.4. Clearly, microparticle aggregation became more and more pronounced when increasing the "microparticle mass:bulk fluid volume" ratio. This is in good agreement with the observed differences in the changes of the glass transition temperature of the polymer (Fig. 6): after 10–14 d, the Tg of PLGA decreased more rapidly at higher "microparticle mass:bulk fluid volume" ratios. This can be attributed to the observed differences in the decrease of the pH of the bulk fluid shown in Fig. 2(b). With increasing "microparticle mass:bulk fluid volume" ratio, the pH of the release medium more rapidly decreases, resulting in accelerated PLGA degradation. With decreasing polymer molecular weight, the glass transition temperature of the microparticles decreases (Siepmann et al., 2005; Klose et al., 2006, 2008). Thus, with increasing "microparticle mass:bulk fluid volume" ratio the Tg more rapidly decreases, resulting in accelerated microparticle agglomeration. After 10–14 d, also the effects of the "microparticle mass:bulk fluid volume" ratio on the changes in the internal and external system morphology became significant. Fig. 7 shows for example surfaces of initially porous, lidocaine-loaded microparticles upon 14 d exposure to phosphate buffer pH 7.4 (left hand side: lower magnification, right hand side: higher magnification). Clearly, the microparticles' porosity significantly increased with increasing "microparticle mass:bulk fluid volume" ratio (from the top to the bottom). This further confirms the accelerated polymer degradation at higher "microparticle mass:bulk fluid volume" ratios, resulting in increased drug mobility and, eventually, increased drug release rates (Fig. 2(a)).

4. Conclusions

Great care has to be taken when defining the conditions for *in vitro* drug release measurements from PLGA-based microparticles: the "microparticle mass:bulk fluid volume" ratio may or may not significantly affect the observed drug release rates. Changes in the pH of the release medium can alter decisive system properties, such as the agglomeration behavior, inner and outer morphology and glass transition temperature of the polymer.

Acknowledgements

The authors are grateful for the support of this work by the French Association for Cancer Research "ARC" ("Association pour la Recherche sur le Cancer": postdoctoral fellowship for Dr. Florence Siepmann and doctoral fellowship for Mrs. Diana Klose), the "Nord-Pas de Calais" Regional Council (in the frame of the PRIM: "Pôle de Recherche Interdisciplinaire pour le Médicament", Interdisciplinary Research Center on Drug Products) and the "Agence Nationale de la Recherche" (ANR, National Research Agency; in the frame of the BIOSTAB project).

References

Anderson, J.M., Shive, M.S., 1997. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv. Drug Del. Rev. 28, 5–24.
Aubert-Pouëssel, A., Bibby, D.C., Venier-Julienne, M.C., Hindre, F., Benoit, J.P., 2002. A novel in vitro delivery system for assessing the biological integrity of protein upon release from PLGA microspheres. Pharm. Res. 19, 1046–1051.
Bain, D.F., Munday, D.L., Smith, A., 1999. Modulation of rifampicin release from spray-dried microspheres using combinations of poly-(D,L-lactide). J. Microencapsul. 16, 369–385.
Berkland, C., Kim, K., Pack, D.W., 2003. PLG microsphere size controls drug release rate through several competing factors. Pharm. Res. 20, 1055–1062.
Blasi, P., D'Souza, S.S., Selmin, F., DeLuca, P.P., 2005. Plasticizing effect of water on poly(lactide-co-glycolide). J. Control. Rel. 108, 1–9.
Bodmeier, R., Chen, H., 1989. Evaluation of biodegradable poly(lactide) pellets prepared by direct compression. J. Pharm. Sci. 78, 819–822.
Brunner, A., Maeder, K., Goepferich, A., 1999. pH and osmotic pressure inside biodegradable microspheres during erosion. Pharm. Res. 16, 847–853.
Burgess, D.J., Hussain, A.S., Ingallinera, T.S., Chen, M.L., 2002. Assuring quality and performance of sustained and controlled release parenterals: AAPS workshop report, co-sponsored by FDA and USP. Pharm. Res. 19, 1761–1768.
Burgess, D.J., Crommelin, D.J.A., Hussain, A.S., Chen, M.L., 2004. Assuring quality and performance of sustained and controlled release parenterals. Eur. J. Pharm. Sci. 21, 679–690.
Conti, C.B., Genta, I., Giunchedi, P., Modena, T., 1995. Testing of "in vitro" dissolution behaviour of microparticulate drug delivery systems. Drug Dev. Ind. Pharm. 21, 1223–1233.
Crank, J (Ed.), 1975. The Mathematics of Diffusion, 2nd ed. Clarendon Press, Oxford.
D'Souza, S.S., DeLuca, P.P., 2005a. Development of a dialysis in vitro release method for biodegradable microspheres. AAPS PharmSciTech. 6, article 42.
D'Souza, S.S., Faraj, J.A., DeLuca, P.P., 2005b. A model-dependent approach to correlate accelerated with real-time release from biodegradable microspheres. AAPS PharmSciTech. 6, article 70.
D'Souza, S.S., DeLuca, P.P., 2006. Methods to assess in vitro drug release from injectable polymeric particulate systems. Pharm. Res. 23, 460–474.
Faisant, N., Siepmann, J., Benoit, J.P., 2002. PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release. Eur. J. Pharm. Sci. 15, 355–366.
Faisant, N., Akiki, J., Siepmann, F., Benoit, J.P., Siepmann, J., 2006. Effects of the type of release medium on drug release from PLGA-based microparticles: experiment and theory. Int. J. Pharm. 314, 189–197.
Fournier, E., Passirani, C., Montero-Menei, C.N., Benoit, J.P., 2003. Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility. Biomaterials 24, 3311–3331.
Giteau, A., Venier-Julienne, M.C., Aubert-Pouëssel, A., Benoit, J.P., 2008. How to achieve sustained and complete protein release from PLGA-based microparticles? Eur. J. Pharm. Biopharm. 350, 14–26.
Grizzi, I., Garreau, S.L., Vert, M., 1995. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 16, 305–311.
Guse, C., Koennings, S., Blunk, T., Siepmann, J., Goepferich, A., 2006. Programmable implants – from pulsatile to controlled release. Int. J. Pharm. 314, 161–169.
Ignatius, A.A., Cleas, L.E., 1996. In vitro biocompatibility of bioresorbable polymers: poly(L,DL-lactide) and poly(L-lactide-co-glycolide). Biomaterials 17, 831–839.
Kang, J., Schwendeman, S.P., 2007. Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins. Mol. Pharm. 4, 104–118.
Klose, D., Siepmann, F., Elkharraz, K., Krenzlin, S., Siepmann, J., 2006. How porosity and size affect the drug release mechanisms from PLGA-based microparticles. Int. J. Pharm. 314, 198–206.
Klose, D., Siepmann, F., Elkharraz, K., Siepmann, J., 2008. PLGA-based drug delivery systems: importance of the type of drug and device geometry. Int. J. Pharm. 354, 95–103.
Lu, L., Garcia, C.A., Mikos, A.G., 1999. In vitro degradation of thin poly(DL-lactic-co-glycolic acid) films. J. Biomed. Mater. Res. 46, 236–244.
Menei, P., Daniel, V., Montero-Menei, C., Brouillard, M., Pouplard-Barthelaix, A., Benoit, J.P., 1993. Biodegradation and brain tissue reaction to poly(D,L-lactide-co-glycolide) microspheres. Biomaterials 14, 470–478.
Menei, P., Jadaud, E., Faisant, N., Boisdron-Celle, M., Michalak, S., Fournier, D., Delhaye, M., Benoit, J.P., 2004. Stereotaxic implantation of 5-Fluorouracil-releasing microspheres in malignant glioma. Cancer 100, 405–410.
Ravivarapu, H.B., Burton, K., DeLuca, P.P., 2000a. Polymer and microsphere blending to alter the release of a peptide from PLGA microspheres. Eur. J. Pharm. Biopharm. 50, 263–270.
Ravivarapu, H.B., Lee, H., DeLuca, P.P., 2000b. Enhancing initial release of peptide from poly(lactic-co-glycolic acid) (PLGA) microspheres by addition of porosigen and increasing drug load. Pharm. Dev. Technol. 5, 287–296.
Shameem, M., Lee, H., DeLuca, P.P., 1999. A short term (accelerated release) approach to evaluate peptide release from PLGA depot-formulations. AAPS Pharmsci. 1, article 7.
Siepmann, J., Goepferich, A., 2001. Mathematical modeling of bioerodible, polymeric drug delivery systems. Adv. Drug Del. Rev. 48, 229–247.

Siepmann, J., Faisant, N., Benoit, J.P., 2002. A new mathematical model quantifying drug release from bioerodible microparticles using Monte Carlo simulations. Pharm. Res. 19, 1887–1895.
Siepmann, J., Elkharraz, K., Siepmann, F., Klose, D., 2005. How autocatalysis accelerates drug release from PLGA-based microparticles: a quantitative treatment. Biomacromolecules 6, 2312–2319.
Siepmann, J., Siepmann, F., Florence, A.T., 2006. Local controlled drug delivery to the brain: mathematical modeling of the underlying mass transport mechanisms. Int. J. Pharm. 314, 101–119.
Siepmann, J., Siepmann, F., 2008. Mathematical modeling of drug delivery. Int. J. Pharm. 364, 328–343.