



Impact of the experimental conditions on drug release from parenteral depot systems: From negligible to significant

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ABSTRACT

The aim of this study was to evaluate the impact of the experimental conditions on drug release measurements from parenteral depot systems. Frequently applied setups were used, including agitated and “non-agitated” flasks and tubes, flow-through cells as well as agarose gels. The bulk fluid volumes and flow rates were varied. Lipid implants (prepared by direct compression or melting & casting) as well as PLGA-based microparticles (prepared by O/W or W/O/W or S/O/W solvent extraction/evaporation methods) were studied. Theophylline, lidocaine, prilocaine, propranolol HCl, dexamethasone and ibuprofen were used as model drugs at different initial loadings. In all cases, the release medium was phosphate buffer pH 7.4, kept constant at 37 °C. Particle size analysis, SEM, X-ray diffraction, DSC analysis and mathematical modeling were applied to better understand the observed phenomena. Interestingly, the importance of the impact of the experimental conditions ranged from negligible to significant, depending on the specific type of drug delivery system and setup. Both, lipid implants as well as PLGA-based microparticles can exhibit more or less sensitive/robust drug release patterns. The observed differences in sensitivity could partially be explained in a mechanistic way, but in many cases they are not yet fully understood. A thorough understanding of the underlying drug release mechanisms can be very helpful. If the devices are poorly characterized and treated as “black boxes”, great care must be taken when drawing conclusions from *in vitro* drug release measurements.

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1. Introduction

Parenteral controlled drug delivery systems can offer major benefits (Benoit et al., 2000; Woo et al., 2001; Menei et al., 2004, 2005; Hiremath et al., 2011; White et al., 2011). For instance, the administration of highly potent drugs with narrow therapeutic windows can be significantly facilitated, or the administration of fragile drugs (e.g. proteins) enabled. Poly(lactic-co-glycolic acid) (PLGA)-based microparticles are often the first choice. However, due to the creation of acidic microclimates, lipid implants might represent an attractive alternative, especially in the case of acid-sensitive drugs (Mohl and Winter, 2004; Koennings et al., 2007a; Schulze and Winter, 2009). Obviously, the release rate of the incorporated drug out of these dosage forms is of crucial importance for the performance of the advanced drug delivery systems. However, yet no regulatory test method has been established, allowing for stan-

dardized and uniform measurements all over the world (Burgess et al., 2002, 2004; Martinez et al., 2008, 2010). In practice, a large spectrum of different techniques is applied to monitor drug release from parenteral depot systems. This includes system exposure to different types of release media in more or less agitated tubes or flasks, flow-through cells, or agarose gels.

It has to be pointed out that yet the impact of the experimental conditions used for drug release measurements from parenteral depot systems is not fully understood. Very interesting studies have been reported. For example, Rawat et al. (2011) showed that risperidone release from commercially available PLGA-based microparticles (Risperdal Consta) was not very much affected by variations in the flow rate when using a USP apparatus 4. Furthermore, they correlated dexamethasone release from PLGA-based microparticles *in vitro* (measured using a flow-through cell) with *in vivo* drug release kinetics measured in rats upon subcutaneous injection (Zolnik and Burgess, 2008). This group also studied in detail the effects of the pH and temperature of the release medium on the resulting drug release kinetics from PLGA-based microparticles (Zolnik et al., 2006; Zolnik and Burgess, 2007) and recently proposed an accelerated *in vitro* test method for implantable PLGA microparticle/polyvinyl alcohol hydrogel composite coatings (Shen

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and Burgess, 2012). DeLuca and co-workers demonstrated a major impact on leuporeline release from PLGA-based microparticles when using agitated versus “non-agitated” tubes (D’Souza and DeLuca, 2005). The same group proposed a dialysis method for drug release measurements and correlated the observed release kinetics with *in vivo* measurements in rats (Kostanski and DeLuca, 2000; Kostanski et al., 2000). They also investigated the impact of elevated temperature on the resulting drug release rates from peptide loaded, PLGA-based microparticles (Shameem et al., 1999). Varying the temperature and pH of the bulk fluid the drug delivery systems are exposed to, can help to speed up drug release and provide accelerated short term tests. This is a very interesting approach, but note that the aim of the present study was to evaluate the impact of the experimental conditions on drug release, while keeping the temperature at 37 °C (body temperature) and using phosphate buffer pH 7.4 (physiological pH) as release medium. The idea was to “best” simulate *in vivo* conditions in these respects. Furthermore, the importance of the ratio “PLGA microparticle mass: bulk fluid volume” was recently studied in more detail (Klose et al., 2010).

To be able to appropriately analyze experimentally measured drug release kinetics from controlled delivery systems, ideally the underlying mass transport mechanisms should be understood. It is well known that the physical and chemical processes involved in the control of drug release from parenteral dosage forms can be highly complex and significantly vary from system to system (Siepmann and Goepferich, 2001; Siepmann et al., 2006; Siepmann and Siepmann, 2008, 2011). In case of lipid implants, for instance diffusional mass transport as well as limited drug solubility might be of major importance (Guse et al., 2006a,b; Koennings et al., 2007b; Siepmann et al., 2008; Kreye et al., 2011a,b,c,d, in press). In case of PLGA-based microparticles, diffusion, polymer degradation and erosion as well as pore creation and closure can play a crucial role (Brunner et al., 1999; Kang and Schwendeman, 2007; Li and Schwendeman, 2005; Fredenberg et al., 2011a,b,c). It has to be pointed out that so far only very little is known on the potential impact of the experimental conditions used for *in vitro* drug release measurements on the underlying drug release mechanisms.

The aim of this study was to better understand the importance of the experimental conditions for the observed release kinetics from parenteral depot formulations (keeping the temperature at 37 °C and using phosphate buffer pH 7.4 as release medium in order to “best” simulate *in vivo* conditions). Various types of lipid implants and PLGA-based microparticles were studied, prepared by direct compression or melting & casting, or using O/W, W/O/W or S/O/W solvent extraction/evaporation methods. Theophylline, lidocaine, prilocaine, propranolol HCl, dexamethasone and ibuprofen were chosen as model drugs (exhibiting different water solubility) and incorporated at different loadings. The idea was to study a broad range of parenteral controlled drug delivery systems. Particle size analysis, scanning electron microscopy (SEM), X-ray diffraction, DSC analysis and mechanistically realistic mathematical modeling were used to better understand the observed phenomena.

2. Materials and methods

2.1. Materials

Glyceryl-trilaurate, -tripalmitate, and -tristearate (Dynasan 112, 116, and 118; Sasol, Witten, Germany); glyceryl-palmitostearate (Precirol ATO 5; Gattefosse, Saint-Priest, France); poly(D,L-lactic-co-glycolic acid) (PLGA, Resomer RG 502H and 504H, 50:50 lactic acid:glycolic acid, acid terminated, inherent viscosities of 0.1% solutions in chloroform at 25 °C=0.16–0.24 and 0.45–0.60 dl/g according to the supplier; Boehringer Ingelheim, Ingelheim, Germany); lidocaine (free base; Sigma–Aldrich,

Steinheim, Germany); propranolol hydrochloride and ibuprofen (Salutas, Barleben, Germany); prilocaine (free base) and anhydrous theophylline powder 200 (BASF, Ludwigshafen, Germany); dexamethasone (Discovery Fine Chemicals, Wimborne, UK) (all experiments with this drug were conducted under protection from light); acetonitrile and dichloromethane (VWR, Fontenay-sous-Bois, France); tetrahydrofuran (HPLC Grade; Fisher Scientific, Illkirch, France); polyvinyl alcohol (Mowiol 4-88; Sigma–Aldrich); agarose (GenAgarose LE; Genaxxon Bioscience, Ulm, Germany).

2.2. Solubility measurements

Excess amounts of prilocaine (free base) or dexamethasone were exposed to 2 or 20 mL phosphate buffer pH 7.4 (USP 32) at 37 °C in an Eppendorf tube or glass flask in a horizontal shaker (80 rpm; GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). Every 24 h, samples were withdrawn, filtered and analyzed by: (i) UV-spectrophotometry in the case of prilocaine ($\lambda = 260$ nm; UV-1650 PC; Shimadzu, Champs-sur-Marne, France), or (ii) HPLC in the case of dexamethasone (ProStar 230 pump, 410 autosampler, 325 UV-Vis detector, Galaxie software; Varian, Les Ulis, France). A reversed phase column C18 (Gemini 5 μ m; 110 Å; 150 mm \times 4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase consisted of acetonitrile:water (33:67, v/v). One hundred microliters filtered samples (PVDF syringe filters – 0.45 μ m) were injected, the flow rate was 1.5 mL/min, the detection wavelength 254 nm. Samples were withdrawn until equilibrium was reached. Each experiment was conducted in triplicate.

2.3. Implant preparation

Compressed implants: lipid powder and drug were sieved (50–100 μ m; Retsch, Haan, Germany) and mixed in a glass vial using a vortex mixer (level 4, 10 s, Vortex-2 Genie; Scientific Industries, Bohemia, NY, USA). The blends were compressed with a Frank Universalpruefmaschine 81816 (Karl Frank, Weinheim-Birkenau, Germany) into cylindrical, flat-faced implants (diameter=height=2 mm). The compression force was 250 or 300 N (as indicated), and held for 10 s.

Molten & cast implants: the lipid was heated until a clear liquid was obtained. The sieved drug (50–100 μ m; Retsch) was homogeneously dispersed within the molten lipid using a magnetic stirrer (250/min, RET basic; IKA, Staufen, Germany). The dispersion was cast into cylindrical plastic molds (diameter=height=2 mm) using heated glass pipettes, and cooled down to room temperature. Excess lipid on the top was removed with a heated blade. The implants were tempered for 3 weeks at 50 °C.

The mean particle sizes of the drugs used for implant preparation were: 12 (± 0.8) μ m (theophylline), 12 (± 1.4) μ m and 68 (± 0.6) μ m (propranolol hydrochloride in compressed and molten implants).

2.4. Microparticle preparation

Non-porous ibuprofen-loaded microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: 46 mg drug and 1 g PLGA (Resomer RG 504H) were dissolved in 9 g dichloromethane. 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). The formed particles were hardened by adding 2.5 L further outer aqueous phase and 4 h gentle stirring (700 rpm). The particles were separated by filtration and subsequently freeze-dried to minimize their residual solvents' content. The particle size range was

narrowed by sieving (average pore sizes of the sieves: 40 and 63 μm ; Retsch).

Porous ibuprofen- and lidocaine-loaded microparticles were prepared by a water-in-oil-in-water (W/O/W) solvent extraction/evaporation technique: 0.5 g de-mineralized water was emulsified into a solution of 46 mg of the drug and 1 g PLGA (Resomer RG 504H) in 9 g dichloromethane using an Ultra-Turrax (60 s, 13,000 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), inducing microparticle formation. The latter were hardened by adding 2.5 L further outer aqueous phase and 4 h gentle stirring (700 rpm). The particles were separated by filtration and subsequently freeze-dried to minimize their residual solvents' content. The particle size range was narrowed by sieving (average pore sizes of the sieves: 40 and 63 μm ; Retsch).

Non-porous prilocaine (free base)-loaded microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: a solution of the drug and PLGA (Resomer RG 502H) in dichloromethane (7 mL) was emulsified within 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). The formed particles were hardened by adding 2.5 L further 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. The particle size range was narrowed by sieving (average pore sizes of the sieves: 50 and 90 μm ; Retsch). The theoretical drug loading was 4% (46 mg of drug and 1 g of PLGA) or 20% (210 mg of drug and 836 mg of PLGA), as indicated.

Non-porous dexamethasone-loaded microparticles were prepared using a solid-in-oil-in-water (S/O/W) solvent extraction/evaporation technique: 320 mg PLGA (Resomer RG 502H) was dissolved within 3 mL dichloromethane and 80 mg of the drug was dispersed into the polymer solution by sonication during 2 min (30% amplitude, Sonopuls UW2070; Bandelin, Berlin, Germany). This dispersion was emulsified within 50 mL of an outer aqueous polyvinyl alcohol solution (5%, w/w; previously cooled to 4 °C), under stirring with a three-blade propeller for 20 min (400 rpm). The formed particles were hardened by adding 200 mL water (4 °C) and 3 min stirring (400 rpm). Then the emulsion was poured into 600 mL water (4 °C) and stirred for 40 min (400 rpm) to promote the extraction of dichloromethane. The particles were separated by filtration and subsequently freeze-dried to minimize their residual solvents' content.

2.5. In vitro drug release measurements

Fig. 1 shows the different types of experimental setups used for drug release measurements. In all cases, the release medium was phosphate buffer pH 7.4 (USP 32) and the temperature 37 °C. If not otherwise indicated, sink conditions were maintained throughout the experiment. In case of microparticles, 5 or 50 mg samples were exposed to the release medium (as indicated). In case of implants, 1 implant was exposed to the release medium. The drug content in the samples was determined by UV measurements (theophylline, lidocaine, ibuprofen, propranolol hydrochloride: $\lambda = 272, 263, 264, 290 \text{ nm}$; UV-1650 PC), or by HPLC analysis in the case of dexamethasone (as described in Section 2.2) and prilocaine. In the latter case, 50 μL filtered samples (PTFE syringe filters – 0.45 μm) were injected and the mobile phase was acetonitrile:phosphate buffer pH 8 (Eur. Pharm. 7) (40:60, v/v). The detection wavelength was 260 nm, the flow rate 1 mL/min. Each experiment was conducted in triplicate.

Flow-through cells: as described in detail by Aubert-Pouessel et al. (2002) the delivery systems were placed into empty HPLC

columns (4.6 mm \times 5 cm; Omega; Upchurch Scientific, Oak Harbor, WA, USA). Syringe pumps (PHD 2000, Harvard Apparatus, Les Ulis, France) assured the continuous flow of release medium at different velocities (as indicated). The columns were placed in a water bath, kept at 37 °C. The *dead volumes* were considered in the calculation of the presented drug release profiles.

Horizontally shaken and vertical, “non-agitated” tubes: the delivery systems were placed in 2 mL Eppendorf or 10 mL glass tubes, filled with 1.5 or 10 mL phosphate buffer pH 7.4 (as indicated). The tubes were either shaken in horizontal position (80 rpm; GFL 3033), or “non-agitated” (in vertical position). At predetermined time points, the release medium was completely exchanged in the case of Eppendorf tubes. In the case of 10 mL glass tubes, 1 mL (ibuprofen and lidocaine) or 2 mL (prilocaine and dexamethasone) samples were withdrawn and replaced with fresh medium.

Horizontally shaken flasks: the devices were placed in plastic flasks, filled with different amounts of phosphate buffer pH 7.4 (as indicated). The latter were horizontally shaken in vertical position (80 rpm; GFL 3033). At pre-determined time points, 1 mL (ibuprofen and lidocaine) or 2 mL (prilocaine and dexamethasone) samples were withdrawn and replaced with fresh medium. Optionally, the flasks contained dialysis bags (molecular weight cut off = 12–14,000 Da; Medicell International, London, UK), into which microparticle suspensions (in 2 mL release medium) were placed. In these cases, 250 mL plastic flasks were used, filled with 48 mL phosphate buffer pH 7.4 (thus, 50 mL total release medium).

Agarose gels: as described in detail by Klose et al. (2009), microparticles (50 mg) were suspended in water (300 μL) and placed in a cylindrical hole (diameter = 1.1 cm) at the center of a 0.6% agarose gel in a Petri dish (diameter = 9 cm). The latter was placed into a water-filled desiccator to prevent water evaporation during the experiments and kept constant at 37 °C. At pre-determined time points, cylindrical gel samples (diameter = 0.5 cm) were removed at different distances from the center, weighed, dissolved in water and analyzed for their drug content (HPLC).

In case of incomplete drug release during the observation period from lipid implants, the latter were removed at the end of the experiment and dissolved in 1 mL cyclohexane. The drug was then extracted into phosphate buffer pH 7.4 (3 times) and quantified to experimentally confirm the 100% value. If indicated, the pH of the release medium was measured using a pH meter (InoLab pH Level 1; WTW, Weilheim, Germany).

2.6. Particle size analysis and morphology studies

Particle sizes were determined by laser diffraction (Mastersizer S; Malvern, Orsay, France). Macroscopic pictures were taken using a Nikon SMZ-U microscope (Nikon, Tokyo, Japan) with a Sony Hyper HAD camera (Sony, Tokyo, Japan). Scanning electron microscopy (SEM) was used to characterize the internal and external morphology of microparticles (S-4700 Field Emission Gun; Hitachi, Hitachi High-Technologies Europe, Krefeld, Germany). Samples were covered under vacuum with a carbon layer. Cross-sections were obtained after inclusion into water-based glue and cutting with razor blade.

2.7. Determination of the initial drug loading

The initial, practical drug loading was determined by dissolving accurately weighed amounts of microparticles in acetonitrile and (i) subsequent UV drug detection (lidocaine, ibuprofen: $\lambda = 263, 264 \text{ nm}$) or (ii) analyzed by HPLC in the case of

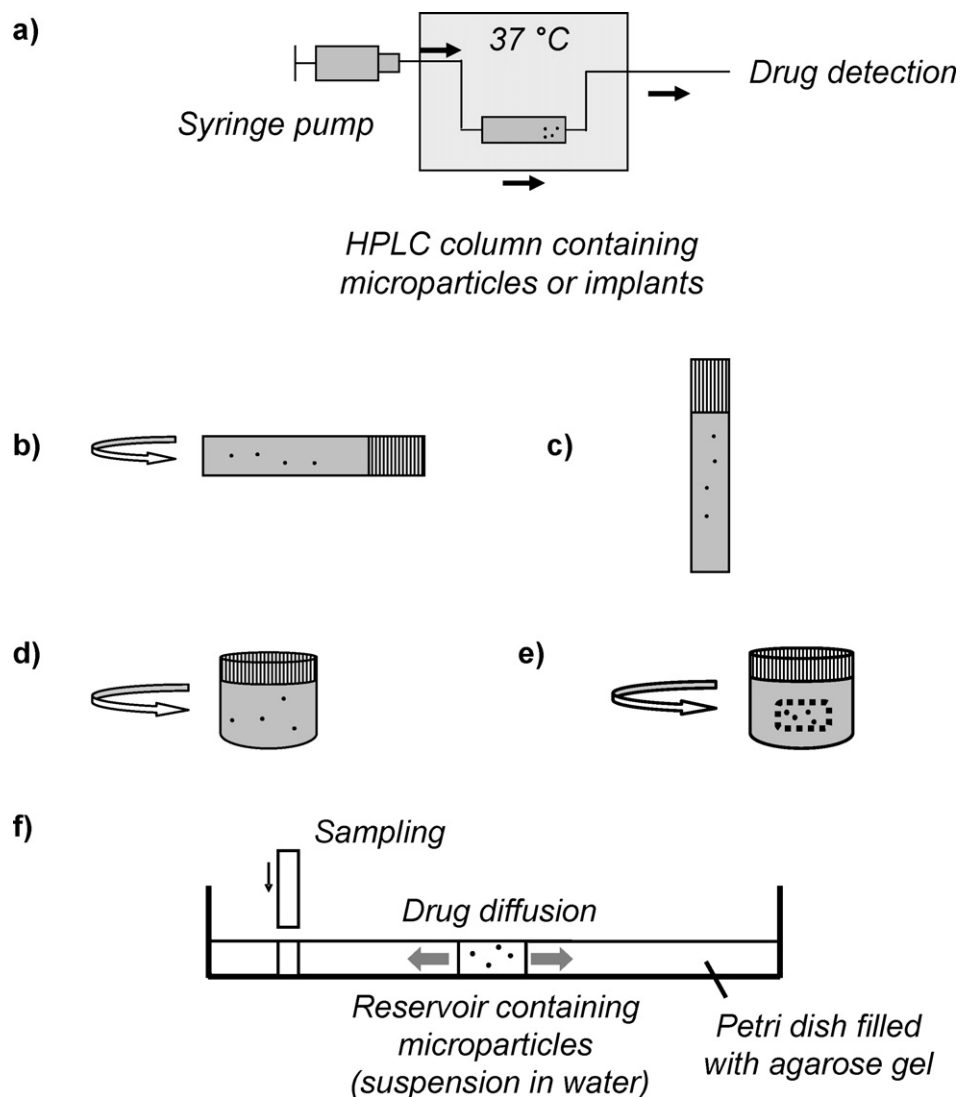


Fig. 1. Experimental setups used for drug release measurements: (a) flow-through cells (at different velocities), (b) tubes in horizontal position, agitated (80 rpm), (c) tubes in vertical position, “non-agitated”, (d) flasks in vertical position, agitated (80 rpm), (e) flasks in vertical position, containing dialysis bags, agitated (80 rpm), (f) agarose gels, “non-agitated”.

prilocaine (as described in Section 2.5, but 20 μL samples were injected). In the case of dexamethasone, the microparticles were dissolved in tetrahydrofuran and analyzed by HPLC (as described in Section 2.2, but 10 μL samples were injected at a flow rate of 2.5 mL/min). Each experiment was conducted in triplicate.

2.8. DSC and X-ray diffraction

The glass transition temperature (T_g) of the polymer was determined by differential scanning calorimetry (DSC1 Star System; Mettler Toledo, Greifensee, Switzerland). Approximately 5 mg samples were heated in sealed aluminum pans (from -10°C to 70°C) at $10^\circ\text{C}/\text{min}$. X-ray powder diffraction analysis was performed with a Panalytical X'pert Pro diffractometer (λ Cu $K\alpha = 1.54 \text{ \AA}$) in Bragg–Brentano θ – θ geometry (PANalytical, Almelo, The Netherlands). The powder samples were placed in a spinning flat sample holder.

3. Results and discussion

3.1. Lipid implants

Fig. 2a shows theophylline release from lipid implants based on glyceryl-trilaurate, which were prepared by direct compression (initial drug loading = 10% theophylline). Drug release was measured in flow-through cells at 1.5 mL/d (triangles), agitated tubes (80 rpm, 1.5 mL, diamonds), or “non-agitated” tubes (1.5 mL, squares). In all cases, the release medium was phosphate buffer pH 7.4 and the temperature 37°C . As it can be seen, the type of experimental setup significantly affected the resulting theophylline release kinetics, being fastest in the case of flow-through cells, followed by agitated and “non-agitated” tubes. This might at least partially be attributed to changes in the morphology of the implants upon exposure to the release medium, as illustrated in Fig. 2b: macroscopic pictures indicate that the cylindrical devices disintegrate upon exposure to phosphate buffer under the investigated conditions (note that also in the case of “non-agitated” tubes the release medium was completely exchanged at each sampling

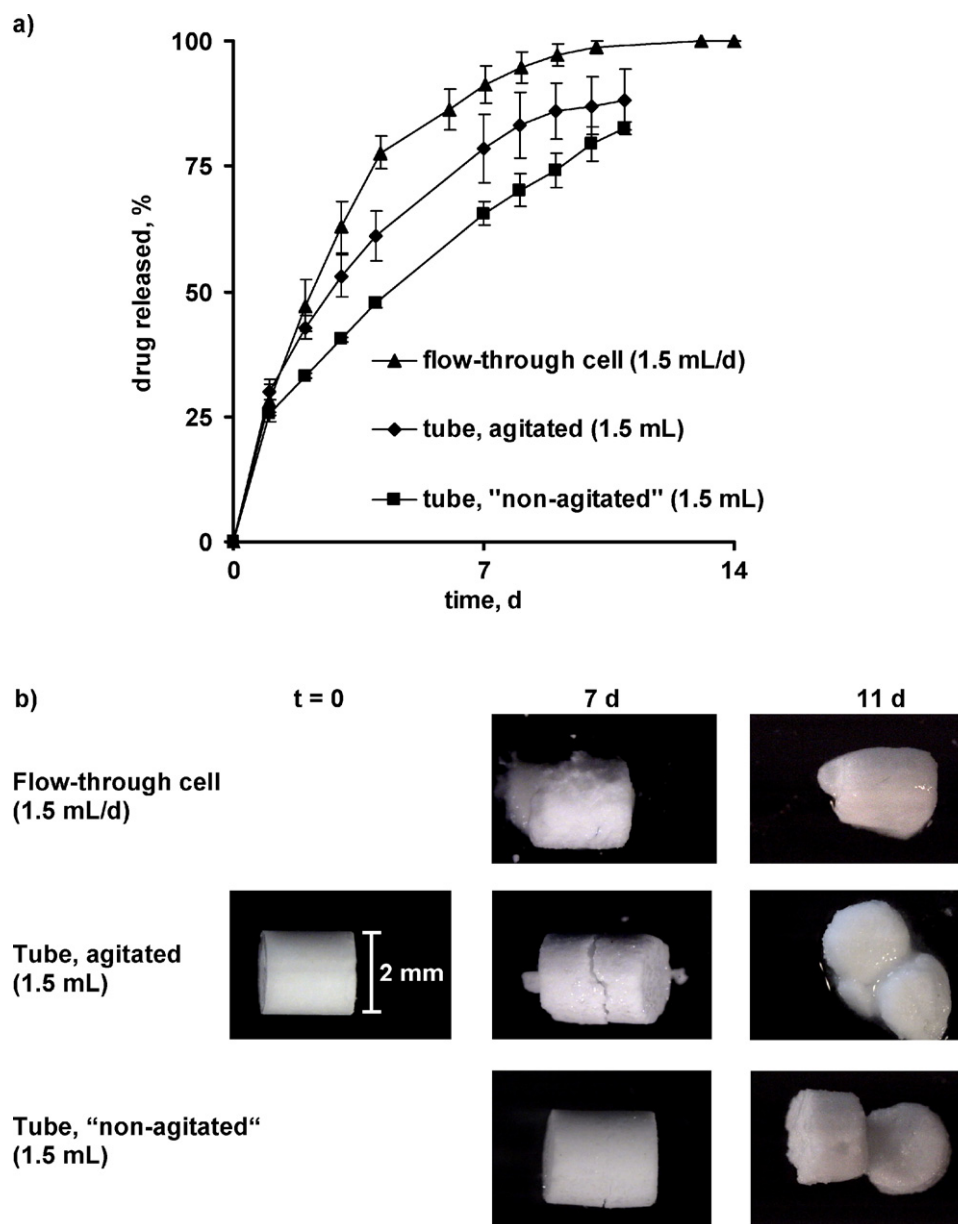


Fig. 2. Compressed lipid implants (force = 250 N) based on glyceryl-trilaurate, loaded with 10% theophylline: (a) drug release kinetics under different experimental conditions (as indicated), (b) macroscopic pictures before and after exposure to the release medium (as indicated).

time point, introducing mechanical stress). Also *in vivo* the implants can be expected to relatively rapidly disintegrate after administration. In case of flow-through cells, it is likely that the release medium partially flows through the created cracks within the lipid matrix, resulting in more pronounced convective drug transport and decreased diffusion pathway length. As previously reported, diffusional mass transport is of major importance in this type of advanced drug delivery systems (Kreye et al., 2011a; Siepmann and Siepmann, *in press*). Consequently, drug release is accelerated upon implant disintegration. In case of agitated tubes this phenomenon is likely to be less pronounced, and in case of "non-agitated" tubes such convective bulk fluid transport through the implant matrix can be considered negligible (except for the sampling time points, at which the release medium was completely exchanged). Hence, in these cases drug release acceleration due to implant disintegration can be expected to be much less important.

In contrast, propranolol hydrochloride release from lipid implants based on glyceryl-tristearate (initial drug loading = 10%), which were prepared by direct compression at 300 N (instead of 250 N), was only slightly affected by the investigated release conditions: the triangles, circles, diamonds and squares in Fig. 3a show the experimentally measured drug release in flow-through cells at 1.5 or 0.75 mL/d, as well as in agitated and "non-agitated" tubes. This difference in sensitivity of drug release to the experimental conditions might at least partially be attributed to the mechanical stability of the latter implants upon contact with the release medium under the investigated conditions: as illustrated in Fig. 3b, all implants remained intact, even after 3 weeks exposure to phosphate buffer pH 7.4. The different mechanical stability of the two types of implants can at least partially be explained by the difference in compressibility of the two lipids, the different properties of the incorporated drugs and/or the difference in the applied

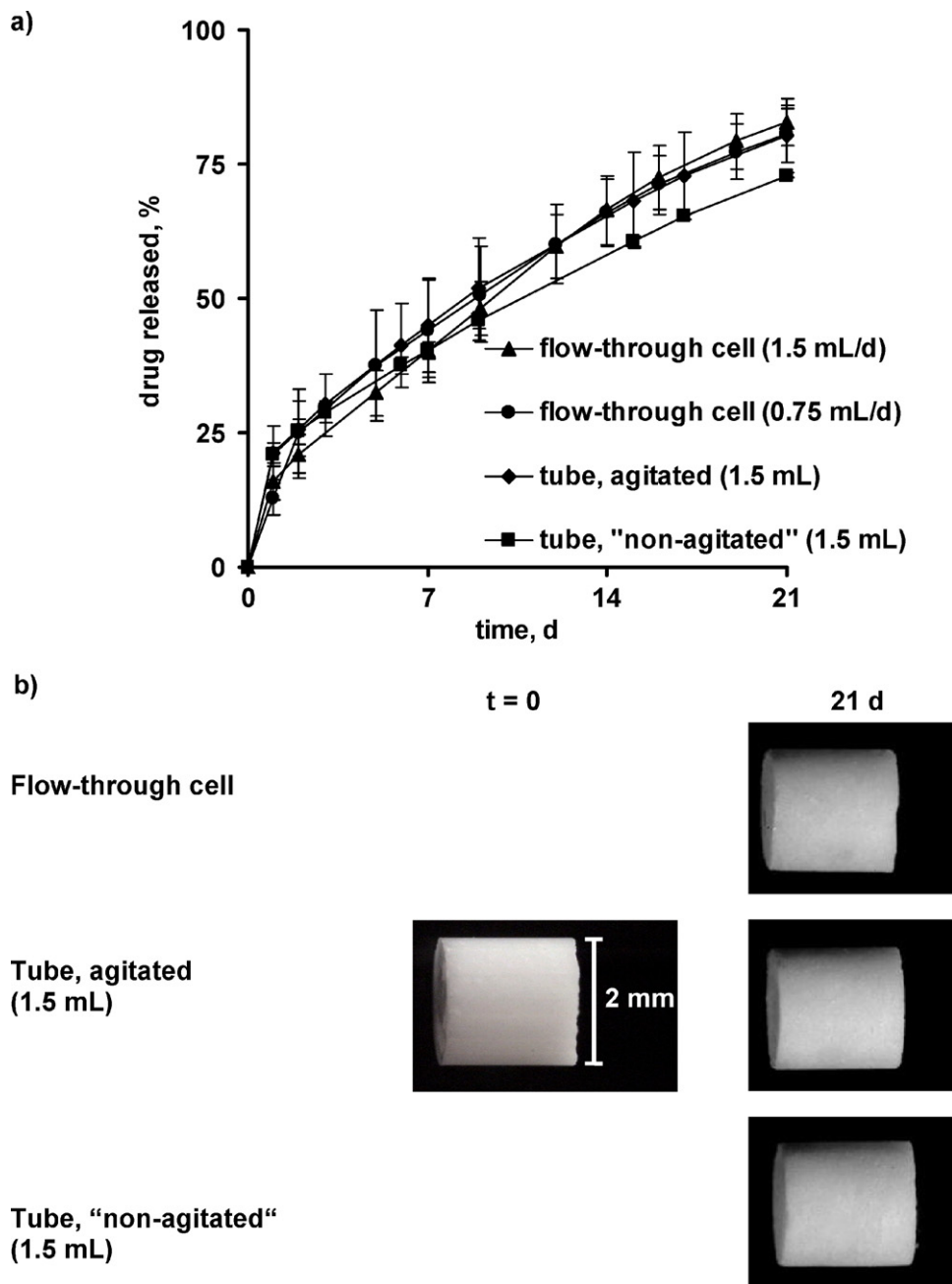


Fig. 3. Compressed lipid implants (force = 300 N) based on glyceryl-tristearate loaded with 10% propranolol hydrochloride: (a) drug release kinetics under different experimental conditions (as indicated), (b) macroscopic pictures before and after exposure to the release medium (as indicated).

compression force (300 *versus* 250 N). Since all implants remained intact during the observation period, convective mass transport through cracks within the lipid matrices can be expected to be negligible in all cases. This is consistent with the observed, very limited impact of the investigated experimental conditions on drug release (Fig. 3a).

In the case of theophylline loaded implants, based on glyceryl-palmitostearate, which were prepared by direct compression (10% drug loading), the investigated experimental conditions (flow-through cell at 1.5 mL *versus* agitated and "non-agitated" tubes filled with 1.5 mL phosphate buffer pH 7.4) did also not very much affect the resulting drug release rate (Fig. 4). The same was true for propranolol hydrochloride loaded implants based on glyceryl-tripalmitate, which were prepared by melting & casting (Fig. 5a, 10% drug loading). In the latter case, the following analytical solution

of Fick's second law of diffusion was fitted to the experimentally determined drug release kinetics to better understand the underlying drug release mechanisms (Vergnaud, 1993):

$$\frac{M_t}{M_\infty} = 1 - \frac{32}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{1}{q_n^2} \cdot \exp\left(-\frac{q_n^2}{R^2} \cdot D \cdot t\right) \times \sum_{p=0}^{\infty} \frac{1}{(2 \cdot p + 1)^2} \cdot \exp\left(-\frac{(2 \cdot p + 1)^2 \cdot \pi^2}{H^2} \cdot D \cdot t\right) \quad (1)$$

where M_t and M_∞ , represent the absolute cumulative amounts of drug released at time t , and infinite time, respectively; q_n are the roots of the Bessel function of the first kind of zero order [$J_0(q_n) = 0$], R and H denote the radius and height of the cylinder,

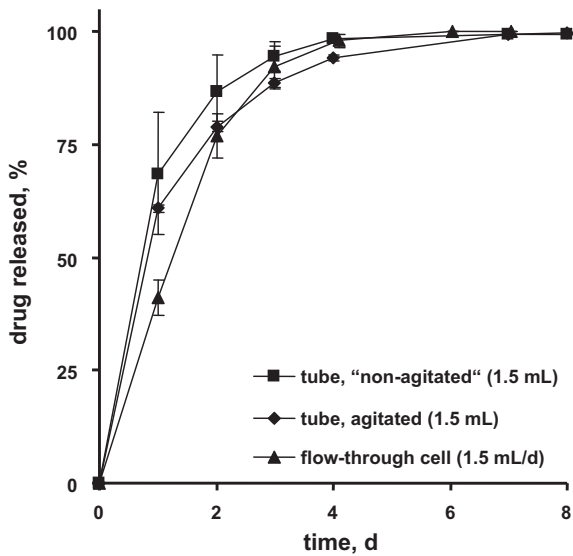


Fig. 4. Theophylline release from compressed lipid implants (force = 250 N) based on glyceryl-palmitostearate under different experimental conditions (as indicated) (10% drug loading).

and D represents the apparent diffusion coefficient of the drug or of water in the lipid matrix. The term “apparent” indicates that it is a “lumped” parameter, including also porosity and tortuosity effects. For the implementation of the mathematical model the programming language C++ was used. Eq. (1) is based on the assumption of pre-dominant drug or water diffusion control, considers axial and radial mass transport in cylinders, homogeneous initial drug and lipid distribution within the systems as well as perfect sink conditions throughout the observation period. As it can be seen in Fig. 5a, good (to rather good) agreement between theory (curves) and experiment (symbols) was observed for all the investigated experimental release conditions. Thus, diffusion is likely to be the dominant mass transport step for the control of propranolol hydrochloride from these devices, whether the implants are placed into flow-through cells or tubes and whether or not the latter are agitated. Based on these calculations the apparent diffusion coefficients of the drug (or water) in these advanced delivery systems could be determined (Fig. 5b). Note that the available results do not allow distinguishing between “drug diffusion control” and “water diffusion control” (Kreye et al., 2011a). Clearly, the diffusivities were similar and the error bars overlapping. Thus, the investigated experimental conditions did neither affect the underlying drug release mechanism, nor the key property of the system (in this case drug or water mobility).

3.2. PLGA-based microparticles

Fig. 6a shows the impact of the investigated release conditions (flow-through cell at 0.7 or 2 mL/d versus agitated and “non-agitated” tubes or flasks) on ibuprofen release from PLGA-based microparticles, which were prepared using an O/W emulsion solvent extraction/evaporation technique (initial drug loading = 4%). As it can be seen, the sensitivity of drug release to the type of experimental setup was limited. The same was true for ibuprofen release from PLGA-based microparticles prepared by a W/O/W solvent extraction/evaporation technique (Fig. 6b). In the latter case, the initial drug loading was also 4%, but the particles were much more porous before exposure to the release medium (Klose et al., 2008, 2010). To better understand the underlying drug release mechanisms from this type of advanced drug delivery systems, the following analytical solution of Fick’s second law of diffusion was

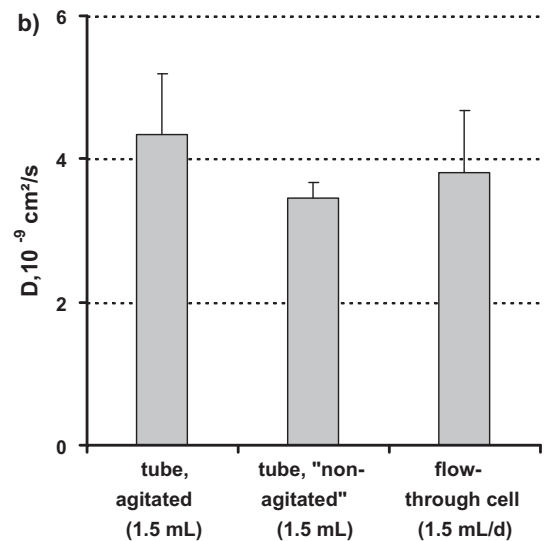
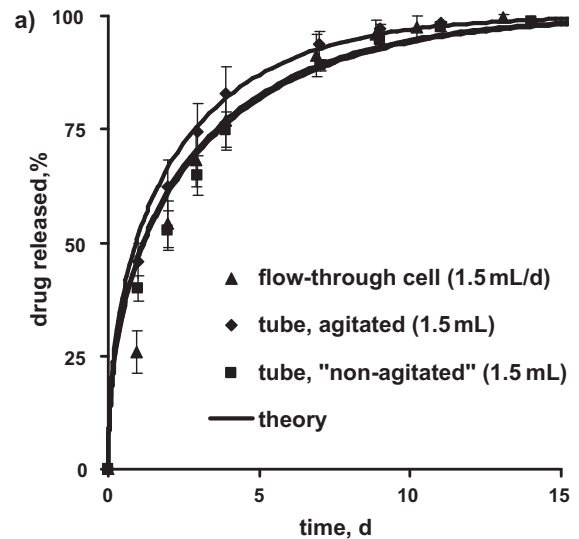


Fig. 5. Lipid implants prepared by melting & casting, based on glyceryl-tripalmitate and loaded with 10% propranolol hydrochloride: (a) drug release kinetics under different experimental conditions (as indicated; symbols: experimental values; curves: theory – Eq. (1)), (b) apparent drug (or water) diffusion coefficient within the implants (determined by the fittings shown in (a)).

fitted to the experimentally determined ibuprofen release kinetics (Crank, 1975):

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

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 systems. Eq. (2) considers diffusional mass transport in spherical microparticles, in which the drug is initially homogeneously distributed. Perfect sink conditions are assumed and the mobility of the drug within the system is considered to be time-independent. As it can be seen in Fig. 6b, good agreement between theory (curves) and experiment (symbols) was obtained in all cases, indicating that drug diffusion is likely to be the dominant mass transport step in these systems, irrespective of the investigated experimental release conditions. Based on these calculations, the following apparent ibuprofen diffusivities could be determined: $D = 2.5 (\pm 0.0)$, $2.5 (\pm 0.0)$, $2.9 (\pm 0.2)$, $2.5 (\pm 0.2)$, and

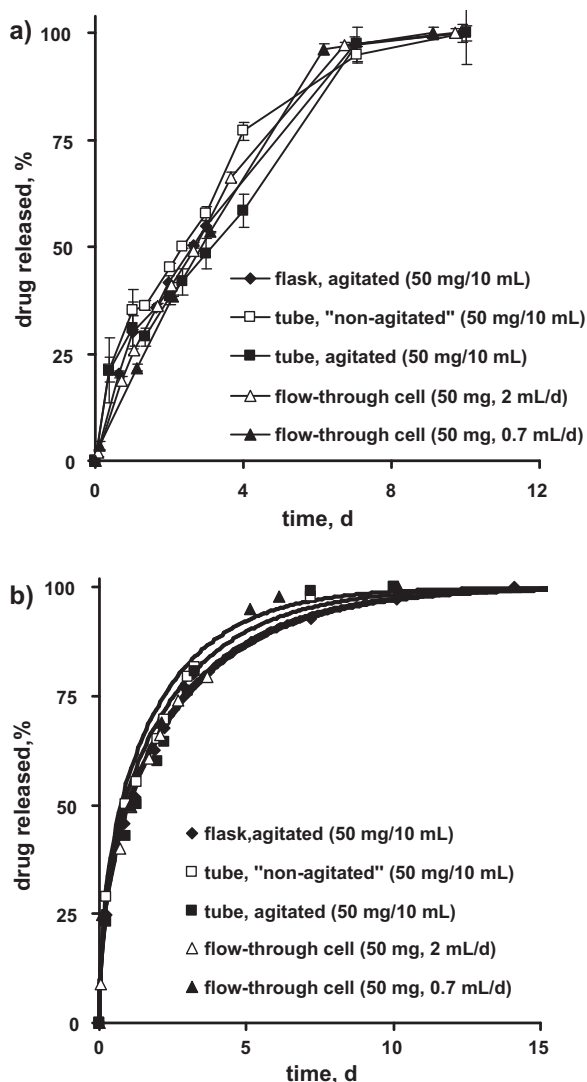


Fig. 6. Impact of the experimental conditions on ibuprofen release from PLGA-based microparticles prepared by: (a) an O/W solvent extraction/evaporation method, (b) a W/O/W solvent extraction/evaporation method (symbols: experimental results; curves: theory – Eq. (2) (4% drug loading).

$3.3 (\pm 0.1) \times 10^{-12} \text{ cm}^2/\text{s}$ in the case of agitated flasks, agitated tubes, “non-agitated” tubes, flow-through cells at 2 mL/d and flow-through cells at 0.7 mL/d, respectively. Note that these diffusivities are “time-averaged” values: since PLGA degrades upon exposure to aqueous media, the polymer molecular weight decreases with time and drug mobility increases. Importantly, also for this type of parenteral controlled drug delivery systems, the investigated experimental release conditions do neither significantly affect the underlying drug release mechanisms, nor the resulting drug release kinetics.

Fig. 7 illustrates the moderate impact of using agitated flasks, agitated or “non-agitated” tubes or flow-through cells at 0.7 or 2 mL/d on the release of lidocaine from PLGA-based microparticles, prepared by a W/O/W solvent extraction/evaporation technique. Also in this case, the drug loading was low (4%). Thus, the drug is likely to be molecularly dispersed within the systems (Klose et al., 2010). Interestingly, the impact of the investigated release conditions (flow-through cells at 0.7 or 2 mL/d versus agitated and “non-agitated” tubes or flasks) was more pronounced in the case of PLGA-based microparticles loaded with $3.3 (\pm 0.1)$ or $14.2 (\pm 0.3)\%$ prilocaine (free base) (Fig. 8). The mean diameters of these

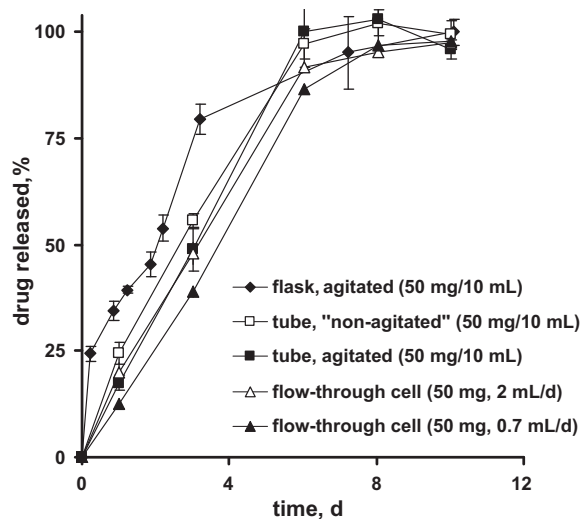


Fig. 7. Impact of the experimental conditions on lidocaine (free base) release from PLGA-based microparticles prepared by a W/O/W solvent extraction/evaporation method (4% drug loading).

particles were $68 (\pm 11)$ and $73 (\pm 22) \mu\text{m}$, respectively. The SEM pictures in Fig. 9 and X-ray diffraction patterns in Fig. 10 indicate that the drug is likely to be dissolved in the PLGA matrix, irrespective of the drug loading. This hypothesis was further confirmed by the experimentally measured decrease in the glass transition temperature of the PLGA (Resomer RG 502H) in microparticles loaded with 3.3 and 14.2% prilocaine free base, as determined by DSC analysis: T_g (onset) = 35 and 24°C . Thus, this drug acts as a plasticizer for PLGA. The DSC thermograms (not shown) did not indicate any drug melting peaks. Importantly, for this type of microparticles the underlying drug release mechanism was fundamentally affected by the investigated experimental setup: the curves in Fig. 8 show fittings of Eq. (2) to the experimentally determined prilocaine release kinetics (symbols). The *solid* curves indicate good agreement between theory and experiment, whereas the *dotted* curves indicate poor agreement. Based on these calculations the apparent and time-averaged diffusivity of the drug in the PLGA matrices could be determined in case of good agreement (Fig. 8). Note that potentially given “non-sink conditions” cannot explain the observed differences: in all cases, except for microparticles with an initial drug loading of 14.2% studied in flow-through cells at 0.7 mL/d, sink conditions were provided (note that in this case the respective D value is biased). Furthermore, prilocaine release from these microparticles was very similar whether 10 mg (data not shown) or 50 mg of the latter were filled into the flow-through cells, resulting in non-sink and sink conditions at 0.7 mL/d (prilocaine solubility in phosphate buffer pH 7.4 at $37^\circ\text{C} = 8.2 \pm 0.1 \text{ mg/mL}$). Interestingly, drug release was much slower, when the microparticles were placed into agarose gels (open diamonds on the right hand side of Fig. 8). This is in contrast to previously reported propranolol HCl loaded, PLGA-based microparticles prepared by a W/O/W solvent extraction/evaporation method: in the latter case, drug release was faster in agarose gels compared to agitated tubes (Klose et al., 2009). Prilocaine release in dialysis bags placed in agitated flasks (stars) was very similar to drug release in flasks without dialysis bags (open circles), indicating that the presence of the artificial membrane did not significantly alter drug transport in this case.

Importantly, the investigated release conditions (agitated/“non-agitated” flasks and tubes, flow-through cells at 0.7 and 2 mL/d) also significantly altered the experimentally measured release kinetics of dexamethasone microparticles, based on PLGA and prepared by

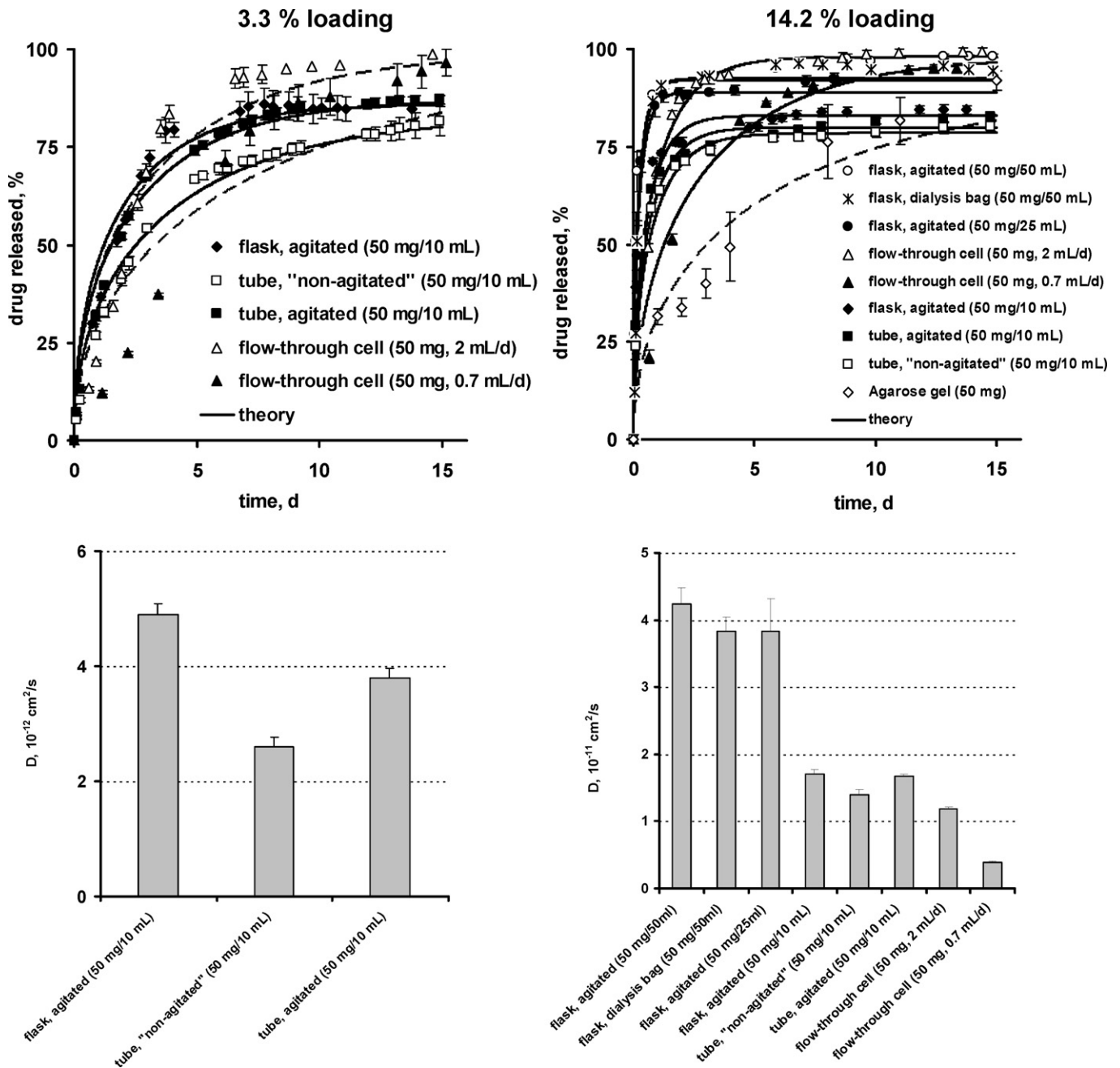


Fig. 8. Drug release from PLGA-based microparticles prepared by an O/W solvent extraction/evaporation method loaded with 3.3 or 14.2% prilocaine (free base) (as indicated) under different experimental conditions (symbols: experimental results; curves: fitted theory – Eq. (2); solid curves: good agreement, dashed curves: poor agreement) (top row). Apparent drug diffusion coefficients within the microparticles (bottom row).

a S/O/W solvent extraction/evaporation technique (Fig. 11). Note that the relative cumulative amount of intact drug release is plotted on the y-axis (drug degraded within the microparticles and/or within the release medium prior to measurement is not taken into account; the 100% reference value is the initial drug loading). As reported by Hickey et al. (2002) dexamethasone can be degraded during drug release measurements. This was confirmed in the present study in the case of microparticle exposure to the release medium in tubes: in the case of agitated tubes, drug release leveled off well below 100% after about 80 d (filled squares in Fig. 11). No intact dexamethasone was detected in the remnants after 120 d exposure (the latter were separated by filtration, filtered, freeze-dried and the drug content measured using the same

analytical method as for the determination of the initial drug loading, described in Section 2.7). In case of microparticles released in "non-agitated" tubes (open squares in Fig. 11), only 1% intact drug was recovered in the remnants after 130 d exposure to phosphate buffer pH 7.4. Thus, under these conditions significant amounts of dexamethasone were degraded during the drug release measurements. This was also the case when the microparticles were released in agitated flasks (open diamonds in Fig. 11). In contrast, when the same type of microparticles was exposed to phosphate buffer in flow-through cells, dexamethasone degradation was negligible (triangles in Fig. 11): irrespective of the investigated flow rate, about 100% of the intact drug was released after about 50 d. This might serve as an indication for the fact that drug degradation

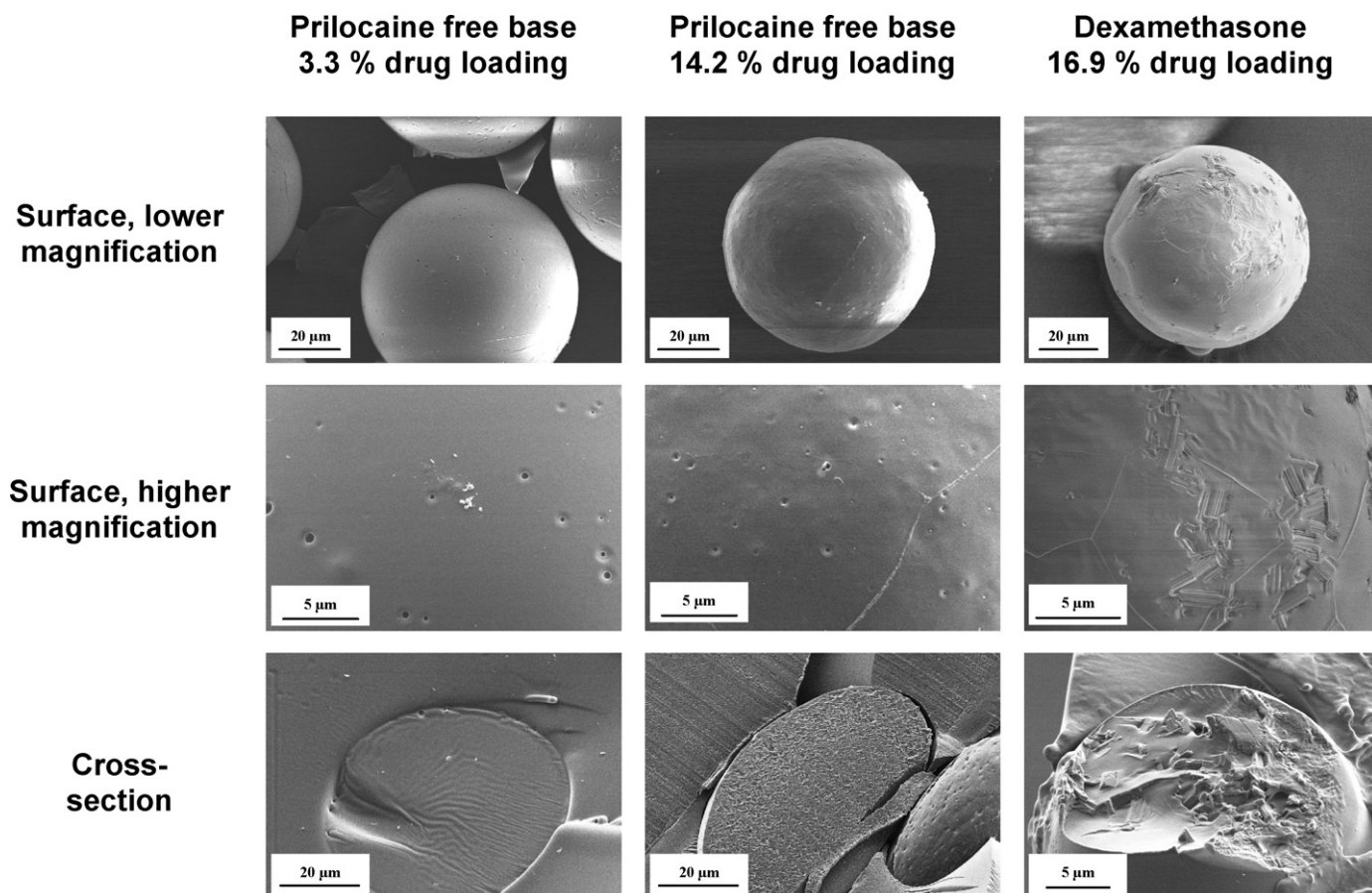


Fig. 9. SEM pictures of surfaces and cross-sections of prilocaine free base and dexamethasone loaded PLGA-based microparticles before exposure to the release medium.

is likely to be much more pronounced in the bulk fluid than within the microparticles.

The practical drug loading of the systems was 16.9 (± 0.2)% and dexamethasone was at least partially present as drug crystals within the microparticles, as evidenced by SEM pictures (Fig. 9), X-ray diffraction (Fig. 10) and DSC analysis (indicating a sharp melting peak, data not shown). The mean particle size was 35 (± 27) μm , the glass transition temperature 35 $^{\circ}\text{C}$. Fig. 12 shows that differences in the pH of the bulk fluid cannot fully explain the observed differences in drug release from the investigated dexamethasone loaded, PLGA-based microparticles. Also, the potential existence of

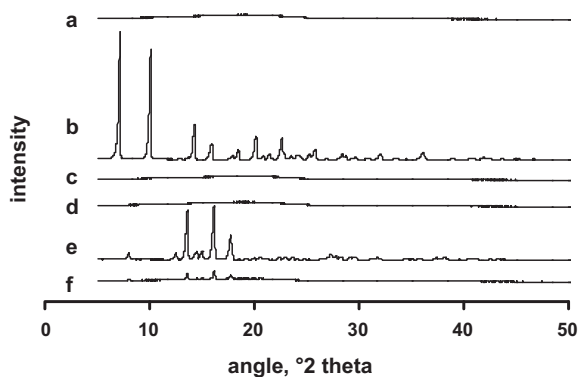


Fig. 10. X-ray diffraction patterns of: (a) PLGA powder (Resomer RG 502H), (b) prilocaine free base powder, (c) PLGA microparticles loaded with 3.3% prilocaine free base, (d) PLGA microparticles loaded with 14.2% prilocaine free base, (e) dexamethasone powder, (f) microparticles loaded with 16.9% dexamethasone.

non-sink conditions is not the (dominant) reason for the observed phenomenon: in case of agitated flasks and flow-through cells at 2 mL/d in Fig. 11 sink conditions were provided throughout the observation period, whereas in case of “non-agitated” and agitated tubes as well as flow-through cells at 0.7 mL/d at least partially

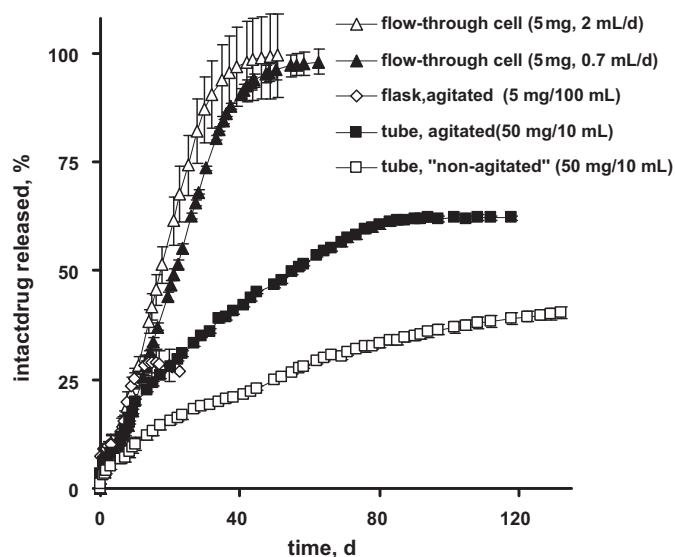


Fig. 11. Impact of the experimental conditions on the release of intact dexamethasone from PLGA-based microparticles prepared by a S/O/W solvent extraction/evaporation method (16.9% drug loading). The 100% reference value for the relative, cumulative amount of drug released is the initial drug loading.

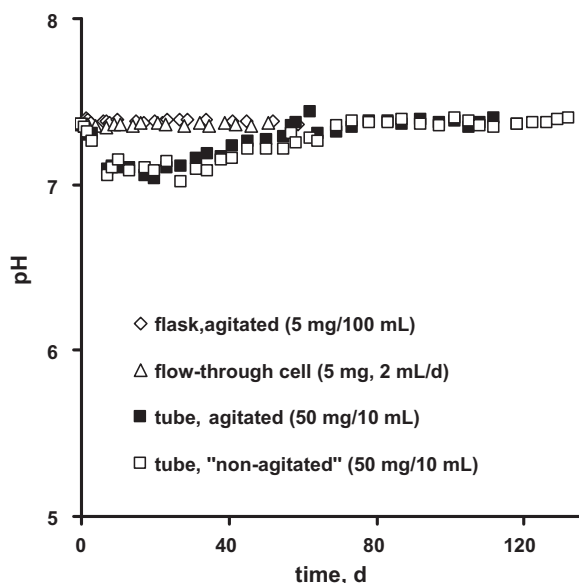


Fig. 12. Impact of the experimental conditions on potential changes in the pH of the release medium during dexamethasone release from PLGA-based microparticles prepared by a S/O/W solvent extraction/evaporation method (16.9% drug loading).

non-sink conditions existed (solubility of dexamethasone in phosphate buffer pH 7.4 at 37 °C = 73.9 ± 0.4 mg/L).

4. Conclusions

The impact of the experimental conditions on drug release from parenteral depot systems might be negligible, moderate or important. The reasons for these fundamental differences in the sensitivity of drug release are not yet fully understood. The knowledge of the underlying drug release mechanism can be very helpful and avoid misleading conclusions from *in vitro* drug release measurements. Great caution must be paid if the depot formulations are poorly characterized and the drug release mechanisms not understood.

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