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Effects of the type of release medium on drug release from PLGA-based microparticles: Experiment and theory

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Abstract

The major objectives of the present study were: (i) to prepare 5-fluorouracil (5-FU)-loaded, poly(lactic-co-glycolic acid) (PLGA)-based microparticles, which can be used for the treatment of brain tumors, (ii) to study the effects of the type of release medium on the resulting drug release kinetics, and (iii) to get further insight into the underlying drug release mechanisms. Spherical microparticles were prepared by a solvent extraction method and characterized using different techniques, including size exclusion chromatography (SEC), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and particle size analysis before and upon exposure to various release media. Interestingly, very different drug release patterns (including mono-, bi- and tri-phasic ones) were observed, depending on the pH, osmolarity and temperature of the release medium. An adequate mathematical theory was used to quantitatively describe the experimentally measured 5-FU release patterns. The model considers the limited solubility of the drug, polymer degradation as well as drug diffusion and allowed to determine system and release medium specific parameters, such as the diffusion coefficient of the drug. In particular, the pH and temperature of the release medium were found to be of major importance for the resulting release patterns. Based on the obtained knowledge the selection of an appropriate release medium for in vitro tests simulating in vivo conditions can be facilitated, and "stress tests" can be developed allowing to get rapid feedback on the release characteristics of a specific batch.

Keywords: Microparticle; Controlled release; PLGA; Release medium; Modeling

1. Introduction

PLGA-based microparticles offer various important advantages compared to conventional pharmaceutical dosage forms, such as: (i) the possibility to accurately control the resulting drug release rates over prolonged periods of time (Chen et al., 1997), (ii) easy administration (using standard needles), (iii) good biocompatibility (even with brain tissue) (Anderson and Shive, 1997; Fournier et al., 2003), and (iv) complete biodegradation (avoiding the removal of empty remnants upon drug exhaust). This is why the practical importance of this type of advanced drug delivery systems is steadily increasing.

Ideally, controlled drug delivery systems provide release patterns that result in optimal drug concentration-time-profiles at the site of action in the human body and, thus, improved therapeutic effects. Obviously, it is of fundamental importance to know at which rate the drug is released from the dosage forms. Unfortunately, it is not straightforward to obtain this information. In vivo, it is generally very difficult to directly measure the amount of drug released into the living tissue. Often, indirect techniques are used, e.g. the amounts of drug remaining within the dosage forms are determined as a function of time. However, this requires the analysis of the entire delivery system, in example the analysis of all administered microparticles, which can be a difficult task in practice. In addition, in vivo experiments are time-consuming and cost-intensive. Thus, it is highly desirable to establish adequate in vitro test methods, simulating the conditions for drug release in vivo (Burgess et al., 2002).

Surprisingly, little knowledge is yet available on the importance of the experimental conditions for the resulting drug release kinetics from PLGA-based microparticles in vitro (Hora et al., 1990; Blanco-Prieto et al., 1999; Bittner et al., 1999).

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Blanco-Prieto et al. (1999) found that vapreotide pamoate release from spray-dried, PLGA-based microparticles into phosphate buffer pH 7.4 was much more slowly than into serum or phosphate buffer pH 7.4 containing 1% bovine serum albumin (BSA). This was at least partially attributed to the lower drug solubility in pure phosphate buffer. Shameem et al. (1999) showed that the temperature of the release medium has a significant effect on leuprorelin release from PLGA-based microparticles prepared by a solvent extraction method into phosphate buffer pH 7.0. Increasing the temperature from 37 to 60 °C resulted in a drastic acceleration of drug release. Thus, release measurements at elevated temperature can be very helpful as "stress tests" during product development and production, providing rapid feedback on the characteristics of a particular batch. Dunne et al. (2000) showed that an increase in the temperature of the release medium from 25 to 45 °C significantly accelerated the degradation of pure PLGA-based microparticles. The effects of the pH of the release medium (1.2, 7.4 and 9.6) on drug release from 5-FU-loaded, poly(D,L-lactic acid) (PLA)- or poly(lactic-co-glycolic acid) (PLGA)-based microparticles was studied by Ciftci et al. (1994, 1996). These systems are intended for the treatment of liver carcinomas upon i.v. administration. Differences in the observed release profiles were attributed to the altered solubilites of the drug within the release media. The degradation of poly(glycolic acid) (PGA)- and poly(lactic-co-glycolic acid) (PLGA)-based absorbable sutures in buffer media of different pH was studied by Chu (1982a,b). Changes in the mechanical properties of the sutures (e.g., breaking strength) were followed at pH 5.3, 7.4 and 10.1 and were attributed to the acid and base-induced hydrolysis of these polymers. Kamijo et al. (1996) showed that the release of the LHRH agonist leuprorelin from PLGA-based microparticles was significantly affected by the pH of the release medium in vitro and that degradation products of the polymer accumulated in vivo (upon s.c. injection of the microparticle in rats).

In the present study, the effects of the type of release medium (including its pH, osmolarity and temperature) on drug release from 5-FU-loaded, PLGA-based microparticles were studied in vitro. These microparticles can be used for the local treatment of brain tumors (Menei et al., 1999, 2004, 2005). The principle of this treatment method is as follows: If the tumor is operable, it is removed from the brain tissue. However, in contrast to many other tumors it is not possible to remove large quantities of surrounding tissue at the same time, because of the risk to affect vital brain functions. Thus, the probability that single tumor cells remain within the neighboring (infiltrated) tissue is elevated, and many patients die due to local tumor recurrences in the direct vicinity of the primary tumor. To reduce this risk, 5-FU-loaded, PLGA-based microparticles can be injected into the wall of the resection cavity (during the same operation, when the crane is still open). The anticancer drug is then released in a time-controlled manner over several weeks at the site of action.

To better understand the effects of the type of release medium on the resulting drug release kinetics, an adequate mathematical model has been used to analyze experimentally measured characteristics of the microparticles and changes thereof upon exposure to various release media. This model was recently proposed (Faisant et al., 2002) and takes into account: (i) the spherical geometry of the investigated microparticles, (ii) the limited solubility of the drug within the release media, (iii) polymer degradation, and (iv) drug diffusion with time-dependent diffusion coefficients. As it is a physicochemically realistic model it allows to determine system and medium specific parameters, such as the diffusion coefficient of the drug within the polymeric matrix. Thus, the effects of the type of release medium on these parameters can be quantified and deeper insight into the underlying mass transport mechanisms can be gained.

The major objectives of the present study were: (i) to prepare 5-FU-loaded, PLGA-based microparticles, (ii) to study the effects of the type of release medium (e.g., its temperature, pH and osmolarity) on the resulting drug release kinetics in vitro, and (iii) to get further insight into the underlying drug release mechanisms.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA; Resomer® RG 506; PLGA 50:50; containing 25% D-lactic units, 25% L-lactic units and 50% glycolic units; Boehringer Ingelheim, Paris, France), 5-fluorouracil (5-FU; Roche, Neuilly sur Seine, France).

2.2. Microparticle preparation

5-FU-loaded, PLGA-based microparticles were prepared with an oil-in-water (O/W) solvent extraction technique, which has previously been described in detail (Faisant et al., 2002). Briefly, 4 g drug were dispersed within 45 mL dichloromethane using an Ultra turrax (13,500 rpm, 4 min; Ika, T25 basic/S25N-10G, Staufen, Germany). The PLGA (5 g) was added to this dispersion, which was subsequently stirred for 4 h to allow complete polymer dissolution. This organic phase was emulsified into 1.5 L aqueous polyvinyl alcohol solution (10% w/w, 2 °C). The emulsion was stirred with a propeller at 375 rpm for 4.75 min. The addition of 4.5 L water and further stirring for 2 min allowed microparticle hardening. The latter were separated by filtration under nitrogen pressure (0.8 bar; filtration system supplied by Sartorius, Palaiseau, France) with a cellulose ester filter membrane (8 μm; Millipore, Saint Quentin en Yvelines, France), freeze-dried and sieved (125 μm). To minimize the amount of residual organic solvent, the microparticles were also vacuum-dried at 37 °C for 72 h.

2.3. Determination of the initial drug loading

Approximately 7 mg drug-loaded microparticles were dissolved in 50 mL dimethylsulfoxide. Upon agitation at room temperature, samples were analyzed UV-spectrophotometrically (λ = 266 nm; Uvikon 922, Kontron, St Quentin en Yvelines, France).

2.4. Particle size analysis

Mean particle diameters were determined using a Coulter Counter (Multisizer, Coultronics, Margency, France). Microparticles (approximately 10 mg) were suspended by sonication for 10 min in an aqueous solution of Tween 80 (0.02% w/v) and assayed after dilution in Isoton® II (Coultronics, Margency, France).

2.5. Scanning electron microscopy (SEM)

Samples were carbon coated (10 nm) using a MED 020 (Baltec, Balzers, Liechtenstein) and observed on a JEOL 6301F field emission microscope (JEOL, Paris, France) (voltage: 5 kV). Cross-sections of the microparticles were obtained after inclusion into epon (Epon 812 Fluka, Saint Quantin Fallavier, France) (liquid monomers served as suspension vehicle and were subsequently polymerized under solidification) and cutting with a diamond knife (Leica Ultracut S, Rueil Malmaison, France).

2.6. In vitro drug release studies

Samples of microparticles were placed within dialysis bags (approximately 20 mg/bag; Spectra/Por® membrane, molecular weight cut-off 6–8 kDa; Prolabo, Paris, France) at the bottom of glass flasks containing 250 mL release medium. The flasks were fixed in an agitated thermostatic water bath (100 rpm; Polytest 30®; Fischer Scientific, Illkirch, France), and protected from light. At pre-determined time intervals, 1 mL samples were withdrawn and analyzed UV-spectrophotometrically ($\lambda = 266$ nm; Uvikon 922). Each experiment was conducted in triplicate (mean values ±1 S.D. are indicated in the figures). Drug release was studied in various types of release media, differing in:

- *Osmolarity*: 280, 370, 560, 700, and 840 mosm/L [phosphate buffer pH 7.4 (Eur. Pharm.), 0.13 M, 37 °C; osmolarities > 280 mosm/L were adjusted by adding adequate amounts of NaCl].
- *Buffer concentration*: 0.02, 0.13, and 0.26 M [phosphate buffer pH 7.4 (Eur. Pharm.), 700 mosm/L (adjusted by adding adequate amounts of NaCl), 37 °C].
- *pH*: citrate buffer pH 1.3, phosphate buffer pH 4.5, phosphate buffer pH 7.4, and carbonate buffer pH 10.8 (Eur. Pharm.) [700 mosm/L (adjusted by adding adequate amounts of NaCl), 37 °C].
- *Temperature*: 37, 45, 53, 60, and 65 °C [phosphate buffer pH 7.4 (Eur. Pharm.), 0.13 M, 280 mosm/L].

In all cases, sodium azide (0.2%) was added to avoid microbial growth. Stability studies showed that 5-FU was stable in all release media during the entire observation periods.

2.7. Drug solubility measurements

Excess amounts of 5-FU were added to the different release media in glass flasks. The latter were fixed in an agitated thermostatic water bath (100 rpm, 37 °C; Polytest 30®). Aliquots of the supernatants were regularly withdrawn and immediately diluted with fresh media (to avoid drug precipitation due to the decrease in temperature). The drug content was measured UV-spectrophotometrically ($\lambda = 266$ nm; Uvikon 922). The plateau values (equilibrium values) were considered as drug solubilities. Each experiment was conducted in duplicate.

2.8. Monitoring of changes within the microparticles during drug release

Samples of microparticles were treated as described in Section 2.6, except that 300 mg microparticles were placed within each dialysis bag (which did not alter the resulting drug release kinetics, data not shown). At pre-determined time intervals, dialysis bags were withdrawn and their contents filtered (0.45 μm). The separated microparticles were washed with distilled water (three times), and then (i) freeze-dried and stored at 4 °C for further analysis [size exclusion chromatography (SEC)], or (ii) deep-frozen and stored for further analysis [differential scanning calorimetry (DSC), in order to measure the glass transition temperature (T_g) of the polymer in water-containing microparticles].

2.9. Differential scanning calorimetry (DSC)

The glass transition temperature of the polymer (T_g) was measured by differential scanning calorimetry (DSC, Mettler Toledo, Viroflay, France). Approximately 5 mg samples were heated in sealed aluminum pans (investigated temperature range: −30 to +330 °C, heating rate: 10 °C/min, two heating cycles).

2.10. Size exclusion chromatography (SEC)

Samples of microparticles were dissolved in dimethylsulfoxide (0.5% w/v). One volume part of this solution was mixed with three volume parts of the mobile phase (tetrahydrofurane:methanol:acetic acid, 85:15:0.8 v/v/v). Approximately 200 μL of this mixture were injected into a size exclusion (gel permeation) chromatography (SEC) apparatus equipped with a pre-column (Shodex KGF, Waters, St Quentin en Yvelines, France), two main columns (Styragel HR1, Waters; PL-gel 5 μm 10^E 4A, Polymer Laboratories, Marseille, France) and a refractometric detector (RID-10A, Shimadzu, Touzart et Matignon, Courtaboeuf, France). All measurements were performed at a flow rate of 1 mL/min at room temperature. The system was calibrated with polystyrene standards (PS-2, Polymer Laboratories, Marseille, France). All indicated molecular weights are weight-average molecular weights (Mw).

2.11. Mathematical modeling

A mathematical model taking into account the limited solubility of the drug, polymer degradation and drug diffusion was used to quantitatively describe the experimentally observed 5-FU release kinetics. The model has previously been described in detail (Faisant et al., 2002). Briefly, the theory is based on the assumption of linear, pseudo-steady state drug concentration

gradients, which are rapidly established within the microparticles upon water imbibition due to the high "initial drug loading:drug solubility" ratio. Higuchi derived the well-known relationship between the relative amount of drug released and the square root of time under these conditions for the case of planar devices (Higuchi, 1961). Later, he extended his model also to spherical geometry, deriving an implicit mathematical equation (Higuchi, 1963). A similar approach was used by Koizumi and Panomsuk (1995), leading to an (approximate) explicit solution describing drug release from non-degradable, spherical devices having the advantage to be easier to handle than the respective equation derived by Higuchi:

$$M_t = 4\pi r^2 \left[\sqrt{2(c_0 - c_s)c_s Dt} + \frac{4c_s Dt}{9r} \left(\frac{c_s}{2c_0 - c_s} - 3 \right) \right] \quad (1)$$

Here, M_t is the cumulative absolute amount of drug released at time t, r represents the radius of the spherical device, c_0 and c_s the initial drug concentration and the solubility of the drug within the system, respectively, and D denotes the constant diffusion coefficient of the drug.

To account for polymer degradation, this approximate pseudo-steady state solution was combined with the following equation, allowing to calculate the 5-FU diffusivity within the microparticles as a function of the average polymer molecular weight of the PLGA at time t [Mw (t)]:

$$D[\mathrm{Mw}(t)] = D_0 + \frac{k}{\mathrm{Mw}(t)} \quad (2)$$

where D_0 is the diffusion coefficient of the drug in the non-degraded microparticles ($t = 0$), and k is a constant. Considering pseudo first order PLGA degradation kinetics upon contact with the release medium, the decrease in polymer molecular weight was calculated as follows:

$$\mathrm{Mw}(t) = \mathrm{Mw}_0 \exp(-k_{\mathrm{degr}} t) \quad (3)$$

where Mw_0 is the initial polymer molecular weight (at $t = 0$, before exposure to the release medium) and k_{degr} is the pseudo first order degradation rate constant of the PLGA.

Eqs. (1)–(3) were fitted to the experimentally determined 5-FU release rates. For the implementation of the mathematical model the programming language C++ was used (Borland C++ 6.0).

3. Results and discussion

3.1. Microparticle size, loading and morphology

The obtained microparticles were spherical in shape, with a mean diameter of 47 μm and an initial drug loading of 26% (w/w). The 5-FU was located in the form of small crystals in different-sized cavities, which were distributed throughout the microparticles. Fig. 1 shows a scanning electron microscopy picture of cross-sections of several microparticles: Black regions represent non-degraded PLGA, bright gray regions drug-filled cavities and scattered regions the epon that was used to obtain the cross-sections.

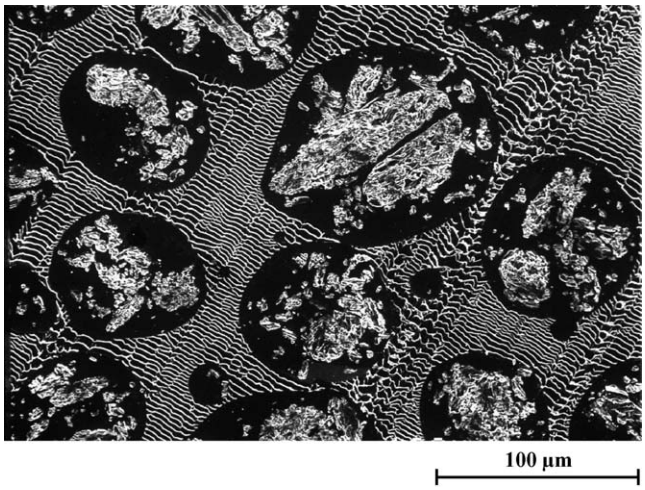

Fig. 1. Scanning electron microscopy (SEM) picture of cross-sections of 5-FU-loaded, PLGA-based microparticles (before exposure to the release media, at $t = 0$): black regions represent non-degraded polymer, bright gray regions drug-filled cavities and scattered regions the epon that was used to obtain the cross-sections.

3.2. In vitro drug release kinetics and mechanisms

Fig. 2 shows the experimentally determined release kinetics of 5-FU from the investigated PLGA-based microparticles in phosphate buffer pH 7.4 (0.13 M, 280 mosm/L) at 37 °C (symbols). Typical, three-phasic release patterns were observed: (i) At early time points, the release rate was high (initial "burst effect"), followed by (ii) a phase with an approximately constant

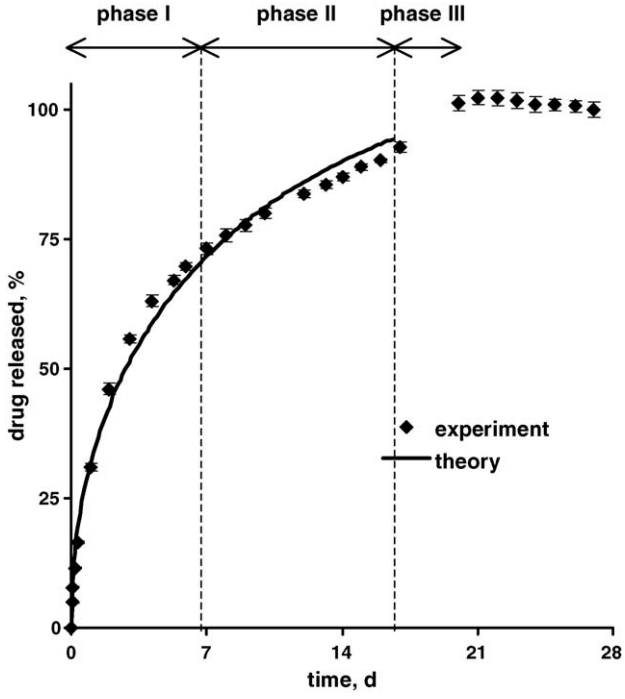

Fig. 2. In vitro release of 5-FU from PLGA-based microparticles in phosphate buffer pH 7.4 (0.13 M, 280 mosm/L, 37 °C): experiment (symbols) and theory (Eqs. (1)–(3), curve).

5-FU release rate (zero order kinetics), and (iii) a final rapid drug release phase, leading to complete 5-FU exhaust.

As previously shown (Siepmann et al., 2002), the initial drug release phase is primarily controlled by 5-FU diffusion and the limited solubility of the drug. In the second release phase, also polymer degradation becomes important: the increase in the length of the diffusion pathways with time (which should lead to a decrease in the release rate) is compensated by an increase in drug mobility (which results from the decrease in the average polymer molecular weight and, thus, increased macromolecular mobilities). The final rapid drug release phase can be attributed to the disintegration of the microparticles (as soon as the average polymer molecular weight reaches a certain, critical threshold value, the polymeric network looses its mechanical stability) (Husmann et al., 2002), resulting in significantly shortened diffusion pathways. The presented mathematical theory is able to describe the first and second phase of drug release (for an adequate description of the final rapid drug release phase, more experimental results would be required). As it can be seen in Fig. 2, good agreement between theory (Eqs. (1)–(3), curve) and experiment (symbols) was obtained. Based on these calculations, the initial diffusion coefficient of the drug in the polymeric system could be determined: $D_0 = 7.7 \times 10^{-12}$ cm^2/s.

3.3. Effects of the osmolarity of the release medium

It is well known that the osmolarity of the release medium can significantly affect different processes which can be involved in the control of drug release from biodegradable dosage forms, such as the rate and extent at which water penetrates into the system. Fig. 3 shows the experimentally determined in vitro release rates of 5-FU from the investigated PLGA-based microparticles (symbols) into phosphate buffer pH 7.4, containing different amounts of NaCl, resulting in osmolarities ranging from 280 to 840 mosm/L (as indicated). Clearly, the drug release rate decreased with increasing osmolarity of the release medium. This can probably be attributed to a decrease in the water uptake rate and extent of the system. With decreasing water content the mobility of the drug decreases and, thus, the release rate decreases. Fitting the presented mathematical model (Eqs. (1)–(3)) to the experimentally determined release kinetics, good agreement between theory and experiment was obtained in all cases (Fig. 3: curves and symbols). Based on these calculations, the initial diffusion coefficient of 5-FU within the microparticles, D_0, could be determined as a function of the osmolarity of the release medium (Fig. 4). Clearly, the D_0 value decreased with increasing osmolarity. Importantly, the following quantitative relationship could be established ($R^2 = 0.98$):

$$D_0 = (-0.0005 \times \text{osmolarity [mosm/L]} + 0.9395) \times 10^{-11}\ \text{cm}^2/\text{s} \quad (4)$$

Based on this equation, the diffusion coefficient of 5-FU can be calculated for arbitrary osmolarities of the release medium. Knowing this value, the resulting drug release kinetics from the microparticles can be predicted in a quantitative way using Eqs. (1)–(3).

As the osmolarity of the release medium was found to affect the drug release patterns from the investigated 5-FU-loaded, PLGA-based microparticles, it was adjusted to 700 mosm/L in the following experiments (except for those described in Section

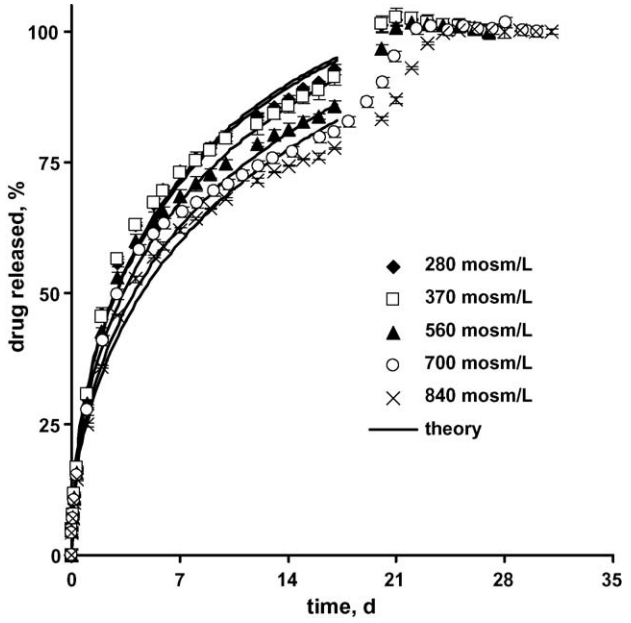

Fig. 3. Effects of the osmolarity of the release medium (indicated in the figure; 0.13 M phosphate buffer pH 7.4, 37 °C) on 5-FU release from PLGA-based microparticles (osmolarities > 280 mosm/L were adjusted by adding adequate amounts of NaCl) [symbols: experimentally determined values; curves: theory (Eqs. (1)–(3))].

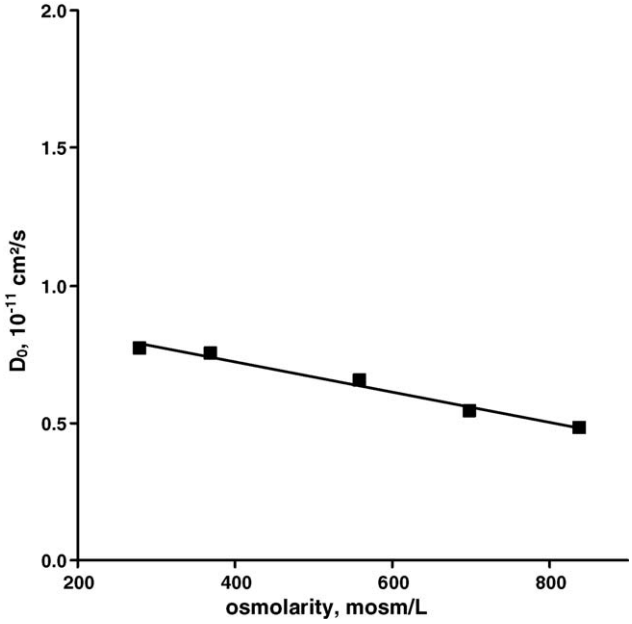

Fig. 4. Dependence of the initial diffusion coefficient of 5-FU within the investigated PLGA-based microparticles on the osmolarity of the release medium (0.13 M phosphate buffer pH 7.4, 37 °C, osmolarities > 280 mosm/L were adjusted by adding adequate amounts of NaCl) [symbols: experimentally determined values; straight line theory (Eq. (4))].

3.6, studying the effects of the temperature in the same medium) by adding adequate amounts of NaCl.

3.4. Effects of the buffer concentration

Fig. 5 shows the effects of the concentration of the phosphate buffer (and, thus, the effects of the buffer capacity of the release medium) on the resulting drug release kinetics from the investigated microparticles (at pH 7.4). Importantly, the 5-FU release rate only slightly decreased when increasing the buffer concentration from 0.02 to 0.26 M. As the osmolarity of all release media was adjusted to 700 mosm/L (by adding adequate amounts of NaCl), osmolarity effects can be excluded. The slight decrease in the release rate might be attributable to the increase in the concentration of bases in the release medium. The latter diffuse into the microparticles and suppress potentially occurring autocatalytic effects (Klose et al., 2006; Siepmann et al., 2005). However, these effects were not very pronounced.

Again, good agreement between theory and experiment was obtained when fitting the mathematical model (Eqs. (1)–(3)) to the experimentally determined 5-FU release kinetics (curves and symbols in Fig. 5). Based on these calculations, the initial diffusion coefficient of the drug in the investigated microparticles was found to (slightly) decrease from 6.5 to 4.5×10^{-12} cm^2/s, when increasing the buffer concentration from 0.02 to 0.26 M.

In contrast, when the concentration of the phosphate buffer was increased from 0.02 to 0.26 M without adjusting the osmolarity of the release medium (to 700 mosm/L by adding adequate amounts of NaCl), the resulting drug release rate significantly decreased (data not shown). This can be attributed to the increase in osmolarity of the release medium and subsequent decrease in the rate and extent of water penetration into the microparticles (as discussed above).

Interestingly, Shameem et al. (1999) found the opposite effect of the buffer concentration on the resulting drug release kinetics from PLGA-based microparticles: when increasing the concentration of the phosphate buffer (pH 7.0) from 0.01 to 0.1 M, the release rate of encapsulated leuprorelin significantly increased. This could be explained as follows: leuprorelin contains basic groups which interact with the acidic end groups of PLGA, slowing drug release down. When increasing the ionic strength of the release medium these interactions are reduced, resulting in increased drug release rates. In the present case, such drug-polymer interactions can be excluded.

3.5. Effects of the pH (and composition) of the release medium

Importantly, the pH (and composition) of the release medium was found to fundamentally affect the resulting drug release patterns from the investigated PLGA-based microparticles (Fig. 6). At high pH (carbonate buffer pH 10.8), 5-FU release was very rapid and complete within only 3 d. In phosphate buffer pH 7.4, an intermediate release rate was observed (complete drug exhaust after 22 d), whereas at low pH (phosphate buffer 4.5 and citrate buffer pH 1.3) 5-FU release was very slow (only 37 and 41% were released after 3 weeks, complete release was observed after 37 d). In order to better understand these tremendous effects, the solubility of the drug, degradation kinetics of the polymer and glass transition temperature of the PLGA-based microparticles were determined in/upon exposure to the different release media.

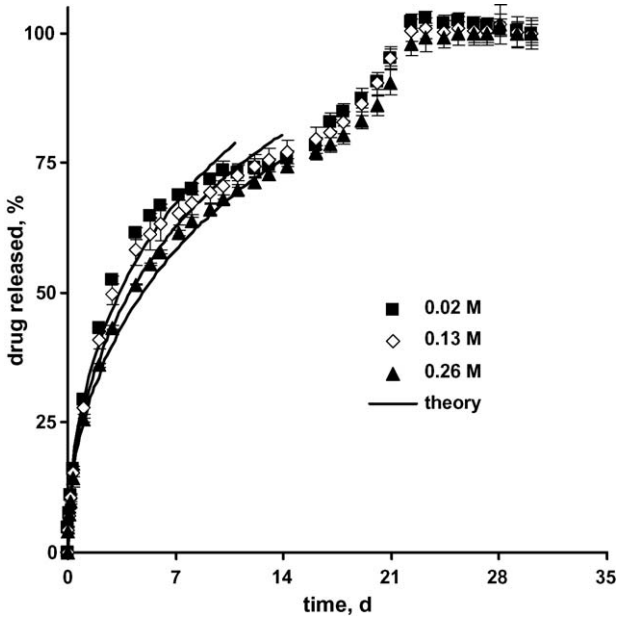

Fig. 5. Effects of the concentration of the phosphate buffer (indicated in the figure) on 5-FU release from PLGA-based microparticles [pH 7.4; 700 mosm/L (adjusted by adding adequate amounts of NaCl), 37 °C] [symbols: experimentally determined values; curves: theory (Eqs. (1)–(3))].

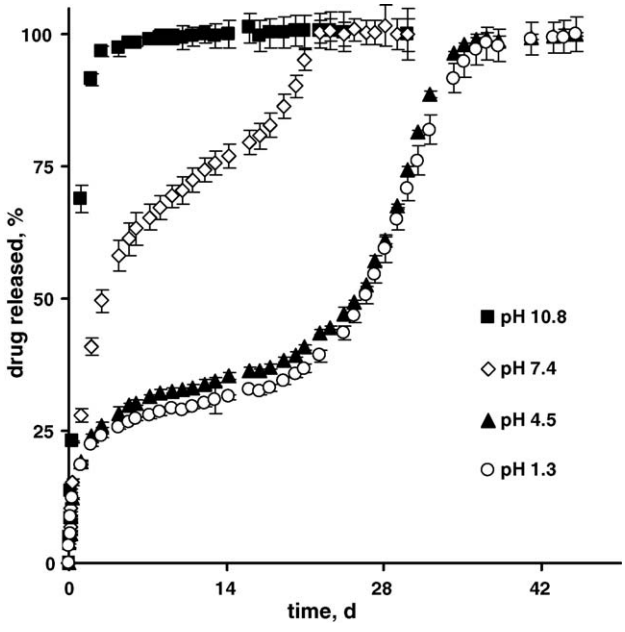

Fig. 6. Effects of the pH (and composition) of the release medium (citrate buffer pH 1.3, phosphate buffer pH 4.5, phosphate buffer pH 7.4, carbonate buffer pH 10.8) on 5-FU release from PLGA-based microparticles [700 mosm/L (adjusted by adding adequate amounts of NaCl), 37 °C].

Table 1
Effects of the type of release medium on the solubility of 5-FU (at 37 °C), pseudo first order degradation rate constant of PLGA (k_{degr}) and glass transition temperature (T_g) of the polymeric microparticles (in the wet state, after 3 d exposure to the release media) (the osmolarity of all media was adjusted to 700 mosm/L by adding adequate amounts of NaCl)

pH	Type of buffer	Solubility (g/L)	k_{degr} (d^{-1})	T_g (°C)
1.3	Citrate	19	0.082	40
4.5	Phosphate	17	0.079	40
7.4	Phosphate	20	0.069	30
10.8	Carbonate	23	0.059	31

The solubilities of 5-FU in the investigated release media at 37 °C are given in Table 1. Although the drug solubility tends to increase with increasing pH value (except for phosphate buffer pH 4.5), the observed (relatively small) differences cannot explain the fundamental increase in the drug release rate (Fig. 6).

The next hypothesis was that the pH of the release medium strongly affects the degradation kinetics of the matrix forming polymer. PLGA is a polyester and its hydrolysis is known to be catalyzed by protons and to be induced by bases. Fig. 7 shows the experimentally determined decrease in the average polymer molecular weight of the PLGA upon microparticle exposure to the different release media at 37 °C. Interestingly, no major affects were visible. In all cases, polymer degradation followed pseudo-first order kinetics. Fitting Eq. (3) to the experimental results, the degradation rate constants shown in Table 1 could be determined. With increasing pH of the release medium the degradation rate constant of the polymer slightly decreased. Thus, changes in the PLGA degradation kinetics cannot explain the observed tremendous effects of the pH (and composition) of the release medium on drug release (Fig. 6) (a decrease in the degradation rate constant should be expected to result in lower macromolecular mobilities and, thus, reduced drug release rates).

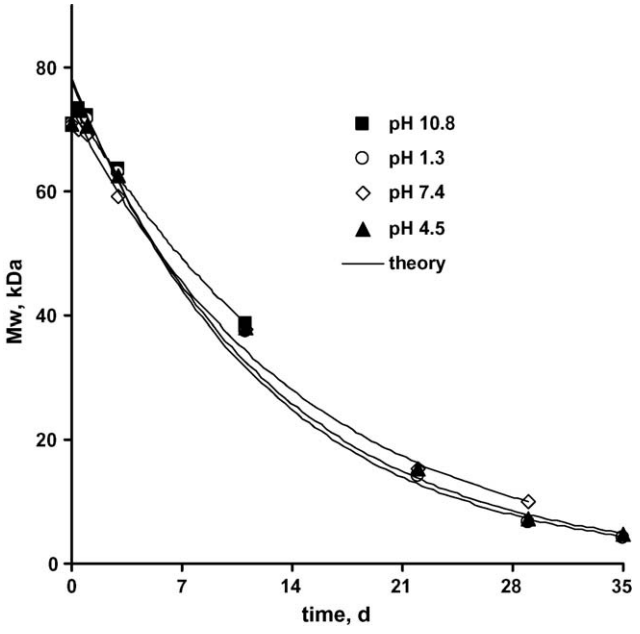

Fig. 7. Effects of the pH (and composition) of the release medium (citrate buffer pH 1.3, phosphate buffer pH 4.5, phosphate buffer pH 7.4, carbonate buffer pH 10.8) on PLGA degradation in 5-FU-loaded microparticles [700 mosm/L (adjusted by adding adequate amounts of NaCl), 37 °C] [symbols: experimentally determined values; curves: theory (pseudo first order kinetics: Eq. (3))].

Next, the glass transition temperature (T_g) of the polymeric microparticles was measured (in the wet state) after 3 d exposure to the different release media (Table 1). Very importantly, the T_g was above 37 °C in the case of citrate buffer pH 1.3 and phosphate buffer pH 4.5 (40 °C), whereas it was below 37 °C in the case of phosphate buffer pH 7.4 and carbonate buffer pH 10.8 (30 and 31 °C, respectively). This clearly indicates that the polymer is in the glassy state at pH 1.3 and 4.5, whereas it is in the rubbery state at pH 7.4 and 10.8. It is well known that the mobility of the macromolecular chains significantly increases when a polymeric system undergoes a glassy-to-rubbery-state transition. In the glassy state, the macromolecules are not very mobile. According to the free volume theory of diffusion, the probability that a drug molecule can jump from one cavity into another is limited in such systems and, thus, the resulting release rate is low. In contrast, if the polymer is in the rubbery state, the mobilities of the polymer chains and drug molecules are much higher, resulting in much higher drug release rates. This fundamental difference in the physical state of the polymer explains the observed *significant* difference in the drug release behavior at pH 1.3/4.5 versus pH 7.4/10.8 (Fig. 6). The difference in the release kinetics in phosphate buffer pH 7.4 versus carbonate buffer pH 10.8 can at least partially be attributed to the altered drug solubility (Table 1). However, also other phenomena seem to be involved.

As only a few experimental data points describing 5-FU release at pH 10.8 were available and as the amount of mobile drug in the first and second drug release phase at pH 1.3 and 4.5 were obviously not equal to the amount initially present within the systems ("plateau values" around only 30% of the total, encapsulated drug amount), the presented mathematical model could not be used to quantitatively describe the observed release patterns.

3.6. Effects of the temperature of the release medium

The effects of the temperature of the release medium (phosphate buffer pH 7.4, 280 mosm/L, 0.13 M) on the resulting drug release kinetics from the investigated microparticles is shown in Fig. 8. Clearly, the release rate significantly increased when increasing the temperature from 37 to 65 °C. Importantly, the drug release profiles became all mono/bi-phasic at and above 45 °C. Thus, 5-FU release was already complete before the polymer particles started to disintegrate. This can be attributed to the increased mobility of the polymer chains and drug molecules at elevated temperature, resulting in increased diffusion rates.

Fitting the mathematical model (Eqs. (1)–(3)) to the experimentally determined 5-FU release profiles, good (to rather good) agreement was obtained (Fig. 8: curves and symbols). Based on these calculations the initial diffusion coefficient of the drug in the PLGA-based microparticles (D_0) could be determined as a function of the temperature (T). As it can be seen in Fig. 9,

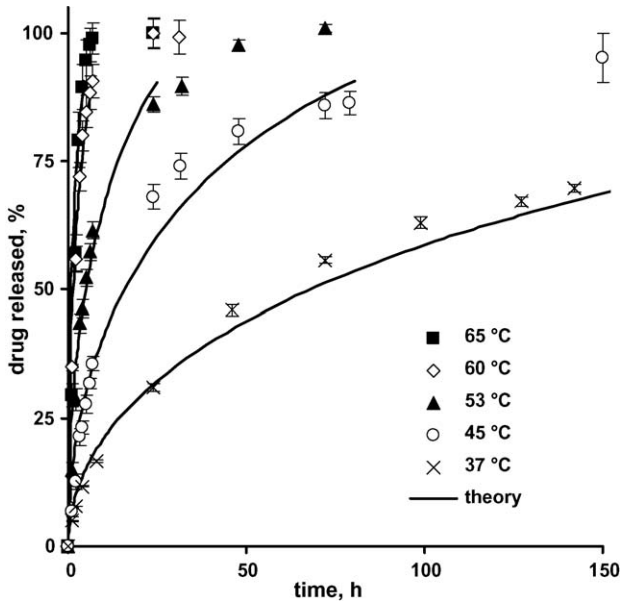

Fig. 8. Effects of the temperature (indicated in the figure) of the release medium (phosphate buffer pH 7.4, 280 mosm/L, 0.13 M) on 5-FU release from PLGA-based microparticles [symbols: experimentally determined values; curves: theory (Eqs. (1)–(3))].

the diffusivity of 5-FU significantly increased with increasing T. Interestingly, the following exponential relationship could be established:

$$D_0 = 2.68 \times e^{0.1557T(^\circ C)} \times 10^{-14}\,cm^2/s \quad (5)$$

The practical benefit of this equation is that it allows to calculate the diffusion coefficient of the drug at arbitrary temperatures (provided that the polymer is in the rubbery state). Knowing this value, the resulting drug release kinetics can be predicted in a quantitative way.

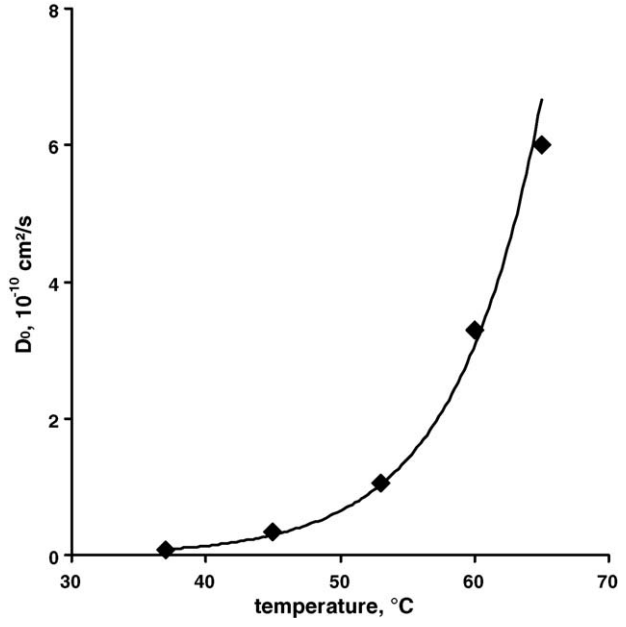

Fig. 9. Dependence of the initial diffusion coefficient of 5-FU within the investigated PLGA-based microparticles on the temperature of the release medium (phosphate buffer pH 7.4, 280 mosm/L, 0.13 M) [symbols: experimentally determined values; curves: theory (Eq. (5))].

4. Conclusion

Great care must be taken when defining the experimental conditions for in vitro drug release measurements from biodegradable microparticles. The present study points out the importance of the type of release medium. For example, the effects of the composition and temperature on the resulting drug release kinetics and underlying mass transport mechanisms were measured and explained. This type of information is important, because the drug release patterns from biodegradable microparticles are crucial for the success of the pharmacotherapies, and drug release measurements in vivo are very difficult. The obtained new knowledge can help to better define the appropriate experimental conditions for in vitro drug release tests. The latter are essential during the development phase of novel products, but also during production (e.g., for quality control). In particular, adequate "stress tests" can be developed allowing to get rapid feedback on the release characteristics of a specific batch. However, caution has to be paid when the underlying drug release mechanisms are altered under "stress conditions".

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