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Pore formation and pore closure in poly(D,L-lactide-co-glycolide) films

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ABSTRACT

Pore formation and pore closure in poly(D,L-lactide-co-glycolide)-based drug delivery systems are two important processes as they control the release of the encapsulated drug. The phenomenon pore closure was investigated by studying the effects of the pH and the temperature of the release medium, and the properties of the polymer. Poly(D,L-lactide-co-glycolide) (PLG) films were subjected to a pore forming pre-treatment, and then pore closure was observed simultaneously with changes in glass transition temperature, wettability (contact angle), water absorption and mass remaining. To further understand the effect of pH, combined pore formation and pore closure were studied at different pH values. Pore closure was increased in a release medium with low pH, with a low-molecular-weight PLG of relatively low degree of hydrophobicity, or at high temperature. Pore closure occurred by two different mechanisms, one based on polymer–polymer interactions and one on polymer–water interactions. The mobility of the PLG chains also played an important role. The surface of the PLG films were more porous at pH 5–6 than at lower or higher pH, as pore formation was relatively fast and pore closure were less pronounced in this pH range. The pH had a significant impact on the porous structure, which should be kept in mind when evaluating experimental results, as the pH may be significantly decreased *in vitro*, *in vivo* and *in situ*. The results also show that the initial porosity is very important when using a high-molecular-weight PLG.

1. Introduction

Poly(D,L-lactide-co-glycolide) (PLG) is a biocompatible polymer that has been used extensively in various areas, such as the controlled release of encapsulated drugs [1], tissue engineering [2], healing of bone defects [3], cancer treatment [4] and vaccines [5]. PLG is the most frequently used biodegradable polymer in the controlled release of encapsulated proteins or peptides. The reasons for its success are its biodegradability, its biocompatibility and the fact that it has been approved for parenteral use by the regulatory authorities around the world. Furthermore, its physico-chemical behavior, and thus the drug release profile, can be tailored by selecting PLGs with the appropriate properties, for example, molecular weight and the lactide:glycolide ratio [6–8]. Blending or co-polymerizing PLG with other materials further extends the possibility of controlling its physico-chemical behavior [9–11].

Encapsulated proteins or peptides diffuse through water-filled pores [12–15]. The release rate is thus very dependent on the porosity of the polymer, and it is important to understand both pore *formation* and pore *closure* when tailoring release from PLG-based formulations. The phenomenon pore closure and also the development of a surface layer less porous than the interior due to heterogeneous degradation, have been observed previously [16–22], but are unfortunately not often mentioned when discussing release mechanisms. Temperature has been shown to affect pore closure [23], and the structural collapse of the polymer has been suggested as one reason for pore closure [20]. Less deep pores have been reported on the surface of microspheres stored at high humidity when a plasticizing agent was added to the polymer [24], and the authors suggested that the polymer chains were rearranged due to the increased mobility of the polymer. However, the phenomenon is far from well understood.

Pore formation has been more discussed in the literature. It has been shown that pores are formed both by water absorption and by degradation/erosion of the polymer [25–27]. These processes, in turn, are influenced by a great number of factors, for example the presence and the concentration of salts, plasticizing excipients used and the properties of the polymer [6–8,21,28,29]. Another such factor is pH. As hydrolysis is acid-catalyzed, the common opinion is that pores are formed faster at lower pH [22,26,30]. However, the pH also affects the degree of polymer terminal carboxyl acid dissociation, which determines the charge of polymer chains. This may be important in the arrangement of polymer chains and thus possibly in pore closure. Both pore formation and pore closure probably take place simultaneously and constantly, and the porosity of the polymer is likely to be affected by both. The domination of these processes may vary with pH, which may decrease significantly from the normal physiological

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value (7.4) during polymer degradation. Inflammatory reactions and formation of a fibrous capsule surrounding PLG microspheres may decrease the local pH *in vivo* [31,32], while acid degradation products from PLG may decrease the pH inside PLG drug delivery systems and of the release medium *in vitro* [33–35].

The purpose of this study was to identify the mechanisms governing the phenomenon of pore closure. The effects of the pH and the temperature of the release medium, and the properties of the polymer were investigated. To our knowledge, no studies have been carried out regarding the effect of the properties of the polymer, such as its molecular weight or hydrophobicity, on pore closure. This study is complementary to previous studies on the effect of the pH and the temperature of the release medium on pore closure [16,23]. Pore closure was studied simultaneously with the changes of the glass transition temperature (Tg), wettability (contact angle), water absorption and mass remaining. To further understand the effect of pH of the release medium, combined pore formation and pore closure were studied at different pH values.

2. Materials and methods

2.1. Materials

Three different PLGs were obtained from Boehringer Ingelheim Pharma KG (Germany), namely RG502H (50:50 lactide:glycolide, with an approximate molecular weight (MW) of 12 kDa), RG504H (50:50 lactide:glycolide, approximate MW 45 kDa) and RG756 (75:25 lactide:glycolide, approximate MW 80 kDa). Polysorbate 80 and sodium Hepes salt were obtained from Sigma-Aldrich Inc. (USA), and Hepes acid from Research Organics (USA). NaCl, $ZnCl_2$ and ethyl acetate were obtained from Merck KGaA (Germany). NaN_3 was obtained from VWR International Ltd (UK) and polyvinylidene fluoride filters (pore size 0.65 μm) were purchased from Millipore AB (Sweden). All salts were of analytical grade.

2.2. Film preparation and sample pre-treatment

Polymer films (about 150 μm thick), were cast on glass dishes from solutions in ethyl acetate (67 mg/ml). Polysorbate 80 was co-dissolved in this solution (1.3 mg/ml) and encapsulated in the PLG film order to mimic a relevant pharmaceutical system utilizing PLG films coated onto microparticles [36]. Solutions of the PLG denoted RG756 also contained 10% (w/w) NaCl particles in relation to the weight of PLG. A polyvinylidene fluoride filter (105 μm thick and pore size 0.65 μm) was encapsulated in all films intended for analysis of pore formation or wettability to provide mechanical support. The filters were placed on the glass dishes and the polymer solutions were poured onto the filters. The filters were completely encapsulated in the PLG films and did not interfere with the analyses. After drying at ambient conditions for 10 days and vacuum drying at room temperature for 7 days, circular samples with a diameter of 1 cm were cut from the film. Pores were created in samples intended for studies on pore closure (see below), while those intended for a study on combined pore formation and pore closure at different pH were not subject to any pore-forming pre-treatment, and were thus smooth and non-porous. Pores were created in samples of PLG denoted RG502H by incubation in Hepes buffer (see Section 2.3) with 1 mM $ZnCl_2$, pH 7.4, for two days at 37 °C. $ZnCl_2$ has been found to increase the rate of pore formation, probably by acting as a Lewis acid and thereby catalyzing degradation [17,28]. Pores formed due to the presence of $ZnCl_2$ during this short period of time were located at the surfaces [17]. Samples of PLG denoted RG504H were incubated for four days in the same way to create pores. The molecular weight of the PLG denoted RG756 was too high for pore forming pre-treatment with $ZnCl_2$. In order for pore formation to occur due to (catalyzed) hydrolysis and erosion within the first few days, a part of the polymer chains must be sufficiently short to reach the molecular weight necessary for dissolution within this time. The pore forming pre-treatment was instead based on a porogen. The encapsulated NaCl particles in samples denoted RG756 were released within four days of incubation in Hepes buffer, as analyzed by scanning electron microscopy (SEM) (see Section 2.4). These pores were located on one of the surfaces of the samples, as the NaCl particles settled to the bottom during drying. $ZnCl_2$ was added to the Hepes buffer during these four days, with the purpose of avoiding unknown effects of $ZnCl_2$ at comparison of the different PLGs. After the pore forming pre-treatment, which was two days for PLG denoted RG502H and four days for the other PLGs, the samples were incubated in Hepes buffer without $ZnCl_2$, and the analysis of pore closure started.

Table 1
Experimental design for the investigation of pore closure.

PLG	MW (kDa)	Relative degree of hydrophobicity	Temperature (°C)	pH of the release medium
RG502H	12	Low	37	7.4
RG502H	12	Low	37	3.0
RG502H	12	Low	9	7.4
RG502H	12	Low	45	7.4
RG504H	45	Average	37	7.4
RG756	80	High	37	7.4

2.3. Incubation

All the samples (those containing pores and those not pre-treated) were incubated in 75 mM Hepes buffer containing 115 mM NaCl and 5 mM NaN_3, pH 7.4, at 37 °C. Samples of different PLGs were incubated in release medium with a pH of 3.0 or pH 7.4, at temperatures of 9 °C, 37 °C or 45 °C. Table 1 presents the experimental design.

Samples intended for studies on the effect of release medium pH on both pore formation and closure were made of PLG denoted RG502H, and were incubated in Hepes buffer with the pH adjusted to 3.0, 5.0, or 6.0 using HCl, or pH 7.4 (no adjustment). The release medium was refreshed continuously to keep the pH constant. At predetermined intervals the samples were investigated with regard to porosity, water absorption and mass loss, and in the study of pore closure the glass transition temperature (Tg) and wettability were also investigated. All the analyses were performed on triplicate samples.

2.4. Scanning electron microscopy

The samples were washed and vacuum dried. The effect of the drying method, i.e. freeze drying or vacuum drying, on the porosity was investigated in an initial experiment. The drying method did not have any effect on the result (data not shown). The porosity was studied using a JSM-6700F field emission scanning electron microscope from Jeol Ltd (Japan). The samples were sputtered with gold prior to inspection. Triplicate samples were analyzed.

2.5. Wettability

The wettability was measured using the captive bubble method to determine the contact angle. PLG samples were mounted in a device allowing the sample to be submerged in water. An air bubble was placed on the downward facing surface of the PLG sample using a hypodermic needle. The equipment was thoroughly cleaned using acids (1:1 HCL:HNO_3) or ethanol in an ultrasonic bath to ensure that contaminants did not interfere with the measurements. The contact angle was measured using a Melles Griot Invaritar P/N 59 LGF 410 camera and the software program Windrop for windows XP. Triplicate samples were analyzed.

2.6. Differential scanning calorimetry

The glass transition temperature (Tg) was analyzed using a DSC 6200 calorimeter (Seiko Instruments Inc., Japan). The samples were washed, vacuum dried and placed in aluminum pans (TA Instruments, USA, ref no. 900790.901). The pans were hermetically sealed and an empty pan was used as a reference. The samples were scanned at a rate of 10 °C/min with a temperature sweep up to 100 °C, starting at −20 °C. The Tg calculated from the second heating cycle using the soft ware program Exstar 6000 (Scientific & Medical Products Ltd. UK). Triplicate samples were analyzed.

2.7. Water absorption and mass loss

Water absorption and mass were determined by weighing the samples in wet state (W_{wet}) and after drying in a vacuum chamber to constant weight (W_{dry}). W_0 denotes the initial weight. Triplicate samples were analyzed.

$$\text{Water absorption} = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100(\%)$$

$$\text{Mass loss} = \frac{W_0 - W_{dry}}{W_0} \times 100(\%)$$

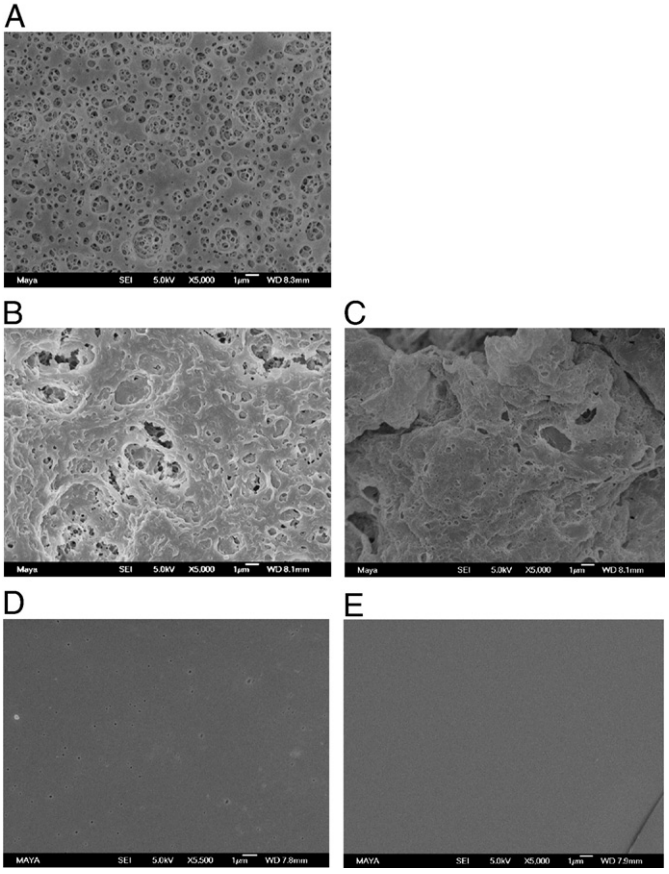

Fig. 1. Porosity directly after pre-treatment (A), after 2 more days of incubation (B and D) and after 12 days (C and E). PLG MW 12 kDa, 37 °C and pH 7.4 (B and C) and pH 3.0 (D and E). Magnification 5000×.

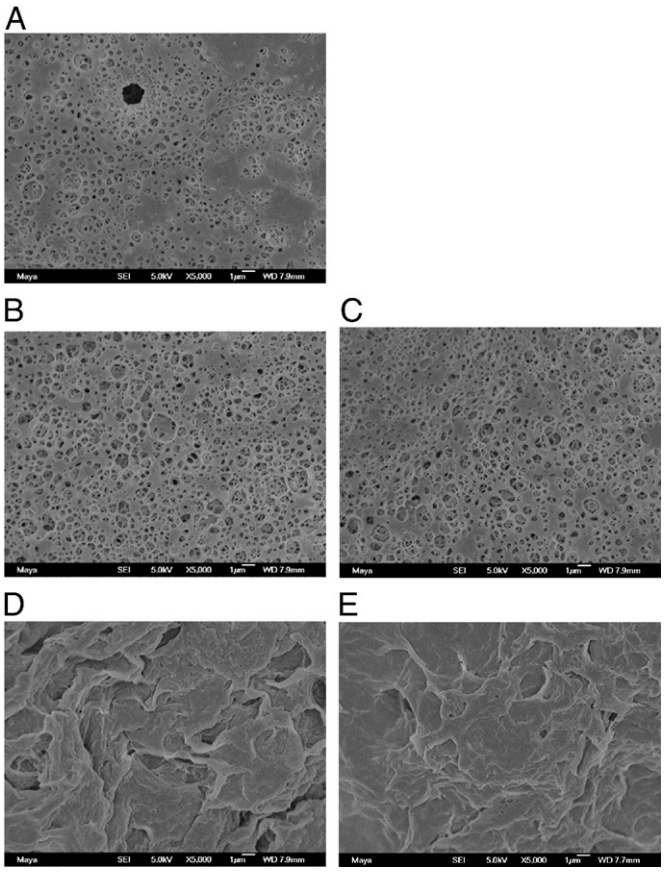

Fig. 2. Porosity directly after pre-treatment (A), after 2 more days of incubation (B and D) and after 12 days (C and E). PLG MW 12 kDa, pH 7.4 and 9 °C (B and C) and 45 °C (D and E). Magnification 5000×. See Fig. 1 for the comparable experiment carried out at 37 °C.

3. Results and discussion

3.1. Pore closure

All the factors investigated, i.e. the pH and the temperature of the release medium, and the properties of the polymer, influenced pore closure, as observed by scanning electron microscopy (Fig. 1–3). Pore closure occurred rapidly at low pH, high temperature and when using a PLG of low molecular weight and a relatively low degree of hydrophobicity. The effect of each factor will be discussed separately below.

3.1.1. Effects of the pH of the release medium

Pore closure was faster at pH 3.0 than at pH 7.4 during the 26 days of observation, although pore closure began within two days at both pH values (Fig. 1). The pores were completely closed at pH 3.0, but not in pH 7.4. Pore formation is commonly believed to be enhanced at low pH due to the well-known acid-catalyzed hydrolysis of PLG [22,26,30]. These results show that pH may affect the polymer in more than one way. In this case, pore closure probably occurred more rapidly than pore formation. Water absorption was slower at the lower pH (Fig. 4), which was somewhat unexpected, as the acid-catalyzed hydrolysis reduces the molecular weight of PLG, which in turn makes the polymer chains more hydrophilic [37]. The samples degraded at pH 7.4 became highly swollen, degraded and sometimes fell apart during the last period of the analyses, which caused the fluctuations seen in Fig. 4A. The results from the pore closure and water absorption analyses can be explained by the lack of dissociation of the terminal carboxyl acids of the polymer chains at pH 3.0, which makes the polymer less charged and more hydrophobic. Measures of wettability confirmed this (Table 2). It is likely that polymer–polymer

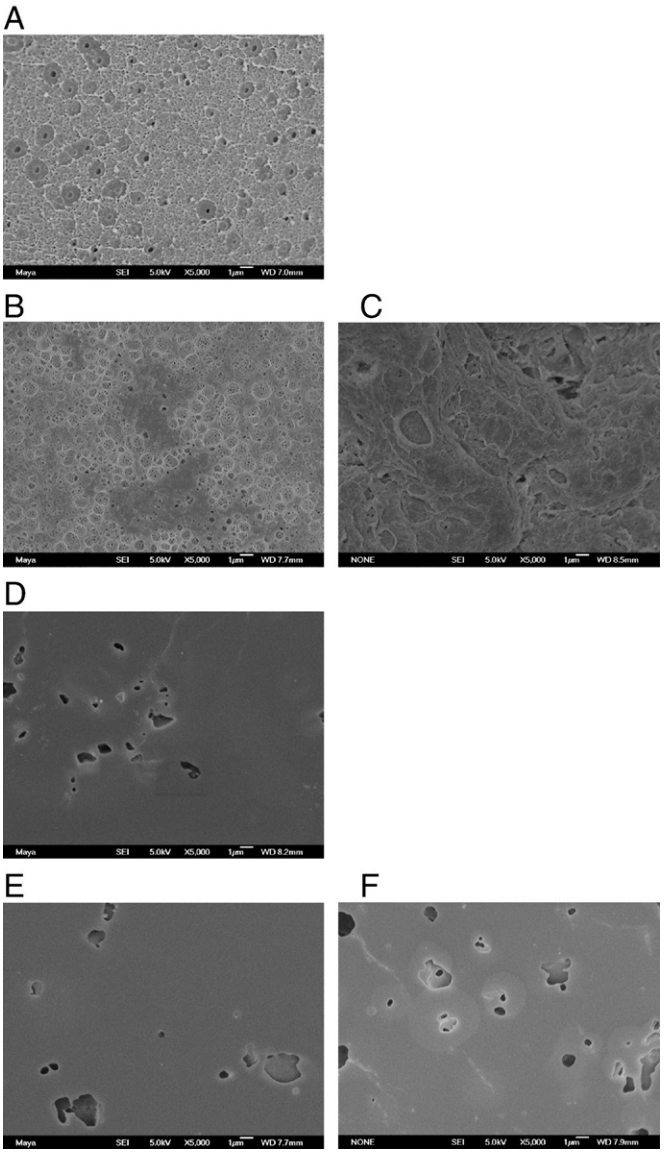

Fig. 3. Porosity directly after pre-treatment (A and D), after 2 more days of incubation (B and E) and after 12 days (C and F). pH 7.4, 37 °C and PLG MW 45 kDa (A-C) and 80 kDa (D-F). Magnification 5000×. See Fig. 1 for the comparable experiment with PLG MW 12 kDa.

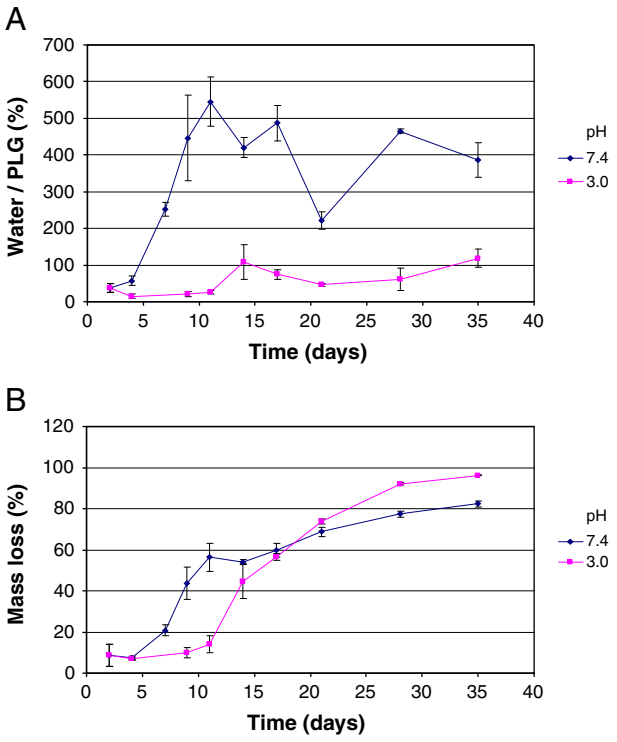

Fig. 4. Water absorption (A) and mass loss (B) at different pH values. The error bars show the standard deviation. The polymer samples were subjected to a pore forming pre-treatment in presence of $ZnCl_2$ at pH 7.4 and 37 °C during the first two days.

interactions resulting from the more hydrophobic nature of the polymer constituted the driving force for pore closure. The higher interfacial tension between water and polymer encourages as small contact area between the two phases as possible, and release of surface bound water from two hydrophobic polymer areas attracting each other is a drive for contraction. This would also explain why the water uptake by the polymer was low. Visually, there was an obvious difference between the samples: those in medium of pH 3.0 contracted into a lump, while those in medium of pH 7.4 swelled and spread out.

Another important factor contributing to the faster pore closure at pH 3.0 was the considerable increase in polymer chain mobility, evidenced by a significant decrease in Tg (Fig. 5). There have also been reports of constant or even increased Tg in acidic environments, however, those PLG matrices contained drugs and, according to our results, the period of degradation was too short for an effect to be seen, [30,38] (we observed no effect until after 4 days of degradation, see Fig. 5). Acid catalysis of the hydrolysis of PLG is well known, as mentioned above, and a lowering of the molecular weight of the polymer chains results in a lower Tg and higher mobility [19,39]. This mobility is important as a rigid polymer will not contract, or will only contract slowly, even if the interfacial tension is strong. This is further discussed in the next section. Rearrangement of polymer chains due to increased mobility has also been suggested as the explanation of the observation that pores on the surface of microspheres stored at high relative humidity became less deep when a plasticizing agent was added [24]. As shown in Fig. 5, the Tg remained constant at pH 7.4, although a slight decrease of Tg was expected. This was probably due to a faster loss of plasticizing substances such as polysorbate 80 and PLG degradation products, which counteracted the decrease in the Tg. At the beginning of the experiment, mass loss was slower at pH 3.0, but by the end of the experiment, mass loss was greater at pH 3.0 (Fig. 4). Mass loss is influenced by both the rate of hydrolysis and the rate of transport of water-soluble degradation products out of the samples, as PLG degradation products become soluble in water when they have been hydrolyzed down to approximately 1100 g/mol [37]. As the porosity was lower at pH 3.0, the transport rate was slower and counteracted the faster hydrolysis.

This rapid and complete pore closure at low pH may play an import role during drug release, as pH may be low *in vivo*, *in vitro* and *in situ*. As mentioned in Section 1, inflammatory reactions and formation of a fibrous capsule surrounding PLG microspheres may decrease the local pH *in vivo* [31,32], while acid degradation products from PLG decrease

Table 2
Wettability, expressed as the contact angle, at the two pH values studied. The standard deviation is given in parentheses. High wettability and low hydrophobicity result in a small contact angle between the air bubble in water and the surface. The polymer samples were subjected to a pore forming pre-treatment in presence of $ZnCl_2$ at pH 7.4 and 37 °C during the first two days.

pH	Day 2 (°)	Day 4 (°)	Day 9 (°)
3.0	36.7 (7.1)	54.7 (3.1)	53.4 (3.1)
7.4	36.7 (7.1)	28.2 (0.44)	34.4 (3.5)

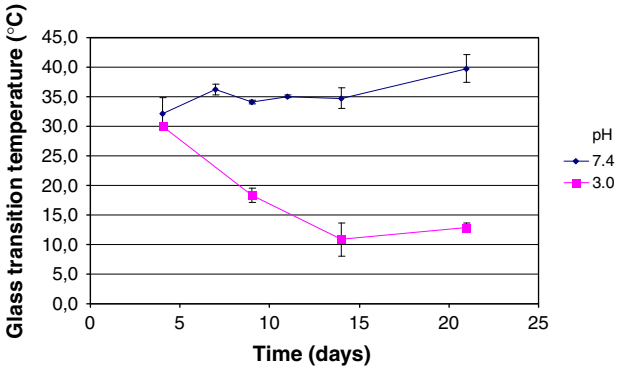

Fig. 5. The change of the glass transition temperature over time at the two pH values studied. The error bars show the standard deviation.

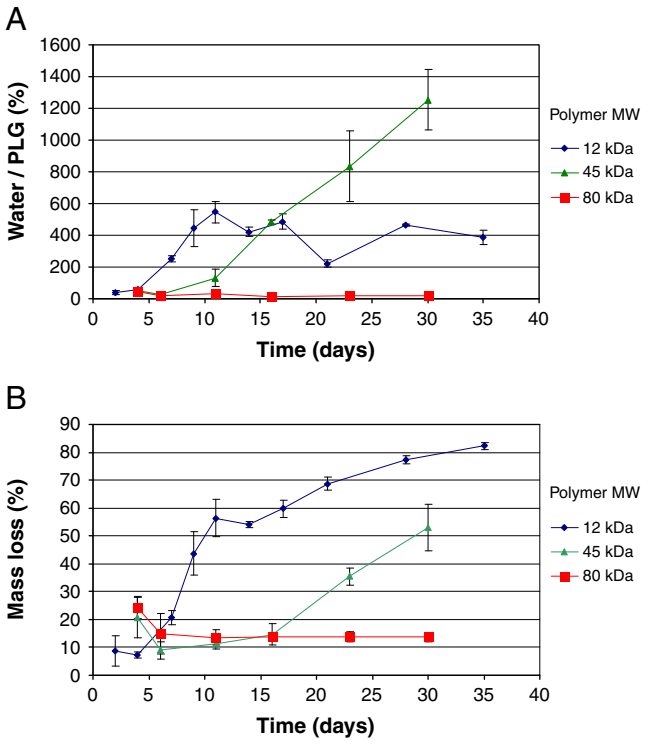

Fig. 6. Water absorption (A) and mass loss (B) for the different PLGs. The error bars show the standard deviation. The polymer samples were subjected to a pore forming pre-treatment in presence of $ZnCl_2$ at pH 7.4 and 37 °C during the first two (12 kDa) or four days (45 and 80 kDa).

the pH of the release medium *in vitro* [33]. The release medium is therefore usually replaced continuously in the studies described in the literature. However, the actual decrease in pH is seldom reported. The pH *in situ*, i.e. inside the PLG particles, has been found to be as low as 1.8, also due to acid degradation products, and the probability the pH being about 3 has been shown to be high [34,35]. The common belief regarding the effect of pH on release rate is that degradation, and thus drug release, is faster at low pH. Both faster [21,40] and slower release have been reported [41]. The effect of pH on drug release is further complicated by the fact that polymer–drug interactions and the rate of drug dissolution, which influence drug release, may depend on the pH. The results of this work show that pH 3.0 may have a retarding effect on drug release due to pore closure.

3.1.2. Properties of the polymer

Pore closure was faster with a low molecular-weight polymer with a relatively low degree of hydrophobicity Fig. 2. Pores began to close within two days in the 12 kDa polymer with the lowest hydrophobicity among the three chosen PLGs. In the 45 kDa polymer with an average hydrophobicity, pores began to close within 7 days, although not clear until 19 days. Pores in the 80 kDa polymer with the highest hydrophobicity were not closed at all. The hydrophobicity depends on the molecular weight, but also the lactide:glycolide ratio and if the polymer chains are end-capped. As expected, a low molecular-weight and less hydrophobic polymer absorbed more water, and the rate of the polymer mass loss was faster (Fig. 6). Thus, a difference in the Tg between the different polymers was expected. The Tg differed initially (Table 3), but after incubation there were no significant differences (data not shown). Plasticizing substances such as polysorbate 80 and PLG degradation products were probably lost at different rates, which could compensate for the effects of the molecular weight and hydrophobicity. The relative degree of hydrophobicity affected the wettability at the beginning of the incubation period (Table 3). Later, the films were unfortunately too degraded and too swollen for reliable measurements.

The more pronounced pore closure associated with low molecular weight and relatively low degree of hydrophobicity can be explained by the mobility and flexibility of the polymer chains and their ability to mix with water. Polymer chains that diffuse easily are more likely to spread and cover pores. Instead of distinct pores, a more swollen and homogeneous polymer structure was formed. Pores were not closed in the high-molecular-weight and highly hydrophobic PLG, although the interfacial tension between water and the hydrophobic polymer would be a driving force for contraction of the polymer, similar to the case of low pH discussed above. The difference was the mobility of the polymer chains, which enabled pore closure in the low-molecular-weight polymer with decreased Tg, but not in the rigid high-molecular-weight polymer.

These results show that the initial porosity of a drug delivery system is very important when using high-molecular-weight PLG of relatively high hydrophobicity. At low initial porosity, the release would be very slow due to slow water absorption and degradation. However, if the initial porosity is high, the pores will not close, and the drug release may be faster than when using a low-molecular-weight PLG. This should be considered when choosing the properties of the polymer.

3.1.3. Temperature

Pores were closed faster as the temperature increased Fig. 3. Pores were not closed at all at 9 °C. Pores began to close within two days at both 37 and 45 °C. However, pores were closed faster during the next 13 days at 45 °C. After that, the samples at 45 °C were too degraded to be analyzed. The faster pore closure at higher temperature was expected and in agreement with a previous report [23]. Increasing the temperature increases the mobility of the polymer, and the polymer chains can diffuse and cover pores more easily, forming a more homogeneous surface layer. A higher temperature also increases the ability of the polymer to mix with water, which results in faster water absorption (Fig. 7). The pores were, however, not completely closed, because of the counteracting process of pore *formation* which also was

Table 3
Initial glass transition temperature (Tg) and wettability (expressed as contact angle) after four days of incubation. High wettability and relatively low hydrophobicity result in a small contact angle. The standard deviation is given in parentheses.

Properties of the PLG	Initial Tg (°C)	Contact angle after four days of degradation (°)
MW: 12 kDa, low hydrophobicity (RG502H)	42 (0.46)	28 (0.44)
MW: 45 kDa, average hydrophobicity (RG504H)	46 (0.46)	38 (0.46)
MW: 80 kDa, high hydrophobicity (RG756)	50 (0.66)	49 (4.1)

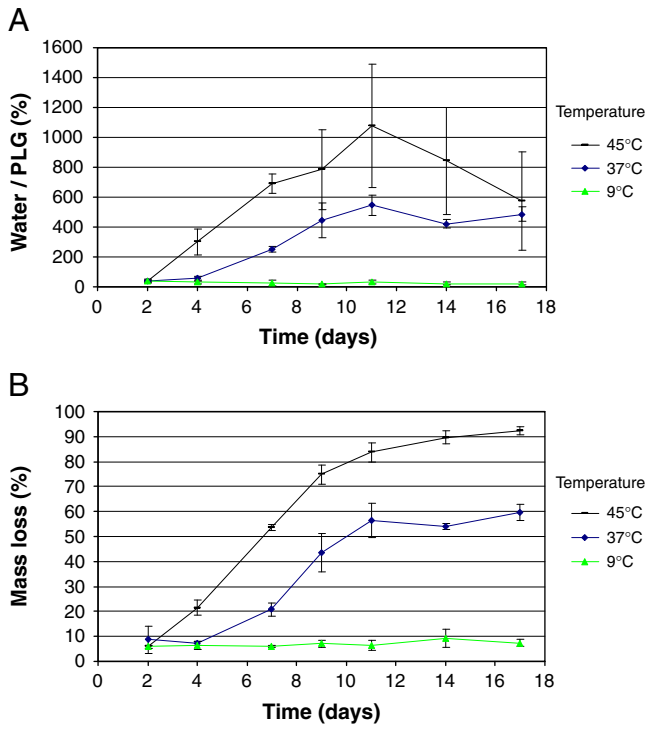

Fig. 7. Water absorption (A) and mass loss (B) at different temperatures. The error bars show the standard deviation. The polymer samples were subjected to a pore forming pre-treatment in presence of $ZnCl_2$ at pH 7.4 and 37 °C during the first two days.

faster. Increasing the temperature increased the rate of hydrolysis and the mass loss (Fig. 7). The highly degraded polymer lost its structure and the surface of the samples could not be completely annealed. The effect of different incubation temperatures could not be seen in measurements of Tg and wettability, probably for the same reasons as described in Section 3.1.2.

3.1.4. The mechanism of pore closure

Pores closure may be caused by at least two different physical events, a polymer–polymer interaction, where polymer–polymer attraction is mainly driven by the hydrophobic effect, and a polymer–water interaction that leads to a more homogenously swollen polymer gel. In both cases polymer mobility will be an important factor.

The results suggest that pore closure at pH 3.0 was driven by the polymer–polymer interaction. Attraction of two hydrophobic polymer areas separated by a water-filled pore releases the surface-bound water and increases the entropy, resulting in a more energetically stable system. In addition, the decrease in the surface tension by separation of water and hydrophobic polymer is more energetically favorable. It was clearly observed visually that the films contracted during the experiments at pH 3.0. The molecular weight of the polymer was low, which promotes polymer chain mobility, allowing separation from water. The high-molecular-weight and highly hydrophobic PLG denoted RG756, would probably also gain considerably in terms of energy by separating the polymer mass from the water-filled pores by contracting, but the polymer chains were too long and rigid.

Pore closure in a highly mobile polymer at pH 7.4 that absorbed water was instead probably driven by swelling of the polymer network. At this pH, where the polymer was charged and thus much more hydrophilic, it had a higher tendency to take up water, as can be seen in Fig. 6. It is thus likely that the polymer chains diffused easily and created a more homogenously swollen polymer network that no longer contained distinct pores. In contrast to the contracting samples at low pH, these samples visually swelled, as evidenced by an increase in thickness and diameter. However, pore closure did not occur when the temperature was lowered to 9 °C, at which the polymer was more rigid. High temperature on the other hand increased the mobility, and thus also the rate of pore closure.

3.2. Pore formation and pore closure at different pH values

The results discussed above show that pore closure occurred at both pH 3.0 and at pH 7.4, but at different rates and as a result of different mechanisms. The porous structure of a PLG matrix will be determined by the combined effects of pore formation and pore closure, which are taking place simultaneously. To understand these processes better, pore formation and pore closure was investigated not only at pH 3.0 and 7.4, as above, but also at pH 5.0 and 6.0. The purpose was to investigate whether there was an optimal pH for pore formation. The SEM analysis showed that pores did not form at pH 3.0, and samples incubated at pH 5–6 had the most porous surfaces (Fig. 8). Pore closure could be seen at all pH values after different periods of time.

Water absorption was faster at high pH and slower at low pH (Fig. 9). The explanation, as mentioned in Section 3.1.1, lies in the degree of dissociation of the polymer terminal carboxyl acids. The polymer chains are more charged and hydrophilic at higher pH. The degree of water absorption and swelling explained the very distinct and different appearances of SEM images of samples at different pH values (Fig. 8). Samples at pH 3 and 5 showed smooth surfaces, while those incubated at pH 6, and even more at pH 7.4, were creased.

The processes of pore formation and pore closure were taking place continuously at all the pH values studied. However, at pH 3.0, pore closure was so dominant that pores were not seen. The polymer–polymer interaction, caused by the lack of dissociated terminal carboxyl groups of the polymer chains, and driving pore closure at pH 3.0, should become much less strong with increasing pH. Pore closure was also rapid at pH 7.4, as shown in Section 3.1.2, due to the diffusion of the highly mobile and hydrophilic polymer chains. This polymer–water interaction should become less strong with decreasing pH. Between pH 3.0 and 7.4, pore closure should thus be less strong, while hydrolysis, which leads to pore formation, is relatively fast due to acid catalysis. This explains the optimal pore formation at pH 5–6, seen in Fig. 8. It should be noted that the effect of the pH of the

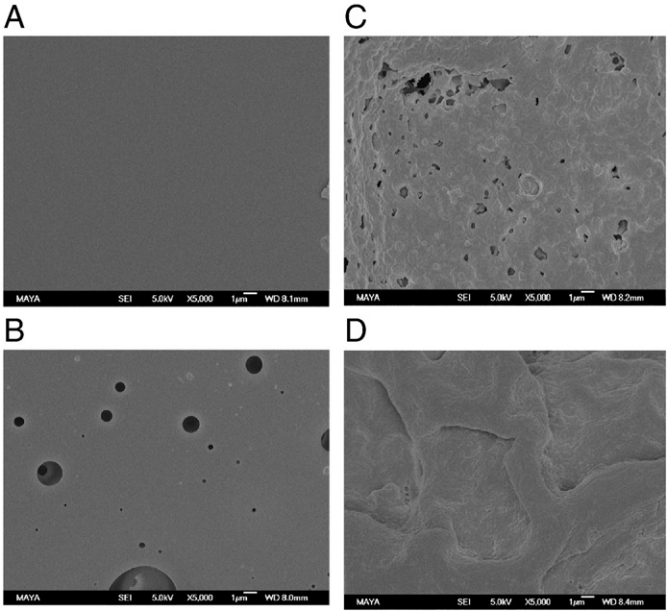

Fig. 8. Pore formation after 10 days of incubation at pH 3.0 (A), pH 5.0 (B), pH 6.0 (C) and pH 7.4 (D).

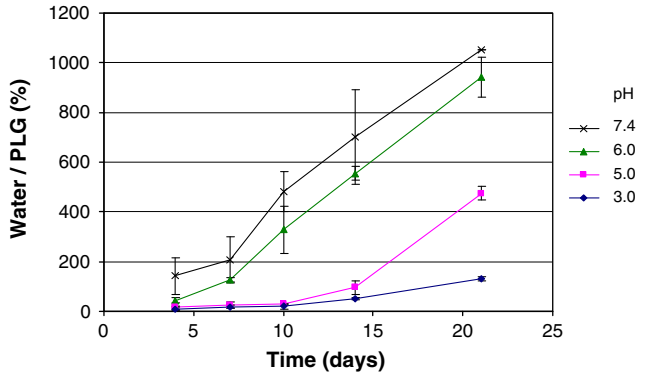

Fig. 9. Water absorption at differentpHvalues. The error bars showthe standard deviation.

release medium on pore formation and pore closure is probably dependent on the molecular weight and the mobility of the polymer chains.

The pH microclimate within a particle or film of PLG can be quite heterogeneous, which means that the porous structure could differ throughout the polymer mass. The formation and closure of pores may be explained by such local pH differences. As mentioned above, it is important to know the pH changes *in vitro*, *in vivo* and *in situ* (e.g. due to dissolved acid polymer degradation products and to inflammatory reactions in the body) as the rate of drug release may depend on this.

4. Conclusions

Pore closure was increased in a release medium with low pH, with a low-molecular-weight PLG of relatively low degree of hydrophobicity, or at high temperature. Pore closure occurred by two different mechanisms, depending on the pH and the degree of dissociated terminal carboxylic acids, which governed the hydrophobicity of the polymer. The results of this study suggest that pore closure at pH 3.0 was driven by polymer–polymer interactions, in which the attraction of two relatively highly hydrophobic areas, separated by a water-interactions caused the release of surface-bound water, increasing the entropy of the system. The surface tension also made the separation of water and hydrophobic polymer more energetically favorable. At pH 7.4, on the other hand, the results suggest polymer–water interactions. The pores in the low-molecular-weight polymer with highly mobile polymer chains and low hydrophobicity, seemed to be closed by diffusion of polymer chains that covered the pores, forming a more swollen and homogeneous polymer structure. This was facilitated at high temperature.

The initial porosity of a drug delivery system is very important when using a high-molecular weight-PLG with a relatively high degree of hydrophobicity, as pores are formed slowly due to slow degradation and water absorption. Pore will not close, and if the porosity is high, the release may be faster than when using a low-molecular-weight PLG.

The highest porosity of the surfaces of the PLG films was seen at pH 5–6. At these pH values, degradation/erosion, and thus pore formation, was relatively fast, while pore closure was less pronounced than at lower and higher pH.

The effect of pH on pore formation and pore closure should be kept in mind, as a significant decrease in pH may occur *in vitro*, *in vivo* and *in situ* due to acid degradation products of the polymer and inflammatory reactions *in vivo*. The pH microclimate within a particle or film of PLG can be quite heterogeneous, which means that the porous structure also may differ throughout the polymer mass. Unexplained formation and closure of pores reported in other studies may be due to local differences in pH. The results of the present study show that conclusions regarding drug release and release mechanisms must be drawn with pH in mind.

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References

[1] B.S. Zolnik, D.J. Durgess, Evaluation of *in vivo – in vitro* release of dexamethasone from PLGA microspheres, J. Control. Release 127 (2008) 137–145.
[2] G. Wei, Q. Jin, W.V. Giannobile, P.X. Ma, Nano-fibrous scaffold for controlled delivery of recombinant human PDGF-BB, J. Control. Release 112 (2006) 103–110.
[3] C. Bertoldi, D. Zaffe, U. Consolo, Polylactide/polyglycolide copolymer in bone defect healing in humans, Biomaterials 29 (2008) 1817–1823.
[4] V.P. Torchilin, Targeted pharmaceutical nanocarriers for cancer therapy and imaging, AAPS J. 9 (2) (2007), Article 15.
[5] W. Jiang, R.K. Gupta, M.C. Deshpande, S.P. Schwendeman, Biodegradable poly (lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens, Adv. Drug Deliv. Rev. 57 (2005) 391–410.
[6] H.B. Ravivarapu, K. Burton, P.P. DeLuca, Polymer and microsphere blending to alter the release of a peptide from PLGA microspheres, Eur. J. Pharm. Biopharm. 50 (2000) 263–270.
[7] H. Kranz, N. Ubrich, P. Maincent, R. Bodmeier, Physicomechanical properties of biodegradable poly(D, L-lactide) and poly(D, L-lactide-co-glycolide) films in the dry and wet states, J. Pharm. Sci. 89 (12) (2000) 1558–1566.
[8] M.A. Tracy, K.L. Ward, L. Firouzabadian, Y. Wang, N. Dong, R. Qian, Y. Zhang, Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro, Biomaterials 20 (1999) 1057–1062.
[9] R.C. Mundargi, S. Srirangarajan, S.A. Agnihotri, S.A. Patil, S. Ravindra, S.B. Setty, T.M. Aminabhavi, Development and evaluation of novel biodegradable microspheres based on poly(D, L-lactide-co-glycolide) and poly(ε-caprolactone) for controlled delivery of doxycycline in the treatment of human periodontal pocket, J. Control. Release 119 (2007) 59–68.
[10] S. Singh, D.C. Webster, J. Singh, Thermosensitive polymer: Synthesis, characterization, and delivery of proteins, Int. J. Pharm. 341 (2007) 68–77.
[11] R.C. Mundargi, V.R. Babu, V. Rangaswamy, P. Patel, T.M. Aminabhavi, Nano/micro technologies for delivering macromolecular therapeutics using poly(D, L-lactide-co-glycolide) and its derivatives, J. Control. Release 125 (2008) 193–209.
[12] Y. Sun, J. Wang, X. Zhang, Z. Zhang, Y. Zheng, D. Chen, Q. Zhang, Synchronic release of two hormonal contraceptives for about one month from the PLGA microspheres: In vitro and in vivo studies, J. Control. Release 129 (2008) 192–199.
[13] A.J. Sansdrap, Moës, In vitro evaluation of the hydrolytic degradation of dispersed and aggregated poly(D, L-lactide-co-glycolide) microspheres, J. Control. Release 43 (1997) 47–58.
[14] C. Guse, S. Koennings, F. Kreye, F. Siepmann, A. Goepferich, J. Siepmann, Drug release from lipid-based implants: Elucidation of the underlying mass transport mechanisms, Int. J. Pharm. 314 (2006) 137–144.
[15] H. Yushu, S. Venkatraman, The effect of process variables on the morphology and release characteristics of protein-loaded PLGA particles, J. Appl. Polym. Sci. 101 (2006) 3053–3061.
[16] J. Wang, B.M. Wang, S.P. Schwendeman, Characterization of the initial burst release of a model peptide from poly(D, L-lactide-co-glycolide) microspheres, J. Control. Release 82 (2002) 289–307.
[17] S. Fredenberg, M. Reslow, A. Axelsson, Effect of divalent cations on pore formation and degradation of poly(D, L-lactide-co-glycolide), Pharm. Dev. Techn. 12 (6) (2007) 563–572.
[18] A. Mochizuki, T. Niikawa, I. Omura, S. Yamashita, Controlled Release of argatroban from PLA film – effect of hydroxylesters as additives on enhancement of drug release, J. Appl. Polym. Sci. 108 (2008) 3353–3360.
[19] T.G. Park, Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition, Biomaterials 16 (1995) 1123–1130.
[20] Y.-Y. Huang, M. Qi, H.-Z. Liu, H. Zhao, D.-Z. Yang, Degradation of porous poly(D, L-lactic-co-glycolic acid) films based on water diffusion, J. Biomed. Mater. Res. 80A (2007) 909–915.
[21] H. Okada, One- and three month release injectable microspheres of the LH-RH superagonist leuprorelin acetate, Adv. Drug Deliv. Rev. 28 (1997) 43–70.
[22] L. Lu, C.a. Garcia, A.G. Mikos, In vitro degradation of thin poly(D, L-lactic-co-glycolic acid) films, J. Biomed. Mater. Res. 46 (1999) 236–244.
[23] J. Kang, S.P. Schwendeman, Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins, Mol. Pharm. 4 (1) (2007) 104–118.
[24] C. Bouissou, J.J. Rouse, R. Price, C.F. van der Walle, The influence of surfactant on PLGA microsphere glass transition and water sorption: Remodeling the surface morphology to attenuate the burst release, Pharm. Res. 23 (6) (2006) 1295–1305.
[25] W.L. Webber, F. Lago, C. Thanos, E. Mathowitz, Characterization of soluble, salt-loaded, degradable PLGA films and their release of tetracycline, J. Biomed. Mater. Res. 41 (1998) 18–29.
[26] R.P. Batycky, J. Hanes, R. Langer, D.A. Edwards, A theoretical model of erosion and macromolecular drug release from biodegrading microspheres, J. Pharm. Sci. 86 (12) (1997) 1464–1477.
[27] A. Matsumoto, Y. Matsukawa, T. Suzuki, H. Yoshino, Drug release characteristics of multi-reservoir type microspheres with poly(DL-lactide-co-glycolide) and poly (DL-lactide), J. Control. Release 106 (2005) 172–180.
[28] S. Fredenberg, M. Reslow, A. Axelsson, Encapsulated zinc salt increases the diffusion of protein through PLG films, Int. J. Pharm. 370 (2009) 47–53.

[29] F. Alexis, Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly[(lactic acid)-co-(glycolic acid)], Polym. Int. 54 (2005) 36–46.
[30] B.S. Zolnik, D.J. Burgess, Effect of acidic pH on PLGA microsphere degradation and release, J. Control. Release 122 (2007) 338–344.
[31] J.M. Anderson, M.S. Shive, Biodegradation and biocompatibility of PLA and PLGA microspheres, Adv. Drug Deliv. Rev. 28 (1997) 5–24.
[32] R.L. Sastre, R. Olmo, C. Teijón, E. Muñíz, J.M. Teijón, M.D. Blanco, 5-Fluorouracil plasma levels and biodegradation of subcutaneously injected drug-loaded microspheres prepared by spry-drying poly(D, L-lactide) and poly(D, L-lactide-co-glycolide) polymers, Int. J. Pharm. 338 (2007) 180–190.
[33] S. Díez, C. Tros de llarduya, Versatility of biodegradable poly(D, L-lactic-co-glycolic acid) microspheres for plasmid DNA delivery, Eur. J. Pharm. Biopharm. 63 (2006) 188–197.
[34] A. Shenderova, T.G. Burke, S.P. Schwendeman, The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecins, Pharm. Res. 16 (2) (1999) 241–248.
[35] A.G. Ding, S.P. Schwendeman, Acidic microclimate pH distribution in PLGA microspheres monitored by confocal laser scanning microscopy, Pharm. Res. 25 (9) (2008) 2041–2052.
[36] M. Reslow, M. Jönsson, T. Laakso, Sustained-release of human growth hormone from PLG-coated starch microspheres, Drug Deliv. Syst. Sci. 2 (2002) 103–109.
[37] T.G. Park, Degradation of poly(D, L-lactic acid) microspheres: effect of molecular weight, J. Control. Release 30 (1994) 161–173.
[38] N. Faisant, J. Akiki, F. Siepmann, J.P. Benoit, J. Siepmann, Effects of the type of release medium on drug release from PLGA-based microparticles: Experiment and theory, Int. J. Pharm. 314 (2006) 189–197.
[39] D. Klose, F. Siepmann, K. Elkharraz, S. Krenzlin, J. Siepmann, How porosity and size affect drug release mechanisms from PLGA-based microparticles, Int. J. Pharm. 314 (2006) 198–206.
[40] S.S. D'Souza, J.A. Faraj, P.P. DeLuca, Model dependent approach to correlate accelerated with real-time release from biodegradable microspheres, AAPS Pharm. Sci. Tech. 6 (4) (2005), Article 70.
[41] W.H. Liu, J.L. Song, K. Liu, D.F. Chu, Y.X. Li, Preparation and in vitro and in vivo release studies of Huperzine A loaded microspheres for the treatment of Alzheimer's disease, J. Control. Release 107 (2005) 417–427.