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International Journal of Pharmaceutics 301 (2005) 294–303
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

In vitro degradation of polyanhydride/polyester core-shell double-wall microspheres

Emily J. Pollauf [a], Cory Berkland [a,1], Kyekyoon (Kevin) Kim [b,c], Daniel W. Pack [a,c,*]

[a] *Department of Chemical and Biomolecular Engineering, University of Illinois, Box C-3, 600 S. Mathews Ave., Urbana, IL 61801, USA*
[b] *Department of Electrical and Computer Engineering, University of Illinois, Urbana, IL, USA*
[c] *Department of Bioengineering, University of Illinois, Urbana, IL, USA*

Received 9 April 2005; received in revised form 6 June 2005; accepted 6 June 2005
Available online 26 July 2005

Abstract

Double-wall microspheres (DWMS), comprising distinct polymer core and shell phases, are useful and interesting for controlled-release drug delivery. In particular, the presence of a surface-eroding polymer core may be expected to limit water penetration and, therefore, delay degradation of the core phase and drug release. In this study, solid microspheres and DWMS were fabricated using a surface-eroding polymer (poly[1,6-bis(*p*-carboxyphenoxy)hexane]; PCPH) and a bulk-eroding polymer (poly(D,L-lactide-co-glycolide); PLG). Erosion of the particles was observed by optical and electron microscopy, while polymer degradation was followed by gel permeation chromatography, during incubation in buffer at 37 °C. Degradation and erosion were very different depending on which polymer formed the particle shell. Nevertheless, the relatively thin (~5 μm) PCPH shells could not prevent water penetration, and the PLG cores completely eroded by 6 weeks of incubation.

Keywords: Microsphere; Double-wall microsphere; Poly(D,L-lactide-co-glycolide); Polyanhydride; Degradation

1. Introduction

Biodegradable polymers have been used for controlled release applications in a number of different morphologies including disks (Heller, 1985; Dang and Saltzman, 1994; Dahiyat et al., 1995; Göpferich, 1997), rods (Zhang et al., 1993; Zhou et al., 1998), particles (Varde and Pack, 2004) and in situ forming depots (Lambert and Peck, 1995; Shively et al., 1995; Hatefi and Amsden, 2002). Two major erosion mechanisms have been identified for biodegradable polymers: bulk and surface erosion. The two mechanisms are distinguished by the relative time scales of polymer hydrolysis and water penetration, which depend on both

* Corresponding author. Tel.: +1 217 244 2816; fax: +1 217 333 5052.
E-mail address: dpack@uiuc.edu (D.W. Pack).
[1] Present address: Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047, USA.

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doi:10.1016/j.ijpharm.2005.06.004

polymer chemistry and size and shape of the device. In bulk-eroding polymers, water penetration is fast compared to hydrolysis. Water therefore diffuses into bulk-eroding polymers inducing swelling and degradation throughout the matrix simultaneously. As more bonds are broken, pores usually develop and the small degradation products are able to diffuse out of the polymer bulk, leading to mass loss. In surface-eroding devices, however, hydrolysis is fast relative to water penetration, and as a result degradation occurs only very near the water-exposed surface.

Polyesters are the most common class of bulk-eroding polymers (Li and Vert, 1994; Park, 1995) while polyanhydrides and poly(ortho esters) are two representative classes of surface-eroding polymers (Tamada and Langer, 1993; Dang and Saltzman, 1994; Shieh et al., 1994; Gopferich, 1996; Heller et al., 2002). In practice, polymers are not perfectly bulk or surface eroding, but one mechanism typically dominates (von Burkersroda et al., 2002). It is possible, for example, that devices formed from typical surface-eroding polymers (e.g., polyanhydrides) can exhibit bulk erosion characteristics as the device size decreases.

Much interest has focused on spherical polymer micro- and nano-particles for drug delivery. Core-shell microcapsules in particular, consisting of aqueous, oil or polymer cores surrounded by a polymer shell, may provide unique opportunities to control drug release rates (Uno et al., 1984; Pekarek et al., 1994; Sanchez et al., 1996; Labhasetwar et al., 1997; Leach and Mathiowitz, 1998; Loxley and Vincent, 1998; Watnasirichaikul et al., 2000; Lee et al., 2002; Yang et al., 2003). For example, particle size and shell thickness have been shown to strongly affect release rates (Berkland et al., 2004a). Further, polymer chemistry and the mechanisms of degradation and erosion of the shell- and core-forming materials add a tunable parameter to such a delivery system.

Several groups have produced two-polymer, double-wall microspheres (DWMS) using modified double-emulsion fabrication methods (Pekarek et al., 1994; Leach and Mathiowitz, 1998; Lee et al., 2002). Most of these DWMS comprise two bulk-eroding polymers, although a few consisted of a bulk-eroding polymer shell encapsulating a surface-eroding polymer core (Pekarek et al., 1996; Leach et al., 1999; Yang et al., 2003). Because of the inherent thermodynamics of these three-phase (e.g., polyester solution/polyanhydride solution/water) systems, it is difficult to produce DWMS with the more hydrophobic, surface-eroding polymers forming the shell. Only very recently, we described a method for fabrication of DWMS of a polyester and polyanhydride in which either polymer can be made to form the shell phase (Berkland et al., 2004c).

Although surrounding one bulk-eroding polymer with another offers greater control of release rates (Berkland et al., 2004a), encapsulation of a bulk-eroding polymer with a surface-eroding polymer may provide improvements for many applications. For example, because surface-eroding polymers such as polyanhydrides degrade primarily at the surface of the capsules, it would be very interesting if a surface-eroding shell could prevent water penetration into the polymer core. With a bulk-eroding polymer core, the absence of water penetration could delay polymer degradation and formation of acidic byproducts (Park, 1995; Fu et al., 2000), potentially protecting fragile therapeutic agents such as proteins and delaying their release in a tunable fashion.

Here, degradation and erosion of DWMS consisting of bulk- and surface-eroding polymers are reported. Poly(D,L-lactide-co-glycolide) (PLG) was chosen as a bulk-eroding polymer, while a polyanhydride with a slow rate of erosion, poly[1,6-bis(*p*-carboxyphenoxy)hexane] (PCPH), was chosen as the surface-eroding material. DWMS and solid microspheres of each polymer were monitored in vitro over a period of several weeks using both microscopy for visualization of particle morphology and quantitative analysis of polymer molecular weight changes.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) in a 50:50 lactide:glycolide molar ratio (PLG, inherent viscosity of 0.82 in hexafluoroisopropanol) was obtained from Birmingham Polymers and poly[1,6-bis(*p*-carboxyphenoxy)hexane] (PCPH) was synthesized and donated by Matt Kipper and Prof. Balaji Narasimhan (Iowa State University). Poly(vinyl alcohol) (PVA,

24 kDa, 88% hydrolyzed)) was obtained from Polysciences. Methylene chloride (DCM, reagent grade), tetrahydrofuran (THF, reagent grade) and chloroform (HPLC grade) were purchased from Fisher Scientific. Isoton II diluent and anionic dispersant type 2 were obtained from Beckman-Coulter (Fullerton, CA).

2.2. Microsphere and microcapsule fabrication

The precision particle fabrication method (Berkland et al., 2001, 2002) was used to create microcapsules of PLG and PCPH as previously described (Berkland et al., 2004a,c). The triple nozzle system generated a compound jet consisting of an inner core-forming stream, annular shell-forming stream and outermost carrier stream (0.5% PVA in de-ionized water). To promote the chosen polymer configuration in the capsules, the core jet consisted of 15% (w/v) PCPH or 30% (w/v) PLG polymer, and the shell stream of a 3% (w/v) polymer solution, all in DCM. The jet was broken up into uniform droplets by acoustic excitation of the nozzle. Nascent particles were collected in 900 mL of 0.5% PVA in water with 5 mL of DCM, and the bath was stirred for 3 h to allow for solvent extraction. The hardened particles were washed with 900 mL of deionized water in a vacuum filtration system prior to lyophilization for 48 h. Samples were stored until use in a −20 °C freezer with desiccant.

2.3. Size distribution

The size distributions of hardened microspheres and microcapsules were determined using a Beckman Multisizer 3. The particles were suspended in Isoton II with two drops of dispersant Type A. A 280-μm aperture was employed. More than 5000 particles were measured for every sample.

2.4. In vitro degradation

Microparticles were incubated in phosphate buffered saline (pH 7.2, PBS) at 37 °C, containing 10 μg/mL rhodamine B when indicated. Samples were removed at pre-determined time points, rinsed twice with deionized water (to remove excess salts from the exterior of the microspheres) and imaged with transmitted light and laser scanning confocal fluorescence microscopies.

Samples were also collected for gel permeation chromatography (GPC), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) analyses. Twenty-five milligram samples were placed in a scintillation vial with 20 mL of PBS and incubated at 37 °C with occasional agitation. Samples of the spheres were removed at the predetermined time points and frozen at −20 °C. At the time of use, the samples were thawed in a water bath and washed three times with deionized water. Samples for scanning electron microscopy were taken from the wet sample and prepared as described below. Chromatography and spectroscopy samples were lyophilized for 48 h, then dissolved in 1 mL of HPLC-grade chloroform.

2.5. Microscopy

Optical and fluorescence images of the particles were obtained using an Olympus Fluoview FV300 Laser Scanning Biological Microscope with a krypton laser (568 nm) to excite rhodamine B. Unless otherwise noted, images of the microcapsules were captured at the particle midline with a 60× oil-immersion objective.

Core encapsulation efficiencies are defined as the fraction of particles in which the core phase is completely surrounded by the shell material. Optical micrographs of several hundred particles were captured at the particle midline. Visual observation was used to determine the number of particles with encapsulated cores relative to the total number of particles imaged. All particles exhibiting partial encapsulation, for which the core is protruding from or contacting the exterior wall of the shell, have been treated as not encapsulated.

The exterior and interior morphologies of the samples were imaged using a Hitachi S-4700 scanning electron microscope (SEM). A razor blade was used to cross-section frozen particles dried on metal sample holders. The samples were then lyophilized overnight and sputter coated for 45 s at 20 mA using an Emitech K-575 Sputter Coater with a gold–palladium target. Images were obtained at 5 or 10 kV.

In some cases, cross-sectioned samples of the particles on an SEM holder were immersed in a bath of tetrahydrofuran (THF) for a period of 40 min. THF dissolved PLG, but is not a solvent for PCPH. Subsequent SEM imaging was performed as above.

2.6. Molecular weight analysis

Gel permeation chromatography (GPC) was used to determine polymer molecular weights. The system consisted of a Waters 1515 HPLC pump, Waters 715plus autoinjector, and Waters 410 differential refractive index detector. Separation was performed in linear Styragel HR 3, HR 4 and HR 4E columns from Waters at 40 °C. Polystyrene standards (10 molecular weights from 580 to 299,400) from Polymer Laboratories were used to generate the calibration curve at concentrations of 1 mg/mL. The semilog calibration curve of molecular weight versus elution volume is linear ($R^2 = 0.997$). HPLC-grade chloroform at a flow rate of 1 mL/min was used as the mobile phase. All injected samples were dissolved in HPLC-grade chloroform. Molecular weights and polydispersities are reported as the average of two measurements.

3. Results and discussion

3.1. Initial characterization

For this study, PLG was selected as the bulk-eroding polymer and PCPH as the surface-eroding polymer. PCPH degrades slowly at a rate of 0.2 μg/(cm^2 h) (Leong et al., 1985). Each polymer was used to produce single-polymer microspheres, and the two polymers were combined in both core-shell configurations (Table 1) using the precision particle fabrication technology (Kim et al., 1989, 1991; Berkland et al., 2004c). Assuming complete polymer phase separation, the shell thicknesses of the microcapsules were calculated to be ∼5 μm based on a volume balance using the mass flow rates of each polymer solution and final particle diameters and assuming equal densities of both phases. Average particle diameters ranged from 44 to 50 μm for the different batches, and the size distributions were very narrow in all cases.

Core encapsulation efficiencies (i.e., the percentage of particles exhibiting cores completely surrounded by shell polymer) were determined for each microcapsule sample using light microscopy as described above. As seen in Table 1, core encapsulations were high (77 and 81%), and most particles exhibited the desired core-shell morphology. Particles with cores protruding from or contacting the outer shell wall were not counted as fully encapsulated, but in all cases these partially encapsulated particles made up the remainder of the sample. In other words, no particles were visible in either DWMS sample that did not have two phase-separated polymer regions.

Polymer location within the microparticles was investigated using SEM. The surfaces of PLG and PCPH microspheres (Fig. 1, A and I) show non-porous, relatively smooth surfaces, while the DWMS (Fig. 1, C and F) exhibit exteriors of smooth polymer disrupted by small hemispherical protrusions. These surface protrusions are more numerous and larger on the PLG(PCPH) DWMS (see Table 1 for nomenclature convention) than with the PCPH(PLG) particles and most likely represent domains of the core polymer that have partitioned to the surface during the fabrication and hardening process. In studies of the miscibility of polyanhydrides and polyesters, Domb found the samples to be macroscopically immiscible (Domb, 1993). However, FTIR and ^{1}H NMR analyses of the layers (both in solution and melt films) found the polyanhydride regions to be purely polyanhydride polymer, whereas the polyester regions were predominately polyester with some polyanhydride (Domb, 1993). The SEM images presented here cannot rule out the possibility of such limited miscibility.

Table 1
Mass composition, shell thickness, size and core encapsulation of uniform PLG and PCPH microspheres and microcapsules

Sample name[a]	Core material (mass%)[b]	Shell material (mass%)[b]	Shell thickness (μm)	Diameter (μm)	Core encapsulation
PLG	PLG (100)	N/A	N/A	44.4 ± 2.2	N/A
PLG(PCPH)	PCPH (50)	PLG (50)	5	44.9 ± 2.0	81%
PCPH(PLG)	PLG (50)	PCPH (50)	5	43.5 ± 2.7	77%
PCPH	PCPH (100)	N/A	N/A	49.5 ± 3.1	N/A

[a] DWMS are named according to the convention P1(P2) where P1 is the polymer forming the shell and P2 is the polymer located in the core.
[b] Mass% was determined from the polymer concentrations and volumetric flow rates of the core- and shell-forming solutions fed into the precision particle fabrication apparatus.

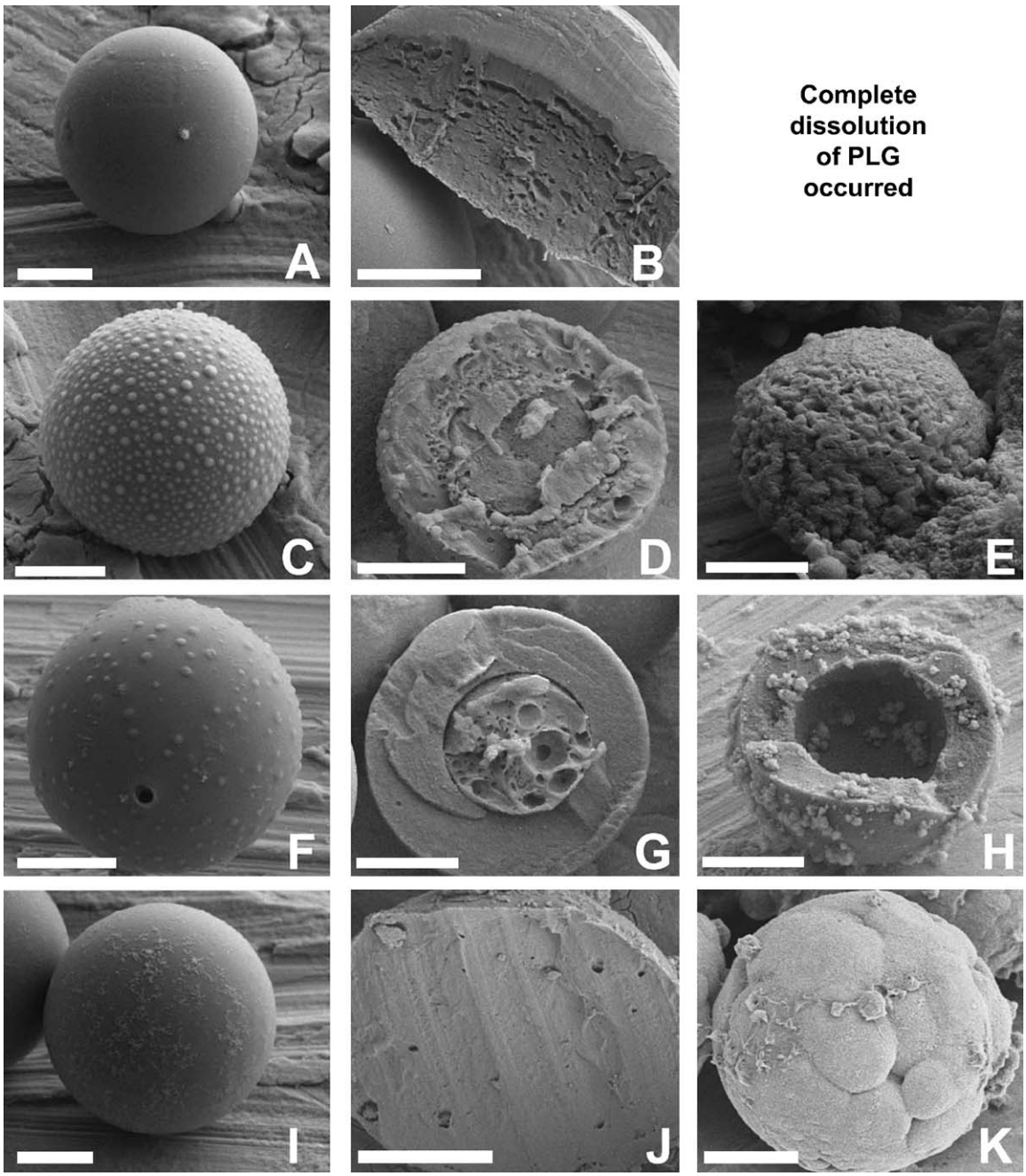

Fig. 1. Scanning electron micrographs of the particle exteriors (first column) and interiors (second column), and of fractured particle morphology after selective dissolution with THF (third column) of (A–B) PLG; (C–E) PLG(PCPH); (F–H) PCPH(PLG) and (I–K) PCPH. Scale bars are 15 μm, except (E) where the scale bar is 10 μm.

To more definitively identify the polymer locations in the DWMS, a selective dissolution technique was employed. Although PLG dissolves on contact with tetrahydrofuran (THF), PCPH is not at all soluble in this solvent. Fractured samples of the microspheres and DWMS were therefore immersed in THF. After dissolution, no PLG microspheres were found, indicating that dissolution had been complete, as expected. SEM micrographs of DWMS showed dissolution of the PLG shell (Fig. 1E), and of the PLG core leaving behind PCPH shells (Fig. 1H). A number of small spheres (presumably composed of PCPH) were found around the remaining PCPH cores and shells. It is thought that the polymers do not completely phase separate, but that the PLG phases may contain a small amount of PCPH dispersed in small pockets throughout it. The small hemispherical protrusions in Fig. 1C and F likely correspond to the small spheres seen in the selectively dissolved images of the same microcapsules in Fig. 1E and H. Nevertheless, in both DWMS samples, the phase thought to be PLG was clearly dissolved, thus indicating that core and shell assignments for the samples are correct.

3.2. Visual evidence of degradation

Optical micrographs of the pure polymer microspheres and core-shell microcapsules can be used to track swelling and major morphological changes in the particles. Freshly prepared particles were spherical in shape (Fig. 2, column 1). Distinct core and shell domains were visible in both the PLG(PCPH) and PCPH(PLG) DWMS (Fig. 2D and H). After 3 weeks of in vitro degradation (Fig. 2B), the PLG microspheres increased in diameter, most likely due to water infiltration and polymer swelling. PLG microspheres showed severe swelling at 4 weeks (Fig. 2C) and a complete loss of integrity after 25 weeks. Pure PCPH microspheres showed only slight swelling and some increase in surface roughness at times up to 4 weeks (Fig. 2M and N).

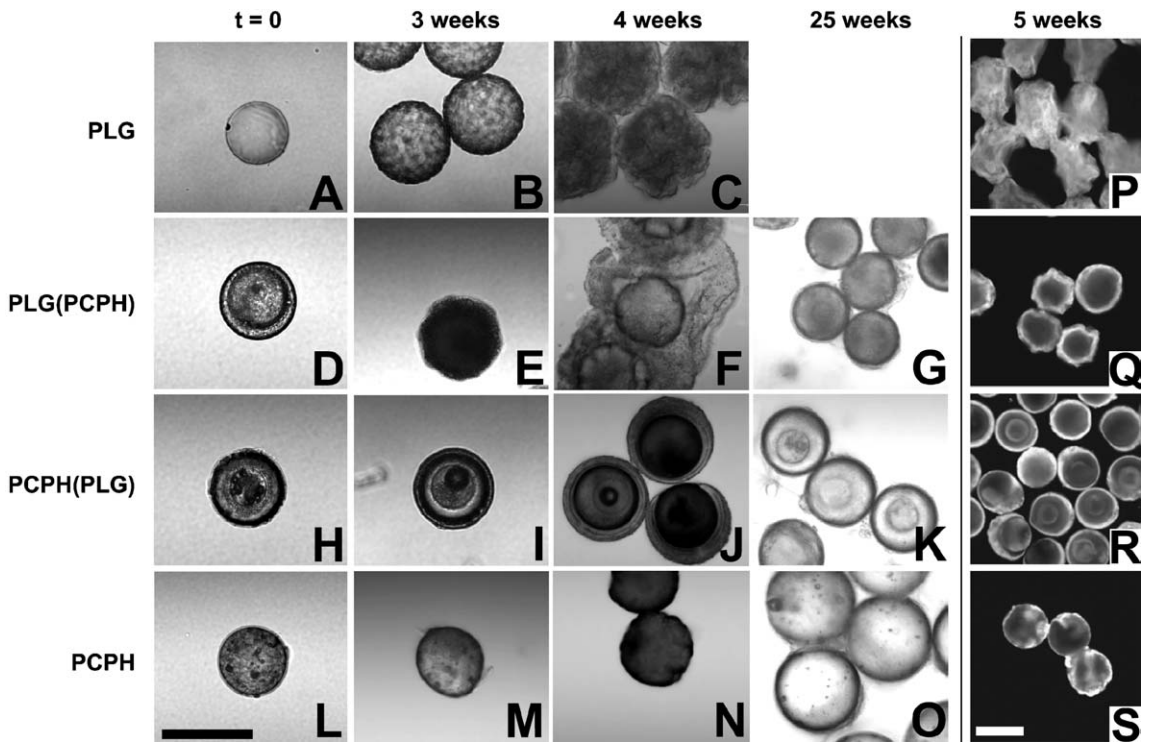

Fig. 2. Optical micrographs at time zero (first column); 3 weeks (second column); 4 weeks (third column) and 25 weeks (fourth column) of in vitro degradation, and confocal fluorescent images of rhodamine B uptake at 5 weeks (fifth column) of in vitro degradation. Images are of (A–C, P) PLG; (D–G, Q) PLG(PCPH); (H–K, R) PCPH(PLG) and (L–O, S) PCPH. Scale bars are 50 μm.

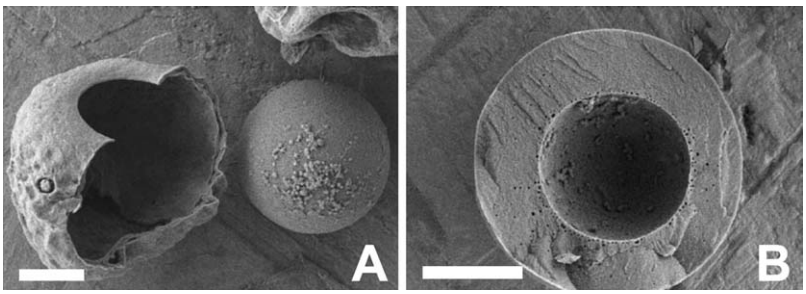

Fig. 3. SEM micrographs of (A) PLG(PCPH) and (B) PCPH(PLG) after 6 weeks of in vitro incubation in PBS at 37 °C. Scale bars are 15 μm.

Even after 25 weeks, PCPH particles remained spherical, but showed significant swelling (Fig. 2O).

The most interesting comparison is between the two types of DWMS. After 3 weeks incubation, the surface of PLG(PCPH) DWMS appeared rough and uneven. At 4 weeks, the PLG shell swelled greatly, and by 25 weeks the PLG shell was apparently lost entirely but the core was mainly intact (Fig. 2G). Thus, the behavior of the PLG shell of PLG(PCPH) DWMS appears to be similar to pure PLG microspheres (Fig. 2A–C). PCPH(PLG) DWMS changed little after 3 and 4 weeks incubation (Fig. 2I and J). PCPH shells were still intact after 25 weeks (Fig. 2K), although their overall size and core diameters were somewhat smaller. While an interior interface was clearly present in PCPH(PLG) DWMS after 25 weeks, no conclusions about the fate of the PLG core can be drawn from these images.

Additional information about water uptake in the particles can be obtained by following penetration of rhodamine B (from the external solution) into the microparticles over time using confocal fluorescence microscopy. The presence of rhodamine in the particle interior, which most likely entered the particle by diffusion, suggests that water is also present inside the particle. (Absence of the dye, however, does not necessarily indicate absence of water.) After 5 weeks of incubation in PBS containing rhodamine, the dye clearly penetrated throughout the swollen PLG microspheres (Fig. 2P) while it was located only near the surface of PCPH particles (Fig. 2S). The fluorescence present "inside" the particles shown in Fig. 2S resulted from "folds" in the particle surface (see Berkland et al., 2004b). Rhodamine penetrated throughout the PLG shell of PLG(PCPH) DWMS with very little fluorescence inside the PCPH cores (Fig. 2Q). Finally, and most importantly, PCPH(PLG) DWMS showed strong rhodamine emission near the particle surface as well as inside the particles (Fig. 2R), suggesting that water is able to penetrate the PCPH shell and reach the bulk-eroding polymer core.

SEM of the PLG(PCPH) and PCPH(PLG) DWMS after 6 weeks of in vitro degradation shows erosion of the PLG phase in both configurations (Fig. 3). The PLG shell separated from the PCPH core and was no longer dense. Rather, a thin wavy structure exists, although a general spherical shape remained (Fig. 3A). The PCPH shell was very dense and clearly defined after 6 weeks, but the PLG core completely eroded within the shell leaving a void in the center of the PCPH(PLG) samples (Fig. 3B).

3.3. Molecular weight loss

The degradation of the microspheres and DWMS over time was tracked by GPC. Based on the optical and confocal fluorescence micrographs, 28 days was determined to be a critical time at which PLG loses integrity. Molecular weight determination, therefore, was focused on the first 4 weeks of incubation

The initial weight-averaged molecular weight of PLG was 87 kDa, while PCPH molecular weight was 33 kDa (Fig. 4). Despite the large numerical difference in average molecular weights of the two polymers, the broad distribution of the PCPH polymer makes determination of the individual polymer molecular weights in DWMS impossible. For that reason the DWMS molecular weights shown in Fig. 4 are the average of both polymers contained in the samples. Using the measured molecular weights of the two microsphere samples, the DWMS (1:1 mass ratio of the two polymers) would be expected to have a molecular weight of 60 kDa with a polydispersity of 1.7.

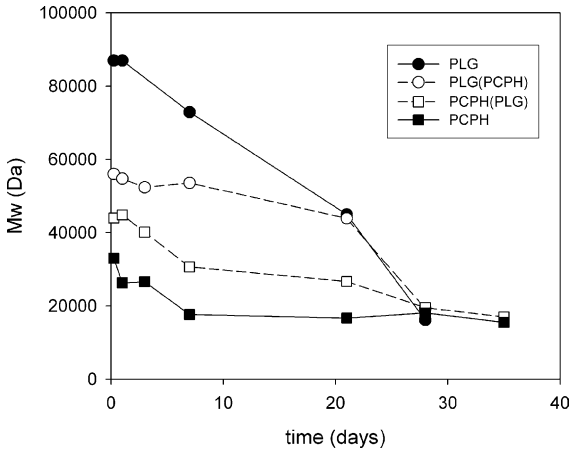

Fig. 4. Weight-averaged molecular weight during in vitro incubation (in PBS, 37 °C) of PLG microspheres (●), PLG(PCPH) (○) and PCPH(PLG) (□) DWMS, and PCPH microspheres (■). DWMS initially consisted of 1:1 mass ratio of PLG and PCPH.

The measured value of 56 kDa for the PLG(PCPH) sample is in close agreement with the expected value. The PCPH(PLG) DWMS, on the other hand, exhibited a measured molecular weight of only 44 kDa with a polydispersity of 1.9, more similar to PCPH than PLG. These variations could indicate incomplete dissolution of the core polymer, but chloroform is a good solvent for both PLG and PCPH and sufficient time was given for dissolution, making this explanation unlikely. Alternatively, low molecular weight fragments present in the initial PLG solutions may have been retained in the PCPH(PLG) DWMS (due to the PCPH shell) relative to either the PLG microspheres or PLG(PCPH) DWMS. Such an effect is expected to lead to a lower weight-averaged molecular weight and high polydispersity, as observed.

Changes in weight-averaged molecular weight during incubation at 37 °C are shown in Fig. 4. PLG microspheres exhibited no change in molecular weight over the first several days, similar to previously reported results (Raman et al., 2005), followed by a steady decrease to 52% of the initial value by 21 days and only 19% by 28 days, corroborating the swelling and visual polymer breakdown observed at this same time point (Fig. 2C). By 35 days of degradation, PLG molecular weight peaks were not detected. Degradation of PCPH microspheres was very different. Molecular weight decreased by ∼50% over the first week and then remained constant during the following 4 weeks. This pattern is consistent with the surface-erosion mechanism.

When comparing the two types of DWMS, it is important to remember that the formulations were prepared with a 1:1 mass ratio of the two polymers. Thus, the PLG(PCPH) and PCPH(PLG) DWMS comprise the same composition; only the relative locations of the two polymers in the particles are different. PLG(PCPH) DWMS degraded slower than PLG microspheres over the first 3 weeks, possibly due to less severe autocatalytic degradation in the relatively thin PLG shell compared to the solid PLG particle (Berkland et al.). A rapid drop in molecular weight was observed between 3 and 4 weeks, again corresponding to the change in particle appearance during that time (cf. Fig. 2E and F). From 4 weeks, the weight-averaged molecular weight of PLG(PCPH) DWMS was indistinguishable from that of the PCPH microspheres. Degradation of PCPH(PLG) DWMS was similar to that of PCPH microspheres except a slow but steady drop in the average molecular weight occurred from 1–4 weeks. Again, by 4 weeks incubation, the weight-averaged molecular weight was indistinguishable from PCPH microspheres. These results, together with the micrographs described above, suggest that the PCPH shell does not prevent water uptake or degradation of the core in PCPH(PLG) DWMS.

4. Conclusions

Double-wall microspheres represent an interesting class of controlled-release drug delivery devices. The ability to generate such particles with control over the core and shell materials and the shell thickness may provide opportunities to enhance control of drug release rates. In particular, it was hypothesized that the presence of a surface-eroding polymer shell may reduce water penetration and, therefore, delay degradation of and drug release from the particle core. The data presented herein show that the core-shell particles do indeed degrade differently depending on the nature of the shell-forming polymer. Results also suggest, however, that although PCPH is a slowly degrading, surface-eroding polymer, water penetrates to the core of PCPH(PLG) DWMS and the PLG core erodes inside the PCPH shell within a time frame of several weeks.

A thicker shell (>5 μm) or a more hydrophobic shell polymer may be required to protect the polymer core from the effects of water penetration.

Acknowledgments

This work was partly supported by NIH grant EB002878. The synthesis of PCPH by Matt Kipper and Balaji Narasimhan at Iowa State University is gratefully acknowledged. Scanning electron microscopy was carried out at the Center for Microanalysis of Materials, University of Illinois at Urbana-Champaign, which is partially supported by the U.S. Department of Energy under grant DEFG02-91-ER45439.

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