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Modeling small-molecule release from PLG microspheres: effects of polymer degradation and nonuniform drug distribution

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Abstract

Modeling release of small molecules from degradable microspheres is important to the design of controlled-release drug delivery systems. Release of small molecules from poly(D,L-lactide-co-glycolide) (PLG) particles is often controlled by diffusion of the drug through the polymer and by polymer degradation. In this study, a model is developed to independently determine the contributions of each of these factors by fitting the release of piroxicam from monodisperse 50-μm microspheres made with PLG of different initial molecular weights. The dependence of the drug diffusivity on polymer molecular weight was determined from in vitro release of piroxicam from monodisperse 10-μm PLG microspheres, and the polymer degradation rate was experimentally measured using gel permeation chromatography. The model also incorporates the effect of nonuniform drug distribution within the microspheres, which is obtained from confocal fluorescence microscopy. The model results agree well with experiments despite using only one fit parameter.

Keywords: Poly(lactide-co-glycolide) microspheres; Modeling; Piroxicam; Diffusion; Degradation; Nonuniform drug distribution

1. Introduction

Development, characterization and analysis of devices for controlled release of therapeutics, such as polymer rods, disks and microparticles, have received considerable attention. Controlled release of therapeutics offers a number of potential advantages over traditional dosage methods, like injections, ointments and pills, including potentially maintaining a constant and optimum drug concentration, reduced dosage frequency, increased patient compliance and site-specific therapy.

Polymeric microspheres are one of the most common types of controlled-release devices because of their ease of fabrication, relatively simple administration and versatility. However, difficulty in controlling release rate profiles is an important

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limitation. A number of studies have shown how various factors, such as polymer molecular weight [1,2], polymer chemistry and monomer ratios [3,4], pH of release media and additives to the release media [5,6], affect the release kinetics of microspheres. Berkland et al. [7] have used an innovative microsphere fabrication technique to demonstrate that the size of the microspheres affects release in several ways. In addition to the obvious effects of surface area–volume ratio, they have shown that microsphere size affects the initial drug distribution within the microsphere, the rate of polymer degradation and the internal morphology of the microsphere [8]. Each of these factors can significantly influence drug release.

A significant amount of work has gone into mathematical modeling to predict drug release rates from microspheres and to provide insight into the fundamental processes that govern release. Higuchi [9] was among the first to present a model for release of medicaments from an ointment suspension. His work was soon extended to slabs, cylinders and spheres with both homogeneous and porous or granular morphologies [10,11]. Higuchi's models, however, assume that the release is purely diffusion controlled. A number of other models have also been proposed to predict drug release when the process is controlled by erosion [12], swelling [13] or dissolution [14]. Chiu et al. [15] have also used percolation theory to describe release from erosion-controlled polymer discs.

Although most models account for only one of the many phenomena governing the release, Faisant et al. [16] and Zhang et al. [17] have proposed models that account for the effect of both erosion and diffusion on the drug release. Faisant's model assumes that the microspheres have a homogeneous morphology during the release without any pores, but Zhang's model assumes the microspheres have an initial porosity.

In this paper, a simple model is proposed to describe the drug release from monodisperse poly(D,L-lactide-co-glycolide) (PLG) microspheres. The model includes diffusion of the drug through a homogeneous polymer phase and the effect of polymer degradation on the diffusion rates. The diffusivities are estimated using initial in vitro release data obtained from 10-μm microspheres where degradation does not play a significant part in the release. The model also accounts for nonuniform distribution of the drug in the microspheres. Here, the use of this model to predict piroxicam release from ≈50 μm monodisperse microspheres fabricated from PLG of different initial molecular weights is reported.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) polymers (50:50 lactide:glycolide, inherent viscosity 0.17–1.08 dl/g in hexaflouroisopropanol) were purchased from Birmingham Polymers. Poly(vinyl alcohol) (88% hydrolyzed) was obtained from Polysciences. Piroxicam was kindly provided by Dongwha Pharmaceuticals (Seoul, Korea). Reagent grade dicholoromethane (DCM) was obtained from Fisher Scientific. All materials were used as obtained.

2.2. Preparation of microspheres

PLG microspheres were prepared using a previously reported technique [7]. Briefly, PLG was dissolved in DCM to make 5% or 30% w/v solutions. Piroxicam was codissolved at 10% of the polymer mass in the 5% solutions and 15% of the polymer mass in the 30% solution. The 5% w/v solutions were used to fabricate 10-μm spheres while the 30% w/v solutions were used to make 50-μm spheres. The polymer solution with the dissolved drug was then injected through a small-gauge needle concentrically with a carrier stream (0.5% w/v PVA in deionized water). The setup was acoustically excited using an ultrasonic transducer (Branson Ultrasonics) controlled by a frequency generator (Hewlett Packard, Model 3325A). The acoustic excitation causes the polymer stream to break up into uniform droplets (containing polymer, drug and solvent), which were collected in a beaker containing approximately 900 mL of a 1% PVA solution. The droplets were hardened to form uniform microspheres by stirring for 3 h to allow for solvent extraction and evaporation. Once hardened, the particles were filtered and rinsed with deionized

water to remove PVA. The microspheres were then lyophilized (Labconco benchtop model) for at least 2 days and stored at −20 °C in the presence of desiccant.

2.3. Piroxicam loading

The piroxicam loading of the microspheres was determined by incubating ≈2 mg of microspheres in 1 mL 0.25 M NaOH at 70 °C for 3 h. An equal weight of blank microspheres (i.e., containing no drug) were also eroded similarly. The absorbance of the supernatant was measured at 350 nm using a UV-Visible Spectrometer (Varian, Cary 50 Scan). The absorbance of the supernatant obtained from the blank microspheres was subtracted and the concentration of piroxicam was determined by comparison to the absorbance of a set of standards.

2.4. In vitro release

Approximately 5 mg of microspheres were suspended in 1.3 mL of phosphate-buffered saline (PBS, pH 7.4) containing 0.5% v/v Tween-20. The microsphere suspensions were inverted at approximately 10 rpm in a 37 °C incubator. At various times, the microspheres were centrifuged and 1 mL of the supernatant was taken out and replaced by an equal volume of fresh buffer. The microspheres were then resuspended by vortexing briefly. The piroxicam concentration in the supernatant was determined by measuring absorbance at 276 nm using a UV-Visible spectrometer (Varian Cary 50 Scan) and compared to a set of standards.

2.5. Microsphere degradation study

To determine the degradation rates of the polymers, microspheres were incubated under the same conditions as the in vitro release. At different time points, a small portion of the microspheres was removed, washed with deionized water to remove buffer salts and frozen. At the end of the study, the microspheres were lyophilized (Labconco benchtop model) and dissolved in 1 mL of Tetrahydrofuran (THF).

Polymer molecular weights were determined by SEC with a Waters 515 HPLC pump, a Spectraseries AS100 autosampler, a Viscotek model 300 triple detector array, and a series of three Viscotek (7.8×300 mm) Viscogel columns (2× GMHHRH and 1× G3000H). Molecular weight data were determined using Viscotek's TriSEC software. The light scattering, mass and viscosity constants were determined from a single 90-kDa narrow polystyrene standard and checked against other known polystyrene standards for accuracy. TriSEC data were obtained in THF solutions at 30 °C with a THF flow rate of 1.0 mL/min. Conventional calibration was performed with linear polystyrene standards.

2.6. Intraparticle drug distribution

The piroxicam-loaded microspheres were examined using an Olympus Fluoview FV300 laser scanning biological microscope. The microspheres were suspended in deionized water, placed on a glass slide and allowed to dry overnight before examination. Piroxicam was excited by a Helium/Neon laser (633 nm) and images were taken at the center line of the microspheres.

The images obtained from confocal microscopy of piroxicam-loaded microspheres were analyzed to obtain drug distributions within the microspheres. The images were converted to intensity plots using ImageJ software and then converted to radial distributions by using a simple computer program to average the intensity at all points at a given radial distance from the center of the microsphere.

2.7. Particle size distribution

A Coulter Multisizer 3 (Beckman Coulter) was used to determine the size distributions of the microspheres after hardening, prior to lyophilization. The 10-μm spheres were sized using a 100-μm aperture while the 50-μm spheres were sized with a 280-μm aperture. Isoton II was the electrolyte used for all cases, and a dispersant was added while sizing the 50-μm particles. At least 5000 microspheres were sampled to obtain each distribution.

3. Mathematical model

The proposed model is a simple diffusion model that incorporates the effect of polymer degradation on

the drug diffusivity in the polymer and the nonuniform distribution of the drug within the microsphere. The equation describing the release is the common diffusion equation:

$$\frac{\partial C}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 D(M_w)\frac{\partial C}{\partial r}\right) \quad (1)$$

where C is the concentration of the drug, r is the radial position, t is time and $D(M_w)$ is the polymer molecular weight dependent diffusivity of the drug. Since M_w changes with time, D is parametrically dependent on time. The above equation is solved using the following boundary conditions:

$$\frac{\partial C}{\partial r}|_{r=0} = 0 \quad (2)$$

$$C|_{r=R} = 0 \quad (3)$$

and the initial condition:

$$C(r)|_{t=0} = f(r) \quad (4)$$

where R is the radius of the microspheres. The initial drug distribution within the microspheres [$f(r)$] was obtained from confocal micrographs of drug-loaded microspheres. In the present model, the diffusivities used to predict the release from 50-μm spheres are estimated from initial release data obtained from 10-μm spheres to give $D(M_w)$. The dependence of the molecular weight on time is also experimentally measured. The model contains one fit parameter, the initial diffusivity (D_0) which is used until the time-dependent diffusivity [$D(M_w)$] is larger than D_0.The equations were solved using an adaptive Runge–Kutta method using the fifth- and sixth-order Runge–Kutta formulas to estimate the error of integration [18,19].

Table 1
Sizes (in microns) and encapsulation efficiencies of fabricated microspheres of different PLG molecular weights

I.V. (dl/g)	Size (μm)	Encapsulation efficiency (%)	Size (μm)	Encapusalation efficiency (%)
0.17	10.0±1.1	43.6	NA[a]	NA[a]
0.39	9.8±1.3	48.4	43.6±0.9	50.7
0.59	11.7±1.9	59.5	43.9±1.0	60.9
0.82	10.2±1.6	55.8	49.2±1.2	59.7
1.08	10.1±0.9	66.0	49.2±0.5	63.8

[a] Not fabricated.

4. Results and discussion

4.1. Preparation of microspheres and drug loading studies

Uniform 10- and 50-μm-diameter spheres with theoretical loadings of 10% and 15% w/w piroxicam, respectively, were fabricated using a method described previously [7]. Both sets of microspheres were relatively monodisperse (Fig. 1, Table 1). The encapsulation efficiencies were between 43.6% and

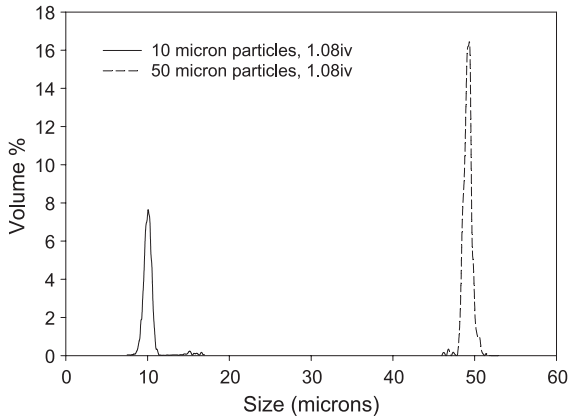

Fig. 1. Size distributions of 10 μm and 50 μm (nominal size), 1.08iv PLG microspheres.

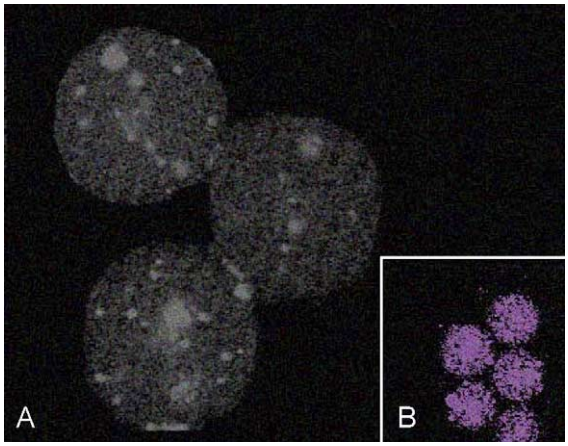

Fig. 2. Confocal images of 1.08iv PLG 50-μm microspheres (A, 40×) and 0.39iv PLG 10-μm (B, 60×) microspheres.

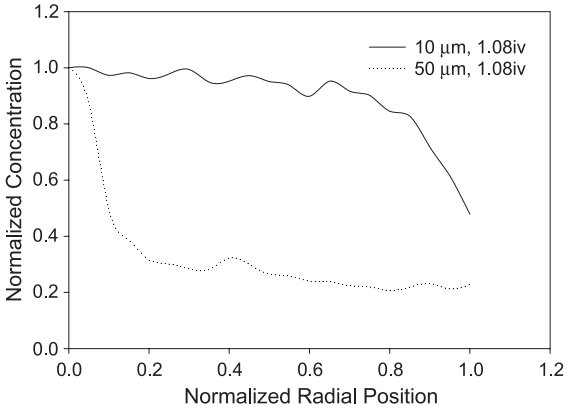

Fig. 3. Average concentration distributions obtained from analysis of 50- and 10-µm 1.08iv PLG particles.

66.0% for 10-µm microspheres and between 50.7% and 63.8% for 50-µm microspheres (Table 1).

4.2. Intraparticle drug distributions

It has been demonstrated that the microsphere size can influence the drug distribution within the microsphere [8]. The model explicitly accounts for the initial nonuniform drug distribution within the microsphere (Eq. (4)). Confocal micrographs of 50- and 10-µm microspheres (Fig. 2) were converted to intensity matrices using ImageJ software and a radial distribution was calculated by averaging the intensity of all pixels at a given radial distance from the center of the microsphere. All images were taken with the focal plane at the center of the spheres and it is assumed

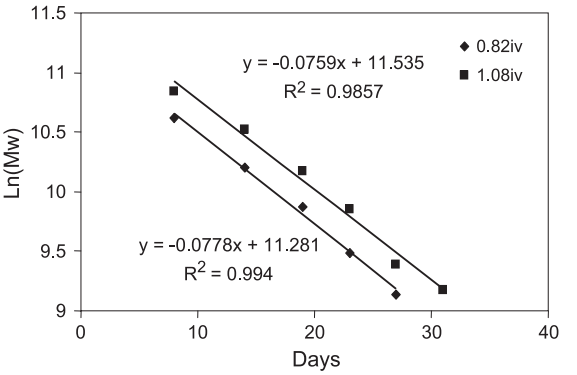

Fig. 5. Data in Fig. 4 plotted as Ln(M_w) versus time.

that this distribution is adequate to describe the fluorescence distribution in the entire microsphere. Although the proportionality constant is not known, it is assumed that the drug concentration is linearly related to the fluorescence.

The initial radial drug distributions used to solve the equations were obtained by averaging the radial drug distribution of at least 5 microspheres for each case, i.e., for each of the 10- and 50-µm microspheres of different polymer molecular weights. The 10-µm spheres showed a more uniform distribution of drug concentration that drops off towards the surface, while the 50-µm spheres showed higher concentrations towards the center of the microspheres as previously reported (Fig. 3) [8]. The concentration distributions for the microspheres of all molecular weights were similar to those shown in Fig. 3.

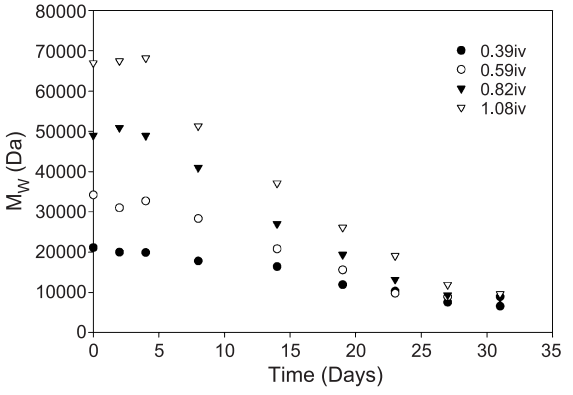

Fig. 4. In vitro degradation of 50-µm, 15% piroxicam-loaded microspheres.

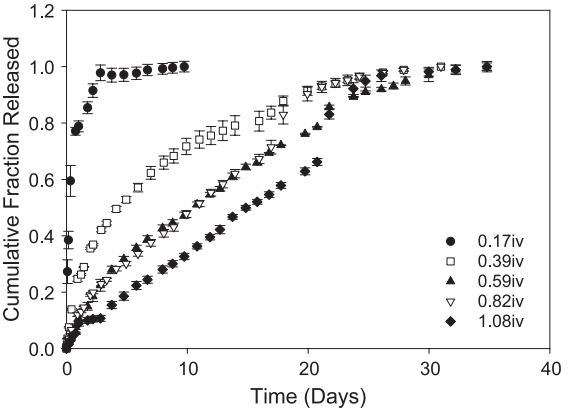

Fig. 6. Release of piroxicam from 10-µm PLG microspheres of different initial polymer molecular weights.

4.3. Microsphere degradation study

As described earlier, the diffusivity of the drug depends on the polymer molecular weight, which in turn depends on time. To measure the dependence of polymer molecular weight with time, a degradation study of the 50 μm piroxicam-loaded particles was performed as described earlier (Section 2.5). It is interesting to note that the molecular weight remains approximately constant for about 4 days before an apparently first-order degradation begins (Fig. 4). While this may seem unusual, similar results have been reported earlier [20]. The observed lag time might possibly be due to the washing out of low molecular weight fractions of the PLG. When the data, excluding the first two data points, are plotted as Ln(M_w) versus time (Fig. 5), the slope of the straight line fit gives the rate constant for the polymer degradation kinetics. The slopes vary slightly, but are still within the range of reported values [16]. For

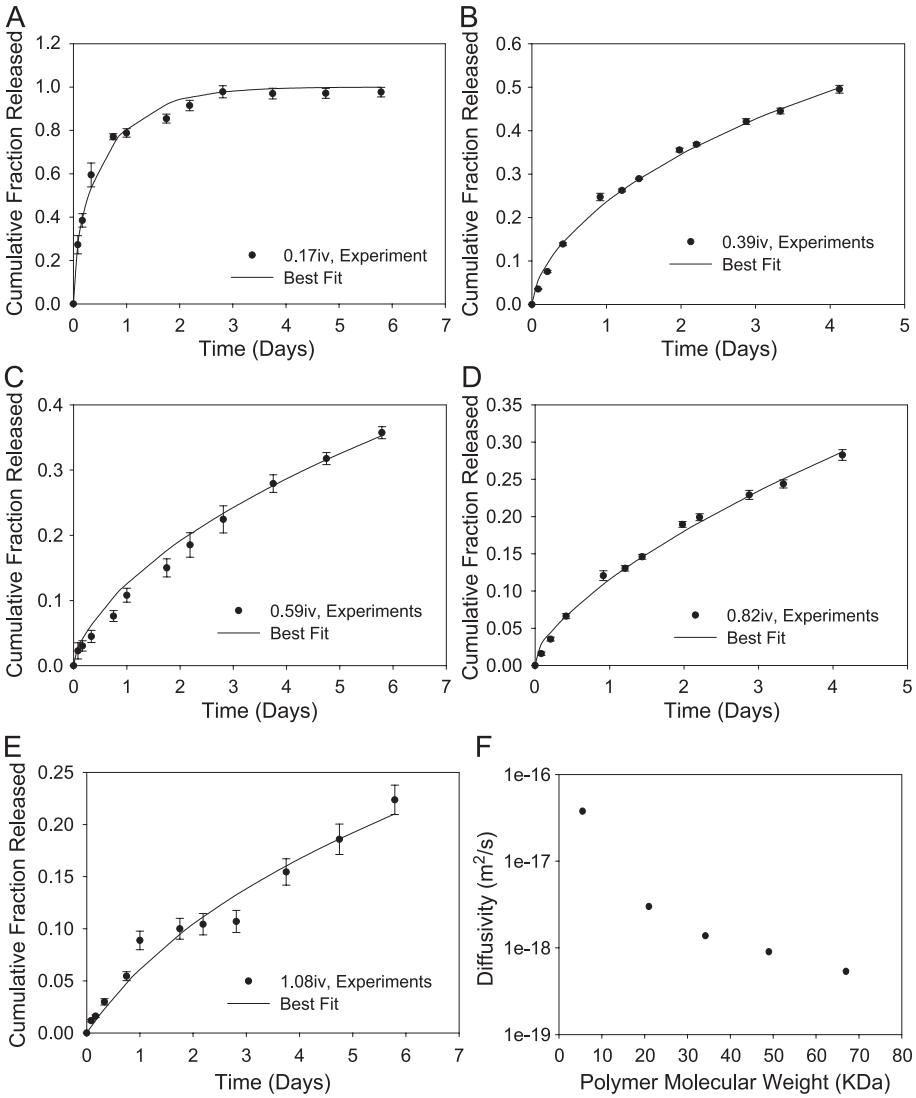

Fig. 7. In vitro piroxicam release from 10-μm 0.17iv (A), 0.39iv (B), 0.59iv (C), 0.82iv (D), 1.08iv (E) PLG microspheres and their respective best-fit diffusion release profiles and the dependence of the diffusivity of piroxicam on PLG molecular weight (F).

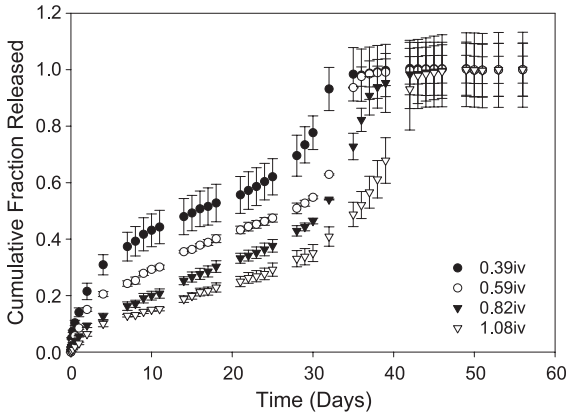

Fig. 8. Release of piroxicam from 50-μm PLG microspheres of different initial polymer molecular weights.

all calculations, the value of 0.0759 day^{-1} was used, as it is obtained from the degradation experiment that spans the entire range of polymer molecular weights.

4.4. Estimation of diffusivities

To obtain the dependence of the diffusivity on the polymer molecular weight, in vitro release of piroxicam from 10-μm PLG microspheres of different initial polymer molecular weights was measured (Fig. 6). The release data were normalized to the total amount released at the end of the study, which was within 20% of the loading reported in Table 1. The diffusion equation (Eq. (1)) was then solved with a constant diffusivity and the initial drug distribution (Section 4.2) until the best fit of the initial release data (≈6 days) was obtained. The use of a constant diffusivity is justified as it has been shown previously that the polymer molecular weight in 10-μm spheres remains fairly constant for up to 2 weeks [8]. Furthermore, it can be seen that using a constant diffusivity describes the in vitro release from the 10-μm spheres well (Fig. 7). The variation of the

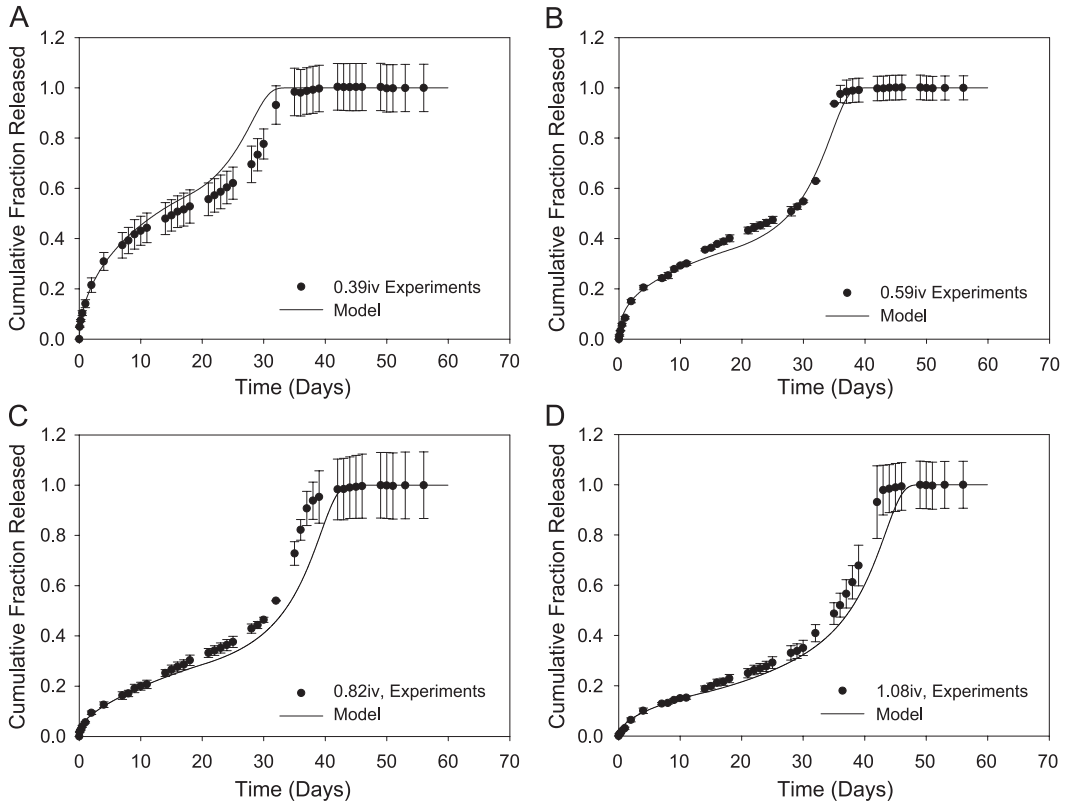

Fig. 9. Model comparison to experimental data of piroxicam release from 50-μm 0.39iv (A), 0.59iv (B), 0.82iv (C) and 1.08iv (D) PLG microspheres with initial diffusivities 15, 6, 4.5, and 1.5×10^{-18} m^2/s, respectively.

diffusivity of piroxicam with PLG molecular weight is shown in Fig. 7F. To include this dependence in the computations, an empirical cubic polynomial fit was used to relate Ln(D) to Ln(M_w), as shown in Eq. (5). In this equation, x is the natural logarithm of the polymer molecular weight (M_w). This dependence was chosen as it fits the data extremely well (R^2=1).

$$\mathrm{Ln}(D) = -0.347x^3 + 10.394x^2 - 104.950x + 316.950 \quad (5)$$

4.5. In vitro release of piroxicam from 50-μm PLG microspheres

In vitro release of piroxicam from 50-μm PLG microspheres of different initial molecular weights was performed (Fig. 8). The release profiles shown in this figure are normalized to the total amount released at the end of the release, which was within 10% of the loading shown in Table 1, to facilitate fitting the model. The release profile from 50-μm spheres was sigmoidal for all cases. In contrast, none of the 10-μm spheres show a sigmoidal release profile (Fig. 6).

4.6. Model results

The diffusion equation (Eq. (1)) was solved with a time-dependent diffusivity and one fit parameter (D_0, the initial diffusivity) to predict the release from 50-μm microspheres. Since the molecular weight does not change for the first 4 days, the molecular weight loss is modeled as follows:

$$M_w = M_{w0} \qquad t<t_{lag} \quad (6)$$

$$M_w = M_{w0} e^{k_d(t-t_{lag})} \qquad t>t_{lag}. \quad (7)$$

In the above equation, M_{w0} and M_w are the polymer molecular weights at times t=0 and at time t, respectively. The degradation rate constant, k_d, was chosen to be 0.0759 day^{-1}, and t_{lag} was set at 4 days as described above (Section 4.3).

Fig. 9 shows the release profiles generated by the model compared to the experimentally obtained release data. The change of molecular weight with time and the dependence of the diffusivity on the molecular weight were both independently determined. The initial diffusivity (D_0) was the only parameter used to fit the model to experimental data.

In the absence of degradation data, the degradation rate constant (k_d) can be used as a fit parameter. As seen in Fig. 10A, a slightly modified value of k_d=0.0740 day^{-1} gives a good fit. The lag time is set to zero days in this case because without degradation data, it would be normal to assume so. Even if the observed lag time of 4 days were to be included, k_d=0.0830 day^{-1} gives a good fit, as seen in Fig. 10B. The initial diffusivity used in these cases is the same as in Fig. 9.

The small differences between the model prediction and experimental data might potentially be due to the following reasons. As mentioned earlier, the degradation rate constant used in the model is the same for all cases despite the GPC data indicating

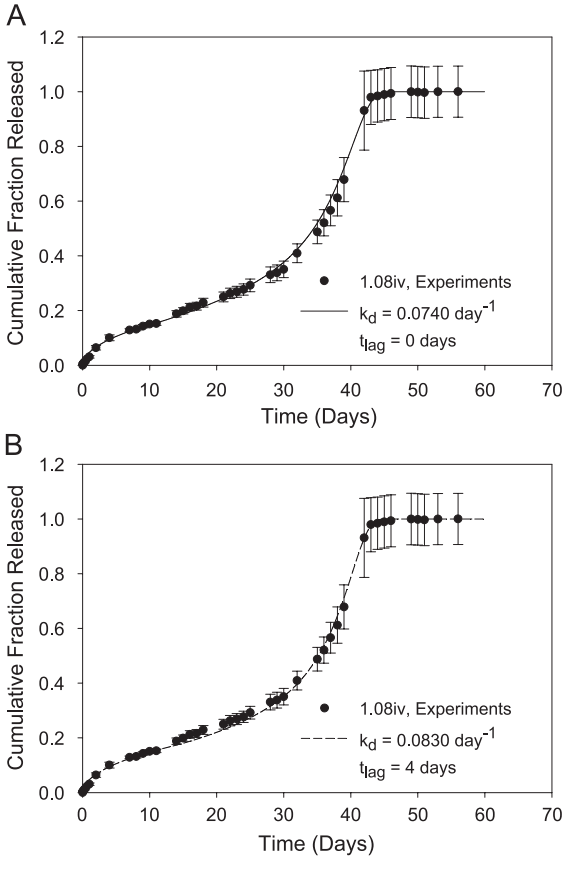

Fig. 10. Model comparison to in vitro piroxicam release data from 1.08iv 50-μm microspheres D_0=1.5×10^{-18} m^2/s and (A) k_d=0.0740 day^{-1}, t_{lag}=0 days and (B) k_d=0.0830 day^{-1}, t_{lag}=4 days.

slight variations with different initial molecular weights. Furthermore, it is possible that piroxicam release can occur by diffusion through pores formed as a result of polymer degradation and erosion which may result in significantly higher effective diffusivities than those predicted by the model. However, since the model agrees reasonably well with experimental data, it can be concluded that drug release through pores is probably not significant in this case and piroxicam release occurs primarily by diffusion through the dense polymer matrix.

5. Conclusions

A model is reported that fits drug release from uniform PLG microspheres that accounts for the dependence of the drug diffusivity on polymer molecular weight and the variation of polymer molecular weight with time due to degradation. It also includes the nonuniform initial drug distribution. Each of these effects were independently determined, thus minimizing the number of fit parameters used in the model. The model results are in good agreement with experimental results, despite using only one fit parameter. Hence, the model may be a useful tool to predict small-molecule drug release from microspheres.

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