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Precise control of PLG microsphere size provides enhanced control of drug release rate

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Abstract

An important limitation in the development of biodegradable polymer microspheres for controlled-release drug delivery applications has been the difficulty of specifically designing systems exhibiting precisely controlled release rates. Because microparticle size is a primary determinant of drug release, we developed a methodology for controlling release kinetics employing monodisperse poly(D,L-lactide-co-glycolide) (PLG) microspheres. We fabricated 20-, 40- and 65-μm diameter rhodamine-containing microspheres and 10-, 50- and 100-μm diameter piroxicam-containing microspheres at various loadings from 1 to 20%. In vitro release kinetics were determined for each preparation. Drug release depended strongly on microsphere diameter with 10- and 20-μm particles exhibiting concave-downward release profiles while larger particles resulted in sigmoidal release profiles. Overall, the rate of release decreased and the duration increased with increasing microsphere size. Release kinetics from mixtures of uniform microspheres corresponded to mass-weighted averages of the individual microsphere release kinetics. Appropriate mixtures of uniform microspheres were identified that provided constant (zero-order) release of rhodamine and piroxicam for 8 and 14 days, respectively. Mixing of uniform microspheres, as well as control of microsphere size distribution, may provide an improved methodology to tailor small-molecule drug-release kinetics from simple, biodegradable-polymer microparticles.

Keywords: Controlled release; Zero-order release; Uniform microspheres; Poly(lactide-co-glycolide); Piroxicam

1. Introduction

In comparison to conventional dosage forms, biodegradable polymeric matrices provide improved delivery methods for small molecules, peptides, proteins and nucleic acids. By encapsulating the drug in a polymer matrix from which it is released at a relatively slow rate over a prolonged time, controlled release affords less frequent administration, thereby increasing patient compliance and reducing discomfort; protection of the therapeutic compound within the body; potentially optimized therapeutic responses and prolonged efficacy; and avoidance of peak-related side-effects by maintaining more-constant blood levels of the drug. Further, because such devices can be administered by injection, one can

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also achieve localized drug delivery and high local concentrations.

The large and growing variety of pharmaceuticals on the market and in development require versatile delivery systems that can adapt to the needs of particular applications [1], especially the capacity to generate the required delivery rates and, perhaps, variation of delivery rate over time. For example, many therapeutics require a constant release rate for varying durations from several days to several weeks [2–6]. Such 'zero-order' release is a long-sought goal of controlled-release drug delivery, but has been difficult to achieve for many pharmaceuticals. In contrast, variable drug release rates can be beneficial for many important indications [7]. Intermittent high doses of antibiotics may alleviate evolution of resistance in bacteria, and discontinuous administration of vaccines often enhances the immune response [2,8,9].

Microparticle drug delivery systems may provide the needed versatility. Drug release rates can be controlled through the choice of polymer chemistry [10,11] (e.g. polymer composition, co-monomer ratios, molecular weight, etc.) or variation of the microparticle formulation parameters, and thus the physical characteristics of the resulting particles [12,13]. Nevertheless, the ability to tailor drug release kinetics is limited. For typical small-molecule therapeutics, as well as some proteins [11,14–17], drug release often exhibits an initial 'burst' phase during which a significant fraction (typically 5–50%) of the encapsulated compound is released in a short time ($<$24 h). The burst is usually undesirable because the drug that is released in this phase is not available for prolonged release, and more importantly for potent therapeutics or drugs with a narrow therapeutic window, this initial bolus may result in toxicity or other side-effects. The burst may be followed by a lag phase exhibiting negligible release and, more typically, a phase in which the release rate decreases with time due to a decreasing driving force as drug is depleted from the matrix. Various strategies for reducing or eliminating the initial burst have been studied including chemistry (block copolymers with hydrophilic regions) [10], variation of microsphere formation parameters [12,13], coating of microspheres (microencapsulated microspheres) [18] and conjugation of drug to the polymer matrix [19].

Microsphere size is a primary determinant of drug release rates. Larger spheres generally release encapsulated compounds more slowly and over longer time periods, other properties (polymer molecular weight, initial porosity, drug distribution within the sphere, etc.) being equal. Thus, controlling sphere size provides an opportunity for control of release kinetics. Numerous studies have been conducted to determine the effects of sphere size on drug release [3,10,11,13,20,21]. However, due to a limited ability to control microsphere size, this approach to modulating release rates has been relatively unexplored.

We have devised a methodology for precisely controlling microsphere size and size distribution [20]. Our spraying technology is capable of generating uniform PLG microspheres ranging in size from about 1 to $>$500 μm. For example, we recently reported fabrication of microspheres with diameters of ~5–80 μm, wherein 95% of the particles had a diameter within 1.0–1.5 μm of the average [20]. Furthermore, the methodology allows fabrication of novel, continuously varying size distributions of any desired shape. We hypothesized that the ability to control particle size afforded by our system would lead to enhanced control of drug release kinetics. Here we report release kinetics for a model compound, rhodamine B, and the non-steroidal anti-inflammatory drug (NSAID) piroxicam from uniform PLG microspheres. While piroxicam is similar in molecular weight to rhodamine, these two compounds were chosen to represent water-soluble (rhodamine, 7.8 mg/ml) and -insoluble (piroxicam, 53.3 μg/ml at pH~7) drugs [22]. We demonstrate that the release kinetics of both compounds are indeed variable depending on the microsphere size, as expected. Further, we show that mixtures of uniform microspheres exhibit release kinetics that are weighted averages of the individual microsphere release kinetics. Based on this finding, we chose appropriate mixtures to generate zero-order release, without an initial burst phase, for both rhodamine and piroxicam.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (50:50 lactic acid:glycolic acid; i.v.=0.20–24 dl/g corresponding

to M_w 10,000–15,000) was obtained from Birmingham Polymers. Poly(vinyl alcohol) (PVA; 88% hydrolyzed) was obtained from Polysciences. Rhodamine B chloride was obtained from Sigma. Piroxicam free base was a gift from Dongwha Pharmaceuticals (Seoul, Korea). HPLC grade dichloromethane (DCM), dimethylsulfoxide and sodium hydroxide were purchased from Fisher Scientific.

2.2. Preparation of microspheres

Microspheres were prepared as described previously [20]. Briefly, PLG solutions (5% (w/v) in DCM) containing rhodamine B or piroxicam at the various concentrations indicated were pumped through a small glass nozzle at various flow rates, while an ultrasonic transducer (Branson Ultrasonics) controlled by a frequency generator (Hewlett Packard model 3325A) disrupted the stream into uniform droplets. A carrier stream (1% (w/v) PVA in distilled water) flowed around the emerging PLG stream. The streams flowed into a beaker containing ~500 ml of 1% PVA, and the particles were stirred at room temperature for 3 h, filtered, and rinsed with distilled water. The microspheres were lyophilized (Labconco benchtop model) for a minimum of 48 h and were stored at −20 °C under desiccant.

2.3. Determination of drug loading

The initial loading of rhodamine B was determined as follows. A known mass (~2–5 mg) of microspheres was dissolved in 50 μl dimethylsulfoxide. PBS (500 μl) was added and precipitated polymer was removed by centrifugation at 12,000 rpm for 10 min. Rhodamine B concentration in the supernatant was determined by measuring the absorbance at 550 nm in a multi-well plate spectrophotometer (Molecular Devices Spectra Max 340PC).

To determine piroxicam loading, a known mass (~5 mg) of microspheres containing piroxicam was dissolved in 1 ml of 0.25 M sodium hydroxide at room temperature for 5 min. Blank (piroxicam free) microspheres of the same size were treated identically. Piroxicam concentration in the resulting solution was determined by measuring the absorbance at 276 nm (Varian Cary 50) in a quartz cuvette and subtracting absorbance values for the blank microspheres.

2.4. In vitro drug release

Rhodamine release was determined by resuspending a known mass of microspheres encapsulating rhodamine B in 2 ml of phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Tween. The suspensions were continuously agitated by inversion (at ~10 rpm) in a 37 °C incubator. At regular intervals the samples were centrifuged, the supernatant was removed, and the spheres were resuspended in fresh PBS. Concentration of rhodamine B in the supernatant was determined using the spectrophotometer as described above. The amount of rhodamine in each sample was summed with the amounts at each previous time point, and the total divided by the amount of rhodamine in the microspheres (experimental loading×mass of microspheres), to arrive at the 'cumulative percent released'.

Piroxicam release was determined by resuspending ~5 mg of microspheres in 1.3 ml of PBS containing 0.5% Tween. Conditions during drug release were the same as described above for rhodamine. After centrifugation, the concentration of piroxicam in the supernatant was determined by measuring the absorbance at 276 nm as described. Average absorbance of the supernatant from tubes containing blank microspheres treated identically was subtracted from all measurements.

2.5. Scanning electron microscopy

Microsphere surface structure and porosity were investigated by scanning electron microscopy (Hitachi S-4700). Samples were prepared by placing a droplet of an aqueous microsphere suspension onto a silicon stub. The samples were dried overnight and were sputter coated with gold prior to imaging at 2–10 eV.

2.6. Particle size distribution

A Coulter Multisizer 3 (Beckman Coulter) equipped with a 100- or 280-μm aperture was used to determine the size distribution of the various sphere preparations. The lyophilized particles were resuspended in Isoton electrolyte and a type I-A

dispersant was used to prevent microsphere aggregation. A minimum of 5000 microspheres was analyzed for each sample.

3. Results

3.1. Microsphere fabrication and characterization

Uniform PLG microspheres were fabricated employing the spraying apparatus described previously [20]. The model drug compounds, rhodamine B and piroxicam (free base form), were encapsulated by co-dissolving the drug with the PLG in DCM. In order to examine the effect of microsphere diameter on drug release kinetics, we fabricated rhodamine-containing particles of 20, 40 and 65 μm, at theoretical loadings of 1, 3, and 5%, and piroxicam-containing particles of 10, 50 and 100 μm with 5, 10, 15, and 20% loading. Rhodamine and piroxicam loading and encapsulation efficiency (e.e.) are reported in Tables 1 and 2, respectively. (While drug loading is less than theoretical, e.e.=10–60%, for simplicity we will refer to the various samples by the theoretical loading.)

The microspheres were very uniform, typically having >90% of the particles within 2-μm of the average diameter (Fig. 1). Microsphere homogeneity is also evident in scanning electron micrographs of the various microsphere preparations (Fig. 2). The

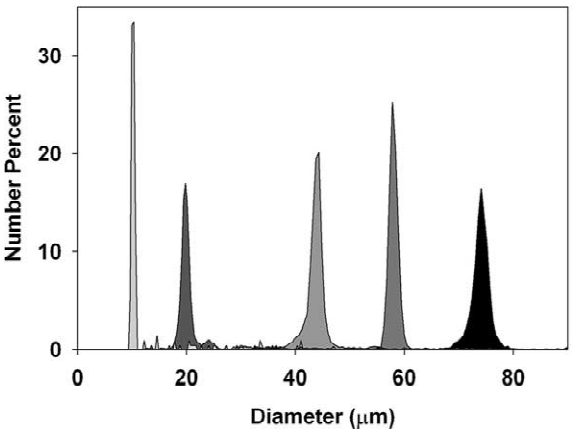

Fig. 1. Typical size distributions of uniform microspheres loaded with rhodamine (20-, 45- and 75-μm diameter) and piroxicam (10- and 55-μm diameter). All distributions are normalized by total area under the curve. Thus, the peak height is also an indication of the relative particle uniformity. Each distribution is colored with a different shade of gray to distinguish where they overlap.

Table 1
Characterization of rhodamine-loaded PLG microspheres

	20 μm			40 μm			65 μm		
Theoretical loading (%)	1	3	5	1	3	5	1	3	5
Experimental loading (%)	0.63	1.80	2.50	0.37	1.05	1.75	0.61	1.29	3.00
Encapsulation efficiency (%)	63	60	50	37	35	35	61	43	60

Table 2
Characterization of piroxicam-loaded PLG microspheres

	10 μm				50 μm				100 μm			
Theoretical loading (%)	f5	10	15	20	5	10	15	20	5	10	15	20
Experimental loading (%)	3.0	4.6	5.6	5.8	1.0	1.0	1.5	3.6	1.0	3.1	3.0	5.8
Encapsulation efficiency (%)	59	46	37	29	19	10	10	18	20	31	20	29

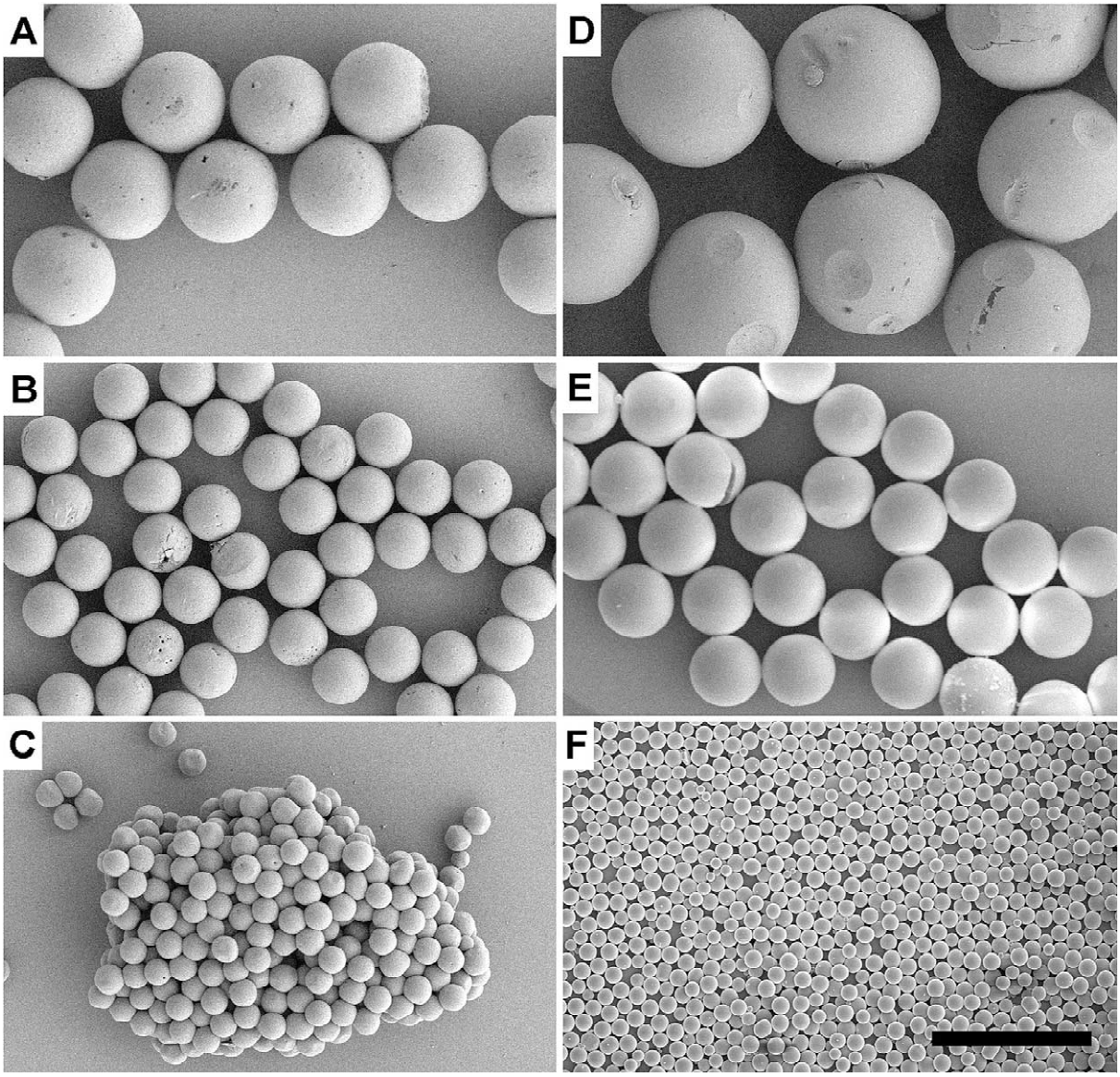

Fig. 2. Scanning electron micrographs of (A) 65- (B) 40- and (C) 20-μm rhodamine-loaded microspheres and (D) 100- (E) 50- and (F) 10-μm piroxicam-loaded microspheres. Scale bar represents 100 μm.

particles exhibit a smooth, slightly porous surface and dense polymer interior similar to microspheres produced using conventional emulsion techniques (Fig. 3) [16,17,23,24]. The average sizes determined by the Coulter counter are ~5–10% larger than the sizes obtained from SEM, but the uniformity is readily apparent. The larger size may be the result of swelling due to water uptake. We refer to the particles according to the smaller sizes obtained from SEM.

3.2. In vitro release from uniform microspheres

To examine the effect of microsphere size and size

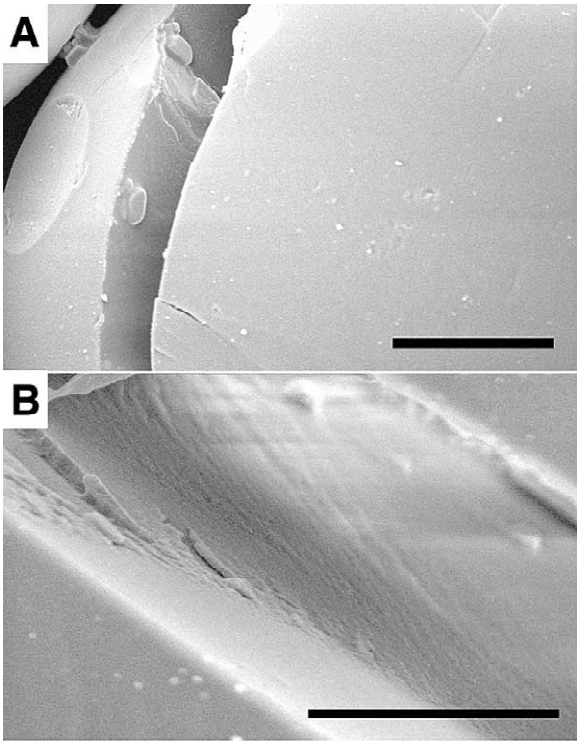

Fig. 3. Scanning electron micrographs of a fractured PLG microsphere depicting the dense polymer matrix of drug-encapsulating microspheres. Scale bars represents 10 μm.

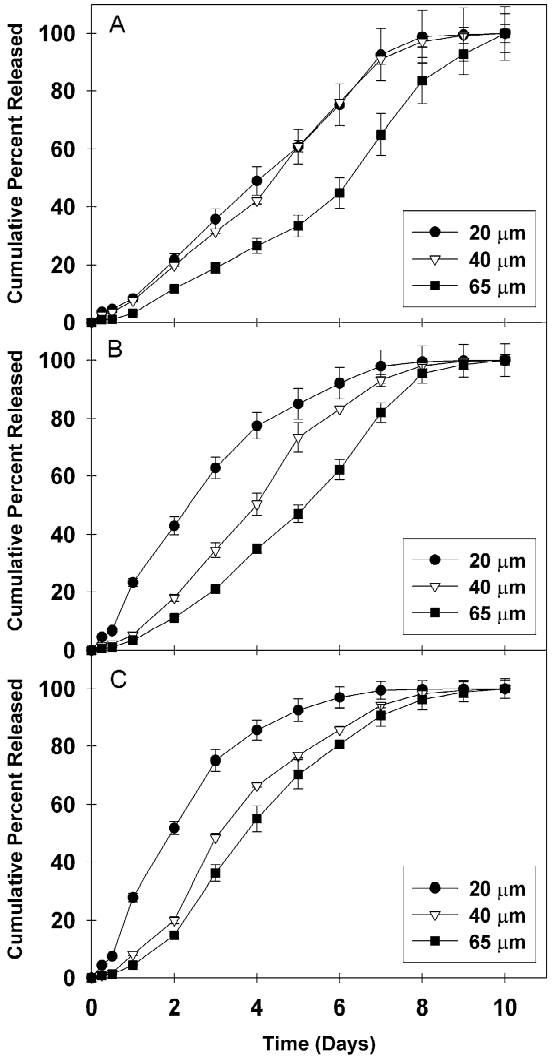

Fig. 4. Effect of microsphere size and drug loading on rhodamine release rates: (A) 1%, (B) 3% and (C) 5% theoretical loading.

uniformity on drug release kinetics, we measured release profiles for both drugs from spheres of all loadings and sizes. Rhodamine release profiles are shown in Fig. 4. As expected, 20-μm microspheres exhibited a faster initial release than 65-μm microspheres, likely due to the increased surface-to-volume ratio of the smaller particles. Further, as drug loading increased, the initial rate of drug release increased. An interesting concave-upward (i.e. sigmoidal) profile was observed with the 65-μm particles and to a lesser extent with the 45-μm particles, wherein drug release was initially slow, then progressed to a more rapid release phase before leveling off [25,26].

Piroxicam release profiles show similar trends (Fig. 5). Samples of 10-, 50- and 100-μm microspheres were studied. The microspheres span a broader size range than the rhodamine-loaded particles, resulting in a more pronounced difference in drug release profiles. The smallest microspheres (10-μm diameter) exhibited a rapid initial rate of release, with 40–60% of encapsulated piroxicam released within the first 24 h. Initial release rates decreased with increasing microsphere diameter for all drug loadings examined. Further, the initial release rate decreased with increasing drug loading. Interestingly, the 50- and 100-μm particles exhibited sigmoidal release profiles similar to rhodamine release from 65-μm microspheres.

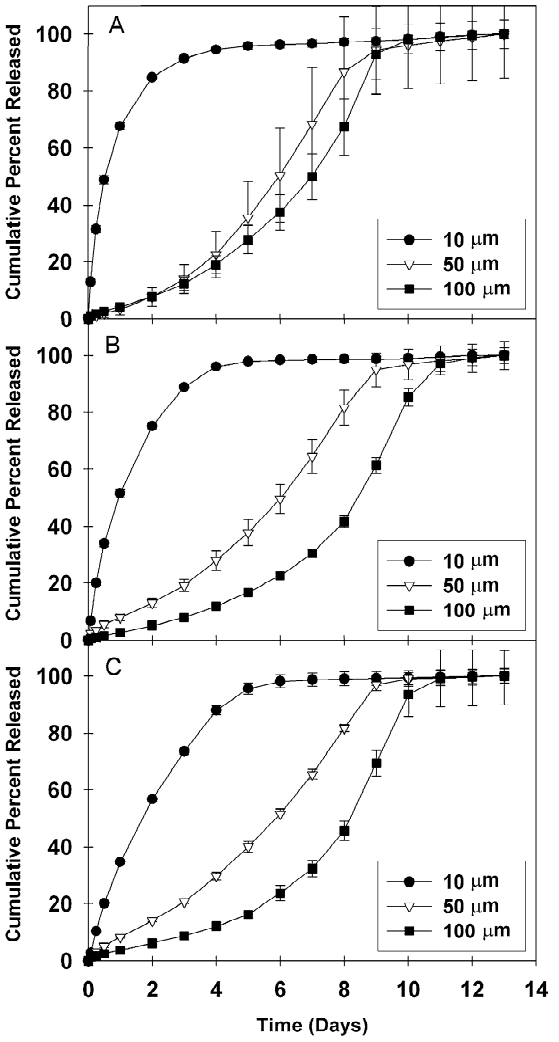

Fig. 5. Effect of microsphere size and drug loading on piroxicam release rates: (A) 5%, (B) 10% and (C) 15% theoretical loading.

3.3. In vitro release from mixtures of uniform microspheres

Based on the different shapes of the uniform microsphere release profiles, and given the reproducibility of our methodology for uniform microsphere fabrication, we reasoned that it may be possible to modulate release kinetics in a desired fashion by mixing appropriate proportions of two or more uniform microsphere preparations [3,10]. To test this hypothesis, we mixed known ratios (1:3, 1:1 and 3:1, w/w) of 20-μm/5% and 65-μm/5% rhodamine-containing microspheres. The release profiles for the microsphere mixtures were intermediate between the two individual microsphere release profiles (Fig. 6A). Further, the mixture release profiles corresponded to a mass-weighted linear combination of the individual release profiles (see dotted lines in Fig. 6A).

Given the agreement between predicted release profiles (i.e. linear combinations) and the experimental data, various linear combinations of individual-microsphere release profiles, based on the nine preparations of varying microsphere size and rhodamine loading, were examined to identify a combination of uniform microspheres that might

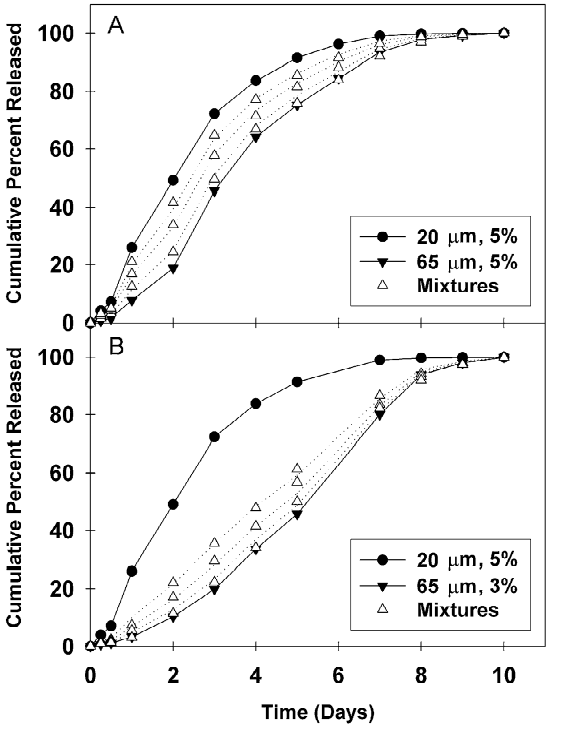

Fig. 6. (A) Rhodamine release from 20-μm/5% microspheres, 65-μm/5% microspheres and 3:1, 1:1 and 1:3 (w/w) mixtures. (B) Rhodamine release from 20-μm/5% microspheres, 65-μm/3% microspheres and 1:4, 1:9 and 1:24 (w/w) mixtures. Filled symbols: experimental data points for individual microspheres. Open symbols: experimental data points for mixtures. Dotted line: weighted average of individual microsphere experimental release data (predicted release). Error bars, typical of those shown in Fig. 4, were removed for clarity.

result in zero-order release kinetics. Based on the predicted release profiles, we chose to mix 20-μm/5% and 65-μm/3% microspheres in various ratios and performed in vitro release studies for comparison to predicted release profiles (Fig. 6B). Again, the experimental release data coincided precisely with the predicted release profile, and the 1:4 (w/w) mixture provided constant release for 8 days.

To investigate the generality of this approach for modulating drug release kinetics, we performed a similar set of experiments to generate zero-order release of the clinically relevant NSAID, piroxicam. Multiple linear combinations of 10-, 50- and 100-μm piroxicam-containing microspheres at various drug loadings were examined computationally to identify a combination resulting in linear drug release. Two possible formulations were found. The first formulation combined 10-μm/15% and 50-μm/15% microspheres in ratios of 3:1, 1:1, and 1:3 (w/w). This formulation resulted in slightly concave downward release profiles for the 3:1 and 1:1 ratios and a linear drug release profile for the 1:3 (w/w) mixture (Fig. 7A). The second formulation comprised 10-μm/20% and 50-μm/10% microspheres in three ratios, 1:6.1, 1:11.5, and 1:39 (w/w). The 1:6.1 (w/w) formulation produced near linear release over the course of the experiment (Fig. 7B).

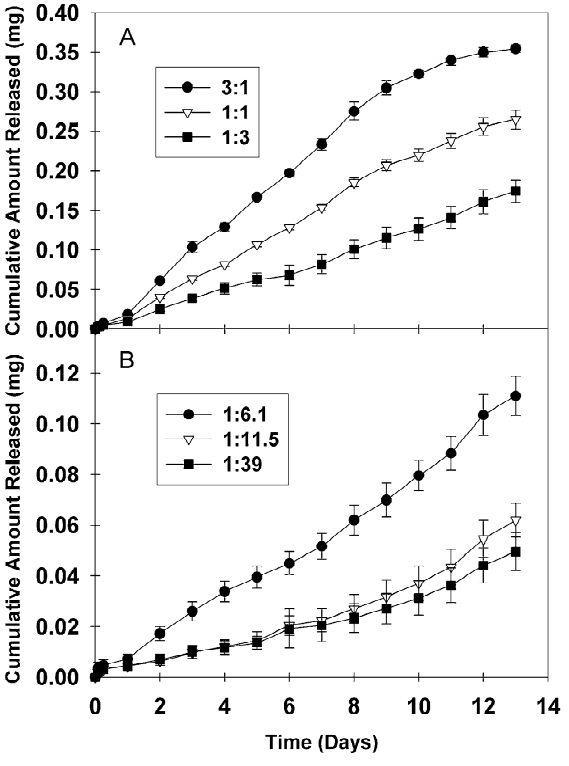

Fig. 7. (A) Piroxicam release from 3:1, 1:1 and 1:3 (w/w) mixtures of 10-μm/15% microspheres and 50-μm/15% microspheres. (B) Piroxicam release from 1:6.1, 1:11.5 and 1:39 (w/w) mixtures of 10-μm/20% microspheres and 50-μm/10% microspheres.

4. Discussion

Long-term zero-order release of small-molecule therapeutics from biodegradable microspheres has been difficult to achieve. Release of model compounds similar to rhodamine B is often rapid and diffusion controlled [27,28]. Similarly, release of NSAIDs, especially piroxicam, encapsulated in polymeric particles typically occurs within 24 h and is dominated by a large initial rate of release (or 'burst'), offering little advantage over conventional oral dosage forms [16,17,23,24,29,30]. In contrast, our results show that simple molecules can be released in a controlled manner over significant durations of time.

The initial release rates of both rhodamine and piroxicam decreased with increasing sphere diameter. This is expected due to the decrease in surface area/volume ratio with increasing size. Furthermore, we observed that the release rates (as percent of total drug released vs. time) from rhodamine-containing microspheres increased slightly with increasing loading for all sphere sizes. For purely diffusion-controlled release, no dependence on drug loading is expected. Other researchers have reported faster release with increased loading, especially for cases in which the drug is phase-separated from the polymer matrix and release can occur through aqueous meso- and macropores created by the drug [31,32]. Such a mechanism may explain the effect of rhodamine loading on release rates. In contrast, we observed that the release rate of piroxicam decreased with increasing loading. Possible explanations for this surprising result may be that the piroxicam, with pK_a of 5.07 and 2.33, is buffering acidification of the intrapolymer environment caused by accumulation of

polymer degradation products, or that the hydrophobic drug is slowing water uptake. Another possibility is that, due to the low solubility of piroxicam, 'sink' conditions were not achieved. The highest piroxicam concentrations measured were ~100 μg/ml while the solubility of piroxicam in the release media at 37 °C is 1.3 mg/ml (the relatively high solubility is due to the presence of Tween 20 at a concentration, 0.5%, above its critical micelle concentration). Because the maximum piroxicam concentration was less than one tenth of its solubility, drug concentration in the release media likely had little effect on the release kinetics.

Small and large particles resulted in qualitatively different release profiles. The smallest microspheres (10- and 20-μm diameter, encapsulating piroxicam and rhodamine, respectively) exhibited concave downward release profiles typical of diffusion-controlled release from PLG microspheres. However, the larger microspheres exhibited a sigmoidal release profile in which the release rate initially increased with time. Similar release profiles have been observed previously. For example, Sansdrap and Moes reported a sigmoidal release profile for a low solubility (10 μg/ml in water) drug, nifedipine [21] using 80-μm (±30 μm) PLG microspheres, while Guiziou et al. found sigmoidal release from poly(lactic acid) (PLA) microspheres loaded with the NSAID indomethacin [24]. Further, Ravivarapu et al. reported a similar release profile for leuprolide acetate-loaded PLG microspheres [33].

The sigmoidal release profiles may result from several mechanisms. Guiziou et al. attributed the upward bending release profile to the microspheres having a more dense polymer matrix with increased stability as compared to microspheres fabricated using lower molecular weight PLA which released in a more diffusion-controlled manner [24]. Alternatively, water uptake, resulting in increased solubilization of the drug and swelling of the polymer matrix [21], may cause an increasing release rate. However, we propose that the sigmoidal shape results from polymer degradation. Erosion was suggested as the cause of sigmoidal leuprolide release profiles described above [33]. Polymer hydrolysis, with or without the subsequent mass loss, is expected to cause both increasing diffusivity of drug through the polymer matrix and an increase in the size of aqueous pores in the polymer (also potentially leading to an increase in the effective diffusivity). Additional studies directly addressing this hypothesis are in progress.

Constant release is highly desirable for many drug delivery applications. Because there is a transition from the concave downward to sigmoidal release profiles as sphere size increases, it appears that nearly linear release may be achieved at a certain size. For example, between 10 and 50 μm, a microsphere size may exist that would provide zero-order piroxicam release over a 4- to 8-day duration (cf. Figs. 4 and 5). Others report linear or near-linear release profiles achieved with microspheres of similar size, ~30–50 μm in diameter [4,5,10]. For example, Woo et al. formulated a leuprolide delivery system using PLA microspheres with an average diameter of 51.7 μm achieving near-linear peptide release for 135 days following a 15-day period of 'diffusion-controlled release' [5]. Our hypothesis suggests that the early phase of release results from the portion of the microspheres in this formulation under ~35 μm, which would be expected to release drug more rapidly. Further, Bezemer et al. used a poly(ethylene glycol)-poly(butylene terephthalate) (PEG-PBT) block copolymer to test the effects of microsphere size on drug release [10]. They also discovered that decreasing the average microsphere size from 108 to 29 μm causes the release kinetics to change gradually from zero-order release to release controlled by Fickian diffusion. The microspheres used for these experiments were not uniform, but the trends are indicative of the trends we observed for uniform PLG microspheres.

Other researchers have suggested that drug delivery rates may be controlled by mixing microspheres of varying sizes or characteristics. For example, Ravivarapu et al. mixed microspheres comprising 8.6- or 28.3-kDa PLG encapsulating leuprolide acetate [33]. The low-molecular-weight polymer resulted in porous, quickly releasing microspheres while the high-molecular-weight formulation resulted in dense microspheres and produced a sigmoidal release profile. By mixing microspheres comprising the two polymers, release rates could be tailored, and the resulting profiles were linear combinations of those resulting from individual microspheres. Bezemer et al. produced linear lysozyme release over

25 days from PEG-PBT microspheres having a bimodal size distribution dominated by 50- and 110-μm particles (in essence a combination of two sizes) [10]. Finally, Narayani and Panduranga Rao combined gelatin microspheres of various size ranges producing zero-order release of methotrexate [3].

Because release kinetics from uniform spheres are very predictable and reproducible, our ability to fabricate uniform microspheres enhances such a technique. We found that upon mixing uniform microsphere preparations, the resulting release profile is a mass-weighted average of the release profiles of the individual microspheres. This demonstrates that the microspheres release their payload independently; there is no interaction between the particles. In these experiments, the shapes of the rhodamine and piroxicam release profiles were such that it was possible to choose appropriate microsphere mixtures that provided zero-order release kinetics (Figs. 6 and 7). However, it may not always be possible to generate a desired release profile from mixtures of only two microsphere sizes. Depending on the desired profile and the shape of the individual release curves, one may need to mix multiple microsphere samples or to fabricate complex microsphere size distributions. Because the reported fabrication method provides a unique ability to generate predefined microsphere sizes [20], this technology may lead to enhanced control of release rates.

5. Conclusions

Microsphere size is a primary determinant of drug release kinetics. Release of model small-molecule drugs can be varied from typical diffusion-controlled profiles to slower, sigmoidal profiles as microsphere diameter is increased in the range of 10–100 μm. Drug release from mixtures of uniform microspheres corresponds to a weighted average of the release from individual uniform microspheres. As a result, it is possible to choose appropriate mixtures to generate desired release rate profiles, in particular constant release. Thus, microsphere mixtures with well-defined size distributions may provide a general methodology for controlling drug release rates.

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