ELSEVIER

Journal of Controlled Release 82 (2002) 137–147

journal of controlled release

www.elsevier.com/locate/jconrel

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

Cory Berkland[a], Martin King[a], Amanda Cox[a], Kyekyoon (Kevin) Kim[b], Daniel W. Pack[a,*]

[a]*Department of Chemical Engineering, University of Illinois, MC-712, Box C-3, 600 South Mathews Avenue, Urbana, IL 61801, USA*
[b]*Department of Electrical and Computer Engineering, University of Illinois, Urbana, IL 61801, USA*

Received 6 April 2002; accepted 17 May 2002

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

*Corresponding author. Tel.: +1-217-244-2816; fax: +1-217-333-5052.
E-mail address: dpack@uiuc.edu (D.W. Pack).

0168-3659/02/$ – see front matter
PII: S0168-3659(02)00136-0

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

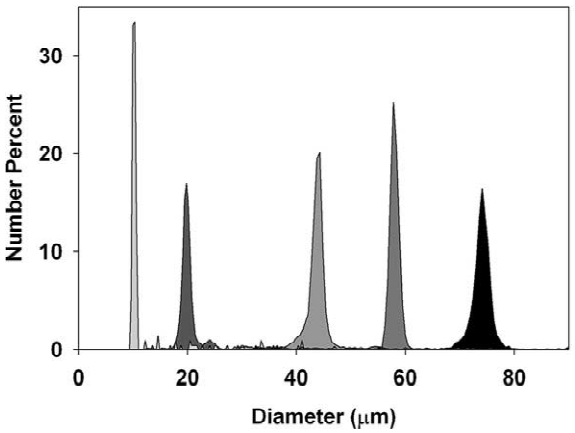

Fig. 1. Typical size distributions of uniform microspheres loaded with rhodamine (20-, 45- and 75-μm diameter) and piroxicam (10- and 55-μm diameter). All distributions are normalized by total area under the curve. Thus, the peak height is also an indication of the relative particle uniformity. Each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

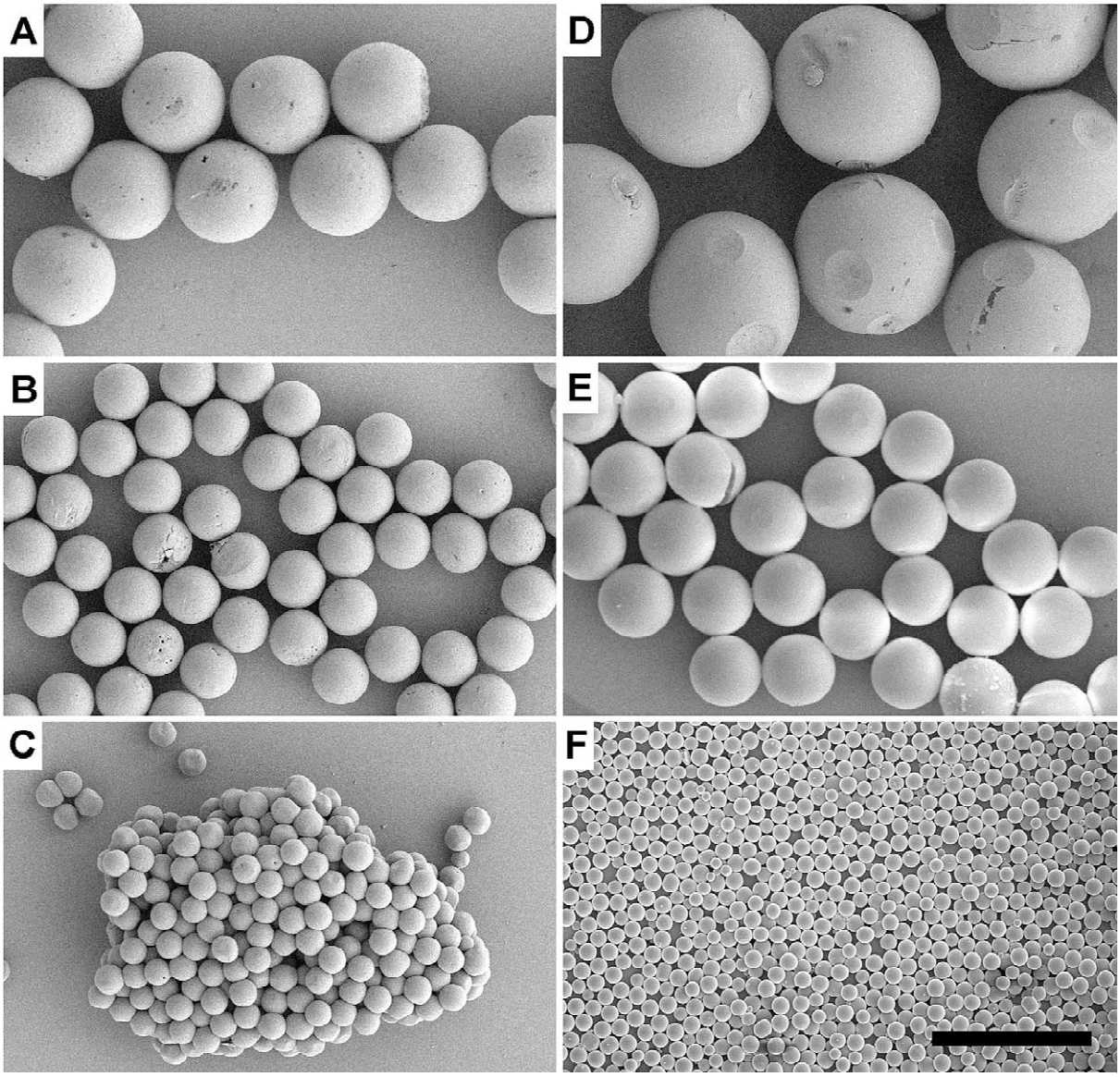

Fig. 2. Scanning electron micrographs of (A) 65- (B) 40- and (C) 20-μm rhodamine-loaded microspheres and (D) 100- (E) 50- and (F) 10-μm piroxicam-loaded microspheres. Scale bar represents 100 μm.

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

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.