Fabrication and Drug Release Properties of Poly(5-benzyloxy-trimethylene-*co*-glycolide) Microspheres

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ABSTRACT: Poly(5-benzyloxy-trimethylene carbonate-*co*-glycolide) random copolymers were synthesized through the ring-opening polymerization of 5-benzyloxy-trimethylene carbonate and glycolide (GA). The copolymers with different compositions, PBG-1 with 17% GA units and PBG-2 with 45% GA units, were obtained. Using these copolymers, microsphere drug delivery systems with submicron sizes were fabricated using an "ultrasonic assisted precipitation method." The *in-vitro* drug release from these microspheres was investigated. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 3451–3455, 2010

Key words: biodegradable; drug delivery systems; polycarbonates; polyesters

INTRODUCTION

Among the most widely investigated and commonly used biodegradable polymers, polyglycolide (PGA) possesses the fastest degradation rate. However, its high crystallinity causes unfavorable properties, such as brittleness and poor solubility, in common organic solvents.[1–3] Aliphatic polycarbonates are a class of biodegradable polymers attracting great interest because of their good biocompatibility and the less acidic degradation product.[4] However, the degradation rate of aliphatic polycarbonates is slow, limiting their applications greatly. To obtain balanced and desirable properties, modification via copolymerization provides an effective way to adjust the properties of the resultant copolymers.[5,6] Incorporating glycolide (GA) units to an aliphatic polycarbonate can obviously accelerate the degradation rate of the resulting copolymers when compared with the polycarbonate homopolymer. However, it was found that the monomeric units of common aliphatic polycarbonates, such as poly(trimethylene carbonate) and poly(2,2-dimethyltrimethylene carbonate), are not able to effectively disturb the regular structure of the polymer chains containing GA units, and thus cannot lower the crystallinity and improve the processiblity of the resultant copolymers efficiently.[5]

In our previous study, biodegradable copolymers of 5-benzyloxy-trimethylene carbonate (BTMC) and GA were synthesized.[7] Compared with the conventional aliphatic polycarbonates, BTMC can modify the properties of the resultant copolymers much more effectively because of the bulky lateral group. The copolymers we obtained can be well dissolved in common organic solvents if the GA content is not very high. The degradation rate of the copolymers can be tailored by adjusting the ratio of two kinds of monomeric units.[7]

The drug delivery systems using polymers as drug carriers are among the most important types of drug delivery systems. Drug-loaded nanoparticles and microparticles attained much importance because of their injectable property which can avoid the inconvenient surgical insertion of large implants. Moreover, they may possess passive targeting property when their sizes are in particular ranges. The drug-loaded microspheres/nanoparticles could be administrated through different routes, such as oral route, injection route, nasal route, and pulmonary route. Clinically, these controlled release drug delivery would be especially useful for drugs that require multiple daily injections and for vaccinations that require additional booster shots at timely intervals. In this work, we fabricated submicron sized microsphere drug delivery systems using an ultrasonic assisted precipitation method, and their *in-vitro* drug release behavior was investigated.

EXPERIMENTAL

Materials

GA was purchased from Beijing Conan Polymer R&D Center, purified by recrystallation from ethyl

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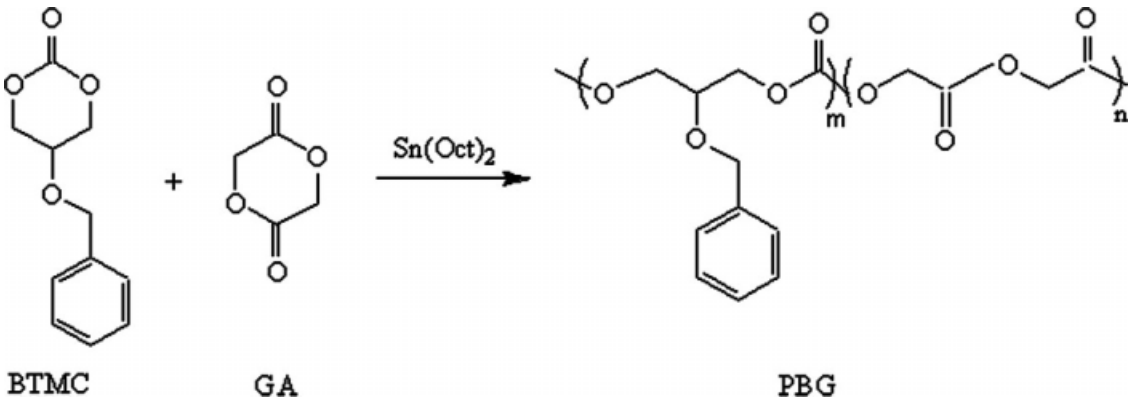

Scheme 1 Chemical structures and the synthetic route of the PBG copolymer.

acetate. BTMC was synthesized according to a published procedure.[7] Stannous octoate was purchased from Aldrich, purified by distillation under reduced pressure and then dissolved in distilled toluene before use. Prednisone acetate was purified from prednisone acetate tablets (Zhejiang Xianju Pharmaceutical, China). All the other reagents (Shanghai Chemical, China) were of analytical grade and used without further purification.

Polymer synthesis

Poly(5-benzyloxy-trimethylene carbonate-*co*-glycolide) (PBG) copolymers were synthesized according to a published procedure.[7] A mixture of BTMC and GA (total amount of 5 mmol) with a specific molar ratio was prepared by grinding the monomers together. The well-mixed monomer mixture was placed in a thoroughly dried glass flask with a magnetic stirring bar. After a certain amount of the catalyst ($Sn(Oct)_2$ in toluene) was added, the flask was evacuated, purged with N_2 three times and sealed, then immersed in an oil bath which was preheated to 150°C to carry out the ring-opening polymerization for 24 h. After the reaction, the product obtained was dissolved in dichloromethane and then precipitated in methanol. The polymer was then isolated by filtration and dried under vacuum.

Polymer characterizations

Fourier transform infrared (FTIR) spectra were obtained on a PerkinElmer-2 spectrometer.

^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Mercury VX-300 spectrometer (300 MHz), using $CDCl_3$ as solvents and TMS as an internal standard.

GPC analysis was performed on a Waters HPLC system equipped with a 2690D separation module and a 2410 refractive index detector. Chloroform was used as an eluent and the flow rate was 1.0 mL/min.

Preparation of drug-loaded microspheres

Prednisone acetate, a hydrophobic anti-inflammatory drug, was used as the model drug in this study. Drug-loaded microspheres were prepared by an "ultrasonic assisted precipitation method." Sixty milligrams of polymer and 6.0 mg drug were dissolved in 3 mL acetone, and then 3 mL distilled water was added dropwise under ultrasonification. The obtained microsphere suspension was subsequently dialyzed against 2 L water for 12 h to remove the organic solvent and free drug, and then freeze-dried.

To determine the drug loading content and entrapment efficiency, the drug-loaded polymeric microspheres were dissolved in DMF. The

TABLE I
Molecular Weights and Molecular Weight Distributions of PBG Copolymers[a]

Polymer	Feed ratio BTMC/GA (mol/mol)	BTMC unit/GA unit in copolymer (mol/mol) (determined by ^{1}H-NMR)	Before degradation		After degradation for 30 days		Yield (%)
			M_w	M_w/M_n	M_w	M_w/M_n	
PBG-1	80/20	83/17	3.73×10^4	1.76	3.18×10^4	2.08	87.3
PBG-2	50/50	55/45	3.20×10^4	1.86	2.58×10^4	3.83	85.8

[a] All polymers were obtained by bulk polymerizations at 150°C for 24 h with the catalyst $Sn(Oct)_2$ content of 0.1 wt %.

TABLE II
Preparation and Properties of PBG Microspheres

Polymer	Loading content (%)	Entrapment efficiency (%)	Particle size (nm)	Polydispersity index (PDI)	Yield (%)
PBG-1	5.0	33.5	638	0.325	67.7
PBG-2	6.4	46.6	890	0.206	58.8

prednisone acetate concentration in the solution was then determined by an ultraviolet–visible spectrophotometer (PerkinElmer Lambda Bio 40).

Characterizations of microspheres

The morphology of drug-loaded microspheres was observed by scanning electron microscope (SEM, Hitachi X650). The samples were prepared by placing a droplet of microsphere suspension onto a glass slide. The samples were then dried overnight and were sputter coated with gold prior to be visualized.

The size of the drug-loaded microspheres was measured by a Zetasizer 3000 (Malvern Instruments).

In-vitro drug release study

Five milligrams of drug-loaded microspheres were suspended in 2 mL phosphate buffer (0.1*M*, pH = 7.4) and then transferred into a dialysis bag. The dialysis bag was sealed and immersed into 30 mL of PBS. The system was shaken in a shaking water bath at 37°C. In predetermined intervals, 3 mL PBS solution was taken out and replaced by fresh PBS. The drug concentration was determined by measuring the absorbance at 243 nm in an ultraviolet–visible spectrophotometer (PerkinElmer Lambda Bio 40). Data were given as mean ± standard deviation (SD) based on three independent measurements.

RESULTS AND DISCUSSION

As shown in Scheme 1, PBG copolymers were synthesized by the ring-opening copolymerization of BTMC and GA, using $Sn(Oct)_2$ as a catalyst. The reason we synthesized the copolymers with feed ratios of BTMC/GA 80/20 (PBG-1) and BTMC/GA 50/50 (PBG-2) is due to the fact that they could be fabricated into microspheres easily using the "ultrasonic assisted precipitation method."

The chemical structures of the copolymers were confirmed by FTIR and ^{1}H-NMR. The characterization details were reported in our previous article.[7] The molecular weights and compositions of PBG copolymers are shown in Table I. From Table I, it is clear that the content of BTMC repeating units in the copolymers is slightly higher than the corresponding BTMC monomer feeding amounts, indicating the BTMC monomer has a higher reactivity comparing with the GA monomer under the reaction conditions we investigated. The molecular weight of the resulting copolymer increases with increasing BTMC content, confirming the same conclusion.

In this study, we used an "ultrasonic assisted precipitation method" to fabricate PBG-1 and PBG-2

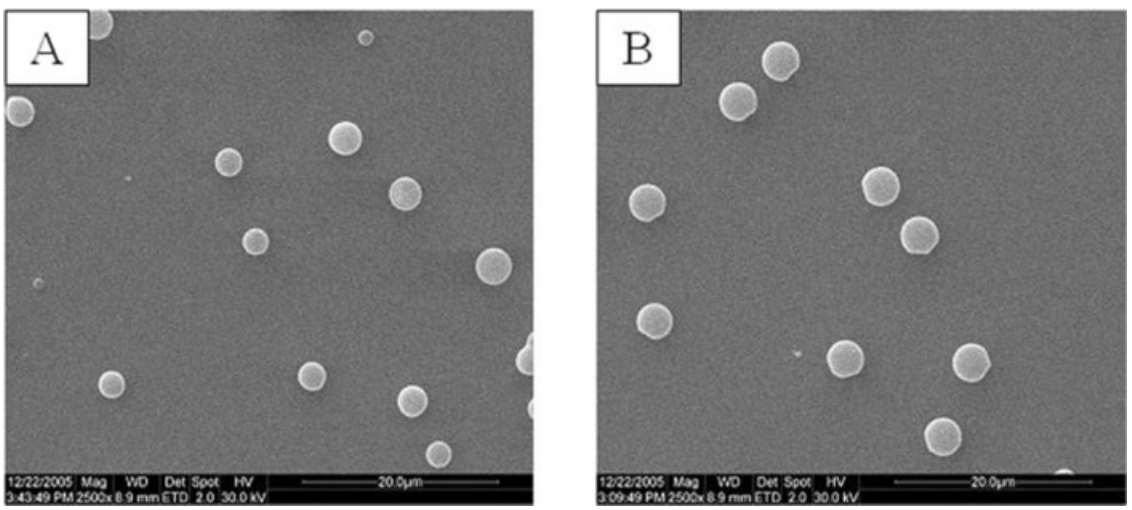

Figure 1 SEM images of (A) PBG-1 and (B) PBG-2 microspheres before drug release.

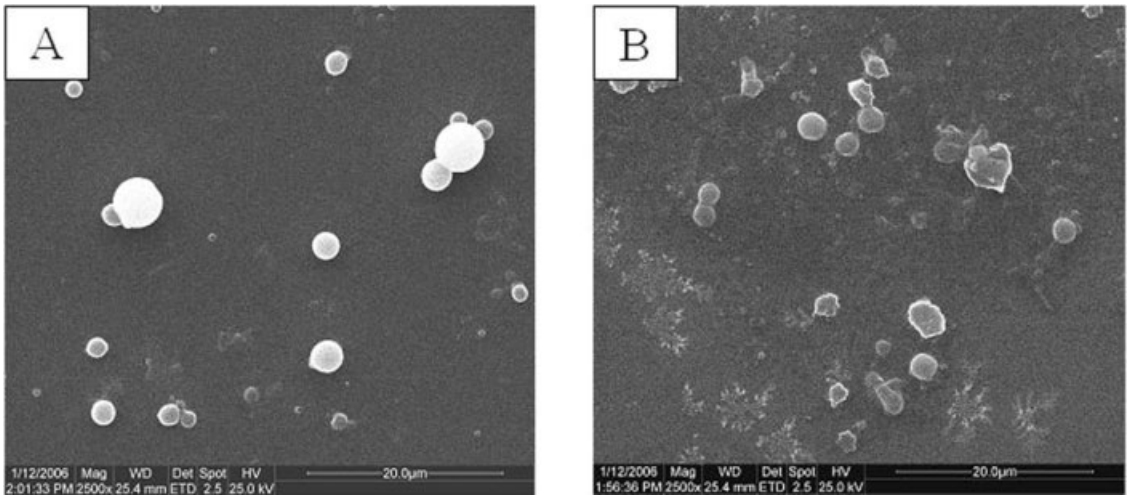

Figure 2 SEM images of (A) PBG-1 and (B) PBG-2 microspheres after drug release for 528 h (22 days).

microsphere drug delivery systems. The drug loading content, entrapment efficiency and properties of the microsphere drug delivery systems are summarized in Table II. As can be seen in Table II, the mean diameters PBG-1 and PBG-2 are in the submicon range. The size distributions of drug-loaded microspheres show that the microspheres have narrow size distributions, with the PI values lower than 0.5.

The morphologies of microspheres before and after the drug release were visualized by SEM. The sizes of the microspheres observed by SEM are in good agreement with the values measured by the Zetasizer. As shown in Figure 1, before drug release, the drug-loaded microspheres we fabricated are regularly spherical in shape, with mean diameters smaller than 1 μm. During microsphere fabrication, the mixture of polymer and drug were dissolved in acetone. Under ultrasonication, the solution was precipitated in water. Because of the reversible dissolution/precipitation process, the dissolution of polymer from the particle surfaces and precipitation of polymer onto the particle surfaces were in equilibrium. As a result, the obtained microparticles had a very regular spherical shape.[8] As the solubility of PBG-1 in acetone/water is higher than that of PBG-2 due to the higher content of BTMC unit of PBG-1, the resultant PBG-1 microsphere has a smaller size when compared with PBG-2. After drug release for 22 days, the PBG-1 microspheres and PBG-2 microspheres were observed by TEM. Compared with PBG-2, PBG-1 has a relatively slower degradation rate. As a result, no obvious morphological change can be observed for PBG-1 microspheres [Fig. 2(A)], while degradation of polymer matrix of PBG-2 [Fig. 2(B)] can be detected clearly.

The *in-vitro* release profiles of prednisone acetate from different types of microspheres are shown in Figure 3. As we know, the drug release from a degradable polymeric matrix is a very complicated process. Generally, several mechanisms, such as diffusion of the drug molecules and degradation of the polymer matrix, may be responsible for the overall release of the drug from the degradable polymeric matrix. The relative importance of these mechanisms for the overall release rate varies considerably from one system to another, depending on the composition, molecular weight, hydrophilicity, and degradation rate of the polymer matrix, as well as the size, porosity and surface character of the drug delivery system. In this study, in the early release stage, PBG-1 microspheres exhibit a faster release rate compared with PBG-2 microspheres. This is due to the following two reasons: (i) Based on our observation, the drug diffusion in homopolymer PBTMC is faster than that in homopolymer PGA. So the drug diffusion coefficient in PBG-1 is higher than that in PBG-2 because PBG-1 has a higher content of BTMC

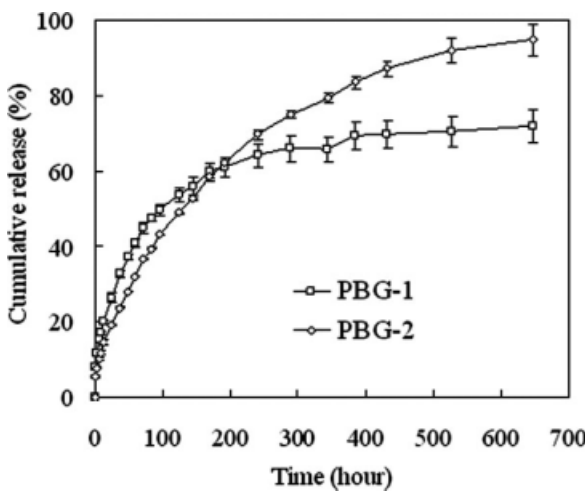

Figure 3 Release profile of prednisone acetate from PBG-1 and PBG-2 microspheres.

units, leading to the faster drug release for PBG-1 in the initial stage of the drug release when the drug diffusion is dominant mechanism of the release; (ii) PBG-1 microspheres have a smaller size, which may lead to the increased surface/volume ratio and the decreased diffusion pathway. In the contrast, in the late release stage, the drug release from PBG-2 becomes faster when compared with PBG-1. This is reasonable because PBG-2 has a faster degradation rate and the polymer degradation is the dominant release mechanism in the late release stage.

CONCLUSIONS

PBG random copolymers were synthesized by ring-opening polymerization. Using these copolymers, the microsphere drug delivery systems with submicron sizes were fabricated. The *in-vitro* drug release from these microspheres was investigated. In the early release stage, PBG-1 microspheres with a higher content of BTMC units exhibit a faster release rate compared with PBG-2 microspheres with a lower content of BTMC units. While in the late release stage, the drug release from PBG-2 microspheres becomes faster when compared with PBG-1 microspheres due to the faster degradation rate of PBG-2 copolymer.

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