Preparation, Properties, and Mathematical Modeling of Microparticle Drug Delivery Systems Based on Biodegradable Amphiphilic Triblock Copolymers

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ABSTRACT: A series of biodegradable amphiphilic A-B-A type triblock copolymers P(BLA-PEG-BLA), composed of hydrophilic poly(ethylene glycol) (PEG) as a middle block component (B) and hydrophobic poly(β-benzyl-L-aspartate) as outer polypeptide block components (A), were synthesized by copolymerization of β-benzyl-L-aspartate *N*-carboxy anhydride (BLA-NCA) and the diaminated PEG with the primary amino groups capped at both ends. These P(BLA-PEG-BLA) copolymers were characterized by ^{1}H-NMR, DSC, and GPC. The triblock copolymers were used to prepare three kinds of drug delivery systems including Norfloxacin (INN)-incorporated P(BLA-PEG-BLA) microparticles and tablets. The morphologies of the microparticles were characterized by SEM. The *in vitro* release properties of the microparticles and tablets in PBS were also evaluated. A mathematical model, which incorporates a linear first-order dissolution term and the transient Fickian diffusion equation, was developed to account for the kinetics of drug release from the INN-incorporated P(BLA-PEG-BLA) microparticles. The results indicated that the overall release process was well controlled by both drug dissolution and diffusion. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 3869–3873, 2004

Key words: drug delivery systems; amphiphiles; block copolymers; computer modeling

INTRODUCTION

For more than a decade, amphiphilic block copolymers have attracted extensive attention because they self-assemble to form micelles in aqueous media, which may be used as potential drug-delivery vehicles. Such copolymers are made up of two segments of different chemical nature, both hydrophilic and hydrophobic components. The hydrophobic segment forms the core of the micelle, whereas the hydrophilic segment surrounds this core as a hydrated outer shell. This core–shell structure enables polymer micelles such as microspheres and nanoparticles to have potential as vehicles for drug delivery, because some hydrophobic drugs can be physically entrapped in the core of block copolymer micelles and then transported at concentrations that can exceed their intrinsic water-solubility.[1–3]

Block copolymers containing poly(amino acid) as one component have been widely studied and reported.[4–6] Polyaspartamide is a biologically well-tolerated synthetic polymer with a proteinlike structure that was proposed as a plasma extender and a drug carrier for it is nontoxic, nonantigenic, and degradable in living systems. It can be modified easily by reactions with the side chains. As reported, antiviral drugs and anti-inflammatory agents were covalently linked to poly-α,β-[*N*-(2-hydroxyethyl)-D,L-aspartamide] (PHEA) forming drug–polymer conjugates capable of increasing drug stability and bioavailability.[7–13] Equally, poly(β-benzyl-L-aspartate) (PBLA) can be used as the hydrophobic segment.[5,6]

It is known that poly(ethylene glycol) (PEG) is nontoxic, nonantigenic, biocompatible, and soluble in water and organic solvents and by itself can have solubilizing properties. Although it is not biodegradable, PEG with molecular weight of 4000 can be 98% excreted in man. Because of these properties, PEG segments can form hydrogen bonds with the aqueous surroundings. As hydrophilic blocks, they can form a tight shell around the micelle core. Moreover, the hydrophilic nature of PEG thus excludes, or inhibits, protein adsorption and makes the micelles more stable in the body.[3,14]

In this work, we synthesized A-B-A type triblock copolymers P(BLA-PEG-BLA), composed of hydro-

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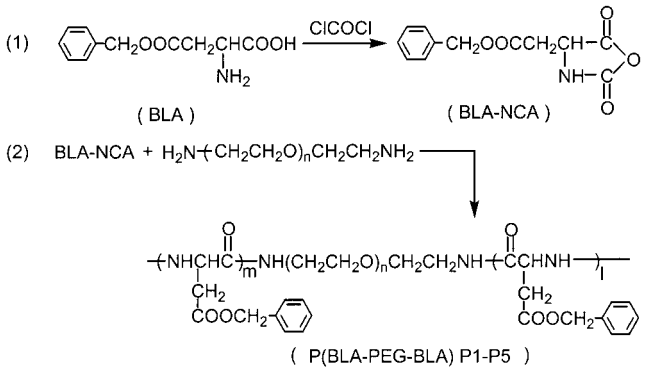

Figure 1 Synthetic route to triblock copolymers P(BLA-PEG-BLA).

philic poly(ethylene glycol) as a middle block component (B) and hydrophobic poly(β-benzyl-L-aspartate) as outer polypeptide block components (A). In the polymerization process, β-benzyl-L-aspartate *N*-carboxy anhydride (BLA-NCA) was initiated by the primary amino groups capped at both ends of PEG as the middle block component (Fig. 1). These P(BLA-PEG-BLA) copolymers were characterized by ^{1}H-NMR, DSC, and GPC. Furthermore, these A-B-A type triblock copolymers were used to prepare three kinds of drug delivery systems including Norfloxacin (INN)-incorporated P(BLA-PEG-BLA) microparticles and tablets. The SEM morphology of the microparticle and the *in vitro* release properties of the microparticles and tablets were also evaluated. A mathematical model, which incorporates a linear first-order dissolution term and the transient Fickian diffusion equation, was developed to account for the kinetics of drug release from the INN-incorporated P(BLA-PEG-BLA) microparticles.

EXPERIMENTAL

Materials

All chemicals and solvents were of analytical grade. PEG (M_n = 2000) with two hydroxyl end groups was purchased from Fluka (Shanghai, China). INN was purchased from Wuhan Jianmin Medical Co. The diaminated PEG[6] and β-benzyl-L-aspartate (BLA)[15] were prepared according to literature.

The copolymers synthesized were characterized by ^{1}H-NMR by using a Varian Mercury-Vx300 NMR spectrometer. The molecular weight (M_n) and polydispersity of the polymers were measured by GPC (Waters 2960D separations module, Waters 2410 Refractive Index Detector, Shodex K802.5 and K805 with Shodex K-G Guard Column, Polystyrene Standard, Chloroform solvent, 1.000 mL min^{-1} flow rate, 323 K Column temperature, and 318 K Detector temperature). The DSC experiment was performed on a Pyris 1 differential scanning calorimeter (Perkin–Elmer). The SEM morphology of the microparticles was studied by using a scanning electron microscope (SEM, Hitachi-X650, Japan) and specimens were coated with gold in SEM coating equipment.

Synthesis of copolymers

Freshly recrystallized BLA (11.16 g, 0.05 mol) was suspended in 200 mL of purified and sodium-dried dioxane and phosgene bubbled through at 60–65°C. After 2 h, a clear, pale-yellow solution was obtained. The solution was concentrated under vacuum at a maximum temperature of 50°C, to a volume of 20 mL. The resultant oil was diluted with 20 mL chloroform whereupon *n*-hexane was slowly added until crystals began to appear. Crystallization was completed in 12 h at 3°C. The precipitate was isolated by filtration and then washed thoroughly with *n*-hexane to remove the excess phosgene. The precipitate was dissolved in a minimum amount of dry ethyl acetate at room temperature, filtered, diluted with *n*-hexane, and allowed to stand at −30°C. After 24 h, the solid was isolated by filtration and dried at 27°C (1 mmHg) for 6 h to yield 8.1 g BLA-NCA (65%).

Triblock copolymers P(BLA-PEG-BLA) with differing amounts of PEG per end group were synthesized according to the following standard procedure. BLA-NCA (2.8 g, 0.0114 mol) was dissolved in 12 mL doubly distilled DMF followed by the addition of 60 mL distilled chloroform. Diaminated PEG (0.5 g, 0.25 mmol) was dissolved in 60 mL distilled chloroform and added to the solution at 35°C under a stream of dry nitrogen. The reaction was carried out for 72 h. The product was precipitated with diethyl ether and freeze-dried from 1,4-dioxane. The solid was reprecipitated from chloroform by using 2-propanol, filtered, and dried under vacuum to yield 2.2 g triblock copolymer P(BLA-PEG-BLA) P3 (66.7%).

Copolymers P(BLA-PEG-BLA) with differing BLA/(OCH_2CH_2) ratios (see Table I) were synthesized to give the following results: P1 (69.9%), P2 (64.2%), P4 (73.4%), P5 (44.8%). Each copolymer gave NMR results matching the following: ^{1}H-NMR ($CDCl_3$, δ ppm): 7.3 (C_6H_5), 5.1 (C_6H_5-CH_2O), 4.7 (N-CH_2CO), 3.7 (CH_2CH_2), 2.9 ($CHCH_2CO$), 1.7 (CH_2CH_2NHCH).

Preparation of INN-incorporated tablets and microparticles

Tablets

INN (5 mg, 0.016 mmol) and triblock copolymer P(BLA-PEG-BLA) P3 (100 mg) were dissolved in 20 mL dichloromethane. The solution was homogenized by sonication for 30 s and then allowed to evaporate. The resulting film was collected and pressed in a tablet press to obtain INN-incorporated P(BLA-PEG-BLA) tablet.

TABLE I
Experimental Data of Triblock Copolymers P(BLA-PEG-BLA)

Polymer	Monomer (BLA-NCA)/initiator (PEG repeat unit) mole ratio[a]	M_n[b]	Polydispersity[b]	Average BLA/(OCH_2CH_2) mole ratio in copolymer[c]	T_g (±1°C)
P(BLA-PEG-BLA) P1	4 : 1	1463	2.8	2.75 : 1	−81
P(BLA-PEG-BLA) P2	2 : 1	2475	1.8	1.51 : 1	−56
P(BLA-PEG-BLA) P3	1 : 1	4541	1.5	1 : 1.44	−48
P(BLA-PEG-BLA) P4	1 : 2	5918	1.5	1 : 2.19	−63
P(BLA-PEG-BLA) P5	1 : 4	2438	2.2	1 : 5.66	−79

[a] Based on monomer (BLA-NCA)/initiator (PEG repeat unit) mole ratio used in the synthesis of these copolymers.
[b] Determined by GPC.
[c] Experimentally determined by ^{1}H-NMR.

Microparticles prepared by Dialysis method[5]

Triblock copolymer P(BLA-PEG-BLA) P3 (40 mg) was dissolved in 10 mL *N*,*N*-dimethylacetamide (DMAc) and stirred at 60°C, whereupon INN (160 mg, 0.5 mmol) was added. This solution was stirred overnight at room temperature. To remove free INN and form INN-incorporated micelles, the solution was dialyzed against 2 L distilled water by using the dialysis membrane (EQ1040-4, 21 mm, MWCO: 8000–12,000). During the first 3 h, the water was exchanged three times (every hour) and then two times during the following 6 h (every 3 h). After a total of 9 h of dialysis, the solution was lyophilized. The content of INN in the microparticles (wt %) was determined to be 8.1% by UV absorption of the INN from the extracted or hydrolyzed particles.

Microparticles prepared by O/W Emulsion method

Typically, INN (6 mg, 0.019 mmol) and triblock copolymer P(BLA-PEG-BLA) P3 (62 mg) were dissolved in 30 mL dichloromethane. The solution was homogenized by sonication for 30 s and added dropwise to 100 mL distilled water under vigorous stirring at room temperature. The mixture was stirred in an air open system to remove dichloromethane by evaporation. Then, the solution was purified by dialysis against distilled water by using the dialysis membrane (EQ1040-4, 21 mm, MWCO: 8000–12,000) and lyophilized.

In vitro drug release study

Ultraviolet (UV) absorptions (A) of 10 solutions of INN in 0.1M phosphate-buffered saline solution (PBS, pH = 7.4) with different concentrations (C) from 1×10^{-5} to $1 \times 10^{-4}M$ were measured (at 273 nm) by a Lambda Bio40 UV/Vis spectrophotometer. According to Lambert–Beer rule, the relationship of A versus C of INN in PBS media was described.

INN-incorporated P(BLA-PEG-BLA) tablets or microparticles (80–100 mg) was suspended in 10 mL PBS. The mixture was added to a dialysis bag. The dialysis bag was sealed and then slowly shaken in 90 mL of PBS at 37°C in a 250-mL Erlenmeyer flask. Aliquots of the solution outside the dialysis membrane (25 mL) were replaced with 25 mL PBS at various time intervals and tested at 273 nm by a Lambda Bio40 UV/Vis spectrophotometer. The change in the concentrations of INN was obtained from curves of A versus C of INN in PBS.

Mathematical model

INN is poorly soluble in water 0.45 mg ml^{-1} at pH 7.5 and 0.40 mg ml^{-1} at pH 7.0. In this work, INN starts dissolving and diffusing through the pores of the polymer toward water when INN-incorporated P(BLA-PEG-BLA) microparticles are placed in PBS. We consider the combination of both drug dissolution and diffusion mechanisms control the overall release process of INN from two INN-incorporated P(BLA-PEG-BLA) drug delivery systems.

Harland et al.[16] developed a mathematical model to account for the kinetics of the dissolution-controlled, diffusional release of drugs from porous, nonswellable polymeric microparticles. Drug release from these systems under perfect sink conditions may be described by Eq. (1) and follow initial and boundary conditions of Eqs. (2)–(4)

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2\partial C}{r\,\partial r}\right) + k(\varepsilon C_s - C) \tag{1}$$

$$t = 0 \quad 0 < r < R \quad C = \varepsilon C_s \tag{2}$$

$$t > 0 \quad r = 0 \quad \partial C/\partial r = 0 \tag{3}$$

$$t > 0 \quad r = \mathrm{R} \quad C = 0 \tag{4}$$

where C is the drug concentration; r is the radial position in the sphere; D is the drug diffusion coefficient; k is a first-order dissolution constant, which characterizes the drug dissolution rate in the absence

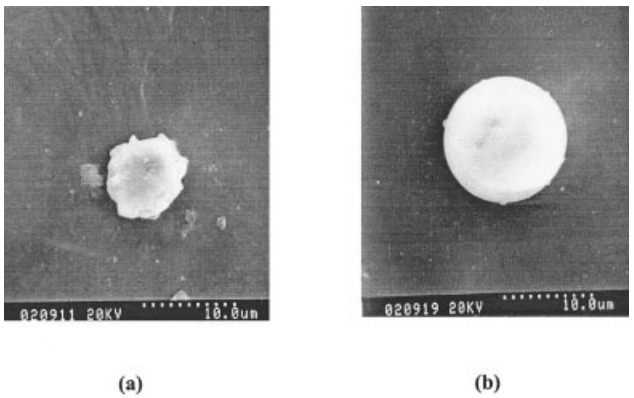

Figure 2 SEM photographs of Norfloxacin (INN)-incorporated microparticles: (a) microparticles prepared by Dialysis (diameter, 8.0 μm; magnification, $\times$3000); (b) microparticles prepared by O/W Emulsion (diameter, 13.8 μm; magnification, $\times$3000).

of polymer; ϵ is the system void fraction; C_s is the drug saturation concentration in the system; and ϵC_s is the equivalent drug saturation concentration in the solution found in the pores. On the right-hand side of this equation, the first term describes the diffusional drug process in the pores, whereas the second term describes the often rate-limiting drug dissolution process. The following dimensionless parameters are defined as

$$\xi = r/R \tag{5}$$

$$\tau = Dt/R^2 \tag{6}$$

$$\psi = (\varepsilon C_s - C)/\varepsilon C_s = 1 - C / \varepsilon C_s \tag{7}$$

$$\mathrm{Di} = kR^2/D \tag{8}$$

where ξ is a dimensionless radial position, τ is the dimensionless Fourier time, ψ is the dimensionless concentration, and Di is the dissolution/diffusion number. By using the dimensionless numbers of Eqs. (5)–(8), Eq. (1) and the boundary conditions of Eqs. (2)–(4) are transformed to

$$\frac{\partial \psi}{\partial \tau} = \left(\frac{\partial^2 \psi}{\partial \xi^2} + \frac{2\partial \psi}{\xi \partial \xi}\right) - Di\psi \tag{9}$$

$$\tau = 0 \; 0 < \xi < 1 \quad \psi = 0 \tag{10}$$

$$\tau > 0 \quad \xi = 0 \quad \partial \psi / \partial \xi = 0 \tag{11}$$

$$\tau > 0 \quad \xi = 1 \quad \psi = 1 \tag{12}$$

According to Fick's law, the flux J and the drug release rate, dM_t/dt, may be expressed as:

$$J = \frac{\partial M_t}{\mathrm{A}\partial t} = -D\frac{\partial C}{\partial r} \tag{13}$$

$$\frac{\partial M_t}{\partial t} = -DA\frac{\partial C}{\partial r} = DA\varepsilon C_s \frac{d\psi}{dr} = D\varepsilon C_s 4\pi r^2 \frac{d\psi}{dr} \tag{14}$$

In the following analyses, *C* programs operating in SumOS 5.6 system was used to solve the equations of the dissolution-controlled, diffusional release of INN from INN-incorporated P(BLA-PEG-BLA) microparticles. The comparison of the simulated INN release results with the drug release experimental data of the INN-incorporated P(BLA-PEG-BLA) microparticles is shown in Figure 3.

RESULTS AND DISCUSSION

Synthesis and characterization

A series of amphiphilic triblock P(BLA-EG-BLA) copolymers containing a hydrophilic PEG segment and a hydrophobic poly(β-benzyl-L-aspartate) segment with different ratios were synthesized and characterized. From Table I, it can be observed that the number molecular weight (M_n) of block copolymers reduces and the polydispersity of copolymers increases as the mole ratio of BLA-NCA/(PEG repeat unit in feed) in the polymerization process rises from 1 : 2 to 4 : 1. However, the molecular number weight of copolymers also drops and the polydispersity of copolymer becomes larger when the mole ratio of BLA-NCA decreases to 1 : 4. In addition, the glass transition of copolymers was measured by DSC and is shown in

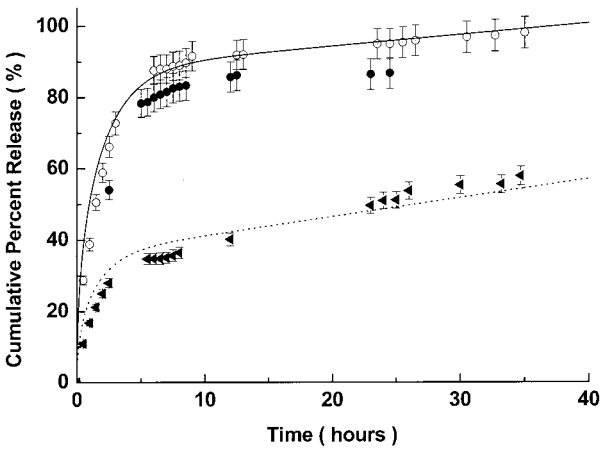

Figure 3 Comparison of simulation results with experimental data of INN release from INN-incorporated P(BLA-PEG-BLA) microparticles in PBS: (○) microparticles prepared by O/W Emulsion; (◀) microparticles prepared by Dialysis; (—) mathematical modeling microparticles prepared by O/W Emulsion; (- - -) mathematical modeling microparticles prepared by Dialysis; (●) INN-incorporated P(BLA-PEG-BLA) tablets.

TABLE II
Parameters Used in Mathematical Model

Parameter	INN-incorporated microparticles prepared by O/W emulsion	INN-incorporated microparticles prepared by dialysis
Radius R (10^{-3} cm)	1.0	0.5
Initial drug concentration C_0 (10^{-3} g cm^{-3})	0.503	1.2
Drug solubility concentration εC_s (10^{-4} g cm^{-3})	4.5	4.5
Drug diffusion coefficient D (10^{-11} cm^2 s^{-1})	5.0	1.0
Drug dissolution constant k (10^{-6} s^{-1})	1.0	4.5

Table I, in which the lower glass transition temperature (T_g) is found.

The SEM morphologies of INN-incorporated microparticles are shown in Figure 2. Compared to INN-incorporated microparticles prepared by O/W Emulsion (average diameter: 12–30 μm), the INN-incorporated microparticles prepared by Dialysis have smaller size (average diameter: 5–17 μm).

In vitro drug release properties and mathematical model

The INN release profiles of INN-incorporated P(BLA-PEG-BLA) tablets and microparticles in PBS are shown in Figure 3. The INN-incorporated P(BLA-PEG-BLA) tablets released INN faster than the INN-incorporated P(BLA-PEG-BLA) microparticles prepared by Dialysis, while tablets released INN slower than the INN-incorporated P(BLA-PEG-BLA) microparticles prepared by O/W Emulsion. Steady release rates of the INN-incorporated P(BLA-PEG-BLA) microparticles prepared by Dialysis could be maintained for more than 80 h.

The analysis to the case of drug release controlled by diffusion and dissolution from polymeric microparticles was described by Eq. (14). The simulated INN release results of INN-incorporated P(BLA-PEG-BLA) microparticles were generated by using the parameters: drug diffusion coefficient D, drug dissolution constant k, initial drug concentration C_0, drug solubility concentration ϵC_s, and radius R, respectively (see Table II). Figure 3 shows that the simulated INN release results fit well to the drug release experimental data of the INN-incorporated P(BLA-PEG-BLA) microparticles. Thus, the diffusional release of INN from INN-incorporated P(BLA-PEG-BLA) microparticles was also controlled by the dissolution of the drug in water. It indicated that the overall release process is well described by a combination of both drug dissolution and diffusion mechanisms.

CONCLUSION

A series of amphiphilic triblock P(BLA-EG-BLA) copolymers, composed of hydrophilic PEG segments and hydrophobic poly(β-benzyl-L-aspartate) segments, were synthesized and characterized. Two types of INN-incorporated microparticles and tablets were prepared on the basis of these amphiphilic triblock copolymers. *In vitro* drug release studies showed that these INN-incorporated P(BLA-PEG-BLA) microparticles and tablets can sustain an *in vitro* release of INN in PBS. A steady release rate of the INN-incorporated P(BLA-PEG-BLA) microparticles prepared by a dialysis method could be maintained for more than 80 h. A mathematical model, which incorporates a linear first-order dissolution term and the transient Fickian diffusion equation, was developed to account for the kinetics of drug release from the INN-incorporated P(BLA-PEG-BLA) microparticles. The results indicated that the overall release process is well described by a combination of both drug dissolution and diffusion mechanisms.

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