Preparation and Characterization of Enalapril Maleate–Loaded Nanoparticles Using Amphiphilic Diblock Copolymers

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ABSTRACT: Nanoparticles with the dimensions of circa 50 nm prepared from the micellar aggregation of diblock copolymers of poly(ethylene oxide) and polycaprolactone (PEO–b–PCL) were explored as a parenteral carrier system for water-soluble organic drugs in salt form. Enalapril maleate (EPM), developed for hypertension and congestive heart failure, was used as a model drug. The nanoparticles from three block copolymers with compositions of $5k$–$7.5k$, $5k$–$5k$, and $5k$–$2.5k$ (PEO–b–PCL) exhibited drug-loading efficiency of 38%, 47%, and 26%, respectively, for an equivalent amount of EPM in a 1% (w/v) micelle solution. Particularly, $5k$–$5k$ micelles could be incorporated with the model drug up to 47% (w/w) of polymer. Furthermore, these nanoparticles possess drug-retaining capability at 25°C or below even after free EPM was eliminated from the aqueous phase by dialysis. A temperature-responsive release behavior was displayed upon heating to the physiological temperature, 37°C. Drug release from the micelles proceeded in a fairly linear fashion for a duration of about 4–7 days, depending on the composition of the block copolymers. Daily average fractional release was consistent regardless of drug contents in the nanoparticles. In a preliminary animal toxicity test the EPM-loaded micelle solutions were intravenously administered to mice of the ICR strain through the tail vein. The animal subjects received 0.7 mL of EPM micelle solution up to six times and showed normal weight gain and food consumption. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 2856–2867, 1999

Key words: micelles; block copolymer; nanoparticles; enalapril maleate; poly(ethylene oxide)–b–polycaprolactone; parenteral delivery

INTRODUCTION

Recently, the application of nanoparticles as a parenteral administration system has attracted a great deal of interest for achieving a controlled input of a drug into the blood pool, with the possibility of extravasation and site-specific delivery either actively or passively.[1–14] Polymeric nanoparticles of aqueous dispersion prepared from amphiphilic block copolymers have unique features including long-term colloidal stability inherently provided by steric repulsion of the surface polymer chains.[11–17] They also demonstrated the minimum plasma protein adsorption, slower cellular uptake by phagocytes, and synthetic feasibility to conjugate functional moieties to impart the targeting capability of delivering them to the specific sites of action.[17] In order to meet the major requirements as a nanoparticulate carrier system, the amphiphilic block copolymers used were nonimmunogenic and nontoxic poly(ethylene oxide) and bioabsorbable hydrophobic compo-

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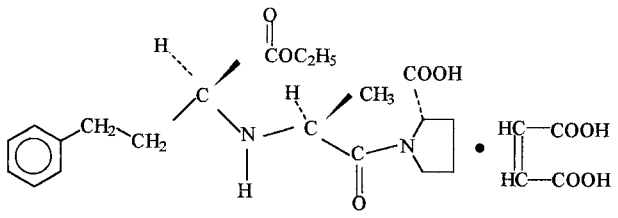
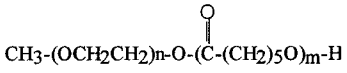


(a)



(b)

Figure 1 Chemical structure of (a) enalapril maleate and (b) poly(ethylene oxide-b-caprolactone).

nents, such as aliphatic polyesters,[12,15,24] polybenzylglutamate,[19] and polyaspartate,[7,10–13] which are capable of forming nanoparticles in aqueous solution.

It was abundantly documented that these amphiphilic copolymers have tremendous advantages over other systems for incorporating therapeutic drugs through covalent bonding,[7,10,22] as well as physical entrapping,[3,12] to prolong blood circulation of the agents. The strategy employing these block copolymers was to incorporate bioactive molecules in the core of micellar colloid systems by forming self-assembling superstructures that otherwise would be insoluble or sparingly soluble in the aqueous medium. Accordingly, studies on the polymeric micelle systems have concentrated on the encapsulation of hydrophobic substances such as steroids, hormones, and anticancer agents employing relatively hydrophilic block copolymers.[1,8,9,12,13,17] It has been well demonstrated that the polymeric micellar system from amphiphilic macromolecules undoubtedly provides an appropriate carrier for the delivery of hydrophobic material. However, a number of organic bioactive agents are developed in acid or base salt forms to render them soluble in physiological fluid and thereby to improve therapeutic efficacy. It has been brought to our attention that there is a need to examine the feasibility of the micellar aggregation system as an effective vehicle for drug types with relatively high water solubility.

Enalapril maleate [EPM, shown in Fig. 1(a)], a model drug employed in this study, is the maleate salt of enalapril, the prodrug of enalaprilat, an angiotension-converting enzyme (ACE) inhibitor. The drug is therapeutically effective for patients with hypertension and congestive heart failure.[25,26] It is soluble in water (1 g in 60 mL · at 25°C) and freely soluble in polar solvents such as methanol and acetone. The recommended daily dose is as low as 5 mg, which may allow for development of a practical injectable dosage form for sustained release. According to the pharmacokinetic studies, bioabsorption through the intestinal tract is approximately 60% in the case of oral administration, and the peak concentration of the enalapril in serum occurs within 3–4 h.[25] However, the drug concentration rapidly decreases below therapeutic levels by excretion in urine. A consistent and steady supply of enalapril at the ACE site is very crucial to the hypertensive patients because of its strong dose dependency. In addition, an injectable dosage form with a sustained-release feature has been desired for patients in emergency situations or in unconscious state when oral medication can not be applied.

This study was intended to evaluate the suitability of PEO–b–PCL polymeric micelles as practical injectable carriers for enalapril maleate in view of the loading capacity, stability, and release profile. The PEO–PCL diblock copolymers of three compositions, $5k$–$2.5k$, $5k$–$5k$, and $5k$–$7.5k$, were employed for the micelle-forming materials and were compared with conventional surfactants with similar HLB values, which ranged over 4.7–16. The diblock copolymers of PEO–b–PCL fit various criteria for particulate drug carriers, such as PEO being blood compatible and excretable when its molecular weight is under around 10,000–20,000 g/mol. Polycaprolactone is also a biocompatible and biodegradable material that is currently used in absorbable surgical sutures. The combination of these two polymers in the form of diblock copolymers may provide an opportunity for therapeutic treatments. Furthermore, all these PEO–PCL block copolymers are readily soluble in acetone, which allows for convenient preparation of micellar aggregates by evaporation of acetone in an acetone and water mixture containing PEO–PCL copolymers with EPM. The latter part of this paper briefly deals with *in vivo* animal toxicity testing after introducing an EPM-loaded micelle solution into the blood circulation of a mouse organism.

EXPERIMENTAL

Materials

Epsilon–caprolactone (MW; 114 g/mol, Aldrich) was purified by distillation under reduced pressure over calcium hydride. Toluene was obtained from Junsei Chemical (Japan) and was purified by fractional distillation over calcium hydride in a nitrogen atmosphere. Other solvents, such as acetone and methanol, were of reagent grade and were used as received. Poly(ethylene glycol) methyl ether (PEOH, MW; 5,000 g/mol) was also obtained from Aldrich and purified by dissolving it in toluene and by subsequent precipitation from methanol. It was dried *in vacuo* for at least 24 h before use. Stannous octoate was obtained from Aldrich and used as received. Enalapril maleate (99.6% MW; 492.5 g/mol), obtained from Dae-Woong Pharmaceutical Co. (Korea), was used as received.

Syntheses

Synthesis of PEO–b–PCL diblock copolymers was carried out via the ring-opening polymerization route. First, 25 g of PEOH was charged into a 250-mL two-neck round-bottom flask and dried under reduced pressure at 100°C for 2 h. Then, purified toluene and stannous octoate (0.2 wt % of caprolactone) were introduced by a syringe to give a 20% (w/v) polymer solution. Finally, an appropriate amount of ε-caprolactone, depending on the target molecular weight of the diblock copolymers, was added to the reactor by a syringe. The reaction mixture was stirred for 16 h at 110°C. The polymer solution was introduced dropwise to vigorously agitated diethyl ether to make polymer precipitates and to eliminate unreacted monomers, which was subsequently recovered by filtration and dried *in vacuo* to a constant weight. The recovered material was carefully weighed to determine the yields of polymerization. Proton NMR spectra of the resulting polymers were recorded on a Bruker DPX-400 MHz spectrometer in a deuterated chloroform solution to determine the composition of the diblock copolymers. Molecular weights and polydispersity of the copolymers were examined by gel permeation chromatography (Waters) equipped with styragel column and refractive index detector using polystyrene standards.

Preparation of Drug Loaded Nanoparticles

Typically, 1 g of diblock copolymer and predetermined amounts of the model drug (300, 500, 1,000, 2,000 mg), EPM, were dissolved in 300 mL of reagent-grade acetone, and then 100 ml of double-distilled water was added slowly to the solution. Subsequently, acetone was selectively distilled off from the solution, exploiting the large difference in volatility of the two solvents, by gradually applying a reduced pressure using a rotary evaporator. The final appearance of the micelle solution was transparent with a bluish tinge. The micelle solution was dialyzed against a large amount of PBS solution (NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g/l, pH 7.4), using 12.5 K molecular weight cutoff tubing to eliminate free drug and residual acetone until the EPM concentration in the outer water reached a constant value.

In Vitro Release Test

Drug-release behavior from the PEO–PCL micelle was studied employing a basket stirrer. Typically, 100 mL of drug-loaded 1% (w/v) micelle solution was placed in dialysis tubing (MWCO = 12,400), which was installed on a basket type grid. Then, the basket was immersed in a container filled with 1,000 mL of releasing media, which was stirred at 50 rpm and 37°C. Alternatively, a dissolution tester was used to exclude the effect of the dialysis membrane. One hundred milliliters of the drug-loaded micelle solution was diluted with 1,000 mL of releasing media (PBS buffer) in a dissolution tester equipped with an overhead stirrer. The solution was stirred at 50 rpm at 37°C. An aliquot of the sample solution (20 μL) was periodically withdrawn from the releasing solution and analyzed by using HPLC. For the release experiments in the presence of human serum and enzymes, 50 mL of the drug-loaded micelle solution was introduced into a dialysis tubing, to which 10–20 mL of enzyme solution (1 mg lipase/mL solution, activity; 46 units/mg) or human serum was added.

Measurements

Particle size and distribution of the nanoparticles in the aqueous dispersion were measured using photon-correlation spectroscopy (Brookhaven BI 90, 5 mW He/Ne laser light source, 90° fixed angle). In general, stock solutions were diluted with double-distilled water to 0.1% (w/v) solution and subsequently filtered with a 0.45 μm Milipore® filter (PP, 25 mm). The measurements were repeated five times, and the average value was

Table I Characterization of PEO–PCL Diblock Copolymers and Corresponding Micelles

Copolymer	Composition PEO–PCL	Mn[a] (g mol^{-1})	MWD[a]	HLB[b]	PS[c] (nm)	PD[c]	CMC[d] (mol/l)
I	$5k$–$2.5k$	7,900	1.25	10.5	34	0.332	1.3×10^{-5}
II	$5k$–$5k$	9,900	1.23	7.0	42	0.232	5.0×10^{-6}
III	$5k$–$7.5k$	11,800	1.15	4.9	53	0.227	2.0×10^{-6}

[a] Mn and MWD were determined by GPC measurements.
[b] HLB values were estimated by group contribution method.
[c] Mean diameter (PS) and polydispersity (PD) were obtained from PCS.
[d] CMC values were determined from ring surface tensionometer.

taken. The EPM concentration in the solution was analyzed by a Waters HPLC system equipped with a Lichrosorb® RP-8 column (4.0 mm × 250 mm) using 0.1*M* phosphate buffer solution or mixture of PBS and methanol (55 : 45 by vol.) as mobile phase. The flow rate and chart speed were 1.0 mL/min and 1.0 cm/min, respectively. EPM in the solution was detected by the UV detector at the wavelength of 212 nm. Critical micelle concentration data were obtained by using a deNuoy ring-method surface tensionometer (Fisher).

Animal Toxicity Test

Drug loaded $5k$–$5k$ micelle solutions were injected into the ICR-strain male mouse through the tail vein, and general symptoms were observed in terms of body weight change and food consumption. For LD_{50} evaluation, 0.7 mL of 2% (w/w) EPM–loaded $5k$–$5k$ micelle solution was administered to a group of 10 mice up to eight times a day at 1 h intervals. For comparison, the control group was given only a blank PBS solution. For the third group of mice the injection was stopped after six times, and general symptoms were observed for the next 15 days. For the fourth group a single dose of drug-loaded micelle solution (0.7 mL) was given once a day for 30 days.

RESULTS AND DISCUSSION

Synthesis of Diblock Copolymers

Diblock copolymers of PEO and PCL, shown in Figure 1(b), were synthesized via anionic ring-opening polymerization of ε-caprolactone using poly(ethylene glycol) methyl ether (PEOH, MW; 5,000 g/mol) as an initiator in the presence of stannous octoate catalyst. The chain length of hydrophobic PCL block in the copolymer was conveniently controlled by the charge ratio of PEOH, monofunctional initiator, and ε-caprolactone monomer. The polymerization route is known to be free of chain transfer reactions to polymers and monomers, giving a quantitative conversion, provided that the reaction material has no active nucleophiles other than growing species. Three block copolymers with segment lengths of $5k$–$7.5k$, $5k$–$5k$, and $5k$–$2.5k$ (PEO–PCL) were prepared to evaluate the composition dependency with respect to the drug-loading and release profile. The number average molecular weights from the GPC and the compositions from the proton NMR spectra showed good agreement with the theoretical values calculated based on the PEO–ε-CL feed ratios. The polydispersity indices of the synthesized diblock copolymers were in the range of 1.13–1.25 by GPC measurements.

Characterization of PEO–PCL Nanoparticles of Aqueous Dispersion

The diblock copolymers of PEO–PCL with the compositions of $5k$–$2.5k$ (copolymer I), $5k$–$5k$ (copolymer II), and $5k$–$7.5k$ (copolymer III) were not homogeneously dispersed in water because of a high content of hydrophobic polycaprolactone block. But it was possible to obtain micellar aggregates through a solvent exchange process. Initially, the copolymer was dissolved in acetone, and then water was introduced slowly to the solution. Then acetone was selectively vaporized in the polymer/water/acetone mixture under reduced pressure, exploiting a marked difference in boiling points between the two solvents. In the course of the solvent exchange from acetone to the aqueous phase, the diblock copolymers aggregated to form micelles. Colloidal stability of the resulting nanoparticles in aqueous dispersion was provided by covalently attached PEO of 5,000 g/mol. Characteristic features of the diblock copol-

ymers and corresponding micelles are summarized in Table I.

Our choice of these three copolymers was based on the structural stability of the nanoparticles, which was evaluated from the time evolution of particle size, generation of precipitates, and dynamic equilibrium. The colloid stability was not maintained for the nanoparticles from diblock copolymers possessing an excessively high content of PCL compared to covalently attached hydrophilic PEO. For example, the micelles prepared from the $5k$–$15k$ copolymer displayed an appreciable size increase and generated a considerable amount of precipitate with time. On the other hand, a conventional equilibrium seemed to exist between associated micelles and nonassociated unimers when the block copolymers were too hydrophilic. Under this circumstance, a considerable fraction of polymer chains was found to be present as free molecules.[5] A preliminary study showed that exhaustive dialysis of PEO–PCL of $5k$–$1k$, by replacing outer water, resulted in a quantitative excretion of polymer mass through the membrane, indicating that micelles are in dynamic exchange with free polymer molecules in solution. This may have a serious influence on the drug-carrying capability *in vivo* under dilution for having high CMC values as was pointed out by Hagan et al.[2] In addition, micelles from hydrophilic polymeric amphiphiles often exhibited much higher release rate *in vivo* than *in vitro*. This may be attributable to these hydrophilic polymeric amphiphiles adsorbing on the surface of hydrophobic substances and being abundantly present in the vascular compartment, thereby, accelerating dissociation of drug-loaded micelles.

The hydrodynamic diameters of the PEO–PCL nanoparticles were measured using photon correlation spectroscopy (PCS). Figure 2 displays the particle sizes and distributions of three nanoparticles. All the micelles showed unimodal distribution. It was observed that the mean diameters of these micelles increased with the increasing molecular weight and hydrophobicity and traceable amount (<2 vol %) of superaggregates. It was postulated that these large and loose complexes are formed by the secondary aggregation of smaller-sized micelles.[18,34] The mean particle sizes, averaged by volume, were 34 nm, 42 nm, and 53 nm for the $5k$–$2.5k$, $5k$–$5k$, and $5k$–$7.5k$ copolymers, respectively.

Micellar aggregation process was followed by particle size measurements in the course of solvent exchange from acetone to water. Figure 3 shows that the change of particle size passes through a maximum when the mixture includes

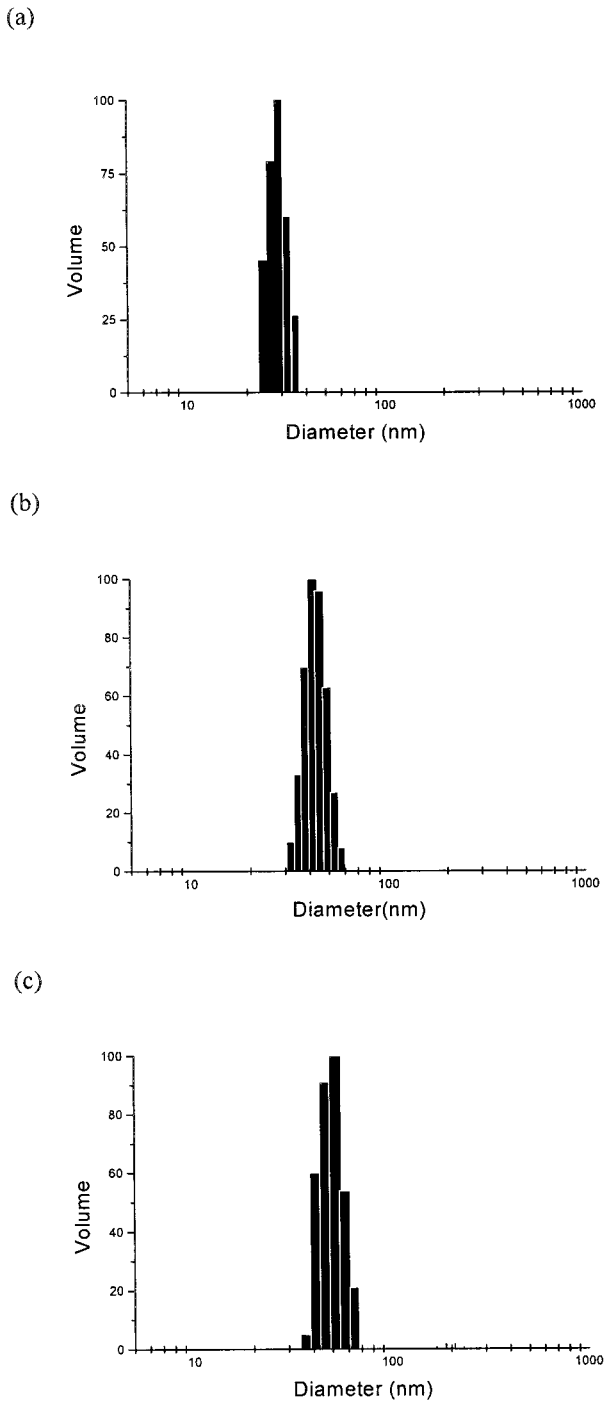


Figure 2 Particle sizes and distributions of micelles from PEO–PCL diblock copolymers measured by photon correlation spectroscopy: (a) $5k$–$2.5k$; (b) $5k$–$5k$; (c) $5k$–$7.5k$.

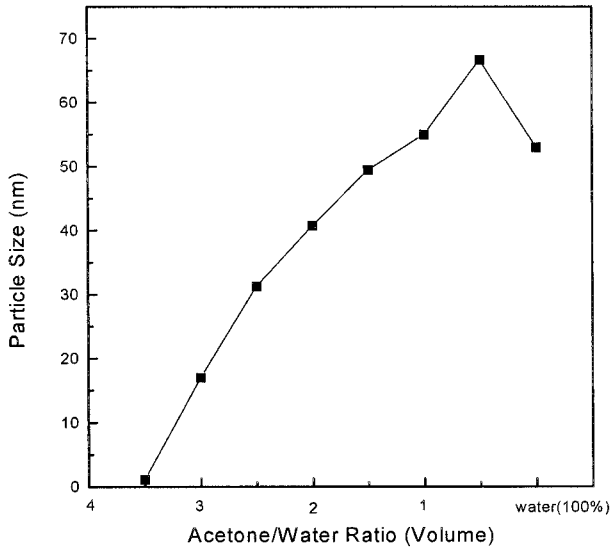


Figure 3 Change of particle size of $5k$–$5k$ micelles with a composition of acetone and water in solution.

the composition of water and acetone. The particle size increased as acetone was depleted to a certain composition and then decreased at a later stage. The progressive increase of particle diameter in the figure may be related to an inherent distribution over the length of hydrophilic and hydrophobic segments in the long-chain block copolymers. As the content of acetone, a good solvent for PCL, is gradually decreased in the solution, the polymeric species with extremely high PCL contents will initiate the nucleation. The next hydrophobic chains will then aggregate onto the nuclei in a sequential manner. Consequently, the resulting nanoparticles could possibly have a transient phase with a spectrum of composition of diblock copolymers across the hydrophilic corona of PEO and the hydrophobic core of PCL, rather than a two-phase structure with a distinct boundary. The subsequent drop in particle size in the latter stage can be attributed to the shrinkage of the swollen micelle interior by complete removal of acetone. In earlier literature Winnik and his coworkers proposed onionlike particles with alternating concentric layers of solvated and nonsolvated blocks for synthetic polymer micelles.[27] This concept of concentric layered structure may well explain the progressive growth of the colloidal dispersion from PEO–PCL block copolymers. The presence of a transient region will make this system very tolerant of encapsulating drugs of intermediate hydrophobicity.

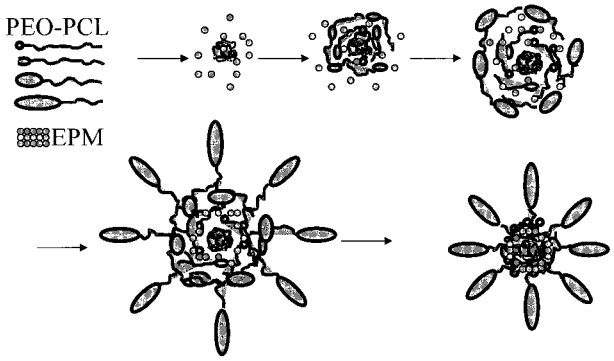


Figure 4 Schematic illustration of micelle formation and drug encapsulation during solvent exchange process from acetone to aqueous phase.

Drug Encapsulation

Both micelle formation and drug encapsulation take place simultaneously by vaporizing acetone from the stock solution, as illustrated in Figure 4. EPM in a water and acetone mixture is partitioned between the aqueous phase and the micelle

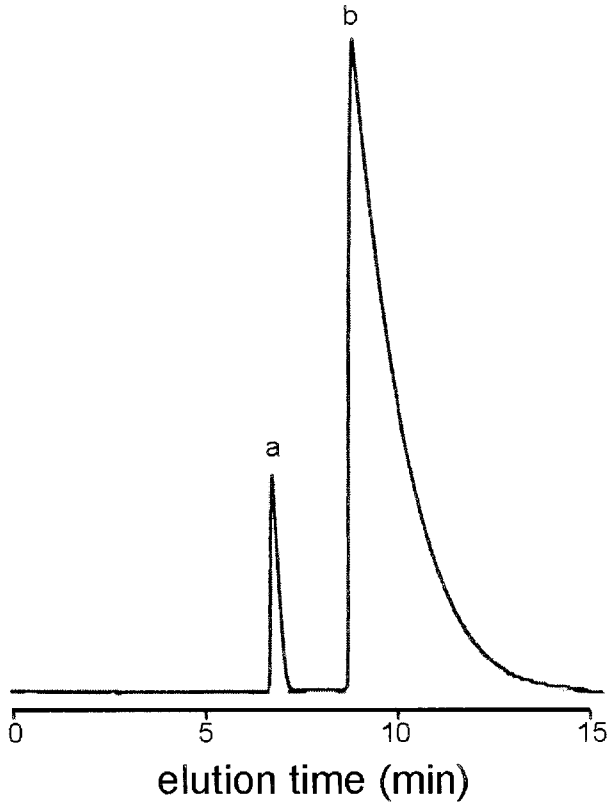


Figure 5 A representative HPLC chromatogram for micelle solution after encapsulation of EPM: (a) free EPM and (b) EPM loaded micelle.

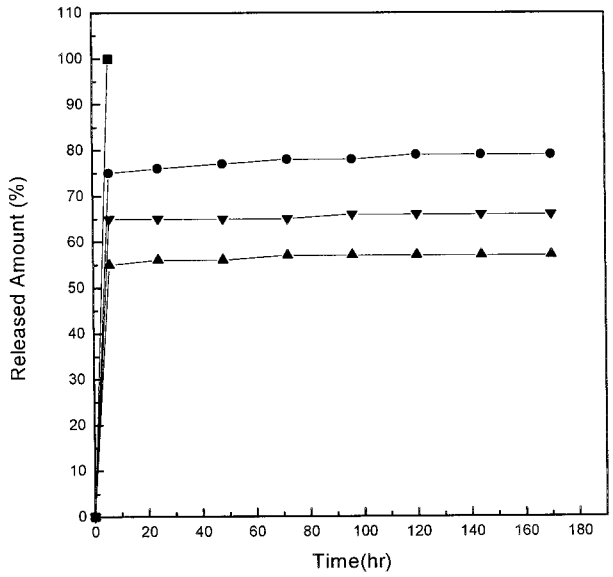


Figure 6 Dialysis of EPM–micelle solution followed by UV spectrometric analysis of outer water (PBS) at 212 nm; data represent the fractions of EPM dialyzed based on initial charge: (■) free drug; (●) 5*k*–2.5*k*; (▲) 5*k*–5*k*; (▼) 5*k*–7.5*k*.

domain during the micellar aggregation because of its mutual solubility in water and organic phase. Figure 5 shows a representative HPLC chromatogram after encapsulation. A free EPM peak is seen along with a strong peak from the micelle containing drug molecules. Employing volatile acetone to dissolve the solutes, diblock copolymers, and the drug presents an advantage over high-boiling polar solvents such as DMF and DMAc that only can be removed by exhaustive dialysis.[13] In that event, a large portion of amphiphilic drug molecules may be excreted to the outer water through the membrane during the dialysis, inevitably resulting in a low yield of encapsulation. The solvent evaporation route could possibly avoid such a loss, giving a higher yield of drug loading.

The free drug remaining in the aqueous phase could be eliminated through dialysis against PBS (pH 7.4), if necessary. At the same time it was possible to evaluate the encapsulation yield by determining the drug concentration in the outer liquid by spectroscopic measurements, which showed good agreement with the values evaluated from the direct analysis of the micelle solution by HPLC before dialysis. In Figure 6 the EPM concentration in the outer water increased linearly with time for several hours of dialysis and then leveled off. It was shown that free EPM was quantitatively removed by dialysis, and encapsulated molecules in the nanoparticles were not retrievable to the aqueous phase of PBS at 25°C. This drug-retaining capability was seen only in buffered solution. In buffer solution with high ionic strength it is likely that enalapril maleate becomes more hydrophobic enalapril and hence, a stronger association in the micelle domain may take place. However, this drug-retaining capability was diminished when the poly-

Table II Efficiency and Amounts of EPM Loading in PEO–PCL Nanoparticles under Various Conditions

Batch No.	Copolymer	Polymer (g)	H_2O (mL)	Acetone (mL)	EPM Charged (mg)	EPM Loaded (mg)	Loading[a] Yield (%)
1	5*k*–7.5*k*	1.0	100	300	1000	350	35.0
2	5*k*–5*k*	1.0	100	300	1000	450	45.0
3	5*k*–2.5*k*	1.0	100	300	1000	250	25.0
4	5*k*–7.5*k*	1.0	100	300	500	174	34.8
5	5*k*–5*k*	1.0	100	300	500	223	44.6
6	5*k*–2.5*k*	1.0	100	300	500	120	24.1
7	5*k*–7.5*k*	1.0	100	300	300	108	35.9
8	5*k*–5*k*	1.0	100	300	300	136	45.3
9	5*k*–2.5*k*	1.0	100	300	300	79	26.4
10	5*k*–5*k*	1.0	50	150	300	189	63.1
11	5*k*–5*k*	1.0	50	150	2000	466	23.3
12	5*k*–5*k*	1.0	200	300	1000	311	31.1
13	5*k*–5*k*	1.0	300	300	1000	271	27.1

[a] Percent of Loaded EPM based on initial charge.

meric surfactants were hydrophilic or short, probably because the depth of transient phase was shallow or excessively solvated.

Table II summarizes the efficiency and amounts of drug encapsulation in the PEO–b–PCL micelles under various conditions. The particle sizes of micelles apparently increased with the incorporation of EPM. Considering the sufficient water solubility of EPM and the low volume fraction of micelle in the solution, partition of the drug in the micelle domain was remarkably large. It's possible that the presence of the extensive transient region with intermediate hydrophilic–hydrophobic balance may be the key feature for these nanoparticles to associate with the amphiphilic drugs effectively. It was shown that the encapsulation efficiency of the micelle was greatly affected by the composition of diblock copolymers. The $5k$–$5k$ micelles, demonstrating highest loading efficiency, appear to have the optimum hydrophilic–lyophilic balance for EPM. There was a strong consistency in EPM partition between water (100 mL) and micelle (1 g) phase over the initially charged drug of 300–1,000 mg. In the case of 2% (w/v) micelle solution, the yield of loading increased to 63%, which was established from a linear relationship between drug encapsulation and micelle concentration. As is shown in the table, the partitioning of 1,000-mg EPM was 550 mg and was 450 mg in water (100 mL) and $5k$–$5k$ micelle (1 g), respectively. The capacity of the micelles to incorporate the EPM was doubled in a 2% (w/v) micelle solution, while there was negligible volume change in the water phase by doubling the micelle concentration. Accordingly, the normalized yield of loading in that case would be given as $(450 \times 2)/(550 + 450 \times 2) = 0.62$, which corresponds closely to the experimental value of 0.63. It was demonstrated that $5k$–$5k$ micelles could be loaded with EPM up to 47% (w/w) of polymer. Further loading in the micelle was not accomplished, even with a higher concentration of EPM in the solution. When 2 g of EPM was charged with 1 g of $5k$–$5k$ diblock copolymer in 100 mL of water, the actual loading remained 466 mg.

Release Behavior of EPM from the Micelles

Table III compares the daily drug release rate from the nanoparticles with various compositions and drug loading. In contrast to the drug-retaining capability at room temperature, EPM in the micelles was steadily liberated to the aqueous phase (PBS) at 37°C until the dosage forms were exhausted. This temperature-responsive release may be in part attributed to the improved solubility of the drug in water at an elevated temperature. More importantly, the change of micelle structure as a result of the characteristic feature of the PEO chain referred to as "LCST behavior" may have a greater influence. In general, the LCST behavior of PEO in diluted aqueous solution appears over 70°C. But the temperature may be lowered significantly under the circumstance that the PEO segment is bound to a hydrophobic chain and/or is present in a highly concentrated state. In addition, the ionic components of buffer solution possibly contribute to the reduction of LCST by depriving water molecules of PEO. Figure 7 shows that the hydrodynamic diameter of the micelle measured by PCS decreases upon heating to 37°C, which reflects the shrinkage of PEO segments at this temperature. Boddé et al. suggested that a lamellar gel structure could be formed from the highly concentrated amphiphilic diblock copolymer comprising PEO and glycerylmonostearate moiety by stacking a lamellar of small crystals of hydrophobic segments in water.[20] The transient phase in the PEO–PCL micelle may be envisioned as having a similar gel structure of short PEO segments and bound water, as well as crystallizable PCL moiety. In the literature it was postulated that the gel structure, comprising flexible oligolipid hydrophobic segments, was easily deformable to expand or reduce the interlamellar distance with the content of the water molecules present in the vicinity of hydrophilic PEO segments. In contrast, the transient phase built up by stacking PCL lamella appeared to be relatively rigid, as was shown from the observation that the particle size remained unchanged after releasing all the loaded drug molecules up to 47% (w/w) of micelle. Therefore, the shrinkage of PEO segments that occupy the interlamellar spacing while maintaining the skeleton of transient phase should contribute to enlarging the interlamellar channel, as schematically illustrated in Figure 8, and thereby increase the permeability of drug molecules in the diffusion process at an elevated temperature.

As can be seen in the table, the fractional release rates were not affected by the initial contents of the drug in the micelles. Thus, a strictly linear relationship could be established between released and loaded amounts. Regarding the effect of micelle compositions, the $5k$–$2.5k$ micelles possessing the highest PEO contents exhibited

Table III Daily EPM Release from the PEO–PCL Nanoparticles at 37°C

Polymer	Days	pH	Released (mg)	Released (%)
5*k*–b–7.5k	Initial	7.40	0	0
(350 mg[a]/100 mL)	1	6.95	65.5	18.7
	2	6.75	54.0	15.4
	3	6.60	50.8	14.5
	4	6.55	46.6	13.3
	5	6.50	43.8	12.5
	6	6.50	36.1	10.3
	7	6.48	27.7	7.9
	8	6.48	4.6	1.3
	9	6.48	0.0	0.0
5*k*–b–7.5*k*	1	7.13	33.6	19.3
(174 mg/100 mL)	2	7.05	27.5	15.8
	3	6.98	24.2	13.9
	4	6.80	23.5	13.5
	5	6.77	20.5	11.8
	6	6.70	18.4	10.6
	7	6.65	15.5	8.9
	8	6.65	2.8	1.6
5*k*–b–5*k*	1	6.79	105.8	23.5
(450 mg/100 mL)	2	6.58	93.6	20.8
	3	6.53	85.5	19.0
	4	6.50	84.6	18.8
	5	6.47	80.6	17.9
5*k*–b–5*k*	1	7.10	55.5	24.9
(223 mg/100 mL)	2	6.80	47.0	21.1
	3	6.71	41.9	18.8
	4	6.61	38.1	17.1
	5	6.56	35.0	15.7
5*k*–b–2.5*k*	1	7.01	79.8	31.9
(250 mg/100 mL)	2	6.73	67.8	27.1
	3	6.52	62.3	24.9
	4	6.51	39.5	15.8
5*k*–b–2.5*k*	1	7.20	36.4	30.3
(120 mg/100 mL)	2	7.10	32.2	26.8
	3	6.99	27.4	22.8
	4	6.80	20.6	17.2

[a] Amount of EPM loaded in 1% (w/v) micelle solution of 100 mL.

the highest release rate, and the 5*k*–7.5*k* with the highest PCL displayed the lowest. Such a compositional dependency may be explained by the degree of swelling in the transient phase of the micelles. The duration of release from the 5*k*–2.5*k* micelles was four days, while the 5*k*–7*k* micelles showed the most prolonged release for seven days. This sustained-release behavior may support the concept of an onionlike micelle because the drug molecules entrapped in the concentric layered region would be liberated by diffusion through a tortuous pathway through variably oriented interlamellar channels. Conventional nonionic surfactants with similar HLB values (4.7–16) to the three PEO–PCL diblock copolymers were tested for comparison purpose. However, those systems were extremely unstable to hold the EPM molecules under simulated physiological conditions, releasing the drug molecules instantly when agitated and heated to 37°C.

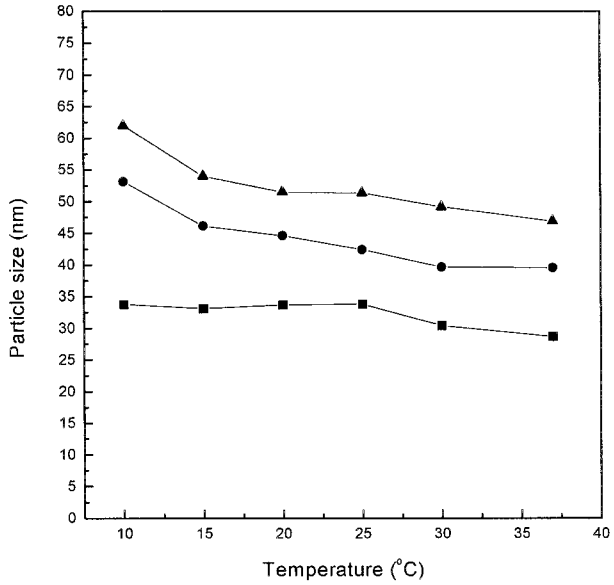


Figure 7 The change of mean diameter of micelle with temperature: (■) 5*k*–2.5*k*; (●) 5*k*–5*k*; (▲) 5*k*–7.5*k*.

When unloaded micelle solution was incubated at 37°C the decrease of pH and the molecular weight of diblock copolymer were negligible over a period of seven days, implying that release of drug from the micelle was not mediated by hydrolysis of PCL. Figure 9 presents the effects of human serum and enzymes such as lipase on the release profiles of EPM from the micelle. As can be seen from the figure, only a slight increase of release rate was observed as the human serum was added in the aqueous phase. Similar results were obtained when lipase was introduced to the micelle solution. This may be accounted for by the absence of dynamic equilibrium and repulsive motion of PEO chains, which prohibits the access of enzymes to the inner domain of micelles.

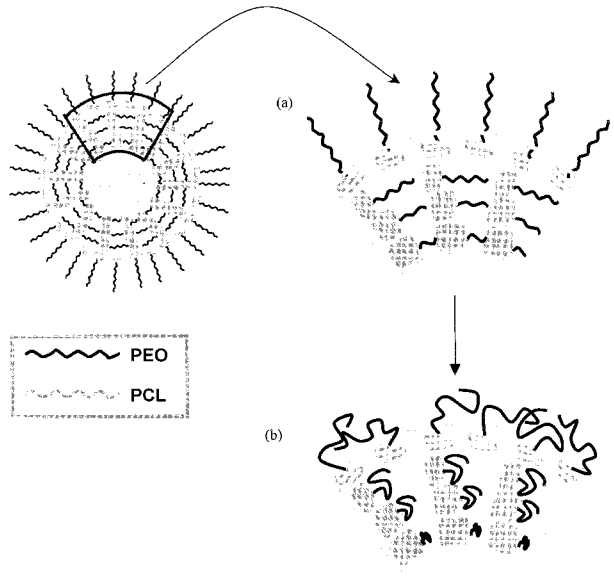


Figure 8 Schematic illustration of interlamellar space with PEO shrinkage upon heating: (a) below LCST; (b) above LCST.

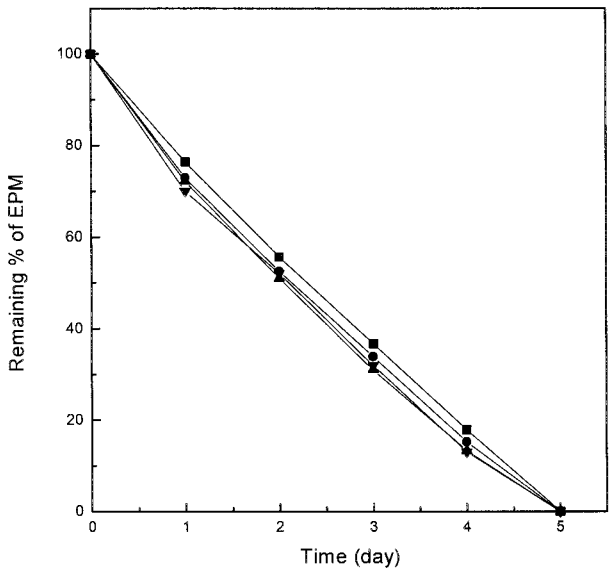


Figure 9 Release profiles from EPM-loaded micelles in the presence of human serum and lipase: (■) 5*k*–5*k* micelle (control); (●) 5*k*–5*k* 50 mL + serum 10 mL; (▲) 5*k*–5*k* 50 mL + serum 20 mL; (▼) 5*k*–5*k* 50 mL + lipase 10 mL.

In Vivo Toxicity

Since aqueous micelle solution is a liquidlike dosage form, the nanoparticles carrying therapeutic agents may be administered orally or via subcutaneous or IV injection, according to the compliance and preference of the patient. As a preliminary evaluation of toxicity of drug-loaded nanoparticles in the case of intravenous injection, animal testing was carried out using male ICR-strain mice. A group of 10 mice were given 0.7 mL of EPM-loaded micelle as a single dose through the tail vein. As shown in Table 4, animal subjects survived after receiving a single-dose injection up to six times at 1 h intervals and were eventually withdrawn after the eighth injection. Stress from the repeated injections was considered responsible for the death of one subject who received the physiological solution. In order to assess the potential toxicity of the micelle solution over this

Table IV Results of Animal Toxicity Test by Repeated IV Injection of EPM Loaded Micelle Solution

	No. of Injection									
Group	0	1	2	3	4	5	6	7	8	9
Control	Sv[a]	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc[b]	—
1	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc	—
2	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc	—
3	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc	—
4	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc
5	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc
6	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc	—
7	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc
8	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc	—	—
9	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc	—	—
10	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Sv	Dc

[a] Survived the test.
[b] Deceased after injection.

extended period, a group of mice were given six times the single dose over a 6-h period, and general symptoms were observed for the next 15 days. The results are shown in Figures 10 and 11, demonstrating normal weight gain and food consumption for 10 days after the injection, except for one subject with low body weight. Another group survived the test after being given a single dose of the EPM-loaded micelle solution on a daily basis for 30 days.

CONCLUSIONS

The nanoparticles formulated with amphiphilic block copolymers were found to be a feasible drug-carrier system for enalapril maleate and possibly for other partially water-soluble therapeutic agents in light of high loading, stability, and a desirable release profile. Stable aqueous dispersion of nanoparticles of a mean diameter of under

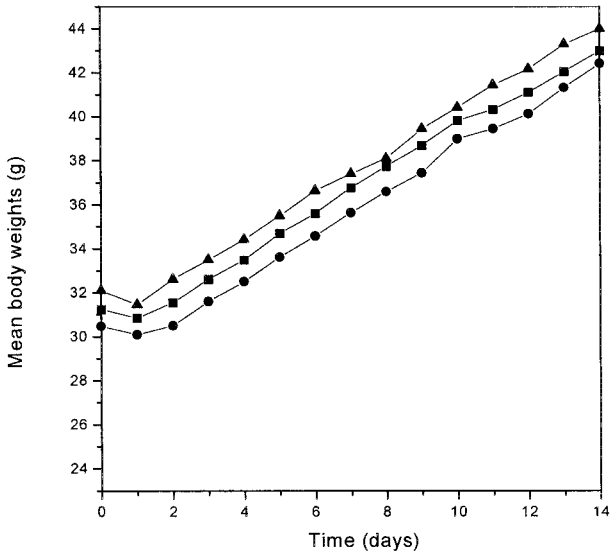


Figure 10 Mean body weight variation of mice after administration six times of single dose: (■) control; (●) 2 w/v % 5*k*–7.5*k* micelle solution; (▲) 1 w/v % 5*k*–7.5*k* micelle solution.

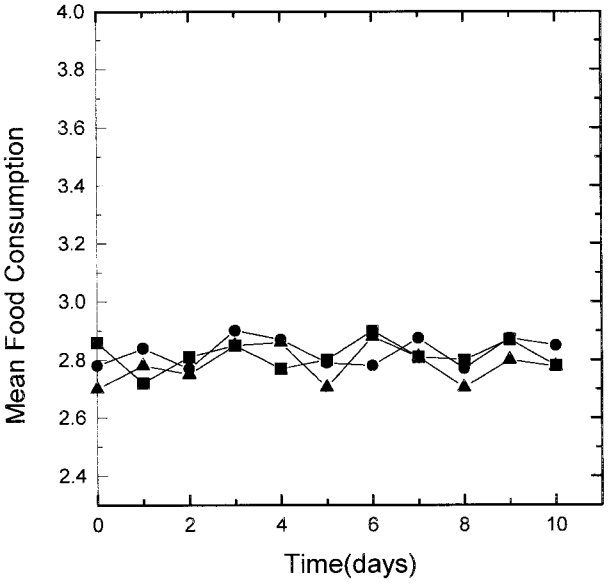


Figure 11 Mean food consumption of mice after administration six times of single dose: (■) control; (●) 2 w/v % 5*k*–7.5*k* micelle solution; (▲) 1 w/v % 5*k*–7.5*k* micelle solution.

100 nm presents a great potential for versatile administration route. Moreover, temperature-triggered release behavior is one of the many exploitable features of micellar aggregation of PEO–PCL diblock copolymers. From the preliminary toxicity test using animal subjects, it was demonstrated that this carrier system is relatively safe, even for parenteral administration.

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