Amphiphilic PEG-Based Ether-Anhydride Terpolymers: Synthesis, Characterization, and Micellization

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ABSTRACT: A series of amphiphilic poly(ether-anhydrides) terpolymers composed of sebacic acid, 1,3-bis(carboxyphenoxy) propane, and poly(ethylene glycol) (PEG) were synthesized via melt-condensation polymerization. The resultant terpolymers were characterized by ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR), Fourier transform infrared spectroscopy, gel permeation chromatography, X-ray diffraction, polarization optical microscope, differential scanning calorimeter, and water contact angle. Biodegradable micelles were prepared via a precipitation method through self-emulsification. The shape of these micelles was uniform and spheric according to the atomic force microscopy and transmission electron microscopy images. The dynamic light scattering measurements indicated that the diameters of micelles were typically in the range of 118–359 nm. The results displayed the PEG-based ether-anhydride terpolymers may be of great potential as nanoscaled carriers for drug delivery system. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 3576–3585, 2010

Key words: biodegradable; amphiphilic; micelles; polymer; nanoparticles

INTRODUCTION

Polyanhydrides are novel biodegradable polymers, and their surface-eroding property in aqueous medium makes them desirable for drug controlled release and functional soft tissue substitute.[1–4] Polyanhydrides have been developed since 1980s' by the Langer's group.[5] Since then, polyanhydrides of aliphatic and aromatic diacids have been extensively investigated as useful biomaterials for controlled drug delivery systems[6,7] because of their good biocompatibility and variable degradation properties. These advantages also make poly(CPP : SA) copolymers composed of 1,3-bis(carboxyphenoxy) propane (CPP) and sebacic acid (SA) good candidates for new drug delivery applications. Hundreds of polyanhydrides have been synthesized in the recent decades, but only poly(sebacic anhydride) and its derivations have been applied in controlled drug delivery system. For example, the US Food and Drug Administration (FDA) has approved the application of poly(CPP : SA) in weight ratio of 20 : 80 to deliver the chemotherapeutic agent BCNU for the treatment of brain cancer.[8]

However, as polylactide (PLA) and polylactide-*co*-glycolide (PLGA) particles widely used in drug delivery, micro/nanoscaled particles fabricated with poly(CPP : SA) possessed hydrophobic surfaces that led to their rapid removal by the immune system and poor resuspension and aerosolization properties.[9] In parallel, polymeric micelles formed by self-assembly of amphiphilic graft or block copolymers exerted many merits, such as biocompatibility, high drug-loaded amount, and markedly improved biodistribution.[10–12] Polymeric micelles have unique core-shell architecture consisted of hydrophobic segments as internal core and hydrophilic segments as surrounding corona in aqueous medium. Poorly water-soluble drugs can be solubilized within the hydrophobic core of the micelles and consequently, polymeric micelles can substantially improve the solubility and bioavailability of various hydrophobic drugs.[13] Polymeric micelles were firstly introduced as drug delivery vehicles in the early 1980s by Helmut Ringsdorf.[14,15] Polymeric micelles must hold several specific properties before they can be used in biomedical field, such as biocompatibility, biodegradability, target specificity, and stability in the body. To date, biodegradable polymeric micelles have found increasing applications as nanoscaled

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carriers in drug delivery system as they can provide several advantages including escaping from reticuloendothelial system (RES) uptake, long circulation in systemic fluid, passive targeting into specific tissues, and simple sterilization.[16] Drug targeting to tumors using ultrasound-activated polymeric micelles was also developed in previous reports.[17–21] The simplicity of micelle formation by self-assembly of amphiphilic block copolymer molecules and drug encapsulation by physical mixing rather than chemical conjugation are of extremely attractive features.

In this article, we synthesized a series of amphiphilic poly(PEG : CPP : SA) terpolymers containing of PEG, CPP, and SA via melt polycondensation. The two-component poly(CPP : SA) copolymers are biocompatible, biodegradable through surface erosion, and have gained increasing attention for sustained drug delivery applications. However, poly(CPP : SA) copolymers are hydrophobic, and their hydrolytic degradation need relatively long time, which results in the limitation of further applications in drug delivery system. PEG is a noncharged, hydrophilic, and nonimmunogenic polymer that possesses wide chemical, biomedical, and industrial applications.[22,23] It was reported that the incorporation of PEG could not only increase the hydrophilicity but also minimize protein adsorption and decrease nonspecific cell adhesion.[24,25] Many studies focused on the amphiphilic PEG-polyesters copolymers in expectation of achieving unique properties and corresponding applications.[26,27] In contrary to the PEG comprising the hydrophilic block, the CPP and SA chains were chosen as the hydrophobic blocks in the backbone of the amphiphilic poly(PEG : CPP : SA) terpolymers. The structure of these resultant terpolymers was investigated by ^{1}H-NMR and Fourier transform infrared spectroscopy (FTIR). The average M_w and its polydispersity index (PDI) were measured with GPC. Intrinsic viscosity ([η]) was determined by Ubbelohde viscometer. The crystallinity and crystallization morphologies were analyzed by XRD and polarization optical microscope (POM), respectively. The melting points of the distinct components in these materials were examined by differential scanning calorimeter (DSC). The water contact angle (CA) was measured to investigate their hydrophilicity.

The biodegradable micelles based on poly(ether-anhydride) were also fabricated by precipitation technology. The properties of micelles were measured by AFM, TEM, and DLS. The objective of this study was to investigate the feasibility of the micelles formation based on these poly(ether-anhydride) terpolymers. We expect that the effective bioavailability of micelles will bring the ether-anhydride family of polymers great potential as nanoscaled carriers for controlled drug delivery.

MATERIALS AND METHODS

Materials

All compounds and solvents were obtained from Chengdu KeLong Chemical Reagent Company (Sichuan, Chengdu, China). They mainly include: succinic anhydride, sebacic acid (SA), poly(ethylene glycol) (PEG, M_w = 1000, 2000, 4000 Da), 1,3-dibromopropane, *p*-hydroxybenzoic acid. Sebacic acid was recrystallized two times from ethanol. Acetic anhydride was refluxed over and distilled from magnesium. All the other solvents were used as received. 1, 3-Bis-(carboxyphenoxy) propane (CPP) was synthesized according to the method described by previous literature.[9]

Synthesis of PEG dicarboxylic acid monomer

Preweighed PEG (40 g), succinic anhydride (10 g), and pyridine (10 g) were dissolved in chloroform (200 mL), and the solution was reacted at 60°C for 72 h. The solution was filtered and evaporated to dryness by rotary evaporation. The concentrated product was then dissolved in 40 mL of 1*N* HCl, washed with diethyl ether, extracted with chloroform, and dried with anhydrous sodium sulfate. Finally, the resultant product was dried under vacuum.

^{1}H-NMR ($CDCl_3$): δ3.66 (s,OCH_2CH_2), 2.47(t,CH_2). IR(KBr,cm^{-1}): 1727 (C=O), 1110 (CH_2OCH_2).

Preparation of acylated prepolymers

Preweighed CPP (10 g) was refluxed in 200 mL of acetic anhydride for 1 h under N_2, followed by the removal of the unreacted diacid by filtration and solvent by evaporation. The CPP prepolymers were deposited with dry ethyl ether, and dried under vacuum.

^{1}H-NMR ($CDCl_3$): δ 7.99, 7.12(d, 4H, Ar-H), 4.26(t, 4H, CH_2O), 2.35(s, 6H, CH_3), 2.27(m, 2H, CH_2). IR (KBr, cm^{-1}): 1801, 1720 (anhydride C=O).

Preweighed SA (10 g) was refluxed in 100 mL of acetic anhydride under N_2 for 30 min and evaporated to dryness by rotary evaporation. The crude prepolymers were washed with a mixture of anhydrous ethyl ether/petroleum ether (1 : 1 v/v), and finally dried under vacuum.

^{1}H-NMR ($CDCl_3$): δ 2.46(t, 4H, C(=O)CH_2), 1.67(m,4H, CH_2), 1.31(m, 8H, CH_2). IR (KBr, cm^{-1}): 1822, 1741(anhydride C=O).

Preweighed PEG dicarboxylic acid (10 g) was refluxed in 100 mL of acetic anhydride for 30 min under N_2 and concentrated to dryness by rotary evaporation. Acetic acid and excess acetic anhydride were removed under vacuum at 60°C. The hot clear viscous residue was dissolved in methylene chloride,

then cooled to 0°C overnight and precipitated with ethyl ether/petroleum ether (1 : 1 v/v). The white precipitate was dried under vacuum at room temperature for 48 h, and stored at −20°C.

^{1}H-NMR ($CDCl_3$): δ3.66 (s,OCH_2CH_2), 2.33(s,CH_3), 2.46 (t,CH_2). IR(KBr,cm^{-1}): 1807, 1743 (C=O anhydride), 1110 (CH_2OCH_2).

Synthesis of the PEG-based ether-anhydride terpolymers

PEG prepolymers, CPP prepolymers, and SA prepolymers were mixed based on predesigned ratios in a three-neck flask under N_2. The flask was then immersed in an oil bath at 180°C for 30 min, the pressure was reduced to 50–60 mm Hg and the prepolymers were allowed to melt. Throughout the polymerization, a strong N_2 sweep was performed for 30 s every 15 min to agitate the reacting melt. After a defined time, the crude polymers were cooled down completely, dissolved in dichloroformethane, and precipitated in petroleum ether. The precipitate was then washed with anhydrous ether and dried under vacuum at room temperature to constant weight. The obtained polymers were stored at −20°C.

^{1}H-NMR ($CDCl_3$): δ6.97, 7.99 (d,ArH), 4.25 (s,CH_2), 3.66 (s,OCH_2CH_2O), 2.45(t,CH_2), 2.33 (m,CH_2), 1.65 (m,CH_2), 1.32 (s,CH_2). IR(KBr, cm^{-1}): ∼ 1812–1773, ∼ 1738 (C=O anhydride), 1112 (CH_2OCH_2).

Preparation of polymeric micelles

The polymeric micelles were prepared using a precipitation method. In brief, 0.05 g terpolymers were dissolved in 5 mL THF in a flask, then the solution of the copolymer was added dropwise using a disposable syringe (21 gauge) into 20 mL deionized water under high speed stirring. The mixed solution was devolved to a beaker and slowly stirred for 24 h at room temperature to facilitate the removal of the THF. Once the THF had been removed completely, the solution was diluted with deionized water to the desired concentration.

Characterization

Infrared spectra were obtained using a Nicolet 5700 spectrometer. The samples were pressed into KBr pellets for analysis.

All samples were prepared by dissolving about 5 mg of polymer in 0.5 mL of a chosen solvent. ^{1}H-NMR spectra were obtained on a Bruker AM 300 apparatus using $CDCl_3$ as a solvent and TMS as an internal reference. Chemical shifts were expressed as parts per million, ppm (δ).

Intrinsic viscosity was measured with an ubbelohde viscometer on 1% (g/dL) solution of polymer at 23°C in chloroform.

Thermal properties of polymer was determined by DSC (Netzsch STA 449C, Bavaria, Germany) from −100 to 200°C with a heating rate of 10°C/min under a nitrogen atmosphere.

The crystallinity of copolymer was characterized using an X-ray diffractometer (XRD, Phlips, X'Pert PRO, Netherlands) and scanning was done in the 2θ angle range of 5–45°.

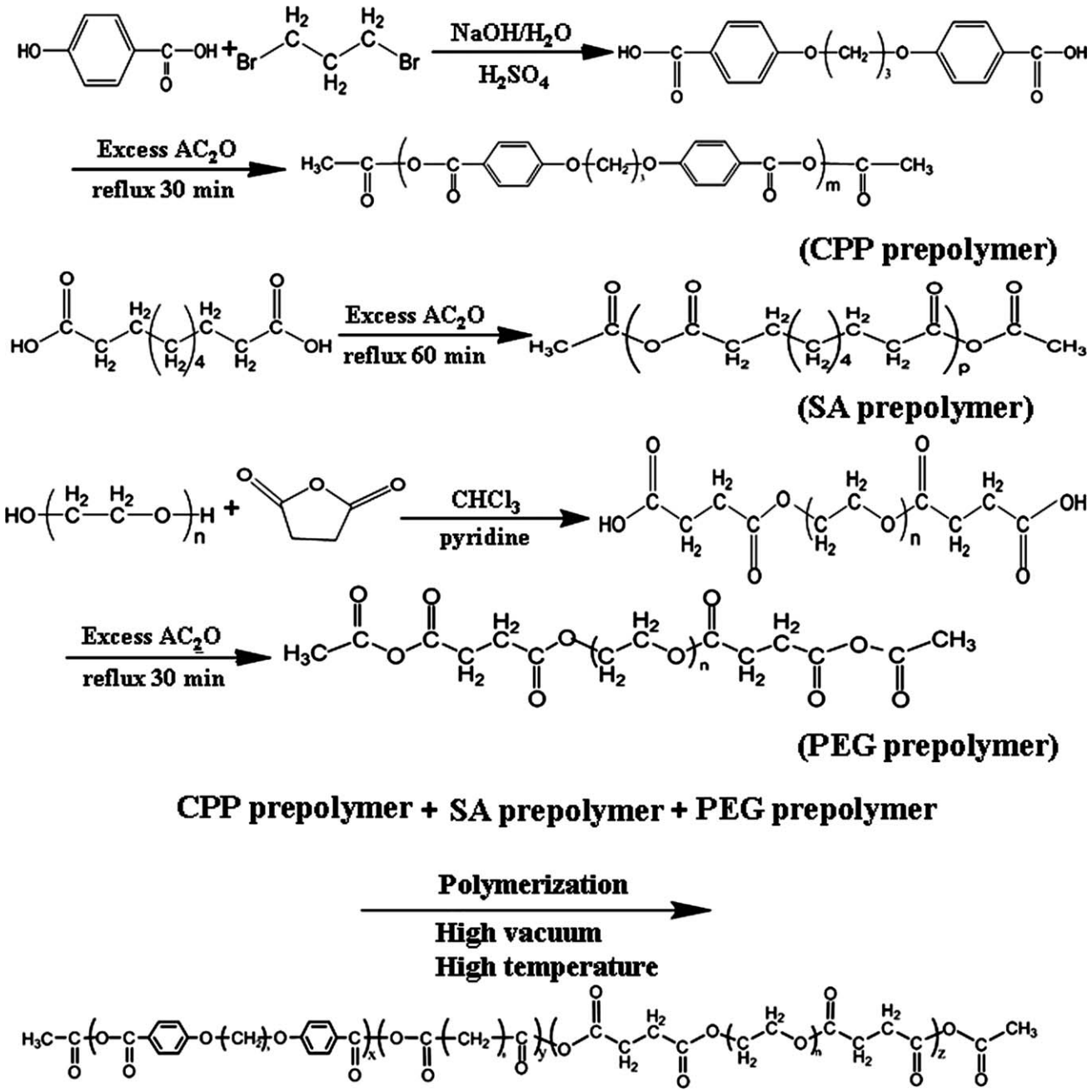
A POM (XPN-203, China) with a hot-stage was used to characterize the isothermal crystallization morphologies of poly(CPP : SA) and poly(PEG : CPP : SA). First, a sample of about 5 mg was placed between two glass slides and was heated to melt completely. Then, the sample was pressed to obtain a sheet with the thickness of 20 μm. Second, the sample was transferred to the hot-stage with the setting temperature of 40°C and maintained at this temperature until the crystallization of the sample was finished completely. The crystallization morphologies of the samples were taken images via a digital camera.

Gel permeation chromatography (GPC) measurements were carried out to measure molecular weight (M_w) and its distribution (PDI) with a Water 2695 separation module equipped with a Styragel HT4DMF column operated at 40°C and series 2414 refractive index detector. Waters millennium module software was used to calculate M_w on the basis of a universal calibration curve generated by narrow molecular weight distribution polystyrene standards.

The water contact angle (CA) was measured using a sessile drop method at room temperature with the contact angle equipment (DSA 100, KRUSS, Germany). CA values of the right side and the left side of the distilled water droplet are both measured, and an average value is used. The contact angle was determined at 10 s after the droplet contacted on the surface of samples. All the CA data were an average of five measurements on different locations of the surface.

Transmission electron microscopy observation was performed with a HITACHI H-700H (TEM, Japan) at the acceleration voltage of 150 kV. TEM sample was prepared by dipping a copper grid with formvar film into the freshly prepared micelles solution. A few minutes after the deposition, the aqueous solution was blotted away with a strip of filter paper and stained with phosphotungstic acid aqueous solution, then dried in air.

The mean size and size distribution were determined by dynamic light scattering (DLS) using a ZETA-SIZER, MALVERN Nano-ZS90 (Malvern, Malvern, UK) Each measurement was also repeated three times and an average value reported.



Scheme 1 Synthesis of PEG, CPP, SA prepolymer and their terpolymer poly(PEG : CPP : SA).

The morphology of the micelles was by tapping-mode atomic force microscopy (AFM) measurements (CSPM5000, Bejing, China**).** The AFM sample was prepared by casting a dilute micelles solution on a slid silicon piece, which was then dried under vacuum.

RESULTS AND DISCUSSION

Synthesis of the PEG-based ether-anhydride terpolymers

A series of PEG-based ether-anhydride terpolymers were synthesized by a melt polycondensation of SA, CPP, and PEG with four different weight ratios (5, 10, 20, and 30%) and three different molecular weight (M_w = 1,000, 2,000, and 4,000 Da), respectively. An outline related to the synthesis of the monomer, prepolymer, and resultant polymer were shown in Scheme 1. First, both hydroxyl end groups of PEG were transformed into carboxylic groups before polymerization. Then, aromatic monomer CPP monomer was synthesized with *p*-hydroxybenzoic acid and 1,3-dibromopropane. Acetic mixed anhydride prepolymers of CPP and SA were prepared by heating corresponding diacids in acetic anhydride. Finally, the resultant terpolymers, poly(PEG : CPP : SA) were

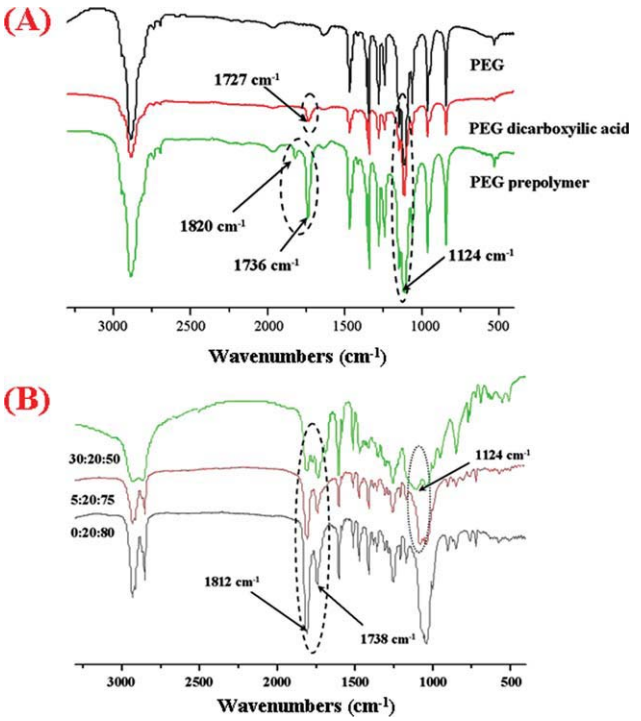


Figure 1 FITR spectra of PEG, PEG dicarboxylic acid, and PEG prepolymer (A), and the poly(PEG : CPP : SA) terpolymers with weight ratios of 0 : 20 : 80, 5 : 20 : 75 and 30 : 20 : 50 of PEG 4000, CPP and SA (B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

synthesized via melt-condensation polymerization of the three component: PEG, CPP, and SA prepolymers without catalyst. The yield of the copolymers ranged from 61.3 to 78.6%.

Characterization of the terpolymers

The structure of poly(PEG : CPP : SA) was confirmed by FTIR and ^{1}H-NMR. FTIR spectrum of PEG, PEG dicarboxylic acid, and PEG prepolymer are illustrated in Figure 1(A). Seen from the figure, peaks at 2854–2840 cm^{-1} is corresponded to the methylene vibrations. The absorption bands at 1124 cm^{-1} are attributed to the characteristic C—O—C stretching vibrations of the repeated —OCH_2CH_2 units of PEG. The absorption band at 1727 cm^{-1} is attributed to the C=O stretching vibrations of the ester carbonyl group, which indicates the successful preparation of PEG dicarboxylic acid. The absorption bands at 1820 cm^{-1} and 1736 cm^{-1} are the characteristic peaks of anhydride bonds, which further indicate that PEG prepolymers can be obtained by the above methods described in Scheme 1.[28]

Poly(CPP : SA) copolymers have been investigated widely. If PEG is introduced into poly(CPP : SA) molecule chains, their chemical structure must be influenced. Figure 1(B) displays the typical FTIR spectra of poly(PEG : CPP : SA) terpolymers with the same molecular weight (4,000 Da) and different content of PEG (0, 5, and 30%). It could be seen clearly that there exists three anhydride carbonyl peaks at 1812, 1740, and 1720 cm^{-1}. The C—H stretching vibrations of long aliphatic alkyl and the PEG are distributed at 2900 and 2850 cm^{-1}. With the amount of PEG in the terpolymers increasing, the intensity of peaks at 1124 cm^{-1} attributed to the characteristic C—O—C stretching vibration also increases. Simultaneously, the absorption band at 1820 cm^{-1} decreases when the content of SA reduced. These phenomena suggests that the poly(PEG : CPP : SA) terpolymers have been synthesized successfully.

^{1}H-NMR spectrum is employed and shown in Figure 2 to further confirm the formation of poly(PEG : CPP : SA) terpolymers. The well resolved chemical shifts at $\delta = 1.32$, 1.66, 2.26, and 2.47 ppm belong to the hydrogen atoms of individual functional groups on poly(sebacic anhydride). The chemical shifts at 1.66, 2.32, 4.25, 6.97, and 7.99 ppm ascribe to the protons of CPP. The sharp single peak at 3.66 ppm is attributed to the methylene protons of homosequences of the PEG units. And the chemical shifts at $\delta = 2.32$ and 2.47 ppm belong to the succinate moieties of PEG units. The peak at 7.25 ppm corresponds to the solvent, deuterated chloroform. These results are consistent with the information obtained from FTIR. The actual weight percentages of PEG, CPP, and SA in the polymer were estimated from the integral height of hydrogen atoms in the ^{1}H-NMR spectra. All the synthesized products have approximate segmental ratios of monomer units to the feeding composition, which are summarized in Table I. As seen from the tables, the amount of PEG in the final products is quite close to the feeding ratios, which indicates that the PEG prepolymers have similar reactive activity with SA and CPP prepolymers.[29]

Molecular weights (M_w) of the copolymers are determined by GPC measurement. The intrinsic viscosities ([η]) of the copolymers are determined by the intrinsic viscosity measurements. As shown in Table I, intrinsic viscosities of the poly(PEG : CPP : SA) terpolymers generally decrease with increasing amounts of PEG in the polymer backbone, which is in good agreement with the result of M_w changing. It is probably because the introduction of PEG may constrain the development of polymer chains following melt polycondensation. For the copolymers with the same PEG content, their M_w increase with the M_w of PEG increasing. However, for the same PEG content, the intrinsic viscosity of the terpolymer with PEG M_w of 2000 is slightly higher than that of polyanhydrides with PEG M_w of 4000. This could also be attributed to the higher chain flexibility of the PEG 4000 in polyanhydride backbone than that of the PEG 2000.[30]

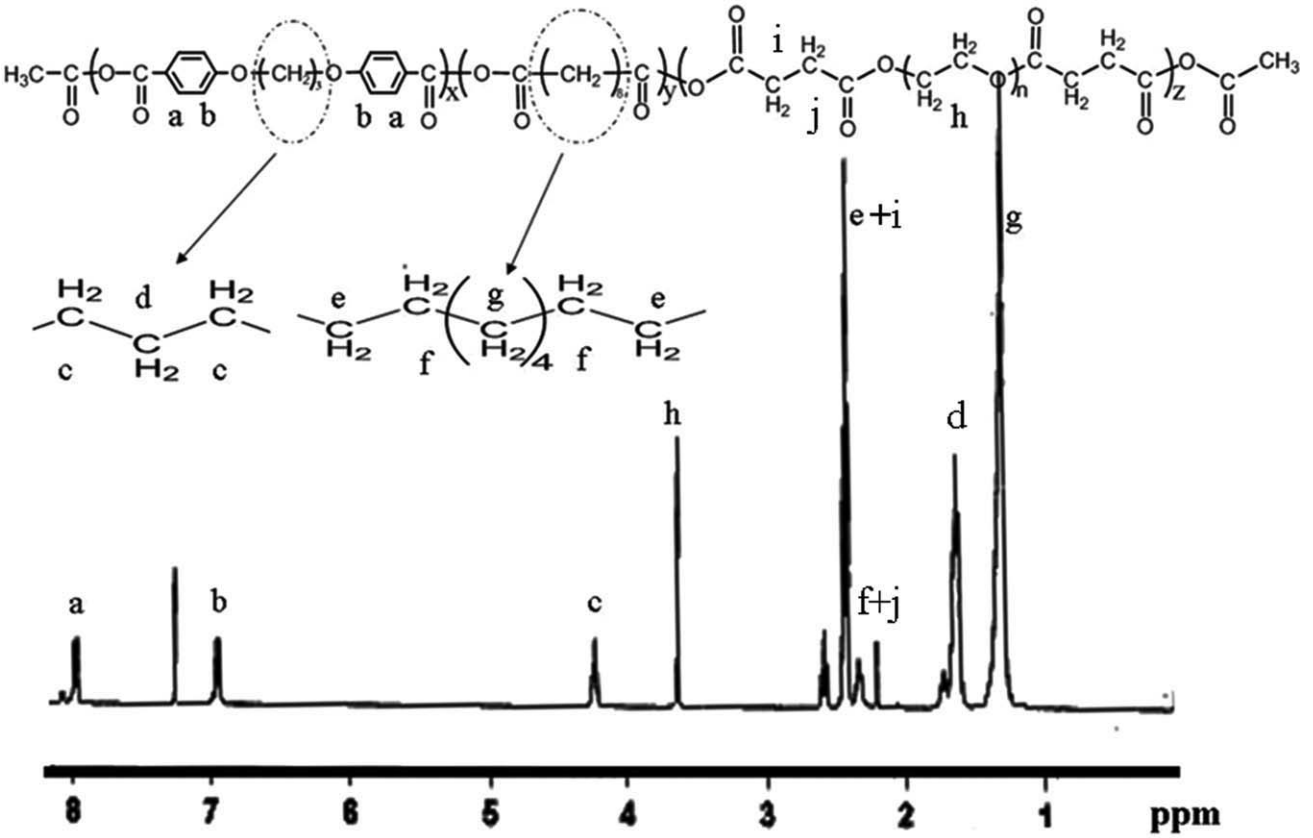


Figure 2 ^{1}H-NMR spectra of poly(PEG4000 : CPP : SA) (20 : 20 : 60).

The terpolymers are also characterized by GPC to determine their Mw (see in Table I). The average M_w varies slightly with the percentage of PEG 2000 and PEG 4000 in the polymer backbone (see in Table IB and C). In this study, terpolymer containing roughly 5 wt % PEG, 20 wt % CPP, and 75 wt % SA has a Mw of 9182 Da, whereas poly(PEG_{2000} : CPP : SA) (30 : 20 : 50) has a M_w of 7100 Da. For the different amount of PEG in the copolymers, their PDI ranged between 1.7 and 2.5, which indicates that the Mw of terpolymers have possessed a slight narrow distribution. Thus, the polymers synthesized in this experiment could be processed into micelles using the precipitation/solvent evaporation method.

For degradable polymers, the crystallinity is an important factor that controls polymer erosion.[31,32] When applied as a drug delivery system, polymeric materials with higher crystallinity may result in a lower rate of release. Therefore, the effect of polymer composition on crystallinity was studied. The propensity of a copolymer to crystallize depends to a considerable extent on whether the monomer units are three-dimensional, similar in shape and size, and on the differences between the lateral dimensions of the molecule chains. Figure 3(A) displays the typical XRD patterns of the poly(PEG : CPP : SA) terpolymers composed of PEG 2000 series. The XRD result of all the terpolymers displays characteristic patterns of the SA chains. As the content of PEG increases, there is not any variety in the angle of the crystalline peaks compared with that of poly(CPP : SA),[33] which suggests that the incorporation of PEG has no effect on the crystallization nature of poly(CPP : SA). When a noncrystallizable unit is introduced into a crystallizable monomer, a decrease in crystallinity may occur. Seen from Table I, the decrease of crystallinity in our case is a direct result of the increase of PEG content and the decrease of SA content in the terpolymers. This result also indicates that PEG chains can hinder the growth of SA and CPP crystals. The shorter the chain of PEG is, the easier PEG chain moves during the crystallization of the copolymer. Therefore, at the same content of PEG, when the Mw decreases, the crystallinity of the copolymers increased. The effect of PEG component on the crystallization of the copolymers was further characterized with POM with a heat stage to observe the crystal morphology. It is noted that upon drying at 45°C, the crystallization of poly(CPP : SA) (20 : 80) and poly(PEG_{4000} : CPP : SA) (5 : 20 : 75) occurs.

Evidence for this phonomenone is provided by POM, as a characteristic spherulite structure of crystal is observed in Figure 3(B). There existed much more little crystal spherulites for poly(CPP : SA)

TABLE I
Characterization of Terpolymers from PEG 1000, PEG 2000, and PEG 4000

Polymer	Feed ratio[a] (wt %)	Yield (w %)	Factual ratio[b] (wt %)	M_w[c] (Da)	PDI[c]	Viscosiy [η][d] (dL/g)	CA degree ± S.D.	T_{m1}[e] (°C)	T_{m2}[e] (°C)	Crystallinity[f]
PEG_{1000} : CPP : SA	5 : 20 : 75	75.3	4.9 : 19.5 : 75.6	4361	2.23	0.1744	68.9° ± 3.0°	g	g	43.0
	10 : 20 : 70	72.5	9.2 : 19.7 : 71.1	3555	2.11	0.1647	64.5° ± 3.7°	43.6	72.2	34.7
	20 : 20 : 60	68.9	17.6 : 18.9 : 63.5	2726	1.86	0.1415	27.9° ± 3.9°	40.5	70.9	30.2
	30 : 20 : 50	65.0	26.7 : 18.7 : 54.6	2052	1.76	0.1253	24.2° ± 4.6°	38.6	68.4	22.8
PEG_{2000} : CPP : SA	5 : 20 : 75	78.6	4.6 : 19.7 : 75.7	9182	2.16	0.4472	68.5° ± 4.0°	g	g	47.8
	10 : 20 : 70	73.2	8.9 : 18.7 : 72.4	8325	2.03	0.4754	64.4° ± 2.7°	53.0	73.1	40.2
	20 : 20 : 60	70.9	18.6 : 17.9 : 63.5	7685	1.87	0.3406	45.2° ± 1.9°	51.2	71.7	33.7
	30 : 20 : 50	66.7	27.7 : 19.7 : 52.6	7100	1.77	0.2720	32.7° ± 4.9°	48.0	67.8	25.3
PEG_{4000} : CPP : SA	5 : 20 : 75	74.3	4.7 : 19.6 : 75.7	9332	2.48	0.3935	50.0° ± 5.0°	g	69.3	53.0
	10 : 20 : 70	71.5	8.7 : 19.9 : 71.4	8997	2.13	0.3731	48.6° ± 1.7°	g	g	46.6
	20 : 20 : 60	64.9	18.6 : 18.8 : 62.6	7787	2.25	0.3600	45.2° ± 1.9°	g	g	35.6
	30 : 20 : 50	61.3	28.7 : 19.7 : 51.6	7601	2.38	0.3162	33.8° ± 2.5°	54.1	66.2	28.2

[a] Poly(ether-anhydrides)were synthesized by melting polymerization.
[b] Estimated from the integral height of hydrogen atoms in the ^{1}H NMR spectra.
[c] Obtained by GPC.
[d] Determined using a viscometer.
[e] Determined by DSC.
[f] Calculated using X-ray diffraction.
[g] Not detected.

(20 : 80) than that for poly(PEG_{4000} : CPP : SA). However, the average size of poly(CPP : SA) and poly(PEG_{4000} : CPP : SA) crystal is 15 and 20 μm, respectively. Almost no crystallization is observed as PEG content is over 5 wt % in the poly(PEG_{4000} : CPP : SA) terpolymers. This indicates that PEG chains would clearly hinder the growth of SA and CPP crystals. The size of the spherulites becomes larger with the 5 wt % introducing of PEG into poly(CPP : SA) backbone, which indicates that the variety of the crystallization could be related to the decrease of relative amount of SA segments or the increasing freedom of polymer chain movement because of the addition of PEG chains. The crystallite structures are determined mostly by the component with the high percentage in the terpolymers,[33] namely, here determined by SA. This property is of great importance, especially in studying erosion phenomena in polymers, crystalline regions will erode more slowly than amorphous regions, and the type of crystals that form the crystalline region may determine the erosion rate.

Thermal analysis was further performed to the terpolymers as shown in Table I. The melting temperature (T_m) of the terpolymers made of PEG 2000 was determined with DSC. The information could easily be obtained by the melting peaks in the DSC curves. No glass transition temperature is observed for any of the polymers ranging from −100 to 200°C, which was consistent with the previous report.[27] Poly(PEG_{2000} : CPP : SA) (10 : 20 : 70) and poly(PEG_{2000} : CPP : SA) (20 : 20 : 60) exhibited melting endotherms at 55.9°C and 73.1°C and at 56.2°C and 71.7°C, respectively. The appearance of two distinct melting points implied that there existed a phase separation between PEG- and SA-rich regions at high PEG percentages.[31] The endotherm at 55.9°C and 56.2°C corresponded to the melting of crystalline regions of PEG, whereas the endotherm at 73.1°C and 71.7°C offered by SA segments. A little decrease in the melting points of the SA segments

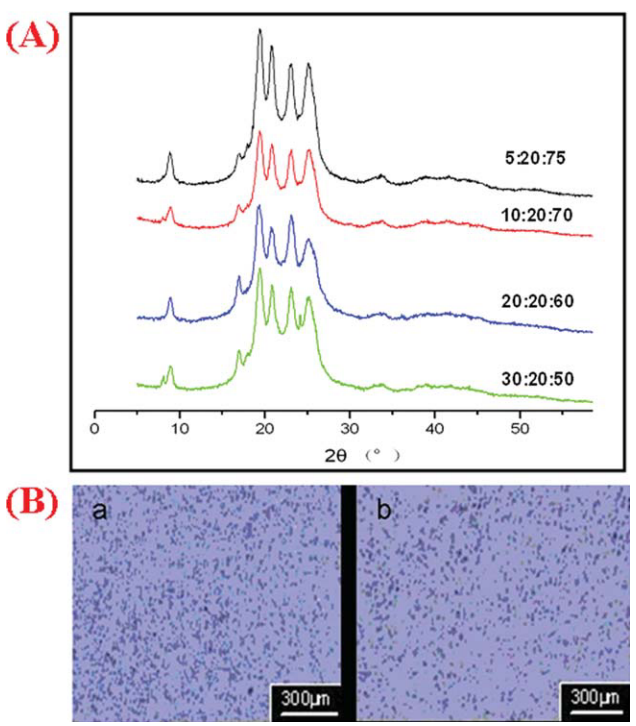


Figure 3 X-ray diffraction patterns of poly(PEG2000 : CPP : SA) terpolymers (A) and the POM images of P(CPP-SA) and poly(PEG4000 : CPP : SA) (5 : 20 : 75) (B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
The Average Particle Diameter of the Polymeric Micelles

Polymer	Feed ratio (wt %)	The Z-average diameter (nm)	Polydispersity
PEG_{1000} : CPP : SA	5 : 20 : 75	359 ± 34.0	0.401
	10 : 20 : 70	299 ± 27.0	0.374
	20 : 20 : 60	274 ± 36.0	0.298
	30 : 20 : 50	161 ± 33.0	0.275
PEG_{2000} : CPP : SA	5 : 20 : 75	304 ± 24.0	0.389
	10 : 20 : 70	231 ± 18.0	0.345
	20 : 20 : 60	205 ± 31.0	0.284
	30 : 20 : 50	151 ± 17.0	0.255
PEG_{4000} : CPP : SA	5 : 20 : 75	274 ± 13.0	0.298
	10 : 20 : 70	198 ± 36. 0	0.189
	20 : 20 : 60	169 ± 18.0	0.177
	30 : 20 : 50	118 ± 23.0	0.156

because of the SA content was reduced. This also indicates that PEG chains depress SA crystallinity. The crystallization trend of the terpolymers made of PEG 1000 and PEG 4000 was almost similar with it. Only the SA melting point (69.3°C) is observed when PEG content was less than 10% for the poly(PEG_{4000} : CPP : SA) (5 : 20 : 75), and poly(PEG_{4000} : CPP : SA) (30 : 20 : 50) exhibited melting endotherms at 54.1°C and 66.2°C. At the same PEG content, a decrease in the melting points of SA portions as the PEG chains became longer, which could have also resulted from the crystallinity of the poly(PEG : CPP : SA) terpolymers with long PEG chains increasing. However, no glass transition temperature (T_g) was detected between −100 and 200°C. Compared the poly(CPP : SA) copolymers with the poly(PEG : CPP : SA) terpolymers, the disappearance in T_g was mainly induced by soft chain segments of PEG.

The hydrophilicity of the poly(PEG : CPP : SA) should be improved if PEG segments have been introduced into p(CPP-SA) chains. The water contact angle (CA) was used to evaluate it, which was displayed in Table I. The CA of the poly(CPP : SA) (20 : 80) was 71.3 ± 5.3°. On the whole, the hydrophilicity of the terpolymers increased with PEG content in copolymer increasing. Especially, for low molecular weight (M_w < 2000 Da) of PEG, the CA values reduce sharply when PEG content arrived at 20%. During the contact angle test, it was noticed that the tiny drop of water gradually expanded on the polymer surface when PEG content in copolymers was more than 20 wt %, which was also a sign of increased hydrophilicity attributed to the introduction of PEG segments into the polyanhydrides backbones successfully. Comparatively, at the same PEG content, the CA values were reduced with the decreasing of Mw of PEG. In other words, the hydrophilicity of the terpolymers was reduced as the decreasing of the length of PEG Chains. This mainly resulted from the higher hydrophilicity of low M_w of PEG than that of high M_w of PEG. The CA values also indicated that the terpolymers hold amphiphilic property and furthermore, the property can be altered by adjusting the Mw of PEG and the content of PEG component. Thus, polymeric micelles could be fabricated based on the amphiphilic structure of poly(PEG : CPP : SA) terpolymers.

Characterization of micelles

Here, a precipitation method was used for preparation of micelles without any other surfactant. The micelles were fabricated from the amphiphilic structure of poly(PEG : CPP : SA) terpolymer, which possessed self-emulsification function as a macromolecule surfactant. The average particles diameter and their polydispersity were measured by DLS and shown in Table II.

As shown in Table II, the diameter of the micelles decreases with the increase of the PEG content. The size distribution of micelles also becomes narrower with the increasing of the PEG content in the terpolymers. The main reason is that with the increasing of the PEG amounts the hydrophilicity of the terpolymers is also improved. A hydrophilic segment can form the outer shell to the micelles surface. A total of 30 wt % hydrophilic PEG blocks of the terpolymers can interact with water molecules by hydrogen bonding more easily than 5 wt % hydrophilic PEG of the polymer. As a result, smaller diameters values were obtained with the 30 wt % PEG content compared with the terpolymer with PEG amounts below 30 wt %. It is also of interest to compare the micellization properties of copolymers synthesized with different M_w of PEG.

The difference may be ascribed to the fact that the change of PEG chains has affected the PEG/(CPP + SA) ratio. The hydrophilicity of terpolymers mainly depends on the PEG/(CPP + SA) ratio which might have much influence on the micelles properties. At

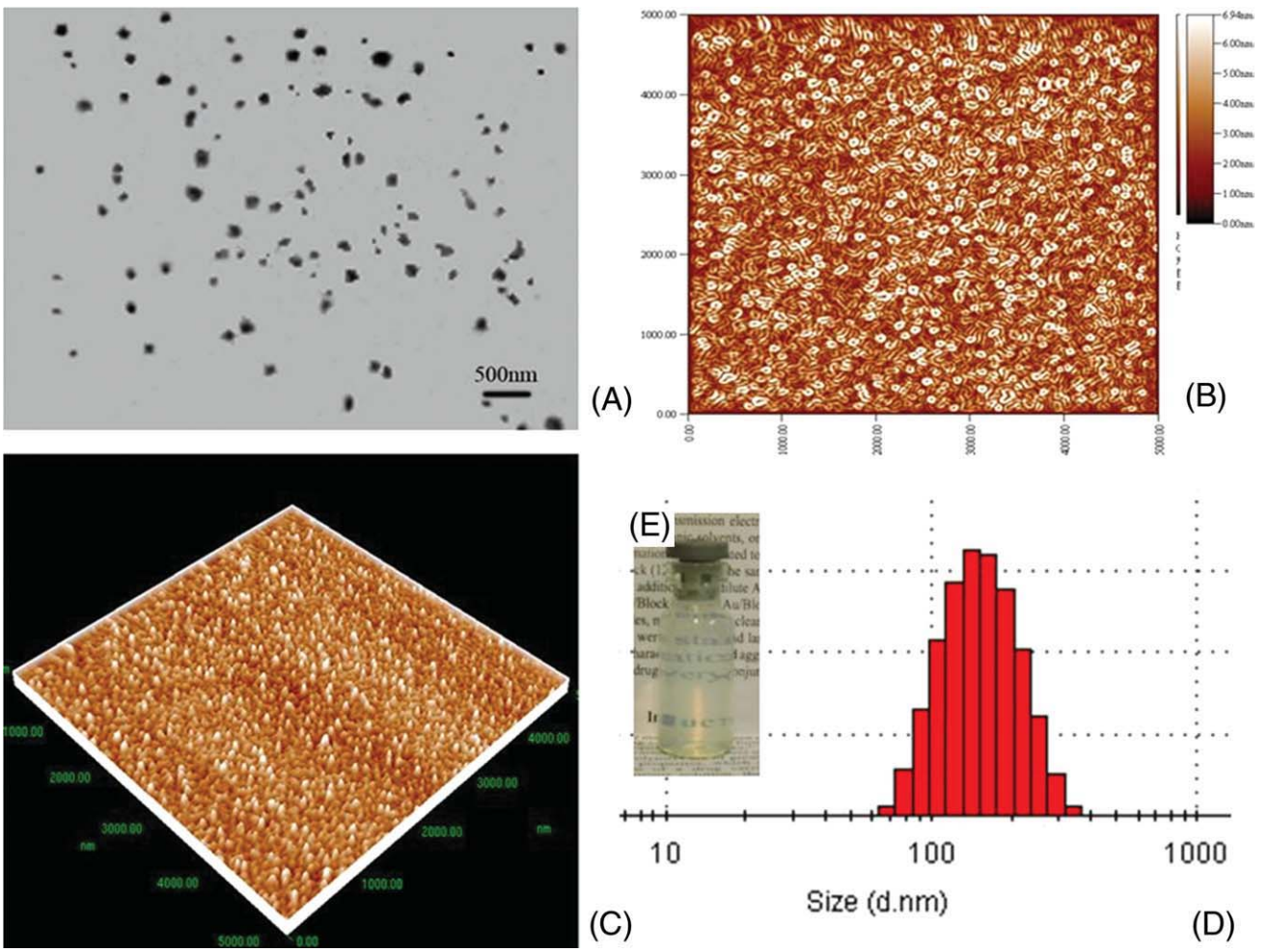


Figure 4 Morphologies of the micelles of poly(PEG4000 : CPP : SA) (30 : 20 : 50) (2 mg/mL): (A) TEM image of the micelles, (B) AFM plane image of the micelles, (C) AFM three-dimension image of the micelles, (D) The *Z*-average diameter obtained by DLS, (E) The photograph of the micelles solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the same PEG content, terpolymers made of the longer PEG chains are more inclined to form smaller micelles than terpolymers with the shorter PEG chains due to the different structures. The reason why smaller size can be obtained from the longer PEG chains could be attributed to the more compact structure with stronger interactions between the longer PEG chains.

This micellles solution (2 mg/mL) was dropped on a clean silicon wafer for AFM observation. The sample (2 mg/mL) for TEM observation was obtained after slow evaporation of solvent at room temperature. The structures of the micelles were examined by TEM and AFM. Polymeric micelles are created by a spontaneous self-assembly of individual polymeric molecules (unimers), which are synthetic amphiphilic copolymers comprised of hydrophilic and hydrophobic blocks. Usually, micelles are spherically shaped core-shell structures with a hydrophobic core and hydrophilic shell.[34,35] Quasi spherical micelles with diameters typically in the range of 90–120 nm are observed in Figure 4(A). At the same time, we can find that their dispersing was very uniform. Figure 4(D) displays that size measurements using DLS yielded a *Z*-average diameter of about 118 nm, which is consistent with TEM image. The micelles based on poly(PEG_{4000} : CPP : SA) (30 : 20 : 50) in water are very stable and almost transparent as shown in Figure 4(E). The micelles formed by poly(PEG : CPP : SA) terpolymers in pure water have a quasi spherical surface structure with *Z*-average diameters ranged from 118 to 359 nm.

AFM technique has been widely applied to obtain surface morphological information of micelles.[36] Figure 4(B,C) also give the tapping mode AFM images of poly(PEG : CPP : SA) micelles. The observed AFM image of the micelles might result from the collapse of micelle during drying process. It can be seen from Figure 4(B) that the collapse of poly(PEG : CPP : SA) micelles also seemed to be spherical, and their dispersion in water is very uniform, which is in accordance with TEM image. The

diameters of spherical micelles are observed typically in the range of 90–120 nm [Fig. 4(C)]. The diameters observed from AFM and TEM images are slightly smaller than a *Z*-average diameter due to the dehydration and shrinkage of the micelles during drying.[37]

The precipitation method relies on hydrodynamic instability to break up an immiscible polymeric solution into droplets, which subsequently form microscaled particles through cross-linking. The size of micelles is generally less than 150 nm. The polymeric micelles with a size of less than 200 nm would reduce nonselective RES scavenging and show enhanced permeability and retention (EPR) effects at solid tumor sites for passive targeting.[38] Furthermore, because of their submicron size (typically between 10 to 200 nm), intravenous administered polymeric micelles provide possibilities to reach the pathological sites, whereas avoiding biological barriers in the human body upon oral administration, such as limited gastrointestinal absorption and high hepatic first-pass effect.[39]

CONCLUSIONS

A series of poly(ether-anhydrides) terpolymers containing various ratios of SA, CPP, and PEG with three kinds of Mw and four kinds of content were synthesized by a melt polycondensation process. The M_w of the terpolymers increased as the PEG amount decreased or as PEG M_w increased. The crystallinity and the hydrophilicity of the terpolymers can be controlled by varying PEG amount in the polymer backbone. The average M_w of the terpolymers could be adjusted by altering the M_w and content of PEG units. The micelles with the size ranged from 118 to 359 nm fabricated with the terpolymers seemed to be spherical, and their dispersion in water was very uniform because of the amphiphilic structure of the terpolymers. The size of the micelles decreased with PEG content increasing. Therefore, the terpolymers could find potential application as a novel vehicle in drug controlled release system. In our future research, it is of great interest to focus on the investigation of the properties of the micelles used for cancer therapy.

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