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Synthesis and characterization of novel amphiphilic block copolymers based on maltoheptaose and poly(ε-caprolactone)

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ABSTRACT

Novel amphiphilic block copolymers consisting of hydrophilic maltoheptaose segments and hydrophobic poly(ε-caprolactone) (PCL) segments were prepared for the first time, and characterized by ^{1}H NMR spectroscopy and GPC analyses. To obtain such a copolymer, the peracetylated maltoheptaose having a hydroxyl group at the reducing end was synthesized from β-cyclodextrin, and then used to initiate the ring opening polymerization of ε-caprolactone in the presence of stannous octoate. By means of fluorescence technique, transmission electron microscopy and dynamic light scattering, it was found that the resulting block copolymers in aqueous solution could self-assemble into nanosize spherical micelles with a narrow unimodal distribution. Moreover, the critical micelle concentration and the size of the self-assembled micelles could be modulated by changing the PCL content in the resultant block copolymer.

1. Introduction

As a kind of important macromolecules in biological systems, oligo- and polysaccharides have shown their multifunctional characteristics in biological systems in the construction of cell walls, cell recognition, immune response and energy storage. In particular, their combination with some synthetic macromolecules can result in an important class of glycopolymers, which have proven to exhibit enhanced physiological and structural properties for numerous biotechnology and pharmaceutical applications (Ladmiral, Melia, & Haddleton, 2004; Miura, Koketsu, & Kobayashi, 2007; Miyagawa, Carmelita, Kasuya, & Hatanaka, 2007; Okada, 2001; Spain, Gibson, & Cameron, 2007; Zhu, Gosen, & Marchant, 2006).

In recent years, the formation of polymeric micelles by the self-association of diblock copolymers consisting of hydrophilic and hydrophobic segments in aqueous medium has received considerable attention due to their attractive uses to various research areas such as drug delivery carrier technology, detergents, oil recovery, surface coating and technology (Bhatia, Mourchid, & Joanicot, 2001; Liggins & Burt, 2002). In the biomedical materials field, polymeric micelles must possess several specific properties to be of use. These include biocompatibility, biodegradability, target specificity and stability in the body. Hydrophobic poly(ε-caprolactone) (PCL) is one of the most commonly used polyesters with good biocompatibility and biodegradability. Up to now, a number of polymeric micelles formed from PCL-based amphiphilic diblock copolymers have been studied for various biomedical applications, in which poly(ethylene glycol) (PEG) is frequently chosen as a hydrophilic segment. For example, Jette, Law, Schmitt, and Kwon (2004) reported the preparation and drug loading of PCL-*b*-PEG micelles through the evaporation of an azeotrope cosolvent; Choi et al. (2006) carried out the synthesis and physicochemical characterization of amphiphilic PEG-*b*-PCL self-aggregates; Forrest, Won, Malick, and Kwon (2006) investigated the in vitro release of the mTOR inhibitor rapamycin from PEG-*b*-PCL micelles; He et al. (2004) studied the synthesis, crystallization and morphology of diblock copolymers based on PEG and PCL. However, a drawback of these block copolymers is the absence of reactive functional groups on their molecular chains, which limits further modification or ligand coupling. In order to overcome this, Mahmud, Xiong, and Lavasanifar (2006) conducted the ring opening polymerization of *a*-benzyl carboxylate-ε-caprolactone with methoxy poly(ethylene oxide) to develop the micelle-forming poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) copolymers bearing functional side groups on the PCL block; Loontjens et al. (2007) copolymerized ε-caprolactone (CL) with benzyl protected hydroxymethyl glycolide (BHMG) in the melt using benzyl alcohol/tin(II) 2-ethylhexanoate as the initiator system to introduce, after deprotection, hydroxyl groups into the polyester; Timbart, Renard, Tessier, and Langlois (2007) used monohydroxylated poly(3-hydroxyoctanoate) oligomers to initiate the polymerization of ε-caprolactone; Bougard, Jeusette, Mespouille, Dubois, and Lazzaroni (2007) combines the controlled ring opening polymerization of ε-caprolactone and atom

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transfer radical polymerization of *N*,*N*-dimethylamino-2-ethyl methacrylate to prepare amphiphilic PCL-based block copolymers. In contrast, oligo- and polysaccharides seem to be attractive alternatives to PEG hydrophilic segments for designing amphiphilic block copolymers.

In this article, we report the synthesis and characterization of novel amphiphilic block copolymers based on the combination of an oligopolysaccharide segment with a synthetic aliphatic polyester segment. To obtain such a block copolymer, the peracetylated maltoheptaose having a free hydroxyl group at the reducing end (AcMH-OH) was first synthesized from β-cyclodextrin, and then used to initiate the ring opening polymerization of ε-caprolactone. The resulting block copolymers were investigated with respect to their self-aggregation characteristics in an aqueous phase by fluorescence technique, dynamic light scattering and transmission electron morphology. To our knowledge, this is the first report on the biodegradable amphiphilic block copolymers consisting of a naturally occurring oligosaccharide segment and a synthetic aliphatic polyester segment.

2. Experimental

2.1. Materials

β-Cyclodextrin (β-CD) was purchased from Sigma and was recrystallized three times from water and dried in vacuum before use. ε-Caprolactone (ε-CL) was purchased also from Sigma and was dried over CaH_2 for 24 h and distilled prior to use. Toluene was distilled under CaH_2. Stannous octoate and other reagents were of analytical grade and used as received.

2.2. Synthesis of AcMH-OH

AcMH-OH was synthesized via a three-step reaction, as shown in Fig. 1. At first, β-CD (**1**) was acetylated with acetic anhydride (Ac_2O) in pyridine in the presence of catalytic amount of 4,4-(dimethylanino)pyridine (DMAP), giving peracetylated β-CD (**2**) (Haleton & Ohno, 2000). Secondly, the acid-catalyzed ring opening reaction of **2** was carried out in acetic anhydride in the presence of $FeCl_3{\cdot}6H_2O$ as the catalyst at 70 °C for 3.5 h, giving *O*-acetylmaltoheptaose (**3**) (Farkas, Jánossy, Harangi, Kandra, & Lipták, 1997). After that, **3** was treated with benzylamine in THF for 24 h at room temperature to selectively deacetylate at the reducing end of the polysaccharide, giving the peracetylated maltoheptaose having a hydroxyl group at the reducing end (**4**, AcMH-OH) (Yoshida et al., 1999).

2.3. Ring opening polymerization of ε-CL in the presence of AcMH-OH

The ring opening polymerization of ε-CL in the presence of AcMH-OH was performed in a previously dried three-necked round-bottom flask, resulting in the protected block copolymer (**5,** AcMH-*b*-PCL), as shown in Fig. 1. In a typical experiment, a solution of AcMH-OH (1 g, 0.482 mmol) in dry toluene (25 ml) was dried with anhydrous Na_2SO_4 and heated at reflux using a Dean–Stark apparatus until about 15 ml of toluene was evaporated. After that, 1.0 g (8.772 mmol) ε-CL and 88 mg stannous octoate (0.217 mmol) were added successively under an argon atmosphere. Then the temperature was raised to 130 °C, the reaction was maintained for 12 h. After the completion of the reaction, the reaction mixture was poured into a large amount of cold diethyl ether. The resultant precipitate was collected by filtration, and then extracted with the mixed solvent of tetrehydrofuran and diethyl ether for 6 h in order to remove the homopolymer of ε-CL and unreacted AcMH-OH. After simple filtration, the purified precipitate was dried in a vacuum at 40 °C for 48 h, resulting in the protected AcMH-*b*-PCL block copolymer. By changing the molar feed ratios of ε-CL to AcMH-OH, the AcMH-*b*-PCL block copolymer with different block lengths could be synthesized in this way.

2.4. Deprotection of AcMH-b-PCL block copolymer

The protected AcMH-*b*-PCL block copolymer with acetylated oligosaccharide block was firstly dissolved in mixed tetrahydrofurane/methanol solvent (v/v = 1/1). After that, a catalytic amount of $NaOCH_3$ (pH 8) was added. After stirring at room temperature for 3 h, the deprotected block copolymer (**6**, MH-*b*-PCL), as shown in Fig. 1, was recovered by neutralization, precipitation, filtration and vacuum-drying.

2.5. Structure characterization

^{1}H NMR spectra were recorded on Mercury-Plus 300 (Varian, USA) spectrometer, using tetramethylsilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. Gel permeation chromatography (GPC) measurements were conducted at 30 °C with a Waters Breeze GPC instrument equipped with a Waters ultrastyragel column and a differential refractometer detector. THF was used as the eluent at a flow rate of 1.0 ml/min. Polystyrenes with narrow polydispersity were employed as the standards to generate the calibration curve.

2.6. Preparation of micelle solution from MH-b-PCL block copolymer

To prepare the micelle solution from MH-*b*-PCL block copolymer, doubly distilled water was added dropwise (at 15 s per drop) to a THF solution (20 ml) of MH-PCL (5 mg) under a mild stirring. The removal of THF in a rotary evaporator (30 °C, 2 h) afforded the micellar solution.

2.7. Fluorescence measurements

All the fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorophotometer. Pyrene was used as fluorescence probe. The pyrene concentration of the samples was 5.0×10^{-7} mol/l. For the measurement of pyrene emission spectra, emission and excitation slit widths were set at 3 and 5 nm, respectively. The excitation wavelength was set at 330 nm. The ratio (I_1/I_3) of the first and third band intensity at 372 and 383 nm was calculated from the spectra.

2.8. Light scattering measurements

Dynamic light scattering measurements were performed using a Brookhaven BI-200SM Goniometer and BI-9000AT autocorrelation. All the measurements were carried out at 25 °C. The sample solutions were purified by passing through a Millipore 0.45 μm filter. The scattered light of a vertically polarized He–Ne laser (532 nm) was measured at an angle of 90° and was collected on an autocorrelator. The COUTIN method was employed to determine the size and size distribution.

2.9. TEM observation

Transmission electron microscopy (TEM) (Japan, JEM-2010HR) was used to observe the morphology of the micelles. Samples were placed onto a copper grid coated with carbon. They were dried at room temperature, and then examined by negative staining with an aqueous solution of phosphotungstic acid.

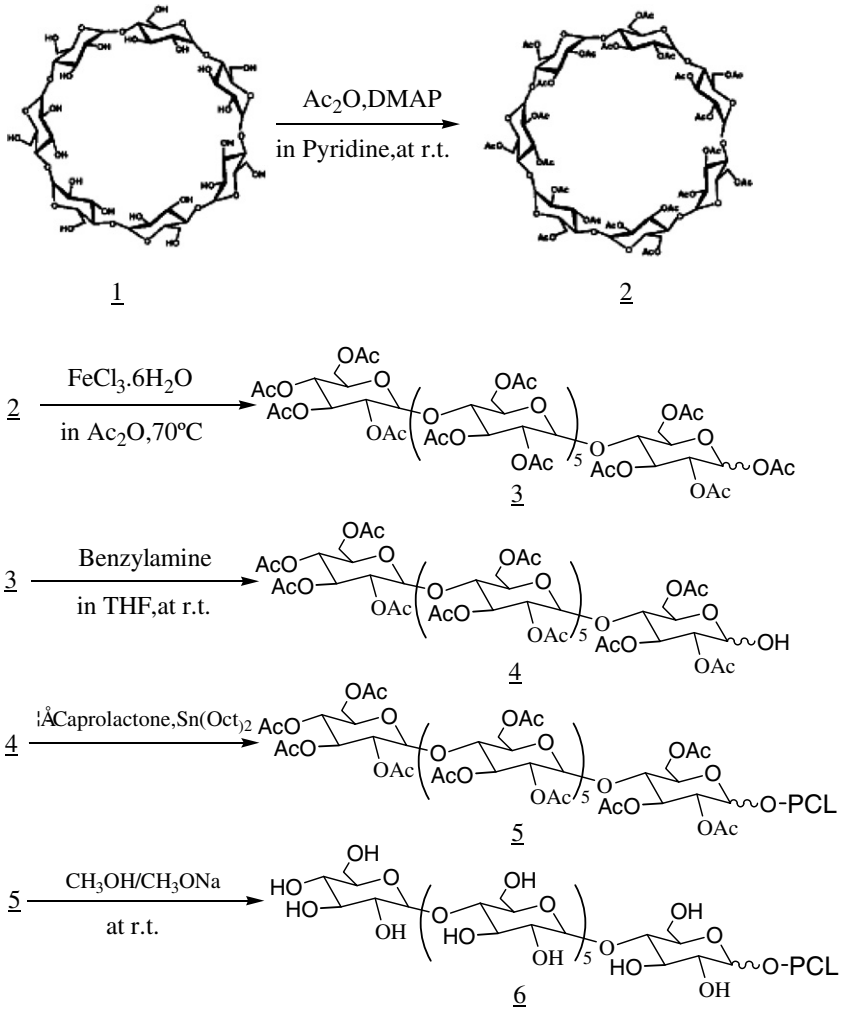

Fig. 1. The synthesis route of amphiphilic MH-*b*-PCL block copolymers.

3. Results and discussion

The synthesis route of maltoheptaose-*b*-poly(ε-caprolactone) (MH-*b*-PCL) block copolymer was shown in Fig. 1. The macroinitiator, AcMH-OH, was firstly prepared from commercially available β-CD. The hydroxyl group at the reducing end of AcMH-OH was used as the initiation site for the ring opening polymerization of cyclic ε-caprolactone (ε-CL) in the presence of stannous octoate as the catalyst. In this work, we synthesized two AcMH-*b*-PCL block copolymers, namely AcMH-*b*-PCL1 and AcMH-*b*-PCL2, by changing the molar feed ratios of AcMH-OH to ε-CL (Table 1). To make sure the ring opening polymerization occurred, the synthesized copolymer was investigated by ^{1}H NMR spectroscopy and GPC analysis. Typical ^{1}H NMR spectrum of AcMH-*b*-PCL block copolymer (AcMH-*b*-PCL2), illustrated in Fig. 2a, shows not only the characteristic resonance peaks of PCL block at 1.40, 1.66, 2.33 and 4.07 ppm but also the characteristic resonance peaks of AcMH block at 1.9–2.3 (acetyl group protons) and 3.5–5.5 ppm. The number-average molecular weights ($M_{n,\ NMR}$) of two AcMH-*b*-PCL block copolymers and the content of the incorporated PCL were calculated from the integration data of ^{1}H NMR spectra, and summarized in Table 1. From the GPC curves shown in Fig. 3, the single peak was observed for two AcMH-*b*-PCL block copolymers and AcMH-OH. This result indicates the absence of unreacted PCL. In comparison with AcMH-OH, two AcMH-*b*-PCL block copolymers show a wider GPC peak and a shorter elution time. This result confirms further the success of the reaction. The GPC-derived number-average molecular weights ($M_{n,\ GPC}$) and the polydispersity index (M_w/M_n) were also included in Table 1 for two AcMH-PCL block copolymers.

To obtain the deacetylated product (MH-*b*-PCL block copolymers), the resulting AcMH-*b*-PCL block copolymers were treated

Table 1
Two AcMH-PCL block copolymers synthesized in this work

Sample	AcMH/ε-CL (feed molar ratio)	ε-CL conversion[a](%)	Yield(%)	M_n, ^{1}H NMR [b]	M_n, GPC	M_w/M_n [c]
AcMH-*b*-PCL1	1.0/11.0	92.6	39.1	7950	9181	1.28
AcMH-*b*-PCL2	1.0/18.2	90.1	41.6	9310	10950	1.35

[a] Estimated by ^{1}H NMR from the integrated areas of δ 1.40 ppm for PCL and δ 2.64 ppm for ε-CL.
[b] Calculated by $M_n = M_{n,\ AcMH}$ (2076) + 114 n, n was determined by ^{1}H NMR from the integrated areas of δ 1.9–2.3 ppm and δ 1.40 ppm.
[c] Estimated by GPC.

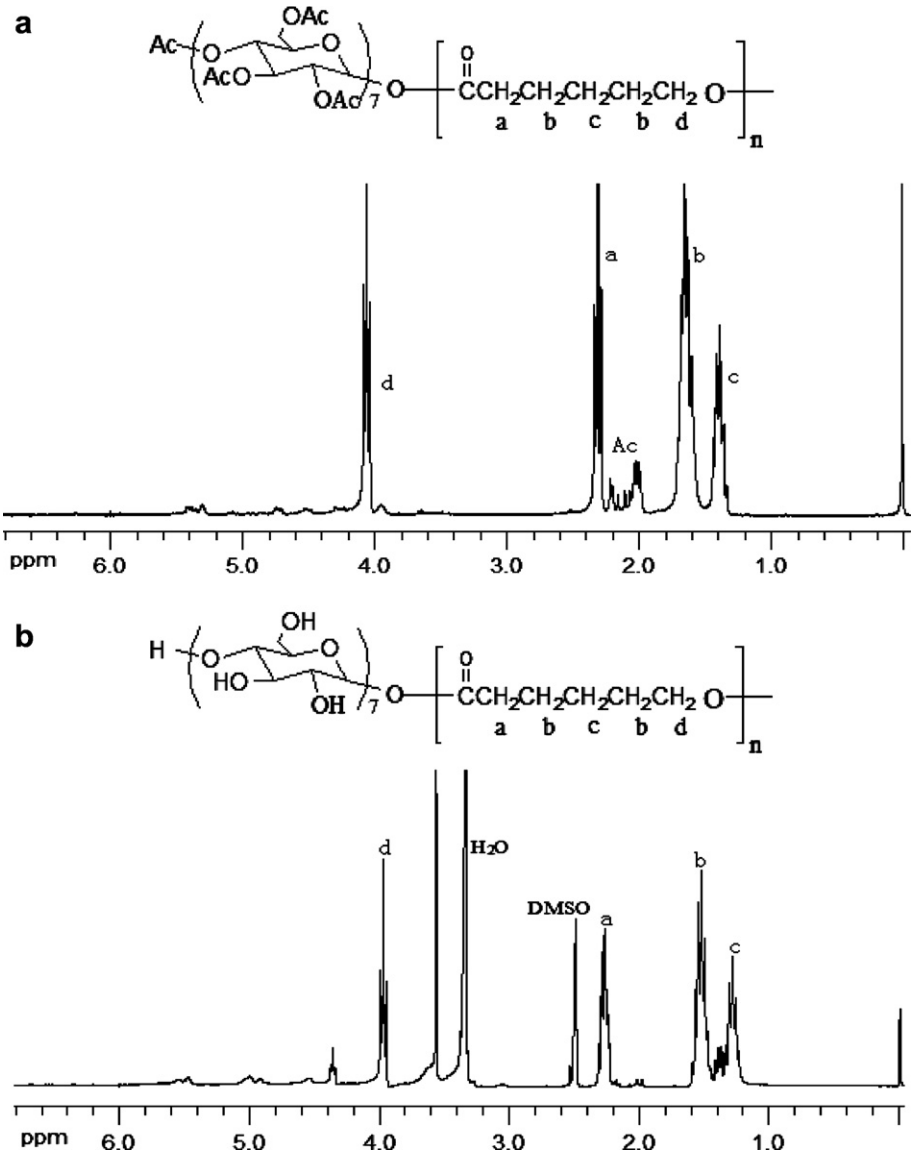

Fig. 2. ^{1}HNMR spectra of AcMH-*b*-PCL2 (a) in $CDCl_3$ and MH-*b*-PCL2 (b) in DMSO.

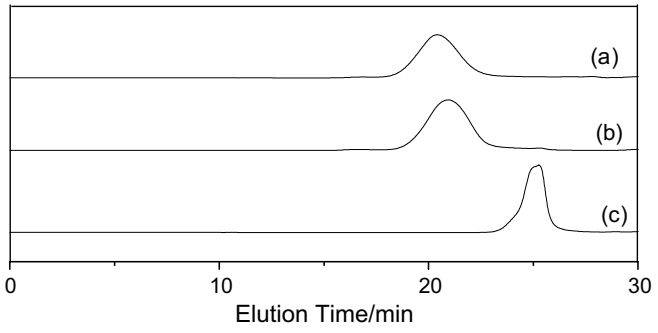

Fig. 3. Gel permeation chromatograms of AcMH-*b*-PCL2 (a) AcMH-*b*-PCL1 (b) and AcMH-OH (c).

with sodium methoxide in a mixture of tetrahydrofurane and methanol to deprotect the acetyl groups from the AcMH block. Fig. 2(b) shows typical ^{1}H NMR spectrum of MH-*b*-PCL2 that corresponds to AcMH-*b*-PCL2. As seen, the signals of acetyl group protons of AcMH-*b*-PCL2 at 1.9–2.3 ppm, shown in Fig. 2a, have completely disappeared in Fig. 2b after the deprotection. In addition, the signals resulting from PCL block and MH block still retain. These results confirm the successful removal of the acetyl groups.

The amphiphilic MH-*b*-PCL block copolymers, consisting of hydrophilic MH and hydrophobic PCL blocks, were investigated with respect to their micelle behavior in aqueous media using a fluorometer in the presence of pyrene as a fluorescent probe. It is known that the variation in the ratio (I_1/I_3) of intensity of first (372 nm) to the third (383 nm) vibronic peaks, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where the pyrene is located (Nouvel et al., 2004). Fig. 4 gives the change of the I_1/I_3 value with the concentration (C) for the two block copolymers prepared in this study. At the lower concentrations, the I_1/I_3 values have a little change. Further increase of the concentration results in a much significant decrease of the intensity ratio, implying the formation of the polymeric micelle. The critical micelle concentration (*cmc*) was determined to be 3.0 mg/l for MH-*b*-PCL1 and 2.4 mg/l for MH-*b*-PCL2, respectively, by the interception of two straight lines as shown in Fig. 4. It seems that the *cmc* value decreases with the increase of hydrophobic PCL content in the MH-PCL block copolymer. Compared with low

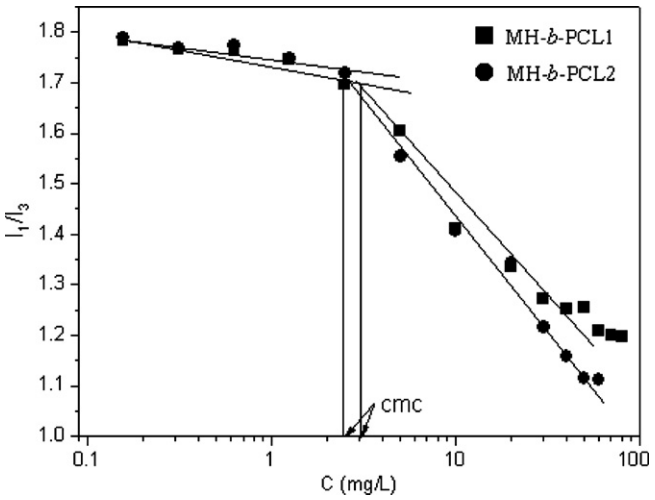

Fig. 4. The change of I_1/I_3 with the concentration for two MH-*b*-PCL block copolymers.

molecular weight surfactants (Zhang, 2001), such an amphiphilic block copolymer has lower *cmc* values.

The morphology of the formed micelles was investigated by the transmission electron microscopy (TEM) technique. Fig. 5 shows the TEM images of polymeric micelles for two MH-*b*-PCL block copolymers. As seen, the shape of the formed micelles in water is near spherical. Further investigation was carried out on the size of polymeric micelles and their size distribution by dynamic light scattering (DLS) measurements. As shown in Fig. 6, all micelle aggregates have a unimodal size distribution, and the mean diameters of MH-*b*-PCL1 and MH-*b*-PCL2 were found to be 117.6 and 123.7 nm, respectively. The micelle sizes measured by DLS are greater than those observed by TEM (Fig. 5), which is probably due to the deformation of the micelles during the drying process for the TEM observation. In addition, the polydispersity factors of the formed micelles were found to be 0.135 for MH-*b*-PCL1 and 0.169 for MH-*b*-PCL2, respectively. These results implied that the synthesized block copolymers had a narrow size distribution.

4. Conclusions

Amphiphilic MH-*b*-PCL block copolymers could be prepared by the ring opening polymerization of ε-CL in the presence of AcMH-OH and subsequent deprotection of the peracetylated maltoheptaose block. The resulting block copolymers could self-assemble into spherical micelles in the aqueous phase, with a narrow unimodal distribution. By changing the content of hydrophobic PCL block in the copolymer, the *cmc* value and the size of the self-assembled micelles could be modulated. It is expected that such a block copolymer may have excellent biodegradability and find numerous biotechnology and pharmaceutical applications.

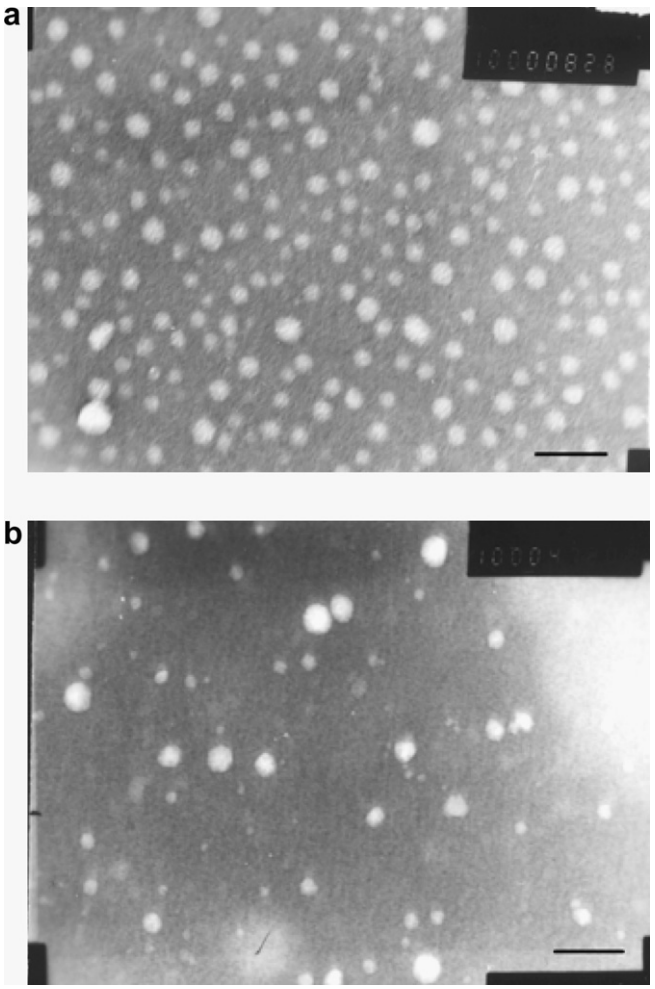

Fig. 5. TEM images of polymeric micelles for MH-*b*-PCL1 (a) and MH-*b*-PCL2 (b).

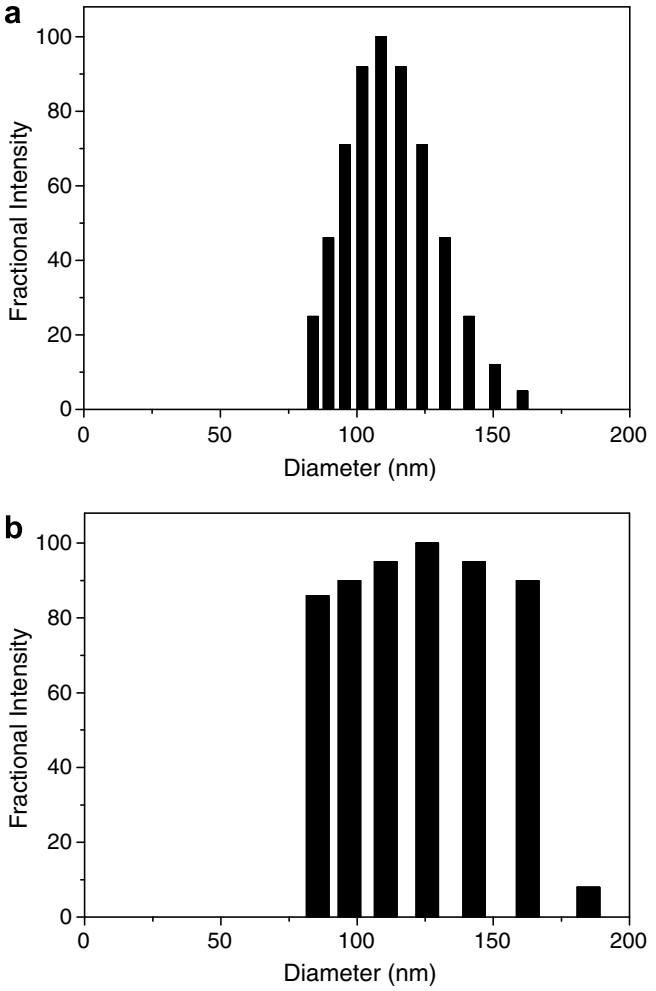

Fig. 6. Size distribution of MH-*b*-PCL1 micelles (a) and MH-*b*-PCL2 micelles (b) in aqueous environment.

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