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Novel biodegradable poly(ethylene glycol)-*block*-poly(2-methyl-2-carboxyl-propylene carbonate) copolymers: Synthesis, characterization, and micellization

Zhigang Xie[a,b], Huili Guan[a,b], Li Chen[a,b], Huayu Tian[a,b], Xuesi Chen[a,b], Xiabin Jing[a,b,*]

[a]*State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China*
[b]*Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China*

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Abstract

A novel amphiphilic biodegradable poly(ethylene glycol)–poly(2-methyl-2-carboxyl-propylene carbonate) (MPEG–PMBC) block copolymer was successfully synthesized by ring-opening polymerization (ROP) of 2-methyl-2-carboxyl-propylene carbonate (MBC) with MPEG as a macroinitiator. ^{13}C NMR and GPC confirmed its structure. It could self-assemble into micelles in aqueous solution with the critical micelle concentration (cmc) as low as $\sim 10^{-3}$ g/l. The cmc decreased with increasing PMBC content. DLS analyses of the MPEG–PMBC micelles revealed their size of less than 100 nm and narrow size distribution. The micelle size depends much on the copolymer composition and micelle preparation conditions. The morphology of micelle observed by ESEM is spherical. The obtained polymeric micelles are expected to be used as biomedical materials such as drug delivery vehicles.

Keywords: 2-Methyl-2-carboxyl-propylene carbonate; Block copolymer; Micellization

1. Introduction

Amphiphilic block and graft copolymers can self-assemble into micelles. The micelles have unique characteristics such as nanosize, a core-shell architecture, and a good thermodynamic stability in physiological condition because of their low critical micelle concentration (cmc) [1–5]. Polymeric micelles are currently a topic of great interest. This interest is motivated by the attractive applications of polymeric micelles in various research areas such as detergents, surface coating, oil recovery, drug delivery carrier technology, and nanotechnology [6–9].

The hydrophilic block is typically poly(ethylene glycol) (PEG), also known as poly(ethylene oxide) (PEO), although other polymers have been investigated, including poly(2-ethyl-2-oxazoline) and poly(*N*-vinyl pyrrolidone) [10,11]. An important aspect of PEG is its tremendous water solubility and hydrophilicity; a significant amount of water molecules can be bound onto its backbone via H-bonding. With a PEG-based shell, the microparticles formed can avoid the adsorption of proteins and adhesion of cells in biological media. Typically, the molecular weight of PEG used in micelles has varied between 2000 and 12,000 g/mol, and is usually of a length that is greater than or equal to the length of the core-forming block.

Up to date, amphiphilic block copolymers containing poly(acrylates) or poly(acrylic acid) have been studied [12–17]. Recently, biodegradable and biocompatible polymers such as poly(lactide) (PLA) [18–21], polycaprolactone (PCL) [22–26], poly(γ-benzyl L-aspartate) (PLBA) [27,28] and poly(γ-benzyl L-glutamate) (PLBG) [29,30] have been used as the core material of micelles. We now propose the use of block copolymers containing a hydrophobic polycarbonate block for preparation of micellar delivery systems. To our knowledge, amphiphilic diblock

* Corresponding author. Address: State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China. Tel.: +86 431 5262112; fax: +86 431 5685653.
E-mail address: xbjing@ciac.jl.cn (X. Jing).

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Table 1
Related data on MPEG-b-PMBC diblock copolymers

Sample	[M]/[MPEG][a]	W_{PEG}/W_{PMBC}[b]	M_n(g/mol)[b]	M_n(g/mol)[c]	M_w/M_n[c]	M_w(g/mol)[d]	cmc (g/l)
PMBC23	6	77/23	6500	9000	1.06	1.104×10^4	7.44×10^{-3}
PMBC30	10	70/30	7100	11,000	1.21	1.511×10^4	3.00×10^{-3}
PMBC48	40	52/48	9600	13,000	1.10	2.628×10^4	0.82×10^{-3}

Polymerization was carried out at 110 °C for 4 h. Molar ratio of MBC to $ZnEt_2$ was 200.
[a] Molar ratio of monomer (MBC) to MPEG in feed.
[b] Determined by ^1H NMR in $CDCl_3$ solution.
[c] Determined by GPC in $CHCl_3$ at 35 °C.
[d] Determined by light-scattering in $CHCl_s$ at 25 °C.

copolymers containing a hydrophobic polycarbonates block have been rarely reported [31].

During the past decade, increasing attention has been paid to aliphatic polycarbonates because they are biocompatible, biodegradable, and nontoxic [32–36]. For example, poly(trimethylene carbonate) (PTMC) has been synthesized by solution or bulk polymerization with a variety of initiators [37]. In addition, TMC has been copolymerized with glycolide, lactide, caprolactone and other carbonates [38,39]. Recently, a few groups have also reported on the synthesis of amphiphilic block copolymers containing polycarbonate blocks, such as ABA triblock copolymers with PEG as the central block and PTMC, poly(5-methyl-5-methoxycarbonyl-1,3-dioxane-2-one) (PMMC) or poly(5-benzyloxy-trimethylene carbonate) (PBTMC) as the lateral blocks [31,40].

In this paper, we report the synthesis of diblock copolymers containing PMBC as the hydrophobic block. The ring opening polymerization of 2-methyl-2-carboxyl-propylene carbonate (MBC) was performed using monomethoxy-terminated poly(ethylene glycol) (MPEG) as a macroinitiator and diethyl zinc ($ZnEt_2$) as a catalyst. The molecular structure and amphiphilic properties of the diblock copolymer obtained were characterized.

2. Experimental section

2.1. Materials

Monomethoxy-poly(ethylene glycol) with a molecular weight of 5000 was purchased from Aldrich. Prior to use, MPEG was dried by an azeotropic distillation in toluene. Diethyl zinc ($ZnEt_2$) was kindly supplied by Prof Wang Xianhong in Changchun Institute of Applied Chemistry. MBC was prepared according to Ref. [41]. Toluene were dried and distilled from sodium/benzophenone under a nitrogen atmosphere before use. Other reagents were commercially available and used as received.

2.2. Synthesis of MPEG–PMBC

The diblock copolymers of MPEG–PMBC were synthesized using $ZnEt_2$ as the initiator precursor. Under the protection of argon, prescribed amounts of MPEG, MBC were added to a round-bottom flask and dried by toluene azeotropic distillation. A stoichiometric amount of $ZnEt_2$ in toluene solution also added to the flask. The reaction mixture was then heated to 110 °C in an oil bath and stirred at this temperature for 4 h. The block copolymer was precipitated into an excess of cold hexane. The copolymer was then isolated by filtration and dried under vacuum at room temperature.

2.3. Characterization of the block copolymer

^{13}C NMR and ^1H NMR spectra were recorded on a Bruker AV 300M in $CDCl_3$ at 25 °C. Chemical shifts were given in parts per million from tetramethylsilane. The percentage of PMBC was calculated from the peak intensities of the phenyl proton signal (7.3 ppm) of PMBC and the ethylene proton signal (3.6 ppm) of MPEG in the ^1H NMR spectra. To prove the micellar structure, ^1H NMR of MPEG–PMBC was also measured in D_2O. Gel permeation chromatography (GPC) measurements were conducted with a Waters 410 GPC with $CHCl_3$ as eluent (flow rate: 1 ml/min, at 35 °C). The molecular weights were calibrated with polystyrene standards. The absolute molecular weight was determined by light scattering in $CHCl_3$ at 25 °C with a vertically polarized He–Ne laser (DAWN EOS, Wyatt Technology, laser wavelength of 690 nm).

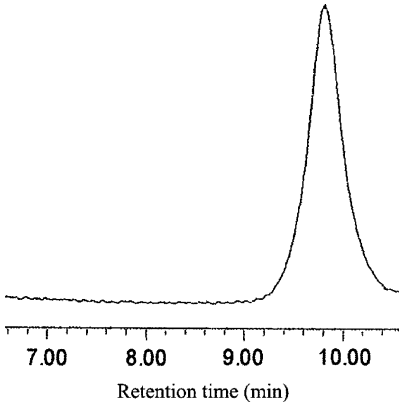


Fig. 1. Typical GPC trace of MPEG–PMBC (Table 1, PMBC23).

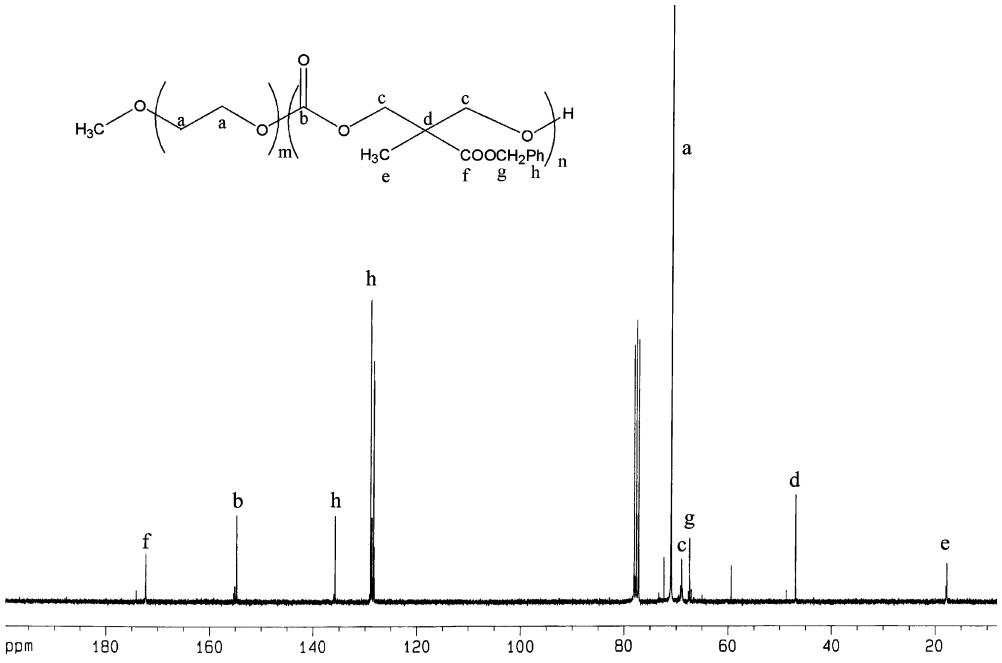


Fig. 2. ^{13}C NMR spectrum of MPEG–PMBC in $CDCl_3$ (Table 1, PMBC30).

2.4. Preparation of micelles

The micelles were prepared using a solvent displacement method with a tetrahydrofuran/water (THF/H_2O) system. The block copolymer micelles were prepared as follows [42]: A copolymer (0.1 g) was first dissolved in tetrahydrofuran (THF) (10 ml) in a 100 ml volumetric flask and 40 ml of doubly distilled water was added with gentle agitation, followed by removing THF (25 °C, with a water aspirator) for 4 h. The micellar solution was then diluted to obtain a desired concentration from 10^{-4} to 1.0 g/l. To study the effect of micelle preparation conditions on the property of micelles, we used *N*,*N*-dimethylformamide (DMF) instead of THF as the common solvent.

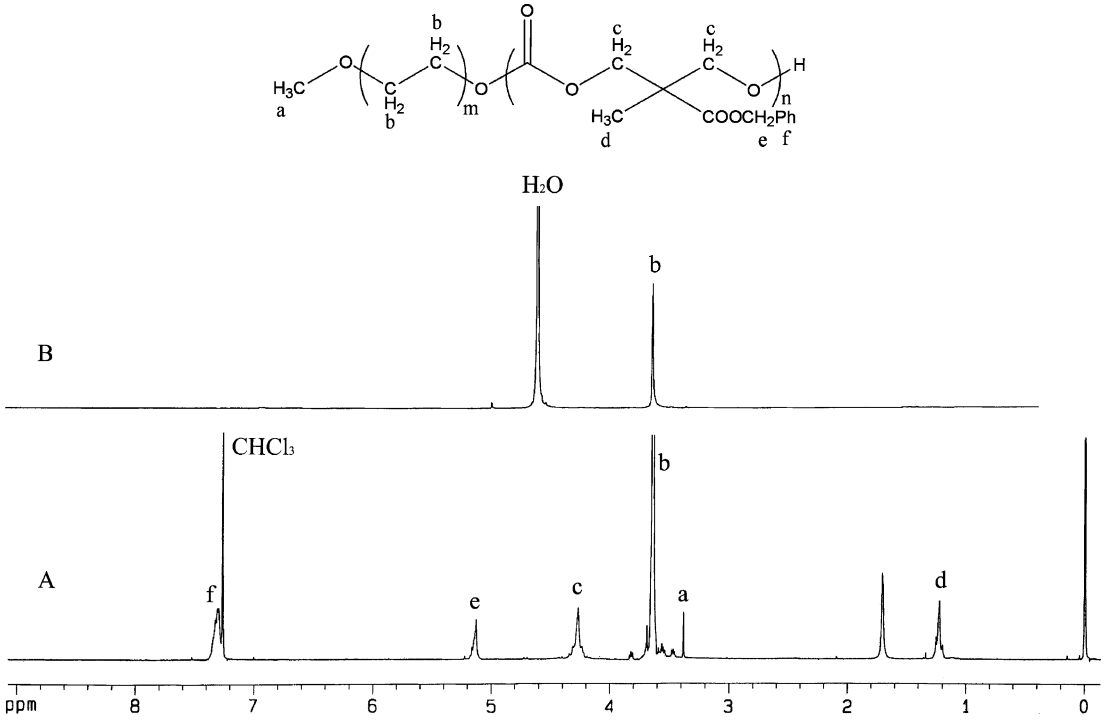


Fig. 3. ^{1}H NMR spectra of MPEG–PMBC in $CDCl_3$ (A) and in D_2O (B) (Table 1, PMBC30).

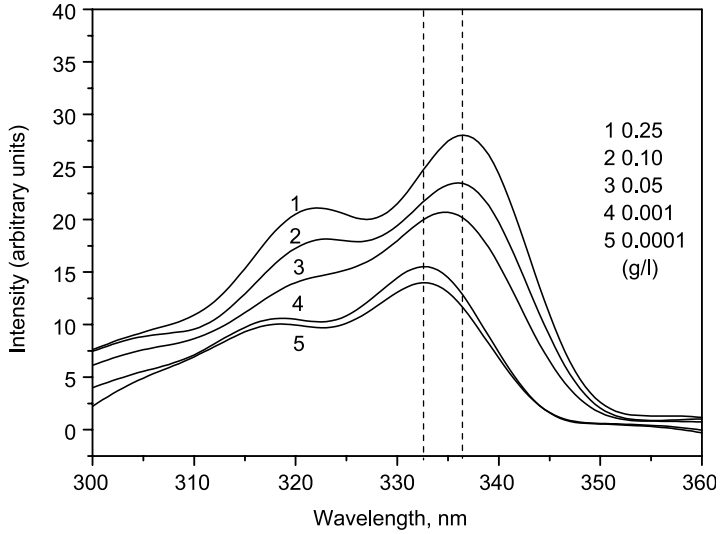


Fig. 4. Fluorescence excitation spectra of pyrene in aquous MPEG–PMBC solution. Emission wavelength 391 nm and temperature 20 °C.

2.5. Measurement of cmc

The formation of micellar structures was confirmed by a fluorescence technique by using pyrene as a probe. Steady-state fluorescence spectra were obtained by a Perkin–Elmer LS50B luminescence spectrometer. The sample solutions were prepared by first adding known amounts of pyrene in acetone to a series of flasks. After the acetone had evaporated completely, the micelle solutions with various concentrations of MPEG–PMBC were added to each of the flasks and mixed by vortexing. The concentration of pyrene in the final solution was 6×10^{-7} mol/l, similar to the saturation solubility of pyrene in water at 22 °C. The flasks were thermo-stated at 40 °C for about 2 h to equilibrate pyrene partition between the water and micelles, and subsequently were cooled overnight to room temperature. For fluorescence emission spectra, the emission wavelength was 391 nm for excitation spectra and the excitation bandwidth was 4 nm. The spectra were recorded with a scan rate at 240 nm/min.

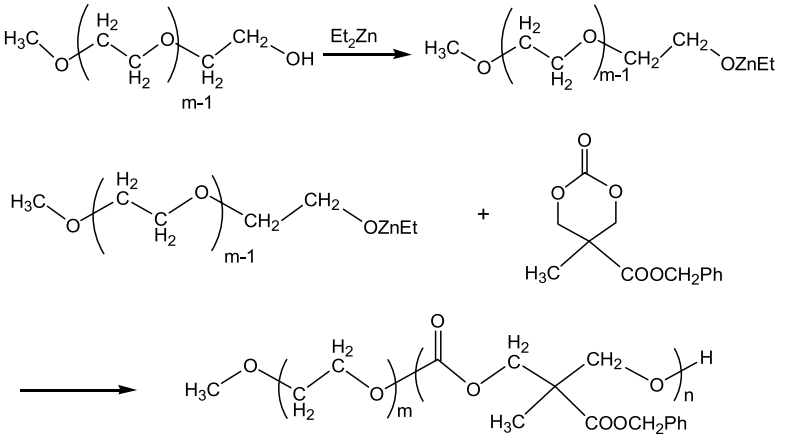
2.6. Determination of micelle morphology and size

2.6.1. Dynamic light scattering

Size distribution of micelles was determined by dynamic light scattering (DLS) with a vertically polarized He–Ne laser (DAWN EOS, Wyatt technology). The scattering angle was fixed at 90° and the measurement was carried out at a constant temperature of 25 °C. The sample solutions were diluted in filtered double-distilled water prior to analysis.

2.6.2. Environmental scanning electron microscopy

The morphology of the micelles was investigated by environmental scanning electron microscopy (ESEM). ESEM was performed on an XL 30 ESEM FEG Scanning Electron Microscope (Micrion FEI PHILIPS). A drop of micelle solution was deposited onto a silicon chip mounted on an aluminum stub. The sample was air dried before measurement.



Scheme 1. Synthesis route to block copolymer MPEG–PMBC.

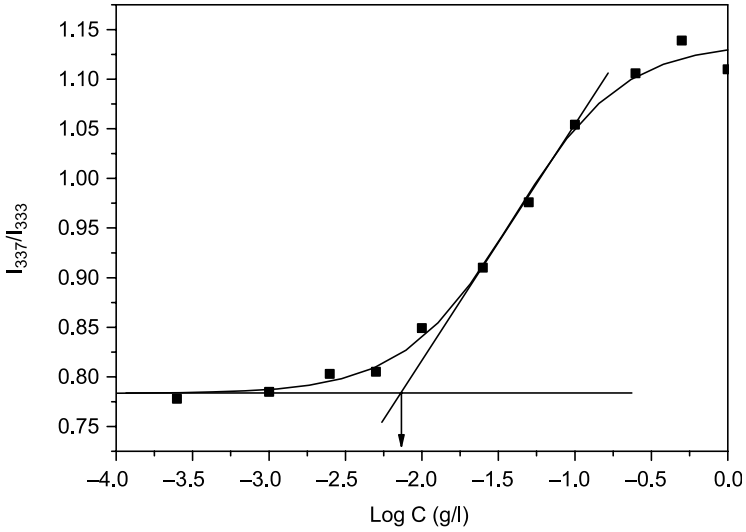


Fig. 5. Plot of I_{337}/I_{333} vs. log C for MPEG–PMBC in deionized water.

3. Results and discussion

3.1. Synthesis and characterization of MPEG–PMBC copolymer

Recently, Jing et al. reported on the synthesis of homopolymer PMBC and the block-copolymerization of MBC with CL using amino isopropoxyl strontium as an initiator [41]. The monomer MBC is a new functionalized cyclic carbonate and can be copolymerized with CL or LA by ROP. Up to date, however, the synthesis of a block copolymer containing PMBC and PEG has not been reported.

It has been demonstrated that PEG was capable of initiating polymerization of LA, lactones and cyclic carbonates to get block copolymers [31]. In our experiments, the MPEG was reacted with $ZnEt_2$ to give the macroinitiator for the polymerization of MBC at 110 °C to yield the block copolymer, as shown in Scheme 1.

Fig. 1 indicates the GPC trace of copolymer MPEG–PMBC. It is a single and sharp peak with a calculated polydispersity of 1.21. It implies that MPEG had reacted with MBC successfully, and no homopolymerization of MBC occurred. As shown in Table 1, the molecular weight of the copolymer increases with increasing ratio of monomer to macroinitiator. This indicates that the polymerization is controllable, and therefore, block copolymer with desired PMBC block length can be prepared by simply varying the ratio of monomer to macroinitiator (Table 1). The molecular weight determined by ^{1}H NMR method is in agreement with the theoretical or calculated one, whereas that measured by GPC deviated from the theoretical molecular weight. This discrepancy may be attributed to the difference in the hydrodynamic property between the block copolymer and the polystyrene standard. The absolute molecular weight determined by light scattering is shown in Table 1 (see the last item in this table). The block copolymers are denoted as PMBC23, PMBC30 and PMBC48 according to their PMBC mass percents.

Fig. 2 includes the ^{13}C NMR spectrum of the block copolymer (Table 1, PMBC30). In addition to the signals corresponding to the MPEG unit (70.90 ppm), signals for the PMBC unit appear at 17.88, 46.89, 67.40, 69.14, 128.21–129.12, 135.80, 155.10 and 172.30 ppm. This provides further evidence for the formation of MPEG–PMBC. The molecular weight of the PMBC block was calculated by comparing the relative intensities of the characteristic resonances of MPEG and PMBC blocks. Curve (A) in Fig. 3 shows the ^{1}H NMR spectrum of MPEG–PMBC in $CDCl_3$. Once again, all characteristic resonances in MPEG and PMBC can be found, indicating the successful preparation of MPEG–PMBC. The characteristic peaks of the phenyl protons and the methylene protons in the benzyl group of PMBC segment appear at 7.3 and 5.1 ppm, respectively. The characteristic peak of ethylene protons of MPEG is shown at 3.6 ppm. These two peaks are used to calculate the overall copolymer composition and the length of PMBC block.

3.2. Formation of the micelles

The amphiphilic nature of the block copolymer MPEG–PMBC provides an opportunity to form micelles in water. The water-soluble MPEG chains serve as the hydrophilic shell stabilizing the nanoparticle, and PMBC constitutes the hydrophobic core. A pyrene probe is used to prove the micelle formation of MPEG–PMBC and to measure its critical micelle concentration (cmc).

Excitation spectra of pyrene in the MPEG–PMBC solutions are shown in Fig. 4. As can be seen, the fluorescence intensity increases with increasing concentration of MPEG–PMBC. Concomitant with the increase in

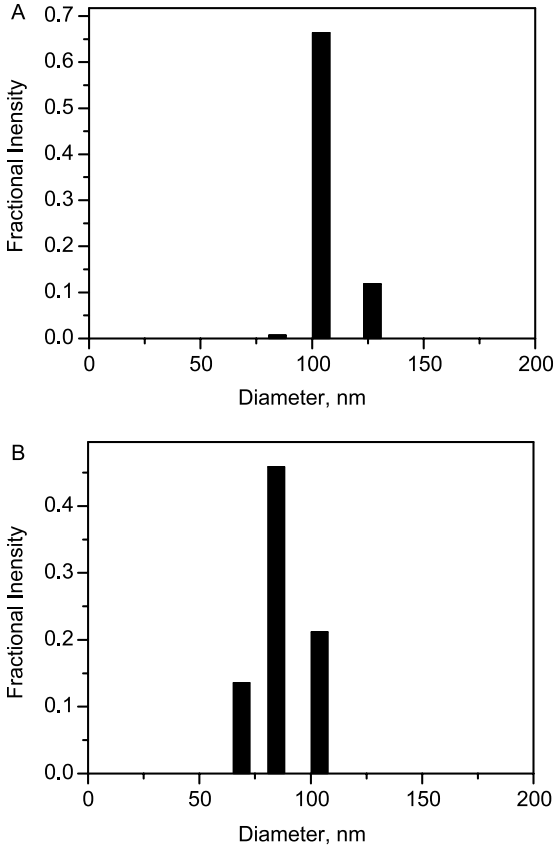


Fig. 6. Size distribution of MPEG–PMBC micelles prepared in THF/H_2O (A) and DMF/H_2O (B) (Table 1, PMBC48).

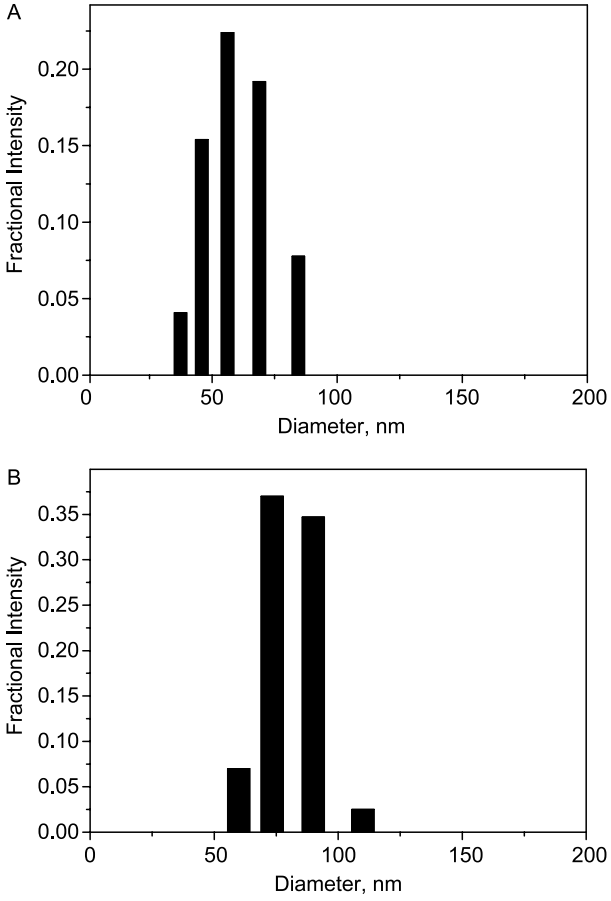


Fig. 7. Size distribution of MPEG–PMBC micelles of PMBC23 (A) and PMBC30 (B) prepared in THF/H_2O.

fluorescence intensity, a red shift from 333 to 337 nm takes place. These results are ascribed to the micellization of MPEG–PMBC. When the intensity ratio I_{337}/I_{333} is plotted against polymer concentration in Fig. 5, cmc value is obtained. As shown in Table 1, the cmc value is in the magnitude of 10^{-3} g/l and decreases with increasing PMBC block length, indicative of the amphiphilic nature of MPEG–PMBC and the high hydrophobicity of PMBC block.

Formation of the micelles is also confirmed by their ^{1}H NMR spectra in D_2O (Fig. 3(B)). It exhibits a striking difference in comparison with that measured in $CDCl_3$. All peaks related to the PMBC block disappear. Only the peak characteristic of the ethylene protons in MPEG is still visible. Such changes imply that the PBMC block is not in the solution state but condensed into the micelle core. Similarly, the MPEG block constitutes the shell of the micelles and thus their protons can be detected by ^{1}H NMR because the MPEG chains are in the solution state.

3.3. Size and morphology of MPEG–PMBC micelles

The size and morphology of MPEG–PMBC micelles in aqueous solution were examined by DLS and ESEM. Fig. 6 presents the size of the micelles formed by different common solvent. A single narrow size distribution is observed no matter what common solvent is used. This indicated that the size of micelle is homogeneous. When DMF is used as common solvent, the average diameter is about 87 nm. However, the average diameter reaches to 107 nm with the THF as the common solvent. As shown in Fig. 7, the size of micelle relates to the molecular structure of the copolymers. The micelle diameter increases from 63 to 80 nm with the increasing PMBC block length from 1500 to 2100 in the copolymer. When the PMBC block length reaches 4600, the average diameter is 107 nm. In short, copolymers with longer PMBC blocks form larger micelle particles.

Fig. 8 presents the ESEM photos of the micelles prepared from DMF/H_2O or THF/H_2O. They are all spherical. The micelle size observed by ESEM is about 50 nm, a little smaller than that determined by DLS, because the micelle diameter determined by DLS represents their hydrodynamics diameter while that obtained by ESEM is related to the collapsed micelles after water evaporation.

4. Conclusion

A novel amphiphilic biodegradable block copolymer with various block compositions was synthesized with

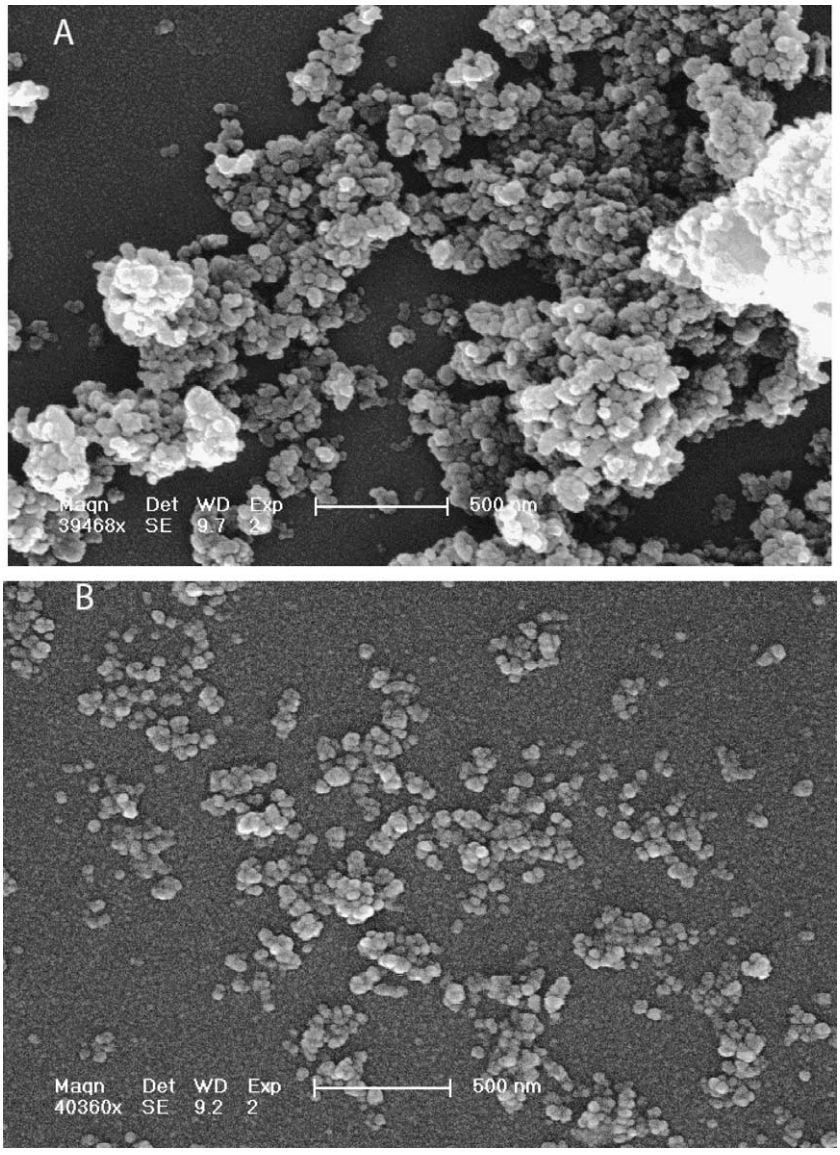


Fig. 8. Typical ESEM micrograph of MPEG–PMBC micelles.

MPEG as the hydrophilic block and PMBC as the hydrophobic block. GPC and ^{13}C NMR studies confirmed its block copolymer structure. It could self-assemble into micelles in aqueous solution. The cmc value measured by fluorescence spectroscopy was in the magnitude of 10^{-3} g/l and decreased with increasing PMBC block length. The average micelle size determined by ESEM and DLS was less than 100 nm. This amphiphilic property would be used in developing new drug delivery vehicles.

Acknowledgements

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