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Synthesis and characterization of PCL/PEG/PCL triblock copolymers by using calcium catalyst

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Abstract

Triblock copolymer PCL–PEG–PCL was prepared by ring-opening polymerization of ε-caprolactone (CL) in the presence of poly(ethylene glycol) catalyzed by calcium ammoniate at 60 °C in xylene solution. The copolymer composition and triblock structure were confirmed by ^{1}H NMR and ^{13}C NMR measurements. The differential scanning calorimetry and wide-angle X-ray diffraction analyses revealed the micro-domain structure in the copolymer. The melting temperature T_m and crystallization temperature T_c of the PEG domain were influenced by the relative length of the PCL blocks. This was caused by the strong covalent interconnection between the two domains. Aqueous micelles were prepared from the triblock copolymer. The critical micelle concentration was determined to be 0.4–1.2 mg/l by fluorescence technique using pyrene as probe, depending on the length of PCL blocks, and lower than that of corresponding PCL–PEG diblock copolymers. The ^{1}H NMR spectrum of the micelles in D_2O demonstrated only the $-CH_2CH_2O-$ signal and thus confirmed the PCL-core/PEG-shell structure of the micelles.

Keywords: Triblock copolymer; Calcium catalyst; Poly(ε-caprolactone)

1. Introduction

Biodegradable aliphatic polyesters, especially poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA) and poly (glycolic acid) (PGA), have received great research interest for their environmental, medical, and pharmaceutical applications [1–5]. PCL is an inexpensive biodegradable polyester. Recently it was found that some enzymes, such as Pseudomonas Lipase (PS), can accelerate its degradation tremendously [6,7]. But generally speaking, it is rather hydrophobic and it degrades very slowly by simple hydrolysis under the human body conditions. If ε-caprolactone is copolymerised with ethylene oxide (EO) or poly(ethylene glycol) (PEG) to prepare PCL/PEG(PEO) block copolymers, their hydrophilicity and biodegradability can be improved, and thus they may find much wider applications.

PEG presents outstanding properties, e.g. hydrophilicity, solubility in water and in organic solvents, nontoxicity, and absence of antigenicity and immunogenicity, which allow PEG to be used for many clinical applications [8]. PEG of low molecular weight can be excreted through the kidney, so its biostability is not a problem [9]. Recently, bioresorbable polyester–PEG diblock or triblock copolymers have been prepared by using a monohydroxy or α,ω-dihydroxy PEG as initiator for the polymerization of lactone monomers [10–19].

Many catalysts have been used in this polymerization [10–19]. Since complete removal of catalyst residue from the polymer is often difficult or impossible, nontoxic catalysts are preferable such as Ca-, Fe- and Mg-based catalysts [20–25], since these metals participate in the human metabolism. Li et al. reported the preparation of PLA/PEG/PLA triblock copolymers using CaH_2 and Zn [11,12]. Although the catalyst residues were nontoxic, their catalytic activity was limited, as evidenced by the high polymerization temperature (140 °C) and long polymerization time (4–7 days).

Amphiphilic block copolymers can self-disperse in certain solvent, which is a good solvent for one block and a poor solvent for the other, to form spherical nonionic micelles [26,27]. Recently, biodegradable polymeric micelles derived from copolymers of polyesters and PEG have attracted much attention because of their bioresorbability, improved biocompability and potential applications in drug delivery [28–32].

In this paper we report on the preparation and micelle

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Table 1
Molecular weights and compositions of the PCL/PEG/PCL triblock copolymers

Sample	M_{PEG}	ε-CL/PEG in feed	CL/EO in product[a]	M_{NMR}[b]	M_n[c]	Polydispersity[c]
1	1000	4.1	4.5	5500	8050	1.1
2	1000	7.7	8.2	9200	11,200	1.3
3	2000	1.1	1.3	4600	6380	1.1
4	2000	2.8	2.9	7700	7720	1.2
5	2000	3.3	3.5	9000	10,500	1.2
6	2000	11.0	10.4	11,400	13,400	1.6
7	5000	1.1	1.1	9550	9150	1.2
8	4600	2.2	2.0	13,800	14,200	1.4
9	5000	2.8	3.1	20,500	17,400	1.4
10	5000	5.5	5.5	32,500	28,900	1.6
11	10,000	0.5	0.4	14,000	12,500	1.2

[a] Calculated according to the integrated area ratio of the resonance peaks due to the PCL block at 4.05 ppm and due to the PEG block at 3.65 ppm.
[b] Calculated from M_{PEG} and the weight ratio of CL/EO.
[c] Determined by the GPC analysis.

system of PCL/PEG/PCL triblock copolymers by using calcium ammoniate as catalyst.

2. Experimental section

2.1. Materials

ε-Caprolactone (Aldrich) was dried over CaH_2 for 1 week and distilled at reduced pressure prior to use. PEG (Aldrich) was dried under reduced pressure for several hours to remove trace of water before use. Xylene was dried by refluxing over Na metal under argon atmosphere. Metal Ca was used without further treatment.

2.2. Polymerization

Gaseous NH_3 was purified by passing it through a sodium sulfate column and a sodium hydroxide column, consecutively, and was liquidized with dry ice. The liquidized NH_3 was introduced into a flask that contained metal Ca and a magnetic stirrer and was kept at −40 °C. Ten minutes later, the excess of NH_3 was evaporated by warming the flask from −40 °C to room temperature. Then the reaction product was transferred into another flask with a magnetic stirrer, which contained known amounts of PEG, ε-caprolactone and xylene. The reaction was carried out at 60 °C under stirring for 24 h. The resulted triblock copolymer was isolated by dissolving in $CHCl_3$ and precipitating into isopropyl alcohol, followed by centrifugation and drying in vacuum at room temperature for 24 h.

2.3. Micelle preparation

To prepare an aqueous solution, a block copolymer solution in tetrahydrofuran (THF) was added dropwise to doubly distilled water with agitation and then the THF was removed using rotary evaporator at 25 °C for 2 h. Pyrene solution in acetone (0.1 mg/ml) was added to doubly distilled water to give a pyrene concentration of 0.25 mg/l, and then the acetone was removed with a water pump for 8 h. These two solutions and doubly distilled water were mixed at such volume ratios that the copolymer concentration varied from 1 g/l to 0.01 mg/l, while the pyrene concentration was 0.05 mg/l. The samples were sonicated for 15 min, heated at 50 °C for 1 h, and cooled to room temperature for overnight to equilibrate the pyrene and micelles.

To prepare PCL/PEG/PCL micelles in heavy water (D_2O), 0.8 ml acetone solution of Sample 7 (10 g/l) was introduced into 10 ml D_2O and then the acetone was removed at reduced pressure. This solution (0.5 ml) was used for ^1H NMR analysis.

2.4. Measurements

NMR spectra were recorded on a Unity-400 NMR spectrometer at room temperature, with $CDCl_3$ as solvent and TMS as internal reference. ^1H NMR spectra of the PCL/PEG/PCL nanoparticles in D_2O were recorded with the same apparatus at room temperature and using trace of water as internal reference. The GPC measurement was conducted with a Waters 410 GPC apparatus equipped with a Styragel HT6E column and a differential refractometer as detector. THF was used as eluent at the flow rate of 1.0 ml/min at 25 °C, and the molecular weights were calibrated with polystyrene standards. Differential scanning calorimetry (DSC) was carried out on a Perkin–Elmer DSC-7 instrument, the heating rate was 10 °C/min. Wide-angle X-ray diffraction (WAXD) was performed using a Philips diffractometer with Ni-filtered Cu Kα radiation. For fluorescence measurements, 2 ml of solution was placed in a 1 cm × 1 cm square quartz cell. All the spectra were recorded on a Perkin–Elmer LS50B instrument. For the excitation spectra, detection wavelength λ_{em} was set to

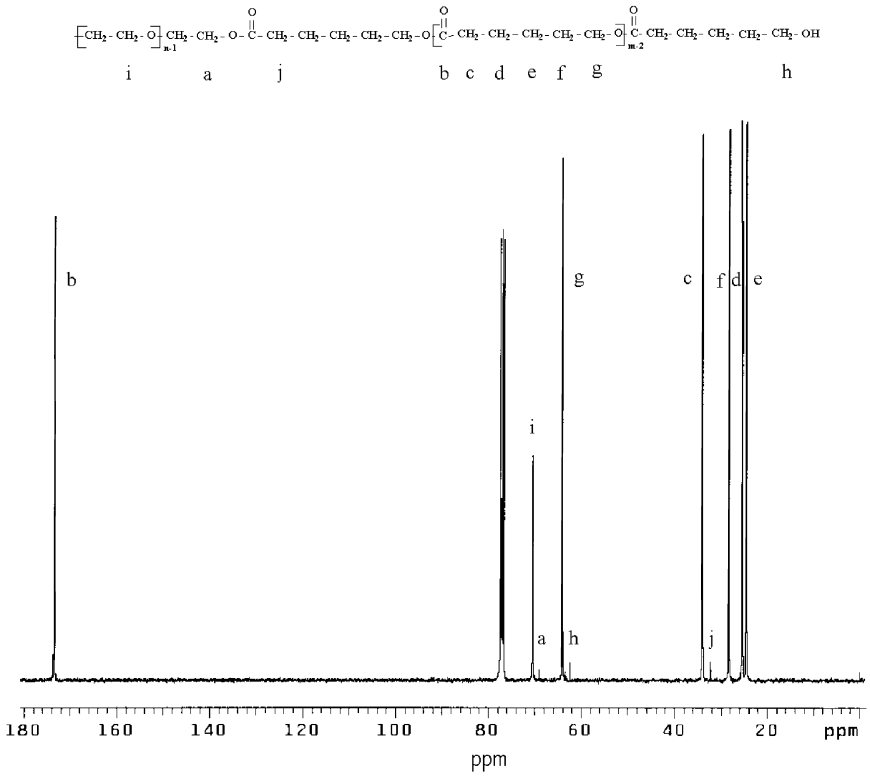


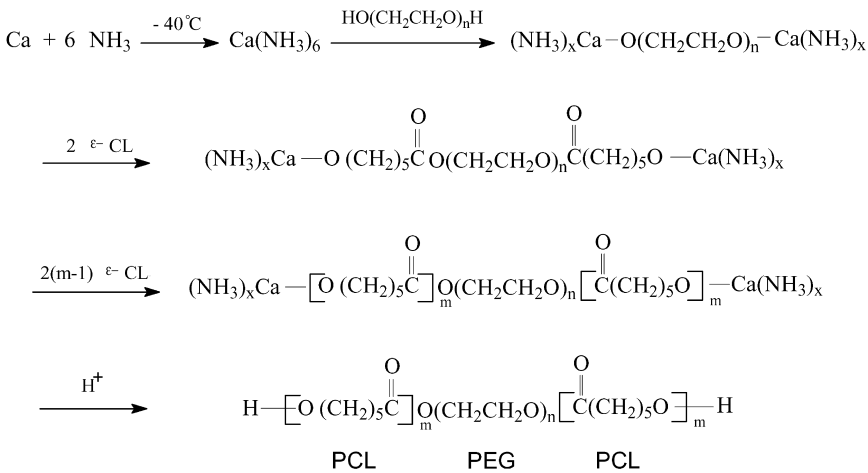
Fig. 1. ^{13}C NMR spectrum of a PCL/PEG/PCL triblock copolymer (Sample 1 in Table 1).

392 nm, the spectral widths of the entrance and exit slits were 5 nm.

3. Results and discussion

3.1. Synthesis and triblock structure

Various PCL/PEG/PCL triblock copolymers were obtained from the ring-opening polymerization of CL catalyzed with calcium ammoniate in the presence of PEGs of various molecular weights (Table 1) and with two functional end-groups, –OH, in each molecular chain. CL/EO weight ratio R in the block copolymer was determined from the area ratio of the ^{1}H NMR peaks at 4.05 ppm (due to the PCL blocks) and 3.65 ppm (due to the PEG block), and from the molecular weights of CL and EO repeat units. The molecular weight of the copolymer was further calculated by $M_{NMR} = (1 + R) \times M_{PEG}$, where M_{PEG} was the molecular weight of PEG used. As shown in Table 1, all copolymers exhibited weight ratios of CL/EO close to the corresponding feed compositions. The GPC curves of the block copolymers were unimodal and had narrow molecular weight distributions. The number average molecular



Scheme 1. Proposed mechanism for the formation of the PCL/PEG/PCL triblock copolymer.

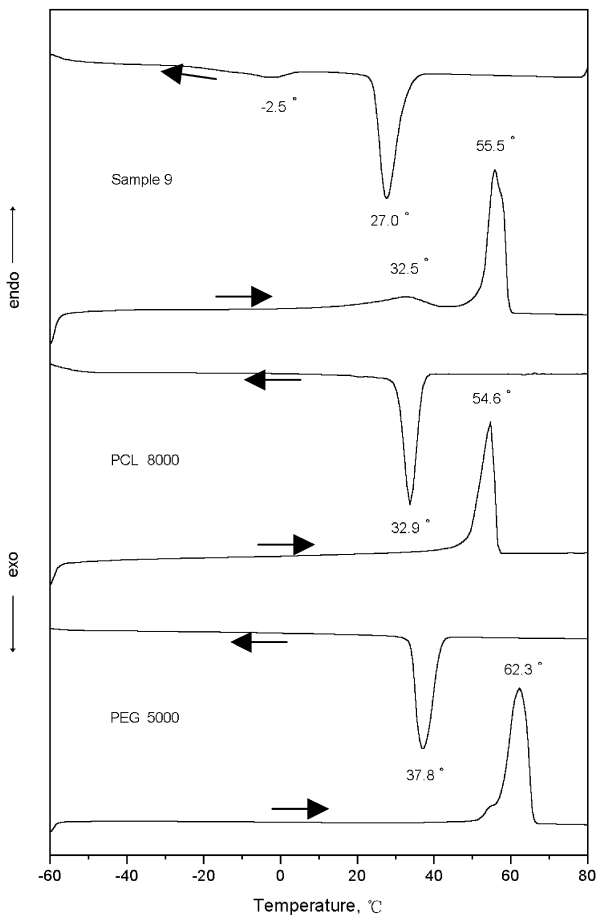


Fig. 2. DSC curves of homopolymers PEG, PCL and triblock copolymer PCL/PEG/PCL for the second heating–cooling run. The specimen was first heated at the rate of 10 °C/min up to 100 °C, kept at 100 °C for 10 min, and then cooled to −60 °C at the same rate.

Table 2
The T_m and T_c of the PCL/PEG/PCL triblock copolymers

No.	Samples		First heating T_m (°C)		Cooling T_c (°C)		Second heating T_m (°C)	
	M_{PEG}[a]	M_{PCL}[b]	PCL	PEG	PCL	PEG	PCL	PEG
1	1000	4500	56.8		27.0		51.4	15.7
2	1000	7700	56.7		30.6		53.9	
3	2000	2600	54.5	40.4	23.5	19.5	48.2	41.1
4	2000	5700	57.6	38.5	28.8	−5.0	52.9	18.0
5	2000	7000	59.1	5.1	29.0	−11.4	53.4	18.8
6	2000	20,300	61.3		29.5		57.1	
7	5000	4550	57.0[c]		21.1	32.6	55.7[c]	
8	4600	9200	58.5[c]		25.7	29.3	57.0[c]	
9	5000	15,500	60.8	38.9	26.9	−3.1	55.7	32.7
10	5000	27,500	56.3	26.8	27.4	−18.9	56.5	25.8
PEG	2000			58.4		23.2		56.2
PEG	5000			66.3		37.5		62.3
PCL		8000	59.5		32.9		54.6	

[a] Molecular weight of the PEG block.
[b] Molecular weight of the PCL block, calculated from M_{PEG} and weight ratio of CL/EO.
[c] Overlapping peaks.

weights (M_n) of the block copolymers determined by GPC were close to the M_{NMR} when the M_{NMR} was higher than 6000, confirming that there were few homopolymers in the final products.

The ^{13}C NMR spectrum of Sample 1 is shown in Fig. 1. The peak at 70.4 ppm is attributed to the methylene carbon atoms (–O–CH_2–CH_2–) of the PEG block, and the peak at 173.4 ppm is attributed to the carbonyl groups (C=O) of the PCL blocks. The peaks at 64.0, 33.9, 28.2, 25.4 and 24.4 ppm are all assigned to the methylene carbon atoms of PCL units. Three additional weak peaks at 69.0, 62.4 and 32.2 ppm are found in Fig. 1. They are assigned to the end groups and linkage groups of the block copolymer: the peak at 69.0 ppm is due to the methylene carbon atom of the PEG block that is bonded directly to the PCL block via an ester linkage; the peak at 62.4 ppm is due to the end group of the PCL block; the peak at 32.2 ppm is due to the methylene carbon atom of the PCL block adjacent to the PEG block.

The above ^{13}C NMR spectrum confirmed the triblock structure of the polymers obtained. The central block came from the PEG used while the other two from the ring-opening polymerization of ε-caprolactone. The neighboring blocks were connected through an ester linkage. Therefore the PEG served as a macromolecular initiator for the polymerization of ε-caprolactone. It is well known that calcium ammoniate is a Ca–NH_3 coordination compound and has a formula of $Ca(NH_3)_6$. Thus the above copolymerization can be depicted as in Scheme 1. In fact, when the catalyst was added to the CL–PEG–xylene mixture, gas bubbles were observed, indicating the reaction of $Ca(NH_3)_6$ with the –OH group of the PEG, with gaseous NH_3 escaping. In a few minutes, no bubbles were generated and the system became more and more viscous. It implied that the polymerization came to the stage of chain propagation. The CL monomer was inserted into the active centers Ca–$O(CH_2CH_2O)_n$–Ca to form the two PCL blocks. It was assumed that these two Ca–O active centers had the same chemical activity, and thus the two PCL blocks should be identical. Finally, the propagation was terminated by hydrolysis of the active centers to form the –OH end-groups.

3.2. DSC and WAXD analyses

DSC curves of PEG, PCL homopolymers and Sample 9 are shown in Fig. 2, and their melting temperature (T_m) and crystallization temperature (T_c) are listed in Table 2. Homopolymer PEG5000 ($M_n = 5000$) showed single melting peak ($T_m = 62.3$ °C) and crystallization peak ($T_c = 37.8$ °C) and corresponding peaks of PCL8000 ($M_n = 8000$) were located at $T_m = 54.6$ °C and $T_c = 32.9$ °C. It is noticed that although both the homopolymers had comparable melting and crystallization temperatures, the typical DSC curves of most PCL/PEG/PCL triblock copolymers exhibited bimodal endothermic and exothermic peaks, as shown in Table 2 for Samples 1,

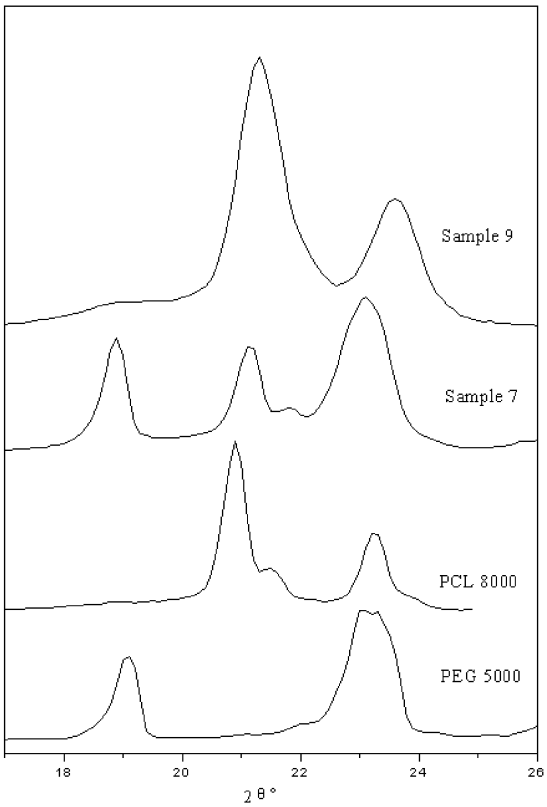


Fig. 3. WAXD curves of homopolymers PEG, PCL and triblock copolymer PCL/PEG/PCL.

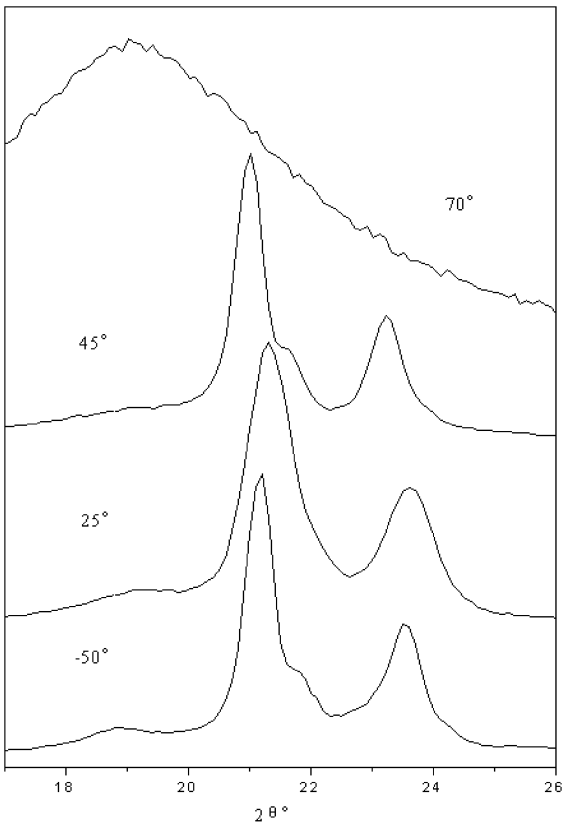


Fig. 4. WAXD patterns of Sample 9 at various temperatures.

3–5, and 9, demonstrating that phase separation took place in the block copolymer to form PCL and PEG domains, respectively. The higher temperature melting and crystallization peaks were attributed to the PCL domain, and the lower temperature ones were attributed to the PEG domain. Exception was found in the following two cases: either the PCL blocks were comparably very long (Sample 2 and 6) or very short (Sample 7 and 8) with respect to the length of the PEG block, where a broad melting peak was observed.

As shown in Table 2, for a given PEG block length, the T_m and T_c of PEG block decreased with increasing molecular weight of the PCL blocks, because earlier crystallization of the PCL block strongly restricted the crystallization of the PEG block, the two ends of which were covalently linked with the PCL blocks. The melting and crystallization peak disappeared when the PCL block was long enough (Samples 2 and 6). Just because the PCL blocks crystallized earlier, T_m and T_c of the PCL domain slightly increased with increasing molecular weight of PCL block, as if the PEG domain did not exist.

For Samples 7 and 8, the PCL blocks were relatively short compared to the PEG block, the T_m and T_c of the PCL domain were no longer higher than those of the PEG domain, and consequently, overlapping melting peaks were observed for them.

WAXD spectra of PCL/PEG/PCL triblock copolymers and PEG, PCL homopolymers are shown in Fig. 3. The crystalline patterns of both PCL and PEG domains were observed in Sample 7, indicating that its broad melting peak in DSC profile was contributed by both the domains. The crystalline pattern of PEG block was not observed in Sample 9, because the WAXD measurement was carried out at room temperature which was above the T_c of PEG domain (Table 2). As shown in Fig. 4, when the WAXD was conducted first at −50 °C, and then at room temperature, the crystalline pattern of PEG domain appeared, because the measurement temperature was below the T_m of PEG domain. When the temperature was further raised, the crystalline peaks disappeared at 45 °C for PEG domain and

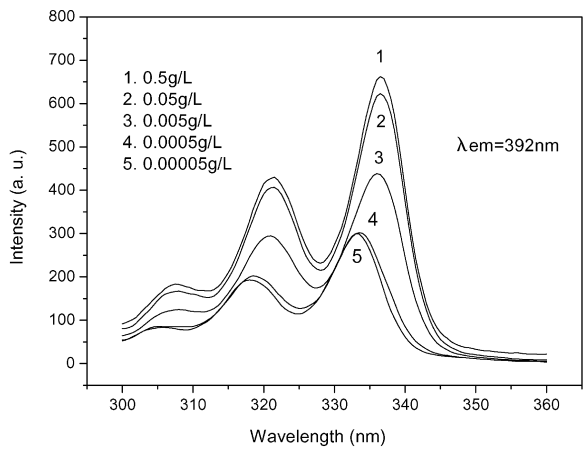


Fig. 5. Excitation spectra of pyrene for Sample 7, as a function of copolymer concentration.

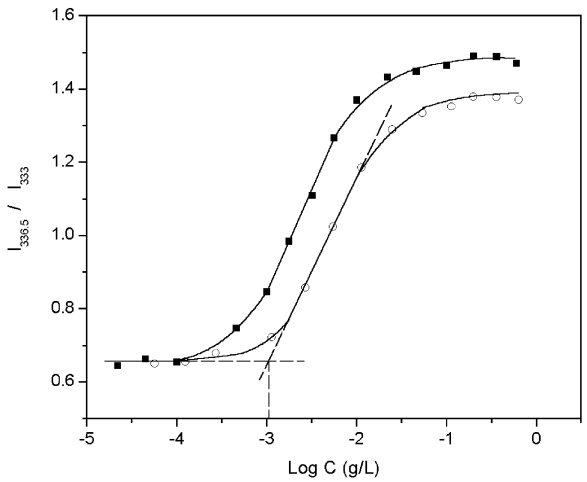


Fig. 6. $I_{336.5}/I_{333}$ vs. log C for Sample 7 (○) and Sample 8 (■), respectively.

at 70 °C for PCL domain, respectively, in agreement with the DSC results in Table 2.

Therefore, it is concluded from the DSC and WAXD analyses that (1) phase separation takes place in PCL/PEG/PCL triblock copolymers to form interconnected PCL domain and PEG domain; (2) crystallization of the PEG block is restricted by the earlier crystallization of PCL blocks that are covalently coupled to the two ends of the PEG block; (3) the longer the PCL blocks, the lower the T_c, T_m and crystallinity of the PEG domain.

3.3. Triblock copolymer micelles

It was well known that amphiphilic block copolymers consisting of a hydrophilic PEG block and a hydrophobic PCL or PLA block can form spherical micelles [28–31]. In an aqueous system, the hydrophobic blocks aggregate to form the core and the hydrophilic blocks constitute the outer shell [32]. Fluorescence techniques based on the selective partition of pyrene in hydrophobic phase against aqueous phase have been used with great success for the measurement of critical micelle concentration (cmc) [33–35] of the micellar systems.

The PCL/PEG/PCL triblock copolymer micelles in water were prepared by the procedure described in Section 2 and investigated by fluorescence spectroscopy and NMR as follows.

Fig. 5 shows the excitation spectra of pyrene as a function of the polymer concentration for Sample 7 in water solution. The intensity increased with increasing polymer concentration, and the characteristic (0,0) band of pyrene shifted from 333 to 336.5 nm when the polymer concentration increased from 5×10^{-5} to 0.5 g/l. In Fig. 6, the intensity ratio ($I_{336.5}/I_{333}$) was plotted against logarithmic concentration of Sample 7 and Sample 8. Below a certain concentration, this ratio was essentially constant, and above this concentration, it increased with increasing concentration, indicating the formation of micelles. The cmc was taken as the intersection of the tangents of the curve in these two parts. They were 1.2 and 0.48 mg/l, respectively, for Sample 7 and Sample 8, much lower than low molecular surfactants, e.g. 2.3 g/l for sodium dodecyl sulfate in water, and lower than PEG/PCL or PEG/PLA diblock copolymers [10]. The longer the PCL blocks were, the lower the cmc was, similar to the case of diblock copolymers [10].

The ^{1}H NMR spectrum of Sample 7 micelles in D_2O is shown in Fig. 7 The micelle solution was prepared by mixing the acetone solution of the copolymer with D_2O, removing the acetone with a rotary evaporator, soliciting and heating–cooling the solution. In order to save the deuterated solvent, relative content of acetone was higher

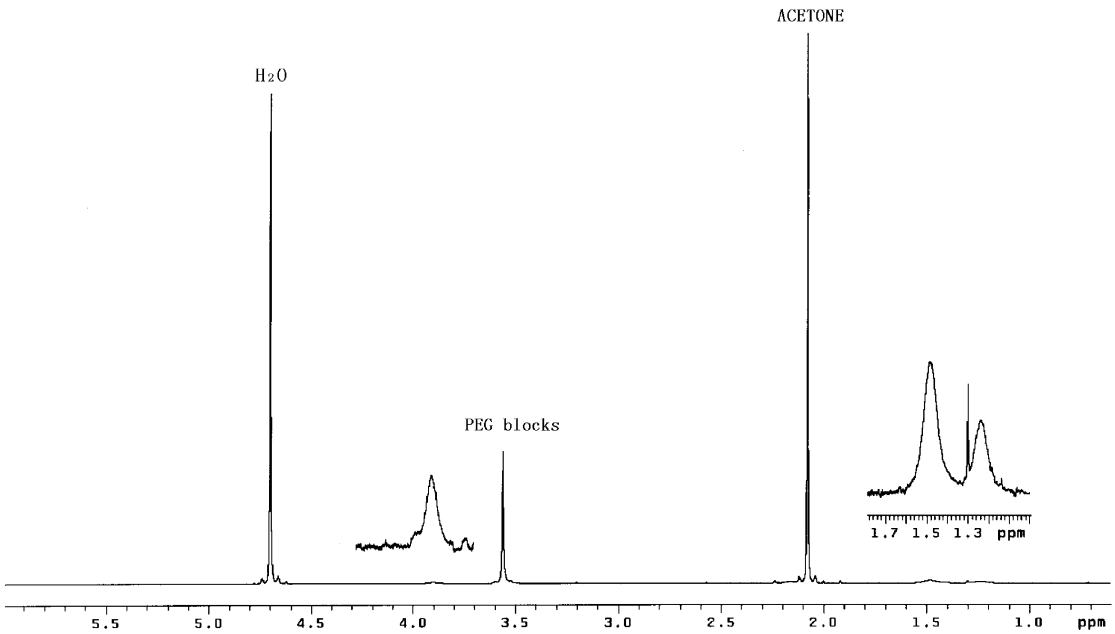


Fig. 7. ^{1}H NMR spectrum of PCL/PEG/PCL triblock copolymer micelles in D_2O.

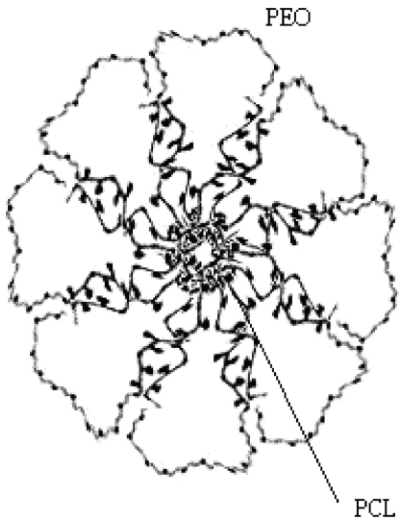


Fig. 8. Schematic picture of the PCL/PEO/PCL triblock copolymer micelles.

than the case of fluorescence measurement. The trace H_2O in D_2O was taken as internal reference and its chemical shift was taken as 4.70 ppm. The peak at 3.56 ppm was due to the methylene protons in the PEG block, and the peak at 2.08 ppm was due to the residual acetone. After extreme magnification, very weak but broad peaks were detected at 3.90, 1.49 and 1.24 ppm. They were assigned to the methylene protons of PCL blocks. The weakness and broadness of these peaks implied that the PCL blocks were not in a solution state, but in a solid state. Therefore, it was reasonably concluded that the PCL/PEG/PCL micelles possessed a core–shell structure: the PCL blocks constituted the core of micelle while the central PEG block constituted the shell of micelle, as shown in Fig. 8.

Acknowledgements

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