# Tunable Thermosensitivity of Biodegradable Polymer Micelles of Poly(ε-caprolactone) and Polyphosphoester Block Copolymers

**ARTICLE** *in* MACROMOLECULES · APRIL 2009

Impact Factor: 5.8 · DOI: 10.1021/ma900288t

# Tunable Thermosensitivity of Biodegradable Polymer Micelles of Poly(ε-caprolactone) and Polyphosphoester Block Copolymers

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*Received February 8, 2009; Revised Manuscript Received March 2, 2009*

ABSTRACT: The development of thermoresponsive and biodegradable polymer nanoparticles with tunable thermosensitivity and biocompatibility is of great interest, especially for in vivo biomedical applications. In this study, biodegradable polymer micellar nanoparticles with tunable thermosensitivity are reported. They are based on various biodegradable block copolymers of hydrophobic poly(ε-caprolactone) and thermosensitive polyphosphoesters, obtained through poly(ε-caprolactone)/stannous octoate coinitiated random ring-opening polymerization of cyclic phosphoester monomers. The thermosensitive micellar shells of nanoparticles turn out to be more hydrophobic and result in aggregates in aqueous solution when the temperature is higher than their lower critical solution temperature (LCST). The phase transition temperatures can be adjusted by controlling the molecular weights and the compositions of biodegradable polyphosphoester blocks. Decreased molecular weights of poly(ethyl ethylene phosphate) (PEEP) lead to higher LCST, whereas copolymerization of PEEP with more hydrophobic component results in lower LCST and sharper response. It has also been observed that increased sodium chloride concentration in micelle solution leads to lower responsive temperature. Therefore, the thermosensitivity of micelles can be conveniently adjusted over a wide temperature range. With good biocompatibility and tunable thermosensitivity, these biodegradable polymer-based nanoparticles are potential stimuli-responsive materials for biomedical applications.



## Introduction

Thermoresponsive biodegradable polymer nanoparticles have attracted great attention because of their potential applications in the biomedical field.[1−3] Thermosensitive polymer nanoparticles can be formed in aqueous solution from amphiphilic diblock copolymers containing thermoresponsive blocks that undergo thermoinduced phase change from the water-soluble to the water-insoluble state.[4−6] A double-hydrophilic block copolymer with thermoresponsive segment intends to self-assemble into nanoparticles (e.g., micelles or vesicles) when the temperature is above its lower critical solution temperature (LCST),[7−10] whereas a diblock copolymer consisting of a hydrophobic block and a thermosensitive block forms micelles below its LCST, but intermicellar aggregation occurs above its LCST.[11,12]

In consideration of thermoresponsiveness, precise control of the LCST and thermosensitivity is crucial and fascinating. Controlled/living radical polymerizations, such as atom transfer radical polymerization or reversible addition−fragmentation chain transfer, have been used to synthesize narrow dispersed thermoresponsive polymers with desired structure and compositions, which enable the LCST to turn accurately in a wide range. This is particularly helpful when it is necessary to adjust a polymer's LCST to a temperature near body temperature for specific biomedical applications.[13−15] However, for in vivo biomedical applications, the main limitations of thermoresponsive polymers obtained by radical polymerizations lay on the nondegradability of the backbone and the biocompatibility problem associated with themselves or residual monomers.[16] Therefore, it is of great interest to synthesize biodegradable and biocompatible thermoresponsive polymers with controllable or tunable thermosensitivity.

Polyphosphoesters (PPEs) are known to be biodegradable and biocompatible and have been widely used in drug and gene delivery and tissue engineering.[17−20] Recently, it has been reported that a series of PPE, namely, poly(ethyl ethylene phosphate) (PEEP), and its copolymers exhibit thermoresponsibility in aqueous solution, which is obtained by ring-opening polymerization with triisobutyl aluminum initiation.[21] Unfortunately, such a synthesis method is not applicable for synthesizing block copolymers of PEEP that can form thermoresponsive nanoparticles for potential biomedical applications. In addition, through such a polymerization method, it is difficult to control the structure, molecular weights, and compositions of PPEs precisely, whereas it is essential to understand how those factors affect the thermosensitivity for further biomedical applications.

In this study, we synthesized a series of diblock copolymers of biodegradable poly(ε-caprolactone) (PCL) with random copolymerized PPE, aiming at the development of thermosensitive polymeric nanoparticles. We adopted well-controlled ring-opening polymerization of cyclic phosphoester monomers initiated by alcohol/tin(II) octoate[22,23] to produce block copolymers with desired molecular structures and compositions. To understand the fundamental aspects of these thermoresponsive nanoparticles, we studied the effects of the molecular weights of PEEP, copolymerization with more hydrophobic or hydrophilic phosphoester monomers, and the concentration of sodium chloride on the thermoinduced phase transition behaviors.

## Experimental Section

**Materials.** Ethyl ethylene phosphate (EEP, 2-ethoxy-2-oxo-1,3,2-dioxaphospholane), isopropyl ethylene phosphate (PEP, 2-isopropoxy-2-oxo-1,3,2-dioxaphospholane), and methyl ethylene phosphate (MEP, 2-methoxy-2-oxo-1,3,2-dioxaphospholane) were similarly

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10.1021/ma900288t CCC: $40.75 
Published on Web 03/31/2009

synthesized, as previously described in the literature,[24,25] and purified with two consecutive vacuum distillations before polymerization. ε-Caprolactone (CL, Acros Organics) and benzyl alcohol (Sinopharm Chemical Reagent, China) were dried over calcium hydride for 48 h at room temperature, followed by distillation under reduced pressure just before use. Tin(II) octoate ($Sn(Oct)_2$, Sinopharm Chemical Reagent, China) was purified as previously described.[26] 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma Chemical. Other solvents and reagents, including triethylamine (TEA), *N*,*N*-dimethylformamide (DMF), and tetrahydrofuran (THF) were used as received.

**Synthesis of Poly(ε-caprolactone) Macroinitiator.** PCL was synthesized through ring-opening polymerization of ε-caprolactone in bulk using benzyl alcohol as the initiator and $Sn(Oct)_2$ as the catalyst, similar to as described in previous literature with slight modification.[27] Briefly, CL (10.43 g, 91.49 mmol), benzyl alcohol (0.32 g, 2.96 mmol), and $Sn(Oct)_2$ (0.073 g, 0.18 mmol) were added to a fresh flamed and nitrogen-purged round-bottomed flask in a glovebox with $H_2O$ and $O_2$ contents of <0.1 ppm. The mixture was maintained at 120 °C for 12 h. The product was dissolved in dichloromethane and precipitated in cold *n*-hexane twice. The precipitate was dried under vacuum to a constant weight at room temperature, affording 89% yield. The degree of polymerization (DP) of the PCL macroinitiator is 25, which is calculated on the basis of the integration ratio of the protons of PCL (4.06 ppm, 2H) and protons of methylene conjoint with the hydroxyl end group (3.65 ppm, 2H) from its $^1$H NMR (data not shown) and further denoted as $PCL_{25}$-OH. The molecular weight distribution of $PCL_{25}$-OH was 1.20 and was determined by gel permeation chromatography (GPC) as described below. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 7.38 (s, $Ar-CH_2-$, 5H), 5.11 (s, $Ar-CH_2-$, 2H), 2.30 (t, $-C(O)-CH_2CH_2CH_2CH_2CH_2O-$, 2H), 1.65 (m, $-C(O)CH_2CH_2CH_2CH_2CH_2O-$, 4H), 1.39 (t, $-C(O)CH_2CH_2CH_2CH_2CH_2CH_2O-$, 2H), 4.06 (t, $-C(O)CH_2CH_2CH_2CH_2CH_2O-$, 2H).

**Syntheses of Diblock Copolymers of PCL and Polyphosphoester (PCL-*b*-PPE).** Block copolymers were synthesized through ring-opening random polymerization of EEP, or EEP and MEP, or EEP and PEP using $PCL_{25}$-OH macroinitiator in the presence of $Sn(Oct)_2$ as the catalyst. In a typical example, EEP (3.50 g, 22.9 mmol), PEP (3.82 g, 22.9 mmol), and $PCL_{25}$-OH (0.52 g, 0.18 mmol) were mixed in a fresh flamed and nitrogen-purged round-bottomed flask, and the polymerization was performed at 90 °C for 2 h with $Sn(Oct)_2$ (0.02 g, 0.05 mmol) in a glovebox with $H_2O$ and $O_2$ contents of <0.1 ppm. The product was dissolved in THF and precipitated in cold ethyl ether/methanol (8/1 v/v) twice. The precipitate was dried under vacuum at room temperature, affording 78% yield.

**Gel Permeation Chromatography.** Number- and weight-average molecular weights ($M_n$ and $M_w$) and molecular weight distributions (polydispersity index, PDI = $M_w/M_n$) were determined by GPC measurements on a Waters GPC system, which was equipped with a Waters 1515 HPLC solvent pump, a Waters 2414 refractive index detector, and three Waters Styragel high-resolution columns (HR4, HR2, and HR1: effective molecular weight range 5000−500 000, 500−20 000, and 100−5000, respectively). HPLC grade chloroform was purchased from J.T. Baker and used as the eluent at 40 °C, delivered at a flow rate of 1.0 mL min$^{-1}$. Monodispersed polystyrene standards obtained from Waters Co. with a molecular weight range of 1310−($5.51 \times 10^4$) were used to generate the calibration curve.

**$^1$H NMR Characterization.** A Bruker AV300 NMR spectrometer was used for the $^1$H NMR spectrum to determine the structure and composition of the block copolymers. Deuterated chloroform ($CDCl_3$) containing 0.03% tetramethylsilane was used as the solvent for NMR measurements.

**Preparation of Micelles.** Micelles were prepared by a solvent evaporation method. Block copolymer (100 mg) was dissolved in 1 mL of THF. To this solution was added 10 mL of Milli-Q water (Millipore Milli-Q Synthesis, 18.2 MΩ) dropwise under gentle stirring. After being stirred overnight to evaporate most of the THF, the residual THF was removed under vacuum at 4 °C.

**Transmission Electron Microscopy (TEM) Measurement.** TEM measurement was performed on a JEOL 2010 high-resolution transmission electron microscope with an accelerating voltage of 200 KV. We prepared the samples by pipetting a drop of the micelle solution (1 mg mL$^{-1}$) onto a 230 mesh copper grid coated with carbon and allowing the sample to dry in air before measurements.

**Critical Micellization Concentration (CMC) Measurements.** A predetermined amount of pyrene in acetone was added to a series of ampules, and the acetone was then removed first by gently flowing $N_2$ and then by vacuum. A predetermined volume of copolymer solutions and ultrapurified water was added to the ampules consecutively to get solutions of different micelle concentrations ranging from $5.0 \times 10^{-6}$ to 1.0 mg mL$^{-1}$, whereas the concentration of pyrene in each flask was fixed at $6.0 \times 10^{-7}$ mol L$^{-1}$, which is slightly lower than the saturation solubility of pyrene in water. Fluorescence excitation spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer at 373 nm emission wavelength and 5 nm slit width.

**Dynamic Light Scattering (DLS) Measurements.** The size and size distribution of micelles in aqueous solution were measured by DLS carried out on a Malvern Zetasizer Nano ZS90 apparatus with a He−Ne laser (633 nm) and 90° collecting optics. All samples were prepared in aqueous solution at a concentration of 10 mg mL$^{-1}$ and filtered through a Millipore 0.45 μm filter prior to measurements. The aqueous solution was kept in the thermostat of the apparatus at various temperatures for 20 min to reach the equilibrium prior to measurements. The data were analyzed by Malvern Dispersion Technology Software 4.20.

**Optical Transmittance Measurements.** Optical absorbance of the copolymer aqueous solution (20 mg mL$^{-1}$) at various temperatures was measured at 500 nm with a UV-2802 PC (UNICO, China) spectrophotometer. The sample cell was thermostatted in a refrigerated circulator bath for 20 min at different temperatures prior to measurements.

## Results and Discussion

**Synthesis and Characterization of Block Copolymers.** The pentavalent nature of the phosphorus allows introduction of various pendant groups such as methyl, ethyl, hydroxyl, ethyl amino groups, and so on.[24,28−30] It is expected that random copolymerization of various five-membered phosphate monomers will enable us to tailor the materials with adjustable physical and chemical properties.[20] In this work, to study the effect of structure on the physicochemical properties of block copolymers, phosphate monomers MEP, EEP, and PEP with methyl, ethyl, and isopropyl pendant groups, respectively, were used for copolymerization to produce random PPE copolymers.

In our previous work, we have synthesized diblock and triblock copolymers of PCL and PEEP with well-controlled composition and narrow PDI.[22,31] The polymerization has been performed in solution with either aluminum isopropoxide as the initiator or $Sn(Oct)_2$/alchohol as the coinitiation system. However, it has been observed that by such a polymerization in solution, it was difficult to produce PPEs with high molecular weight (e.g., higher than 20 000). Our previous study also indicated that a two-substituted phosphate monomer analogue with 4-methyl-2,2-dimethyl-1,3-dioxolane (GEP) can be polymerized at 90 °C in bulk.[29] Earlier work of Penczek et al. has demonstrated that polymerization of lactone in bulk, which is with higher monomer starting concentration, results in higher proportion of polymerized monomer.[32] We therefore performed the polymerization in bulk at 90 °C to synthesize the block copolymer (PCL-*b*-PPE), as depicted in Scheme 1. Biodegradable PCL with a hydroxyl end cap ($PCL_{25}$-OH) was used as the initiator in this study.

Typical $^1$H NMR spectra of block copolymers $PCL_{25}$-*b*-P($EEP_{180}$-*co*-$MEP_{27}$) and $PCL_{25}$-*b*-P($EEP_{177}$-*co*-$PEP_{39}$) are shown in Figure 1. The complete disappearance of resonance at 3.65 ppm indicated that the $PCL_{25}$-OH was completely involved in

**Scheme 1. Synthesis Pathway of Block Copolymer PCL-*b*-PPE**

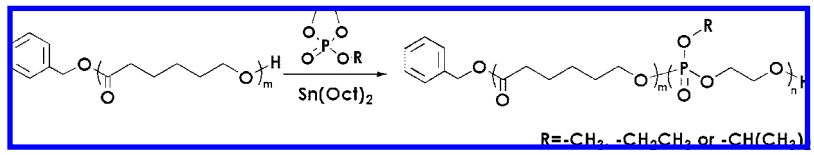


**Table 1. Characterization of Block Copolymers and Their Micellar Nanoparticles**

| code | feed molar ratio EEP/PEP(MEP)/$PCL_{25}$-OH[a] | DP of PEEP/PEP (MEP)[a] | $M_n$[b] | $M_n$[c] | PDI[c] | CMC ($10^{-2}$ mg $mL^{-1}$) | $R_h$ (nm) |
|---|---|---|---|---|---|---|---|
| $PCL_{25}$-*b*-$PEEP_{42}$ | 50/0/1 | 42/0 | 9230 | 14 020 | 1.48 | 2.35 | 58 ± 14.3 |
| $PCL_{25}$-*b*-$PEEP_{103}$ | 150/0/1 | 103/0 | 18 510 | 24 050 | 1.55 | 2.58 | 80 ± 16.3 |
| $PCL_{25}$-*b*-$PEEP_{217}$ | 250/0/1 | 217/0 | 35 830 | 43 580 | 1.54 | 2.75 | 75 ± 10.2 |
| $PCL_{25}$-*b*-$PEEP_{329}$ | 350/0/1 | 329/0 | 52 860 | 57 890 | 1.62 | 3.58 | 156 ± 6.3 |
| $PCL_{25}$-*b*-P($EEP_{177}$-*co*-$PEP_{39}$) | 200/50/1 | 177/39 | 36 230 | 43 090 | 1.54 | 2.38 | 130 ± 4.5 |
| $PCL_{25}$-*b*-P($EEP_{133}$-*co*-$PEP_{60}$) | 150/100/1 | 133/60 | 33 030 | 47 930 | 1.60 | 2.07 | 108 ± 9.5 |
| $PCL_{25}$-*b*-P($EEP_{110}$-*co*-$PEP_{110}$) | 125/125/1 | 110/110 | 37 830 | 46 100 | 1.43 | 1.66 | 150 ± 15.6 |
| $PCL_{25}$-*b*-P($EEP_{180}$-*co*-$MEP_{27}$) | 220/(30)/1 | 180/(27) | 33 940 | 45 350 | 1.52 | 3.27 | 87 ± 12.3 |
| $PCL_{25}$-*b*-P($EEP_{160}$-*co*-$MEP_{41}$) | 200/(50)/1 | 160/(41) | 32 830 | 41 670 | 1.44 | 4.47 | 97 ± 4.3 |
| $PCL_{25}$-*b*-P($EEP_{124}$-*co*-$MEP_{60}$) | 170/(80)/1 | 124/(60) | 30 380 | 42 760 | 1.52 | 4.45 | 134 ± 6.4 |

[a] Molar ratio and degree of polymerization (DP) of MEP were listed in parentheses. [b] Determined by $^1$H NMR. [c] Determined by GPC.



the polymerization. In addition, other than resonances assigned to protons of the PCL block, resonance at 4.28 ppm (a) is a characteristic signal from backbone protons of PPE (−POC$H_2$C$H_2$O−, 4H). Resonances at 4.19 (b) and 1.39 ppm (c) are assigned to protons of methylene (−OC$H_2$C$H_3$, 2H) and methyl protons of PEEP (−OC$H_2$C$H_3$, 3H), respectively. Protons of methyl (−OC$H_3$, 3H) of PMEP exhibit resonance at 3.94 ppm (d). The DP of EEP was calculated on the basis of the integration of peaks c + h after subtraction of the integration of peak j. The DP of MEP was calculated from the integration of peak d by that of the triplet at 2.31 ppm (j). Accordingly, the chemical shifts of PPEP from $^1$H NMR (Figure 1B) are as follows ($CDCl_3$, $\delta$): 4.28 (m, 4H, −POC$H_2$C$H_2$O), 4.67 (m, 1H, −POC$H$($CH_3$)$_2$), 1.39 (m, 6H, POCH($CH_3$)$_2$), and the DP of PEP was calculated from the integration of peak at 4.67 ppm (d) by that of the triplets at 2.31 ppm (j). We synthesized block copolymers with various compositions and molecular weights by tuning the feeding ratio of different monomers and the molar ratio of monomers to $PCL_{25}$-OH initiator. The nomenclature of $PCL_{25}$-*b*-P($EEP_{180}$-*co*-$MEP_{27}$) means it is a block copolymer of PCL (with DP = 25) and random PPE of PEEP (with DP = 180) and PMEP (with DP = 27).

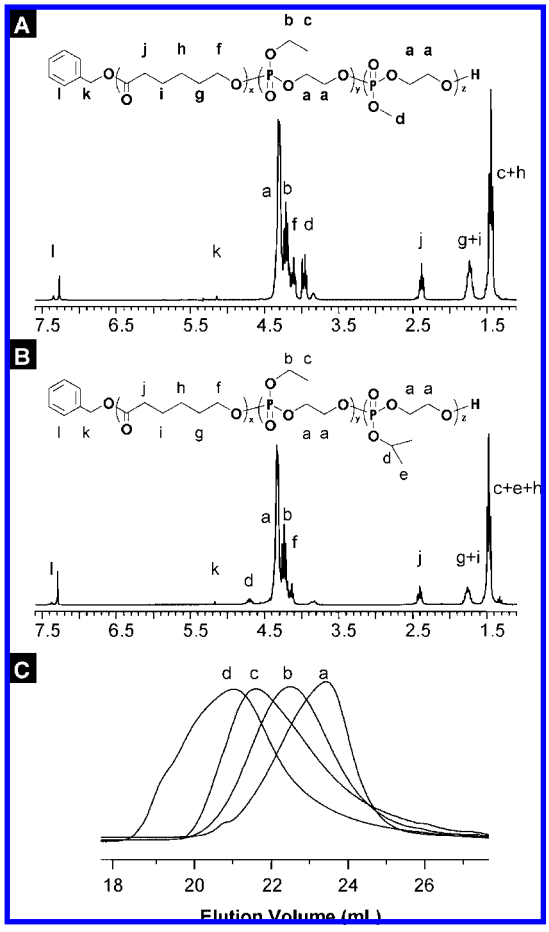


**Figure 1.** $^1$H NMR spectra of block copolymers (A) $PCL_{25}$-*b*-P($EEP_{180}$-*co*-$MEP_{27}$) and (B) $PCL_{25}$-*b*-P($EEP_{177}$-*co*-$PEP_{39}$) (ppm, in $CDCl_3$) and (C) GPC chromatograms of (a) $PCL_{25}$-OH, (b) $PCL_{25}$-*b*-$PEEP_{42}$, (c) $PCL_{25}$-*b*-$PEEP_{103}$, and (d) $PCL_{25}$-*b*-$PEEP_{217}$.

Typical GPC chromatograms of PCL-*b*-PPE are shown in Figure 1C, and all block copolymers show the unimodal peak with decreased retention times compared with the initiator $PCL_{25}$-OH, demonstrating the formation of block polymers. Molecular weights and molecular weight distributions were measured by GPC and summarized in Table 1. The molecular weight distributions of the block copolymers were all ∼1.50.

**Micellization of PCL-*b*-PPE Block Copolymers.** We have previously demonstrated that triblock copolymers of PCL and PEEP (PEEP-*b*-PCL-*b*-PEEP) form micelles with hydrophobic PCL core and hydrophilic PEEP shell.[31] Micellar nanoparticles based on a diblock copolymer of PCL and PEEP have also been utilized for targeted drug delivery to HepG2 cells.[33] In this study, the PCL-*b*-PPE copolymers all formed micelles with diameters in the range of 50−160 nm, depending on the compositions (Table 1). Figure 2A shows the typical TEM micrograph of $PCL_{25}$-*b*-$PEEP_{42}$ micelles. The micelles took a spherical shape. To understand how the composition of PPE affects the formation and property of micelles further, we used a pyrene-probe-based fluorescence technique, as widely reported in the literature.[34] First, to demonstrate the self-assembly of block copolymers, we measured the excitation spectra of pyrene with increased concentration of block copolymer. Typically, as shown in Figure 2B, a red shift of the fluorescence excitation spectra of pyrene with the increased concentration of polymer indicated the transfer of pyrene molecules from a water environment to the hydrophobic micellar core and thus demonstrated the formation of micelles. Figure 2C shows the intensity ratio of the absorbances at 339 and 336 nm ($I_{339}/I_{336}$) as a function of the logarithm of the copolymer concentrations for different

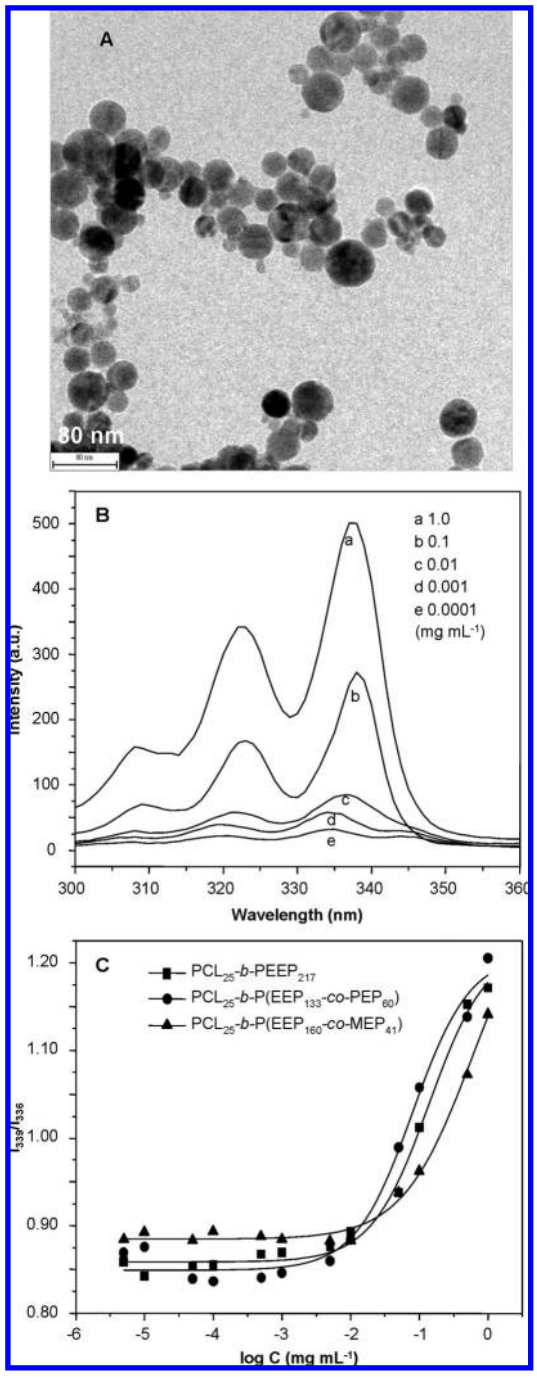


**Figure 2.** (A) Transmission electron microscopic image of $PCL_{25}$-*b*-$PEEP_{42}$ micelles, (B) excitation spectra of pyrene in aqueous solution of $PCL_{25}$-*b*-$PEEP_{217}$ at various concentrations ($\lambda_{em}$ = 373 nm), and (C) the intensity ratio ($I_{339}/I_{336}$) as a function of concentration of various PCL-*b*-PPE.

copolymers. The $I_{339}/I_{336}$ ratio remained constant when the concentrations were below a certain value. When the concentrations were above the value, it generally increased, indicating that the triblock copolymers in water automatically assembled.

The CMC values were taken to be the intersection of the tangents to the horizontal line of intensity ratio with relatively constant values and the diagonal line with rapid increased intensity ratio. In this study, the hydrophobic segments were all $PCL_{25}$; therefore, CMC values of block copolymers will relatively reflect the hydrophilicity of PPE blocks. As summarized in Table 1, when PEP was copolymerized with EEP in the PPE block, the CMC value became lower, indicating the increased hydrophobicity. On the contrast, when MEP was

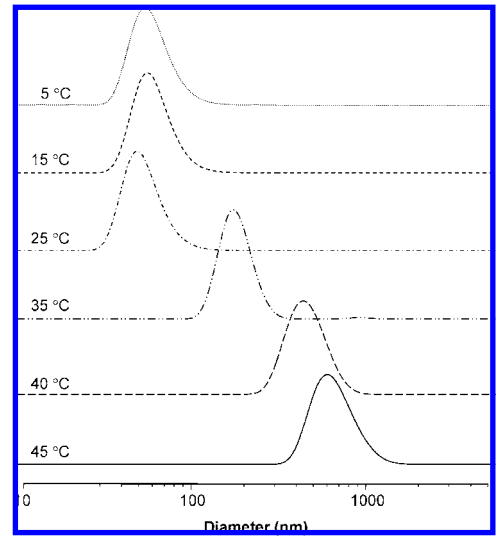


**Figure 3.** Thermoinduced aggregation of $PCL_{25}$-*b*-$PEEP_{217}$ micelles.

copolymerized with EEP in the PPE block, the CMC value became higher, leading to more hydrophilic PPE segments. It has been reported that the incorporation of hydrophobic or hydrophilic monomer to poly(*N*-isopropylacrylamide) (PNIPAAm) can tune the LCST in a wide range.[35,36] On the basis of the observation described above, it is expected that incorporation of different monomers and alternation of the composition of the PPE block would allow us to tune the LCST of micelles.

**Thermoinduced Micelle Aggregation Determined by Dynamic Light Scattering.** It is proposed that when the temperature increases, the micellar outshell of thermoresponsive micelles will turn to be more hydrophobic; therefore, the micelles will thermoresponsively aggregate because of the strengthened hydrophobic interaction, which can be indicated with the increased diameters by DLS measurements.[4,37] In our experiments, we observed that micelles that formed from all block copolymers listed in Table 1 were thermoresponsive. As a typical example shown in Figure 3, the DLS-measured diameter of $PCL_{25}$-*b*-$PEEP_{217}$ micelles in water (10 mg $mL^{-1}$) at 5 °C was ~80 nm. The radius of micelle was almost constant when the temperature was <25 °C. However, a significant increase in measured size and distribution was observed from 25 to 40 °C. When the temperature was >40 °C, the micelles aggregated to 400 nm in diameter. Such aggregation of $PCL_{25}$-*b*-$PEEP_{217}$ micelles at higher temperature should be an indication of PEEP dehydration and collapse due to the occurrence of the hydrophilic-to-hydrophobic transition of the PEEP block.

**Transmittance Measurements of Micelles with Alternated Temperature.** The thermosensitivity of the micelles was further examined through determining the optical transmittance changes of micellar solution as a function of temperature. As reported, the extent of dehydration or collapse of the shell of thermoresponsive micelles was dependent on the environmental temperature, and alternation of temperature would result in micelle interaction at a different extent, which causes the optical transmittance changes with alternated temperatures.[38,39] The transmittance change of $PCL_{25}$-*b*-$PEEP_{217}$ in aqueous solution (20 mg $mL^{-1}$) is plotted against temperature and shown in Figure 4. It is obvious that $PCL_{25}$-*b*-$PEEP_{217}$ micelle exhibits sharp transitions with alternated temperature. When it was heated from 37 to 53 °C, the transmittance sharply decreased from about 43 to 50 °C. Interestingly, a cooling cycle from 53 to 38 °C reversed the aggregation. The heating and cooling cycles are roughly comparable, and no obvious hysteresis is observed, indicating that the phase transition of copolymer is reversible.

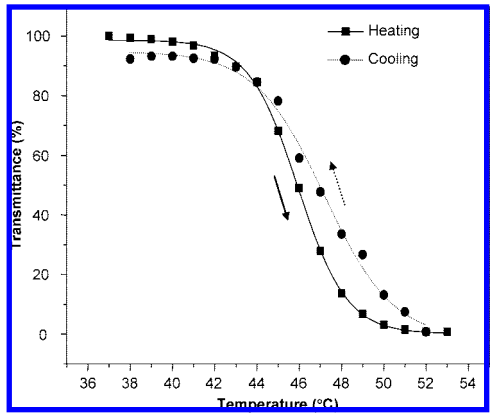


**Figure 4.** Thermoresponsive behavior of $PCL_{25}$-*b*-$PEEP_{217}$ micelles with a heating/cooling cycle.

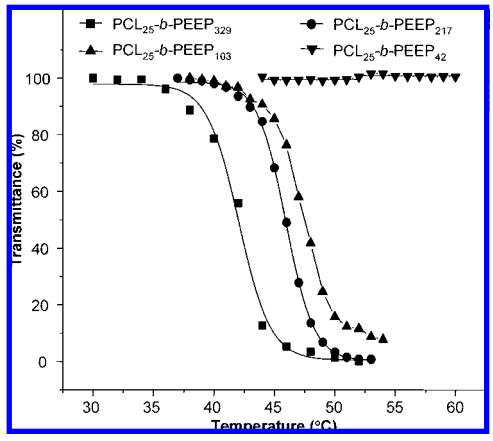


**Figure 5.** Thermosensitive behavior of PCL-*b*-PEEP (2 wt %) with various PEEP molecular weights.

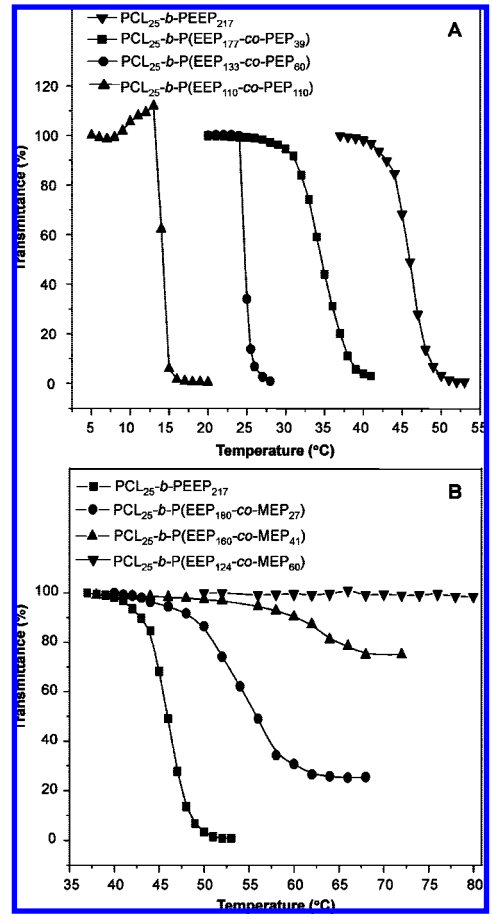


**Figure 6.** Influence of (A) PEP and (B) MEP contents on the thermoresponsive behavior of PCL-*b*-PPE.

**Influence of Molecular Weight of Polyphosphoester on the Lower Critical Solution Temperature.** LCSTs are expected to decrease with increasing polymer molecular weight on the basis of the changes in the polymer−solvent interaction.[40] It has been reported that LCST of narrow-dispersed PNIPAAm is directly dependent on its molecular weight. Aqueous solutions of narrow-disperse PNIPAAMs show a strong decrease in the phase transition temperature with increasing molecular weight.[41] In this study, the LCST is defined as the temperature exhibiting a 50% decrease in optical transmittance of thermoresponsive polymer micelles at 500 nm according to the literature.[37] To understand how the molecular weights affect the LCST of PCL-*b*-PEEP micelles, we determined the LCSTs with various PEEP molecular weights by measuring the transmittance change with temperature. As shown in Figure 5, as the DP of PEEP increases from 103 to 217 and 329, the LCST drops from 47.5 to 46.0 and 42.0 °C. However, when the DP of PEEP was 42, no detectable LCST was observed from the turbidity measurements. Therefore, it is concluded that LCSTs decreased with increasing PEEP polymer molecular weight.

**Influence of Composition of Polyphosphoester on the Lower Critical Solution Temperature.** It is well known that the LCST of thermoresponsive polymer can be tuned by adjustment of its composition.[36,42] For biomedical applications, it is essential for convenient adjustment of LCST, particularly when a LCST close to body temperature is expected. In general, incorporation of hydrophobic or hydrophilic units to thermoresponsive polymers can lead to lower or higher LCST. For example, it is reported that copolymerization of *N*-isopropylacrylamide with a more hydrophilic monomer (e.g., acrylamide) increases its LCST, but copolymerization of *N*-isopropylacrylamide with a more hydrophobic comonomer (e.g., *N*-*t*-butylacrylamide) decreases its LCST.[43] To understand how the composition of PPE affects the LCST, we first determined the effect of PEP copolymerization into PEEP segments. It has been mentioned that copolymerization with PEP increased the hydrophobicity of the PPE block (Table 1). As shown in Figure 6A, with increased molar ratio of PEP of the PPE block, the LCST shifts to a significantly lower temperature, and the phase transition is sharper. Typically, when the molar ratio of PEP is the same as that of EEP ($PCL_{25}$-*b*-P($EEP_{110}$-*co*-$PEP_{110}$)), its LCST is 14 °C, which is much lower than that of $PCL_{25}$-*b*-$PEEP_{217}$. On the contrary, copolymerization with more hydrophilic MEP resulted in increased LCST. As shown in Figure 6B, copolymerization of 13% MEP (in molar ratio) in the PPE block ($PCL_{25}$-*b*-P($EEP_{180}$-*co*-$MEP_{27}$)) leads to significantly higher LCST at 54 °C. In addition, the polymer micelles with MEP incorporation become less sensitive to temperature changes. Moreover, further increasing the molar ratio of MEP to 20% lead to a higher LCST of 62 °C, and copolymerization of 33% MEP even resulted in the loss of thermosensitivity in the measured temperature range to 80 °C. It is possible that dehydration of PEEP segments preferably occurs with the addition of more hydrophobic PEP units but not hydrophilic MEP units. However, by copolymerization with PEP or MEP, the LCST can be tuned over a wide range to meet the requirements.

**Influence of Sodium Chloride on the Lower Critical Solution Temperature.** Salts are known to influence the phase behavior of thermoresponsive polymers. It has been reported that some salts increase the LCST ("salting-in" effect), whereas

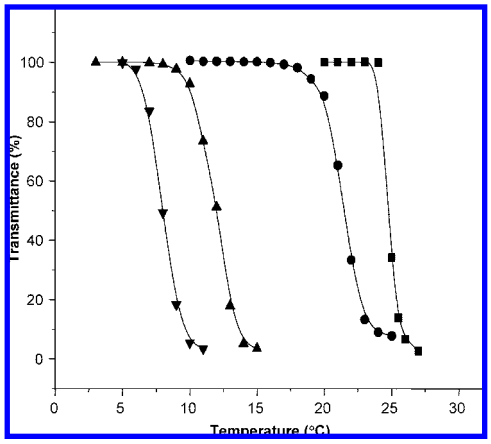


**Figure 7.** Influence of sodium chloride concentration (■, 0; ●, 1; ▲, 5; ▼, 9 mg $mL^{-1}$) on the thermoresponsive behavior of $PCL_{25}$-*b*-P($EEP_{177}$-*co*-$PEP_{39}$) in aqueous solution.

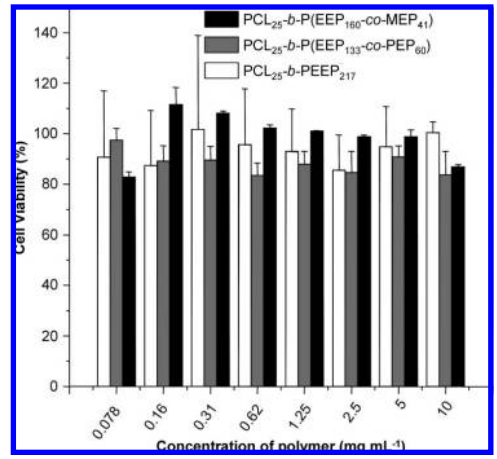


**Figure 8.** Cell viability of HEK293 cells incubated with PCL-*b*-PPE block copolymers for 72 h.

some salts decrease the LCST ("salting out" effect).[37,44] Sodium chloride is a typical example of what is called a water structure-maker, which implies that the hydration sheath near the polymer chains becomes partially destroyed. Figure 7 shows the influence of NaCl on the thermoresponsiveness of $PCL_{25}$-*b*-P($EEP_{177}$-*co*-$PEP_{39}$) by measuring the transmittance of polymer micelles in aqueous solution. With the increase in NaCl concentration, significant decrease in LCST was observed. For example, a dramatic decrease in LCST from 25 to 7 °C was observed with 9 mg $mL^{-1}$ of NaCl, indicating a typical salting-out effect. It is explained that the presence of NaCl increases the hydrogen bonding among water molecules and decreases that among water and hydrophilic chains, which may lead to a partial dehydration of PPE and results in a stronger tendency of association of PPE that decreases its LCST. However, the addition of NaCl will undoubtedly increase the polarity of aqueous media and thus enhance the hydrophobic−hydrophobic interaction and subsequently lead to the stronger tendency for copolymer association, which is indicated by the decrease in LCST.

**Biocompatibility of the Block Copolymers.** It is of great importance to evaluate the potential toxicity or biocompatibility of the polymeric materials for biomedical applications. For example, it is reported that PNIPAAm is biocompatible at the low temperature of 23 °C, whereas it induces significant cellular cytotoxicity at 37 °C when the concentration is >5 mg $mL^{-1}$ after incubation with cells for 24 h.[45] Moreover, the monomer *N*-isopropylacrylamide shows dramatically higher cytotoxicity with respect to the corresponding polymers.[45] In our previous study, we demonstrated that the PEEP with relatively low molecular weight showed good biocompatibility.[31] Here the in vitro cytotoxicity and biocompatibility of the block copolymers was evaluated by MTT assay according to our previous protocol.[31] We used three polymers as representatives, which bore methyl, ethyl, and isopropyl pendent groups. As shown in Figure 8, at all tested concentrations up to 10 mg $mL^{-1}$, the viabilities of HEK293 cells were around 80 to 100% after 72 h of incubation, demonstrating the good compatibility to cells.

## Conclusions

Amphiphilic block copolymers of PCL and PPE have been synthesized, and their tunable thermosensitivities of self-assembled micelles in aqueous solution have been investigated. Cyclic phosphoester monomers with methyl, ethyl, and isopropyl groups have been used for polymerization of PPE to adjust the hydrophobic−hydrophilic balance of the copolymer and thus tune their thermosensitivity in a wide range. It has been revealed that the phase transition of PCL-*b*-PPE micelles is reversible, and the thermosensitivity is affected by the molecular weight, composition of PPE block, and sodium chloride concentration in the medium, which in turn allows convenient adjustment of their thermosensitivity. Additionally, MTT assay has revealed that the block copolymers are biocompatible to cells, rendering these micellar nanoparticles with tunable thermosensitivity potential biomedical applications.

**Acknowledgment.** This work was supported by grants from the National Natural Science Foundation of China (50733003, 20874095), the Ministry of Sciences and Technology of the People's Republic of China (2006CB933300, 2009CB930300), and "Bairen" Program of Chinese Academy of Sciences.

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MA900288T