Preparation and Self-Assembly Behavior of Thermosensitive Polymeric Micelles Comprising Poly(styrene-*b*-*N*,*N*-diethylacrylamide)

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ABSTRACT: The amphiphilic block copolymer poly(styrene-*b*-*N*,*N*-diethylacrylamide) (PSt-*b*-PDEA) was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization of styrene and *N*,*N*-diethylacrylamide. The results of ^{1}H-NMR and GPC analysis showed that the synthesized PSt-*b*-PDEA copolymer had controlled molecular weight and narrow polydispersity. Polymeric micelles having a unimodal size distribution with an average diameter of 40 ± 0.5 nm were obtained from purified PSt-*b*-PDEA using a dialysis method. And the results showed that the clearance of homopolymer in copolymer is the key to prepare the unimodal distribution core-shell nanomicelles. The micelles were thermodynamically stable in aqueous media with a low critical micelle concentration value (0.1 mg L^{-1}). The aqueous micelles solution underwent a reversible dispersion/aggregation transition at around 29°C, which corresponds to the lower critical solution temperature (LCST) of the outer shell PDEA block chains. It is hoped that with their unique characteristics, those thermoresponsive polymeric micelles would found application in drug delivery. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 900–907, 2008

Key words: diblock copolymers; micelles; self-assembly; thermal properties; drug delivery systems

INTRODUCTION

In drug therapy, the toxicity of therapeutic molecules often causes serious side effects as healthy cells are exposed to the attack of the drugs as well. To solve this problem, many efforts have been made to design drug carriers (e.g., microspheres, liposomes) for site-specific drug targeting. However, most of such carriers are subjected to nonselective scavenging by the reticuloendothelial system (RES). Moreover, conjugation of hydrophobic drugs with polymeric carriers often leads to precipitation because of the high local concentration of the drugs. It is expected that a polymeric micelle drug carrier system could solve these problems by utilizing several preferable characteristics of polymeric micelles.[1]

Amphiphilic block copolymers self-assemble into core-shell micelles in selective solvents.[2] The polymeric nanomicelles less than 100 nm are reported to be less susceptible to RES clearance, and are of enhanced permeability and retention (EPR) effect,[3,4] which prolong the circulation time of the micelles in bloodstream and increase the micelles' chance of successfully reaching their target sites through a passive targeting mechanism.[5] In the spherical micelles formed by the block copolymers in an aqueous environment, the hydrophobic segment constitutes the core of the micelle, creating a microenvironment for the incorporation of lipophilic drugs, while the corona or outer shell formed by the hydrophilic segment provides a stabilizing interface between the hydrophobic core and the aqueous medium, which enhances dispersion, inhibits aggregation and interactions with other hydrophobic components. The polymeric micelles maintain their solubility above the critical micelle concentration (CMC) irrespective of the high content of hydrophobic drug inside the inner cores.[6,7] Besides, polymeric micelles can decrease bloodstream viscosity and vascular resistance.[8]

To achieve site-specific delivery of drugs, the drug carriers should be able to recognize selectively their physiological objective. For this purpose, novel stimuli-responsive micelles have been designed as active targeting drug delivery systems. If one or several stimuli-responsive segments are introduced into block copolymer, drug carriers with both passive and active targeting mechanism would be achieved.[9,10]

Temperature is a widely used stimulus for active targeting drug delivery. It is well known that some polymers, such as poly(*N*-isopropylacrylamide) (PNIPAAm), possess the temperature-responsive property as they undergo a reversible coil-to-globule phase transition at a certain temperature, which is termed as the lower critical solution temperature (LCST).[11]

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Therefore, several amphiphilic block copolymers incorporating the PNIPAAm segment have been synthesized, and their self-assembly behavior in solvents were investigated.[12–16] The micelles formed by these temperature-responsive amphiphilic polymers are stable below the LCST and are destroyed above the LCST. When these micelles are loaded with drugs, they will accumulate at target sites by the passive targeting mechanism and release drugs in a local hyperthemia method.[13]

Polymeric micellar size ranges are tailored by careful selection of block segments and their chain lengths. The favorable micelles size can inhibit nonselective scavenging by the RES, and can be utilized as targeting drug carriers based on the EPR effect. The controlled/living polymerization such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization can prepare block copolymers with controlled molecular weight and narrow polydispersity, which are particularly suitable for controlling polymeric micelle size ranges. Thus, various controlled/living radical polymerization attracts much attention of scientists.[17–21]

Poly(*N*,*N*-diethylacrylamide) (PDEA) is a another thermoresponsive polymer, which has a LCST in the range of 25–35°C.[22] However, the synthetic study and characterization of thermoresponsive block copolymer micelles comprising PDEA segment have been scarcely reported. In this work, we synthesized the amphiphilic block copolymer poly(styrene-*b*-*N*,*N*-diethylacrylamide) (PSt-*b*-PDEA) by RAFT polymerization, and investigated the self-assembly behavior of PSt-*b*-PDEA in solution. The LCST of the micelles in aqueous solution was also measured. These micelles have small size and hydrated thermoresponsive out shell, and are expected to show both passive and active targeting characters.

EXPERIMENTAL

Materials

N,*N*′-Azobisisobutyronitrile (AIBN) was provided by Shanghai Chemical Reagent Co., and used after recrystallization from methanol. Benzyl dithiobenzoate (BDTB) and DEA were prepared according to the reported procedure.[23,24] Styrene was obtained from Shanghai Chemical Reagent Co. and used after distillation under reduced pressure. Tetrahydrofuran (THF, Tianjin Chemical Reagent Co.) was refluxed over sodium and distilled prior to use. Anhydrous methanol, petroleum ether was obtained from Tianjin Chemical Reagent Co. and used without further purification.

Preparation of PSt chain transfer agent

PSt chain transfer agent (CTA) was prepared according to the reported procedure.[25] St (5.200 g), BDTB (0.122 g) and AIBN (8.200×10^{-3} g) were added into a septa-sealed vial. Nitrogen was bubbled into the mixture for about 30 min. Then the vial was immersed in an oil bath thermostated at 110°C. Polymerization was carried out under a nitrogen atmosphere for desired time and stopped by placing the vial in an ice water bath. THF (5 mL) was added into the vial and the polymer was precipitated by pouring the mixture into an excess of methanol. The precipitation procedure was repeated for three times. The precipitates were collected and dried in a vaccum oven at room temperature overnight.

Preparation of PSt-*b*-PDEA

DEA (2.83 g), PSt CTA (1.00 g), AIBN (4.20×10^{-3} g), and THF (8 mL) were added in a septa-sealed vial, and nitrogen was bubbled into the mixture for about 30 min. Polymerization was conducted at 90°C under a nitrogen atmosphere for desired time and stopped by placing the vial in an ice water bath. The polymer was precipitated by pouring the reaction mixture into excess petroleum ether. The polymer collected was put into excess distilled water to remove PDEA and then washed with cyclohexane to remove PSt homopolymer. The copolymer was dried in a vacuum oven at room temperature overnight.

^{1}H-NMR measurement

^{1}H-NMR spectra of PSt CTA and PSt-*b*-PDEA were measured in $CDCl_3$ using a AV-300*M* NMR spectrometer. The ^{1}H-NMR (300 MHz) of polymeric micelles measurement was performed in D_2O.

GPC measurement

The molecular weight and molecular weight distribution were determined using GPC equipment (Alliance GPCV2000, Waters Co., America). THF was used as eluent, flow rate being 1 mL min^{-1} and the calibration was carried out with polystyrene monodisperse standard.

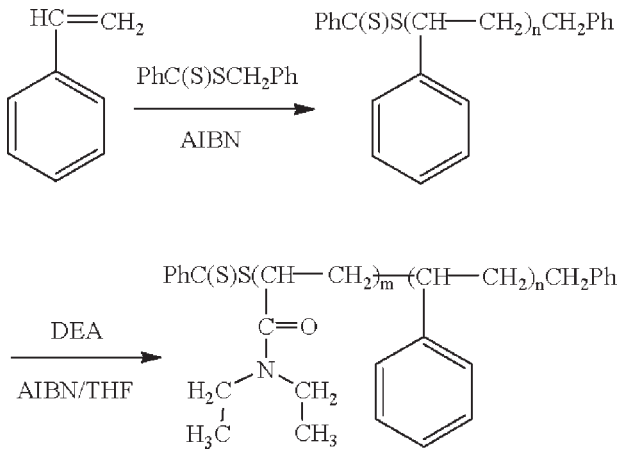
Micelles preparation

Direct solvation method

PSt-*b*-PDEA (0.07 wt %) was added into the cosolvents of THF and water with different ratios. The solution was kept standing still for 48 h after being sonicated for 2 min.

Dialysis method

Distilled water was added slowly (1 drop min^{-1}) into a THF solution of polymer (5 g L^{-1}) under vigorous stirring until turbid. Then the solution was



Scheme 1 Preparation of PSt CTA and PSt-*b*-PDEA.

put into a dialysis bag (MWCO = 3500) and dialyzed against distilled water at 4°C for 48 h, with the distilled water being changed every 8 h. The aqueous solution contained in the dialysis bag was lyophilized to leave a white powder of micelles.

Micellar size distribution measurements

The size distribution of micelles was determined by dynamic light scattering (DLS) (BI-200SM, Brookhaven). The micellar solution (1 g L^{-1}) was measured at 20°C.

Fluorescence measurements

Fluorescence spectra were recorded on a spectrofluorometer (Perkin–Elmer LS55, America). Pyrene was used as a hydrophobic fluorescence probe. Aliquots of pyrene solutions (2×10^{-4} M in diethyl ether, 25 μL) were added to containers, and the ether was allowed to evaporate. Aqueous polymer solutions at different concentrations were then added to the containers containing the pyrene residue. The samples containing pyrene ($\sim 1 \times 10^{-6}$ M) were kept at 4°C for 24 h before measurements. Excitation was carried at 336 nm, and emission spectra were recorded ranging from 350 to 500 nm. Excitation and emission bandwidths were 10 and 3 nm, respectively. From the pyrene emission spectra, the intensity (peak height) ratios (I_1/I_3) of first band (374 nm) to third band (385 nm) were analyzed as a function of polymer concentration. The CMC value was determined at the onset of a decrease in the plot of the polymer concentration versus ratio of I_1/I_3.[12]

Lower critical solution temperature

Transmittance of both PSt-*b*-PDEA copolymer micelles and PDEA homopolymer in aqueous solution (1 g L^{-1}) were measured with a UV-VIS spectrometer (Perkin-Elmer Lambda 35, America) at 500 nm. Sample cells were thermostated with a thermostated container (TB-B5, Shimadzu Co., Japan). The LCST was defined as the temperature at the inflection point in the plot of the transmittance versus temperature. The temperature ranged from 26 to 32°C, and the transmittance of polymer solution was measured at 0.2°C intervals. The solution was kept for 10 min at each temperature before measurement.

Transmission electron microscopy

The TEM micrographs were obtained using a transmission electron microscope (TEM) (H-600, Japan). The samples were prepared by casting a micelles solution (1 g L^{-1}) onto copper grids.

Scanning electron microscopy

The sample obtained by dialysis the polymer without PSt clearance in THF solution against distilled water was examined with a scanning electron microscope (SEM) (JSM-6701F, Japan). The sample was coated with gold before examination.

RESULTS AND DISCUSSION

To prepare PSt-*b*-PDEA copolymer with favorable polymeric micelle size ranges, RAFT polymerization was performed as shown in Scheme 1. BDTB was used as chain transfer agent and AIBN as initiator. The ^{1}H-NMR spectrum of PSt CTA is shown in Figure 1(A). The signals at δ = 7.9 and 4.8 ppm are ascribed to aromatic protons of dithiobenzoate group and methine proton adjacent to sulfur respectively, indicating the successful synthesis of PSt CTA.[25,26] As a macro chain transfer agent, PSt CTA reacted with monomer DEA in the presence of AIBN, leading to the formation of block copolymer. The obtained PSt-*b*-PDEA copolymer was treated sequentially with distilled water and then cyclohexane to remove PDEA and PSt homopolymers. The ^{1}H-NMR spectrum of the purified PSt-*b*-PDEA is shown in Figure 1(B). The characteristic signals of PSt at δ = 6.4–7.2 ppm and the signals at δ = 3.2–3.5 ppm corresponding to methene protons in ethyl group of PDEA unit demonstrate the formation of PSt-*b*-PDEA.

The preparation condition and the GPC results of PSt CTA and PSt-*b*-PDEA are summarized in Table I. It is shown clearly that the synthesized PSt-*b*-PDEA copolymer has a narrow polydispersity (M_w/M_n), which is well bellow the theoretical lower limit of 1.50 for a conventional free radical polymerization.

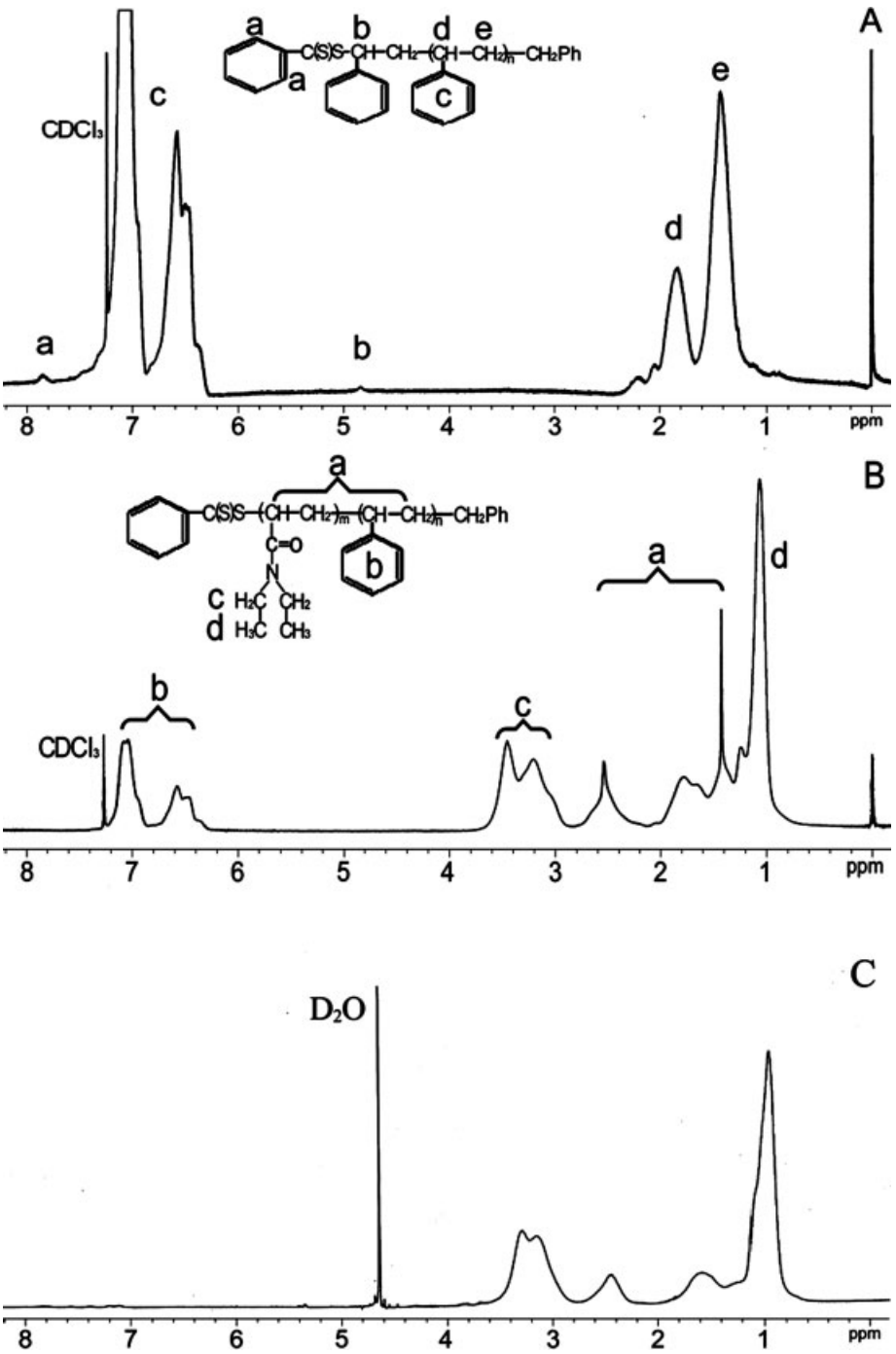


Figure 1 The ^{1}H NMR spectrums of PSt CTA (A), PSt-*b*-PDEA (B), and polymeric micelles (C).

Generally, amphiphilic block copolymers could self-assemble into core-shell micellar structure in a proper milieu. Thus, the polymeric micelles of PSt-*b*-PDEA were prepared using directly solvation method, and THF and H_2O were selected as the cosolvents considering the solubility of the segments of the copolymer. PSt-*b*-PDEA (0.07 wt %) was added into the mixture of THF and H_2O. The solution was transparent when the ratio of THF to H_2O is 11 : 4 (V/V). When the solution was cast onto a copper grid, the nanoparticle morphology was observed using a transmission electron microscope [Fig. 2(a)]. The solution became opaque when the ratio of THF to H_2O rose to 10 : 5 (V/V), and no nanoparticles were detected on TEM micrographs [Fig. 2(b)].

To be used as drug carriers, the polymeric micelles must be present in aqueous environment. To remove the organic solvent, the micelle solution

TABLE I
The Preparation Condition and Results of PSt CTA and PSt-*b*-PDEA

Sample	Material[a]			T (°C)	Time (h)	Conv.[b] (%)	M_n[c]	M_w/M_n[c]
	Monomer	CTA	Initiator					
PSt CTA	St	BDTB	AIBN	110	7	56	4400	1.30
PSt-*b*-PDEA	DEA	PSt	AIBN	90	8	74	11300	1.35

[a] PSt CTA prepared with molar ratio St/BDTB/AIBN = 1000 : 10 : 1; PSt-*b*-PDEA prepared with molar ratio DEA/PSt/AIBN = 980 : 7 : 1.
[b] Conversion was obtained via gravimetry.
[c] M_w, Weight-averaged molecular weight; M_n, Number-averaged molecular weight; The molecular weight of the polymers was determined by GPC.

is prepared using a dialysis method. Distilled water was added slowly (1 drop min^{-1}) into a solution of block copolymer in THF (5 g L^{-1}) under vigorous stirring until turbid, then the solution was put into a dialysis bag (MWCO = 3500) and dialyzed against distilled water at 4°C for 48 h. The formation of micelle is supported by DLS measurement. As shown in Figure 3, polymeric micelles have a unimodal size distribution with an average diameter of 40 nm. It is noteworthy that the micelle distribution could be controlled in a very low range (±0.5 nm), which probably resulted from the controlling chain lengths of block segments and a strict micelle formation procedure. The small size of the micelles corresponds to the size of natural carriers such as viruses, and would be favorable for the PSt-*b*-PDEA micelles to reach the specific sites by a passive targeting mechanism.[27]

The self-assembly of the PSt-*b*-PDEA forming core-shell structure in aqueous solution was verified by ^{1}H-NMR [Fig. 1(C)]. In the measurement, the white powder of micelles obtained by lyophilization method was dissolved in D_2O directly. In contrast to the ^{1}H-NMR of PSt-*b*-PDEA in $CDCl_3$, the characteristic signals of PSt block at δ = 6.4–7.2 ppm disappears. This phenomenon can be interpreted as follows: the PSt segments shrink in aqueous solution because of its' water-insoluble property and form the cores of the micelles. So, the mobility of the PSt segments has been restricted. Further more, the shield of the PDEA outer shell also lead to the disappearance of the PSt block signals.

The formation of micelles from PSt-*b*-PDEA copolymer in water was further verified by fluorescence spectroscopy using pyrene as a fluorescence probe. The result is shown in Figure 4. The intensity ratio (I_1/I_3) of first band to third band is shown as a function of polymer concentration. A dramatic decrease in I_1/I_3 was observed when polymer concentration reached 0.1 mg L^{-1}, indicating the formation of micelles and the transfer of pyrene into the hydrophobic cores of the micelles.[16] This concentration can be defined as the CMC. This block copolymer shows a relatively low CMC in water, which could be attributed to the highly hydrophobic polystyrene block. It is believed that a copolymer system with a low CMC value is advantageous when used in drug delivery system for a perfect *in vivo* stability of the micelles.[7]

To investigate the influence of PSt or PDEA homopolymer on self-assembly behavior of PSt-*b*-PDEA, the polymer without homopolymer clearance were used. The micellar size distribution of polymer

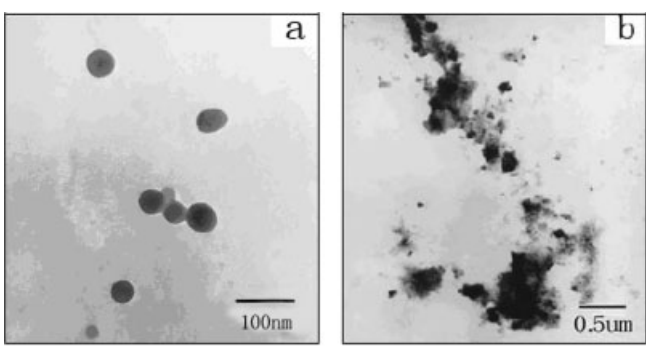


Figure 2 TEM micrograph of PSt-*b*-PDEA (0.07 wt %) specimen cast from (a) THF/H_2O 11 : 4 (V/V) solution; (b) THF/H_2O 10 : 5 (V/V) solution.

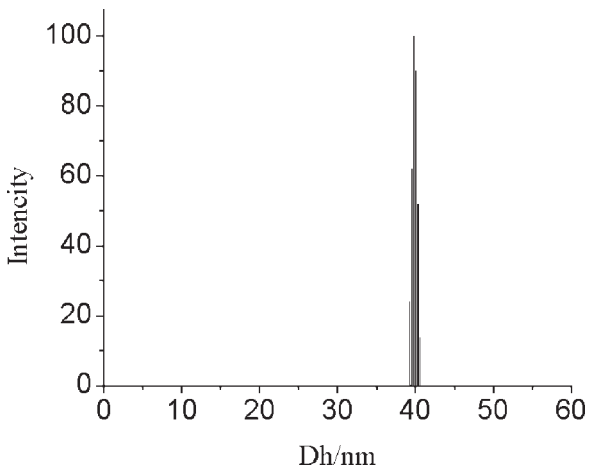


Figure 3 Size distribution of purified PSt-*b*-PDEA micelles prepared using dialysis method determined by using DLS.

without PSt homopolymer clearance is shown in Figure 5. The aggregates had a bimodal size distribution, containing nanomicelles (<50 nm) and larger aggregates (150–450 nm), which probably resulted from the entrance of PSt homopolymer.[28,29] The PSt homopolymer entered into the inner core of nanomicelles and entangled with PSt segment of PSt-*b*-PDEA, thus increase the average diameter and broadened the size distribution of the partition of nanomicelles. On the other hand, the entrance of PSt homopolymer enhanced the hydrophobic-hydrophobic interaction between the cores of the micelles. Thus, several primary nanomicelles combined with each other, forming the larger aggregates. The micellar size distribution of the polymer without PDEA homopolymer clearance is consistent with the results of purified PSt-*b*-PDEA. The explanation is that PDEA is soluble in water below LCST, and PDEA chains have no exact light scattering signals when dispersing in aqueous solution.

Morphology of the polymeric micelles prepared using dialysis method was investigated. As presented in Figure 6(a–c), the aggregates morphology of PSt-*b*-PDEA copolymer without PSt homopolymer clearance takes various forms. Microspheres, rod-like micelles, and vesicles coexist with nanospheres observed by TEM. These mixtures of morphologies can be explained by the force balance effect mentioned by C. Allen et al.[7] In the process of the entrance of PSt homopolymer into inner core, the interfacial energy between the core and the solvent increases. The system tends to decrease the total interfacial area by increasing the aggregation number, thus forming the larger aggregates, microspheres. In addition, the degree of stretching of the core-forming blocks increases and the intercorna

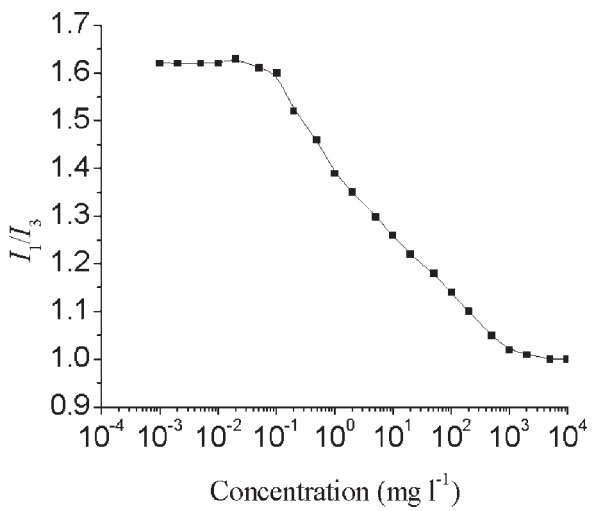


Figure 4 Plot of the intensity ratio (I_1/I_3) of first band (374 nm) to third band (385 nm) in the pyrene fluorescence spectrum as a function of polymer concentration of PSt-*b*-PDEA (λ_{ex} = 336 nm, [pyrene] = 1.0×10^{-6} M).

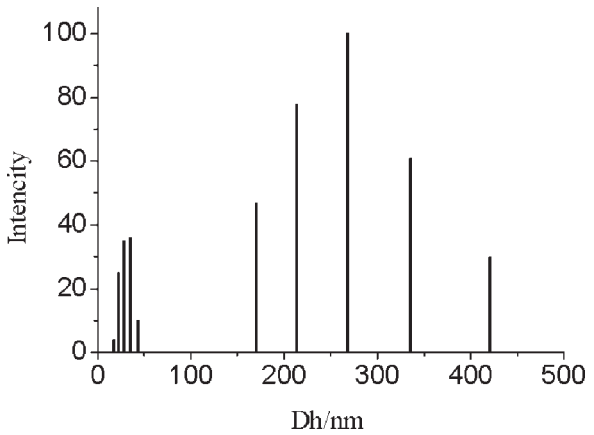


Figure 5 Size distribution of PSt-*b*-PDEA copolymer without PSt clearance.

repulsions increase with the aggregation number. In the case of the degree of stretching is too large to support the spherical morphology, the morphology change to produce rod-shaped aggregates and even to vesicles so as to ensure a decrease in the free energy of the system. The aggregates with different size and various forms can also be observed by SEM measurement (Fig. 7). If PDEA homopolymer was not weeded out, the aggregates would be nanospheres wrapped in PDEA film [Fig. 6(d)]. As shown in Figure 6(e), spherical nanomicelles of a perfect purity were obtained using the purified PSt-*b*-PDEA copolymer. The result suggests that the clearance of homopolymer in copolymer is the key to prepare unimodal size distribution core-shell nanomicelles.

To determine whether PSt-*b*-PDEA copolymer micelles exhibit a thermal response similar to the corresponding pure PDEA, the optical transmittance of an aqueous solution of PSt-*b*-PDEA polymeric micelles as a function of temperature was measured (Fig. 8). The micelles underwent phase transition at a temperature corresponding to the LCST of pure PDEA ($\sim$ 29°C) irrespective of hydrophobic PSt segments incorporation. This result demonstrates that the PSt-*b*-PDEA forms core-shell micelles structure that minimizes the contact of hydrophobic chains with water.[29] The LCST of the micelles results mainly from the uninterrupted long continuous chain of PDEA in PSt-*b*-PDEA copolymer, especially when the hydrophobic PSt segment is buried in the micelles. The hydrophilic (—CON=) and hydrophobic ($—CH_2CH_3$) groups exist together in the shell-forming PDEA segment. H-bonds between hydrophilic group and H_2O molecule contributes mostly below LCST. The highly hydrated outer shell separates the hydrophobic inner core with the external medium, preventing the strong chain–chain aggregation. As temperature increases, the interaction between hydrophobic group and H_2O molecule reinforces and

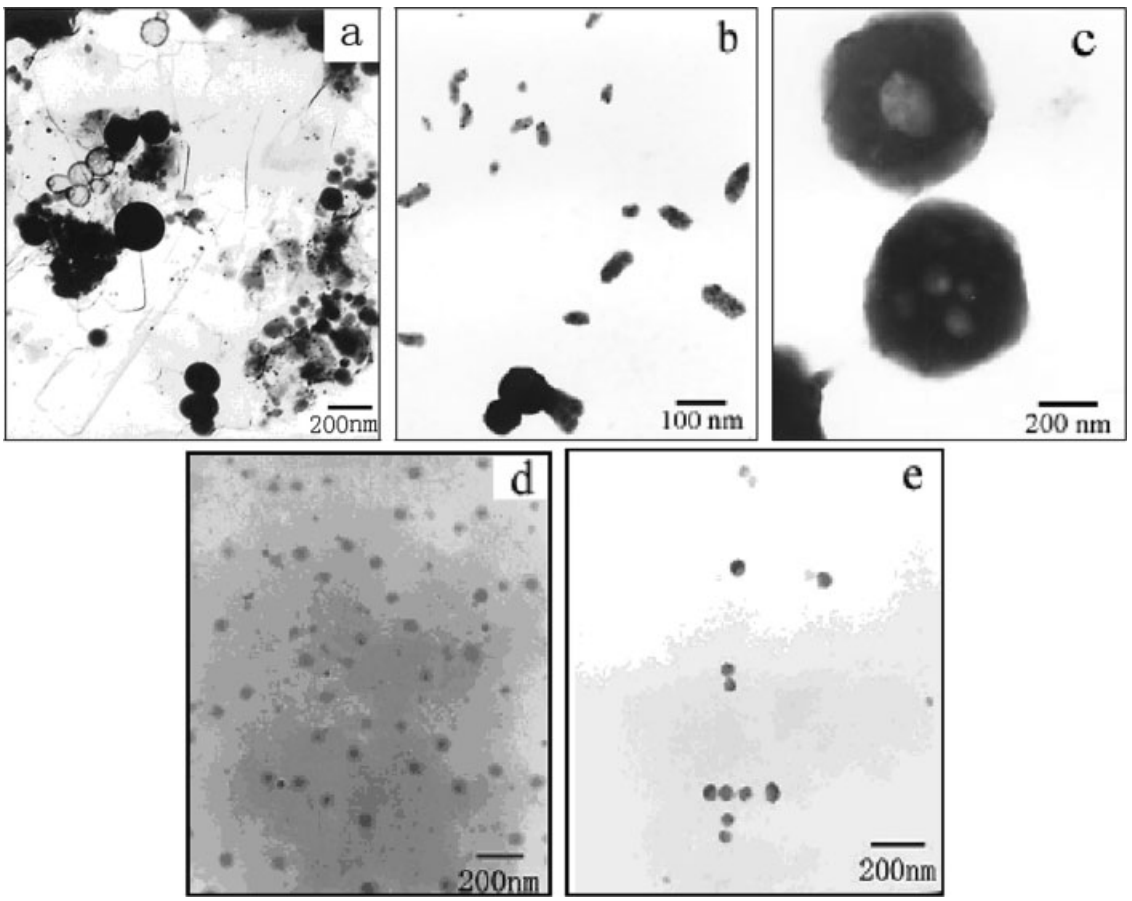


Figure 6 TEM micrograph of polymeric aggregations prepared using dialysis method. (a–c) polymer without PSt homopolymer clearance; (d) polymer without PDEA homopolymer clearance; (e) both PDEA and PSt homopolymers are weeded out.

the hydrated outer shell is destroyed. Without the protection of the micelle shell, micelles aggregate due to inner molecular interaction, which results in a sharp decrease of optical transmission. Moreover, the aqueous solution of the micelles shows reversible change in optical properties: transparent below the LCST and opaque above the LCST. The result is

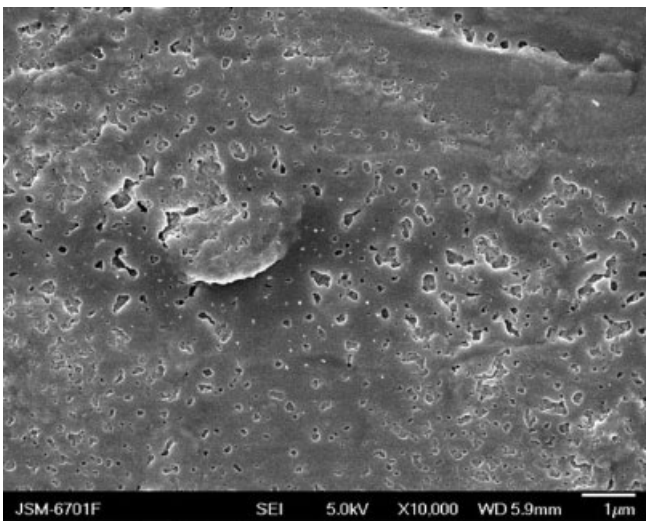


Figure 7 Surface morphology of polymeric aggregations without PSt homopolymer clearance observed by SEM (×10,000).

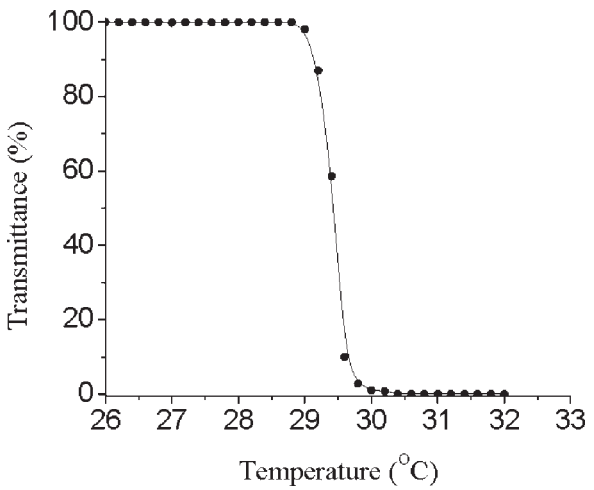


Figure 8 Temperature dependences of optical transmittance at 500 nm in aqueous solution for PSt-*b*-PDEA polymer micelles (1 g L^{-1}).

consistent with the DLS measurements before and after a thermal cycle (20–40°C to 20°C cycling): micelles show no change in their average diameter with a unimodal size distribution before and after the heating period. Similar results were obtained by Okano et al. in their work on PSt-*b*-PNIPAAm micelles, and it was proposed that the phase transition of the micelles is caused by the change in the conformation of the outer shell PDEA chains rather than destruction of the micellar structure.[27] The reversible and sensitive thermoresponse of the PSt-*b*-PDEA micelles might provide opportunities to construct an active drugs delivery system.

For the thermal-responsive polymeric micelles to be of practical value, it is necessary to modulate the LCST a little above the human body temperature, otherwise the micelles would be rapidly destroyed once they enter the bloodstream. At the present time, the improvement to modulate the LCST of polymeric micelles by copolymerization DEA with hydrophilic monomer in the sequentially RAFT polymerization process, as well as the drug-loading and release behavior is under investigation.

CONCLUSIONS

PSt-*b*-PDEA was synthesized by RAFT polymerization of St and DEA monomers. The purified PSt-*b*-PDEA copolymer self-assemble into nanomicelles with a unimodal distribution of 40 ± 0.5 nm. And the clearance of homopolymer in copolymer is absolutely necessary to prepare the unimodal distribution core-shell nanomicelles. The micelles had a low CMC value (0.1 mg L^{-1}), which would be more advantageous when used in drug delivery system for a perfect *in vivo* stability. In addition, the aqueous micelles solution undergoes a reversible phase transition in response to temperature cycles at around 29°C. It is hoped that with such unique characteristics, those thermoresponsive polymeric micelles would find application in drug delivery.

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