



Synthesis and Micellization of Thermo/pH-Responsive Block Copolymer Poly(2-(diethylamino)ethylmethacrylate)-*block*-poly(N-isopropylacrylamide) Prepared *via* RAFT Polymerization

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(Received: 27 March 2012;

Accepted: 14 January 2013)

AJC-12698

Double hydrophilic block copolymer, poly[2-(diethylamino)ethyl methacrylate]-*block*-poly(N-isopropylacrylamide), PDEAEMA-b-PNIPAM, was successfully synthesized with good control *via* reversible addition fragmentation chain transfer radical polymerization (RAFT) with 4-cyanopentanoic acid dithiobenzoate (CPADB) as chain transfer agent in 1,4-dioxane. The diblock copolymer forms core-shell micelles in aqueous solution in dependence of pH and temperature. The behaviour of the double responsive block copolymer in solution was investigated by ¹H NMR and UV-visible. Cloud points measurements indicated that the lower critical solution temperature (LCST) of poly(N-isopropylacrylamide) segment was decreased with increasing pH value of the solution, whereas the pK_a value of PDEAEMA segment was about pH 7.5.

Key Words: Stimuli-responsive polymers, Poly[2-(diethylamino)ethyl methacrylate], Poly(N-isopropylacrylamide), Block copolymer.

INTRODUCTION

Double hydrophilic block copolymers (DHBCs), which represent a class of copolymers with two or more water-soluble blocks made of different chemical species, have recently received the attention of many research groups due to switchable amphiphilic characteristics, the water solubility and the ability for controlled interactions with other nanosized building blocks¹⁻⁵. Double hydrophilic block copolymers (DHBCs) often undergo physical or chemical transformations for one hydrophilic segment becomes hydrophobic, whereas another hydrophilic segment remains soluble in water. The transformation is often induced by subtle adjustment of the temperature, pH, or ionic strength in solutions as well as complexation with appropriate molecules. Thus, double hydrophilic block copolymers provide a wide range of applications including biomimetic chemistry⁶, molecular switch⁷, drug carriers⁸ and gene delivery⁹.

Poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA), a weak polybase with a pK_a of *ca.* 7.3¹⁰, is a pH-responsive polymer. Above its pK_a, PDEAEMA is hydrophobic as its tertiary amine groups are deprotonated. Below its pK_a, PDEAEMA becomes a cationic hydrophilic polymer, as its tertiary amine groups are protonated. PDEAEMA can bind

effectively with negatively charged DNA and thus is one of the desired components to be incorporated in the design of drug and gene delivery systems¹¹. Poly(N-isopropylacrylamide) (PNIPAM), shows lower critical solution temperature (LCST) behaviour in aqueous solution, which is observed at 32 °C¹². In addition, the LCST of PNIPAM-based polymers can be tuned *via* copolymerization with hydrophilic or hydrophobic polymers^{13,14}. Thus, the PNIPAM containing copolymers have been prepared and self-assemble into thermo-responsive micelles as drug carriers.

In recent years, there has been considerable interesting on synthesizing the block copolymer contained pH-responsive segment and thermo-responsive segment by controlled/living polymerization^{2,5,15}. Muller *et al.*¹⁵ synthesized the pH/thermo double responsive block copolymer of poly(acrylic acid)-*block*-poly(N-isopropylacrylamide) *via* reversible addition fragmentation chain transfer polymerization (RAFT). Liu *et al.*¹⁶ synthesized the pH/thermo-responsive diblock copolymer of PNIPAM-b-PDEAEMA *via* RAFT polymerization using 2-cyanoprop-2-yl dithiobenzoate (CDPB) as chain transfer agent and it was subsequently synthesized using the PNIPAM homopolymer as macro-CTA.

Herein, we report the synthesis of well-defined pH/thermo double responsive diblock copolymer of PDEAEMA-b-

PNIPAM via RAFT polymerization using PDEAEMA as macro-CTA in 1,4-dioxane. The pH/thermo double responsive behaviour of the PDEAEMA-*b*-PNIPAM diblock copolymer in aqueous solution was studied by UV-visible and ¹H NMR spectra. These polymers might be applied in drug carriers and gene delivery and biotechnology.

EXPERIMENTAL

2-(Diethylamino) ethyl methacrylate (DEAEMA) (Aldrich, 99 %) was purified by passing through a basic alumina column and distilling from CaH₂ under reduced pressure. N-Isopropylacrylamide (NIPAM) (Aldrich, 97 %) was purified by recrystallization from a mixture of benzene and hexane (3/7, v/v) before use. 4, 4'-Azobis(4-cyanopentanoic acid) (ACPA) (Acros, 97 %) was dried under vacuum prior to use. 4-Cyanopentanoic acid dithiobenzoate (CPADB) was synthesized according to the methods reported in the literature¹⁷. All solvents were redistilled before use. Other reagents were used as received.

Synthesis of PDEAEMA homopolymer: DEAEMA (0.92 g, 4.97 mmol), ACPA (5.8 mg, 0.021 mmol) and CPADB (46.3 mg, 0.17 mmol) were dissolved in 1,4-dioxane (4 mL) in a flask. The solution was degassed by three freeze-vacuum-thaw cycles. The polymerization was carried out at 70 °C for 24 h and stopped by cooling the solution in ice water. The polymer solution was poured into a large excess of *n*-hexane to precipitate PDEAEMA homopolymer. The viscous polymer was dried under vacuum to constant weight. The obtained polymer (0.38 g, 40.8 % conversion) could be used as a macro-CTA.

Synthesis of PDEAEMA-*b*-PNIPAM block copolymer: PDEAEMA (0.146 g, 3.84 × 10⁻² mmol), NIPAM (0.317 g, 2.81 mmol) and ACPA (2.8 mg, 1.0 × 10⁻² mmol) were dissolved in 1,4-dioxane in a flask. The solution was degassed by three freeze-vacuum-thaw cycles. The block copolymerization was carried out at 70 °C for 48 h and stopped by cooling the solution in ice water. The block copolymer was purified by dialysis against distilled water for a week, changing the water twice a day (MWCO 7,000) and recovered by a freeze-drying technique. The results of the homopolymer and diblock copolymer listed in Table-1.

TABLE-1

CHARACTERISTICS OF PDEAEMA HOMOPOLYMER AND PDEAEMA-*b*-PNIPAM DIBLOCK COPOLYMER

Samples	Conversion	M _n ^a	M _n ^b	M _w ^b	PDI ^b
PDEAEMA	40.8	7.3	3.8	4.3	1.12
PDEAEMA- <i>b</i> -PNIPAM	43.2	20.9	11.5	14.3	1.24

^aDetermined by ¹H NMR. ^bDetermined by GPC.

Per cent transmittance: The transmittance of aqueous solution of polymer was determined at λ = 600 nm by Shimadzu UV-2101PC UV-visible spectrophotometer equipped with a Julabo F12 temperature control unit. The LCST of diblock copolymer was defined as the temperature inducing a 10 % decreasing in optical transmittance. The polymeric concentration was kept at 0.2 wt % in all cases.

Potentiometric titration: The titration was performed under constant stirring at 25 °C of 0.2 wt % polymeric solution.

The concentration of HCl was 4.65 × 10⁻³ M used in the experiment.

Detection method: ¹H NMR measurements were performed on a Varian UNITY-plus 400M nuclear magnetic resonance spectrometer using CDCl₃ and D₂O as the solvents, respectively. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) and polydispersity (M_w/M_n) of the polymers were estimated by gel permeation chromatograph (GPC) at 35 °C with a Waters 1525 chromatograph equipped with a Waters 2414 refractive index detector. THF was used as the mobile phase at a flow rate of 1 mL/min. Polystyrene standard was employed for calibration.

RESULTS AND DISCUSSION

Synthesis of the PDEAEMA-*b*-PNIPAM block copolymer: PDEAEMA-*b*-PNIPAM was synthesized via RAFT polymerization, with PDEAEMA as chain transfer agent. The obtained polydispersity is very low. Table-1 summarizes the results of the homopolymer and diblock copolymer.

Fig. 1A exhibits a time-conversion relationship along with the pseudo-first-order kinetic plot for the polymerization of NIPAM in the presence of PDEAEMA macro-CTA and ACPA at 70 °C under N₂ atmosphere in 1,4-dioxane, where the monomer/CTA/initiator ([NIPAM]₀/[PDEAEMA]₀/[ACPA]₀) molar ratio is 520/4/1. The polymerization mixture was subjected to NMR measurements as a function of polymerization to obtain the time-conversion curve. The NIPAM conversion was determined from the ratio between the area of vinyl proton peak at 6.1 ppm and that of the methine proton at 3.9 ppm. Values of M_n and PDI (M_w/M_n) for PDEAEMA macro-CTA used were predetermined to be 3.8 × 10³ between the area of the end benzene protons at 7.4-8.0 ppm and that of the methylene protons at 4.0 ppm and 1.12, respectively. The pseudo-first-order kinetic plot was consistent with a "living" polymerization mechanism over the 28 h; the concentration of active propagating radical species remains nearly constant during this part of the polymerization. Beyond 28 h, downward curvature was observed, which may indicate a decrease in the concentration of propagating radicals. As shown in Fig. 1B, the GPC traces of PDEAEMA-*b*-PNIPAM taken at increasing reaction time showed a continuous shift to higher molecular weight. The GPC elution profiles were unimodal, which indicated that no lower molecular weight PNIPAM homopolymer newly formed. Fig. 1C shows the evolution of M_n and PDI for the polymers obtained by RAFT polymerization of NIPAM as a function of the NIPAM monomer conversion. The M_n and polydispersity and monomer conversion were estimated from GPC and ¹H NMR, respectively. The evolution of M_n with conversion was found to be linear over the 28 h and therefore indicated a controlled polymerization. The PDI increased slightly with the conversion but remained in the range of 1.1-1.3. These observations indicated that the polymerization is reasonably well controlled as "living" in nature.

Thermo-responsive of the PDEAEMA-*b*-PNIPAM diblock copolymer: The thermo-responsive DHBCs contained PNIPAM segment could be investigated by ¹H NMR, for the protons of the micellar core could not be observed in the ¹H NMR spectrum and the protons of the shell remained visible both

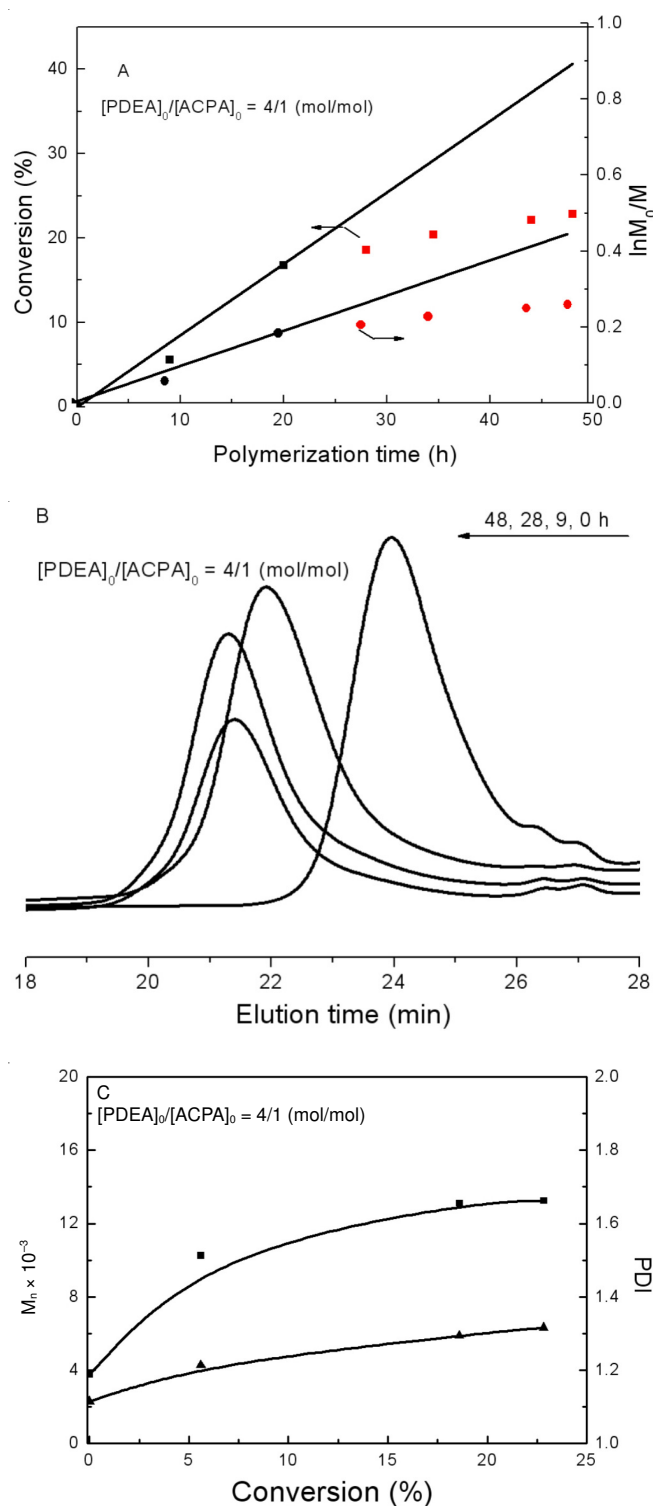


Fig. 1. (A) Conversion-time (squares) and the pseudo-first-order kinetic plots (circles) for the polymerization of NIPAM with ACPA in the presence of PDEAEMA in 1,4-dioxane at 70 °C using a [PDEAEMA]₀/[ACPA]₀ molar ratio of 4/1. (B) GPC traces of PDEAEMA-b-PNIPAM samples taken at different polymerization time. (C) Number average molecular weight (squares) and polydispersity (M_w/M_n) (triangles) of PDEAEMA-b-PNIPAM as a function of conversion at 70 °C

before and after micellization¹⁸. Heat-induced association of PDEAEMA-b-PNIPAM was observed in ¹H NMR spectra measured at different temperatures in D₂O. Fig. 2 shows the ¹H NMR spectra of PDEAEMA-b-PNIPAM measured at pH

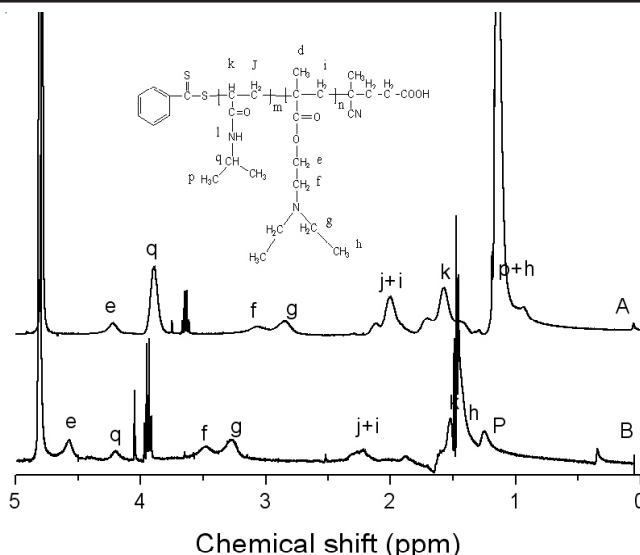


Fig. 2. ¹H NMR spectra of PDEAEMA-b-PNIPAM in D₂O at pH 3.0 under 25 °C (A) and 50 °C (B)

3.0 under 25 °C (A) and 50 °C (B). The resonance peaks in the 2.8 ppm (g), 3.1 ppm (f) and 4.2 ppm (e), observed at 25 °C, were attributed to the side-chain methylene protons in the PDEAEMA unit. The resonance peaks at 3.9 ppm (q) was attributed to the methine protons in the pendent isopropyl group in the NIPAM unit. The composition of PDEAEMA-b-PNIPAM was calculated from the intensity ratio of the resonance bands due to the methylene protons in the side chain of the DEAEMA unit at 4.2 ppm (e) and the methine protons in the side chain of NIPAM unit at 3.9 ppm (e) at 25 °C. The PDEAEMA-b-PNIPAM protons resonance peaks shift to higher fields at elevated temperatures. It is an important observation that the intensities of the resonance peaks due to the isopropyl protons in the NIPAM unit (q and p) relative to the intensity of the resonance peak of due to the side methylene protons in the DEAEMA unit (e) are considerably lower at 50 °C than 25 °C.

The diblock copolymer is completely soluble in aqueous solution at 25 °C. The transmittance of the buffer aqueous solutions (0.05 M phosphate buffer solution with pH 7.4 and citrate buffer with pH 3.0) of PDEAEMA-b-PNIPAM (0.2 wt %) was determined at various temperatures by UV-Vis spectrophotometer at a wavelength of 600 nm (Fig. 3). At pH 3.0, the measured LCST is about 37 °C, which is higher than LCST of pure PNIPAM homopolymer¹². The shift of LCST to higher temperature is attributed to the hydrophilic PDEAEMA segments at pH 3. At pH 7.4 (blood normal pH), the LCST value decreases to *ca.* 33 °C. It is apparent that the LCST values decrease with increasing the pH values. Turbid solutions above LCST become clear again when the solutions are cooled below the LCST of PNIPAM.

pH-Responsive of the PDEAEMA-b-PNIPAM diblock copolymer: The degree of neutralization (α) of the amine group of DEAEMA is defined as

$$\alpha = \frac{([\text{Acid}] - [\text{H}^+] + [\text{OH}^-])}{[\text{Amine}]}$$

where [acid], [H⁺] and [OH⁻] are the concentrations of added acid, free H⁺ and OH⁻, respectively and [amine] is the total

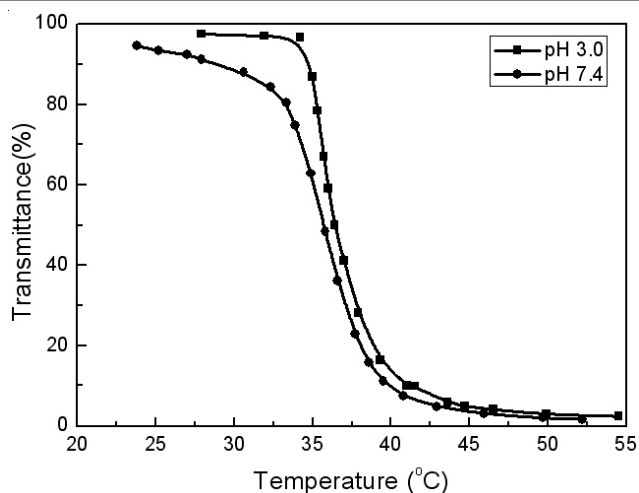


Fig. 3. Temperature dependence of transmittance of the buffer aqueous solutions of PDEAEMA-b-PNIPAM (0.2 wt %) at pH 3.0 (square) and pH 7.4 (circle)

concentration of amine groups. The negative logarithm dissociation constant (pK_b), which provides additional information on the polymer morphology at various pH conditions, can be described by the Henderson-Hasselbalch equation:

$$pK_b = 14 - pH + \log\left(\frac{1 - \alpha}{\alpha}\right)$$

Fig. 4 shows the pH and pK_b curves of the PDEAEMA-b-PNIPAM block copolymer solution (0.2 wt %) as a function of α . As shown in Fig. 4A, the pH value decreased with increasing α and it exhibited a plateau for $0.15 < \alpha < 0.40$. The conformational transitions were also demonstrated by the change in the slope of the pK_b profile (Fig. 4B). The negative logarithm of the intrinsic dissociation constant (pK_0) could be determined by extrapolating the pK_b curve to $\alpha = 0$ and the pK_0 value for the polymer solution (0.2 wt %) is ca. 8.2, as was reported by Tam *et al.*¹⁹. The pK_b curve can be divided into three regions. The slope in the pK_b curve ($\alpha < 0.15$) is negative, which indicates that the amine groups of PDEAEMA are favorable protonation and the PDEAEMA segments are hydrophobic. As α increases ($0.15 < \alpha < 0.40$), the PDEAEMA chains become protonated and the chain conformation becomes more extended.

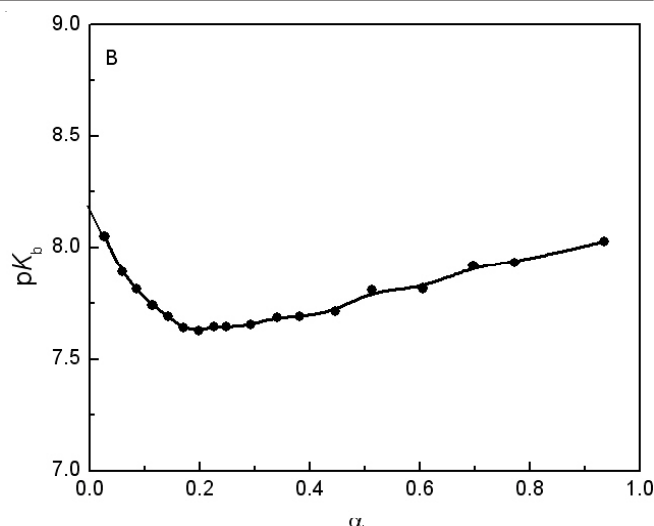
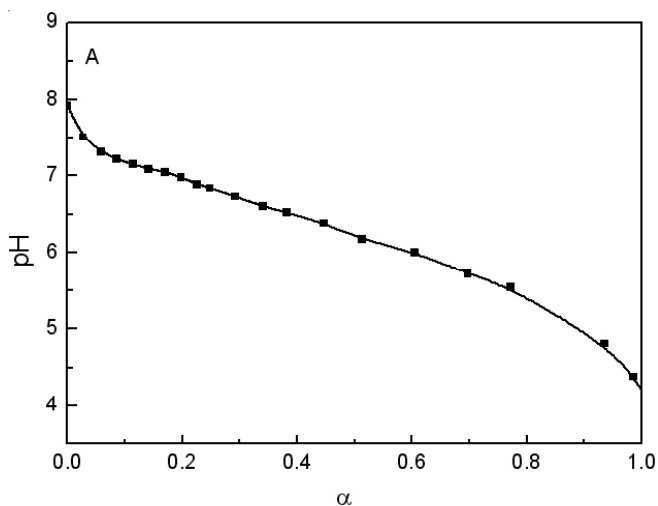


Fig. 4. Titration curves of (A) pH and (B) pK_b for the polymer solution (0.2 wt %)

pH-Induced association of PDEAEMA-b-PNIPAM was also observed in 1H NMR spectra, for the protons of the micellar core could not be observed in the 1H NMR spectrum and the protons of the shell remained visible both before and after micellization²⁰. Fig. 5 shows the 1H NMR spectra of PDEAEMA-b-PNIPAM measured at pH 3.0 (A), pH 7.4 (B) and pH 11.0 (C) under 25 °C. The gradual shift of the peaks at 3.3-3.4, 3.6 and 4.4-4.5 ppm corresponding to the protons of the PDEAEMA side groups to lower area was clearly observed due to the less effective shielding of the electron pair of the deprotonated nitrogen at pH 3 and the resonance peaks of PDEAEMA were almost disappearance at pH 11 due to the forming micellar core of the deprotonated PDEAEMA segment and micellar shell of the hydrophilic PNIPAM segment.

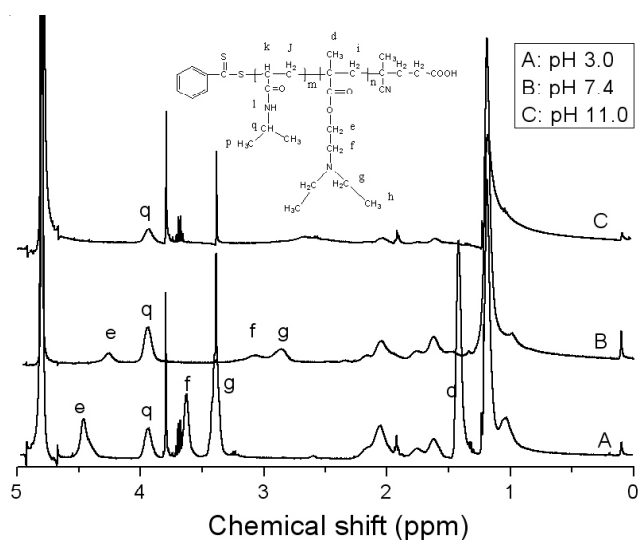


Fig. 5. 1H NMR spectra of PDEAEMA-b-PNIPAM in D_2O at 25 °C under pH 3.0 (A), pH 7.4 (B) and pH 11.0 (C)

To further determine the pH-responsive behaviour of the PDEAEMA-b-PNIPAM diblock copolymer, the transmittance of the polymer solution (0.2 wt %) with various pH values was determined by UV-Vis spectrophotometer at a wavelength of 600 nm (Fig. 6). The transmittance of the solution was

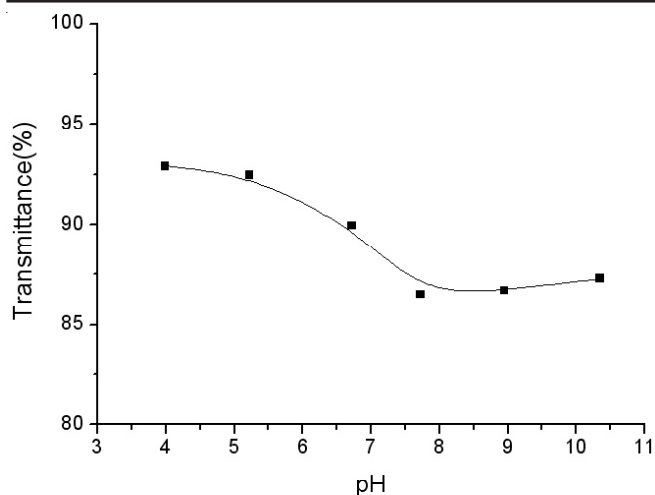


Fig. 6. pH dependence of transmittance of the PDEAEMA-b-PNIPAM solution (0.2 wt %) at 25 °C

decreased with increasing the pH value and it exhibited a plateau for pH > 7.5 due to forming stable core-shell structure with the PDEAEMA block as the core and the PNIPAM block as the shell.

Conclusion

Well-defined PDEAEMA-b-PNIPAM diblock copolymer was successfully synthesized with good control *via* RAFT polymerization with macro-CTA PDEAEMA as chain transfer agent in 1,4-dioxane. The block copolymer forms core-shell micelles in aqueous solution in dependence on pH and temperature. Cloud points measurements indicated that the LCST of PNIPAM segment was decreased with increasing pH value of the solution, whereas the pK_a value of PDEAEMA segment was *ca.* pH 7.5.

ACKNOWLEDGEMENTS

The authors thank the National Natural Science Foundation of China (No. 21106090) and China Postdoctoral Science Foundation (2011M500990) for financial support.

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