

Synthesis and Characterization of Poly(dimethylamino ethyl methacrylate)–Poly(ethylene oxide)–Poly(dimethylamino ethyl methacrylate) Triblock Copolymers

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ABSTRACT: Novel, monodispersed, and well-defined ABA triblock copolymers [poly(dimethylamino ethyl methacrylate)–poly(ethylene oxide)–poly(dimethylamino ethyl methacrylate)] were synthesized by oxyanionic polymerization with potassium *tert*-butanoxide as the initiator. Gel permeation chromatography and ¹H-NMR analysis showed that the obtained products were the desired copolymers with molecular weights close to calculated values. Because the poly(dimethylamino ethyl methacrylate) block was pH-

and temperature-sensitive, the aqueous solution behavior of the polymers was investigated with ¹H-NMR and dynamic light scattering techniques at different pH values and at different temperatures. The micelle morphology was determined with transmission electron microscopy. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 114: 1551–1556, 2009

Key words: anionic polymerization; block copolymers; self-assembly

INTRODUCTION

Poly(ethylene oxide) (PEO)-modified polymers have been developed in past years because they have shown great potential as biomaterials.^{1–9} Poly(dimethylamino ethyl methacrylate) (PDMAEMA) is a complicated but exceptionally unique block with combined pH and temperature sensitiveness in addition to biocompatibility and an ability to be cross-linked.^{10,11} These pH- and temperature-sensitive and biocompatible block copolymers can be used as potential carriers for hydrophobic drugs or DNA.^{1,12–16}

Lascelles et al.¹⁷ used oxyanionic polymerization to synthesize PEO and poly(tertiary amine methacrylate) diblock copolymers. In a previous article,¹⁸ we

reported the synthesis of a PEO–PDMAEMA diblock copolymer bearing an amino moiety at the PEO chain end, and its aqueous pH- and temperature-sensitive behavior was investigated. Although diblock copolymers form core–shell micelles, triblock copolymers may have more complicated and interesting solution behavior. Ni et al.¹⁹ synthesized PDMAEMA–poly(propylene oxide)–PDMAEMA triblock copolymers, and the solution behavior was investigated. However, PEO is mostly used in drug delivery systems, and to our knowledge, the ABA triblock PDMAEMA–PEO–PDMAEMA has not been addressed in the literature. Here, PDMAEMA–PEO–PDMAEMA ABA triblock copolymers with different molecular ratios were synthesized with potassium *tert*-butanoxide (K *t*-Bu) as the initiator, and the self-association behavior in aqueous solutions was carefully characterized with ¹H-NMR, dynamic light scattering (DLS), and transmission electron microscopy (TEM) techniques. They probably have potential applications in drug delivery systems, tissue engineering, and biomimetic mineralization.

EXPERIMENTAL

Materials

Anhydrous tetrahydrofuran (THF; Shanghai Chemical Reagent Co., China, Shanghai) was dried in the presence of benzophenone until a purple color was

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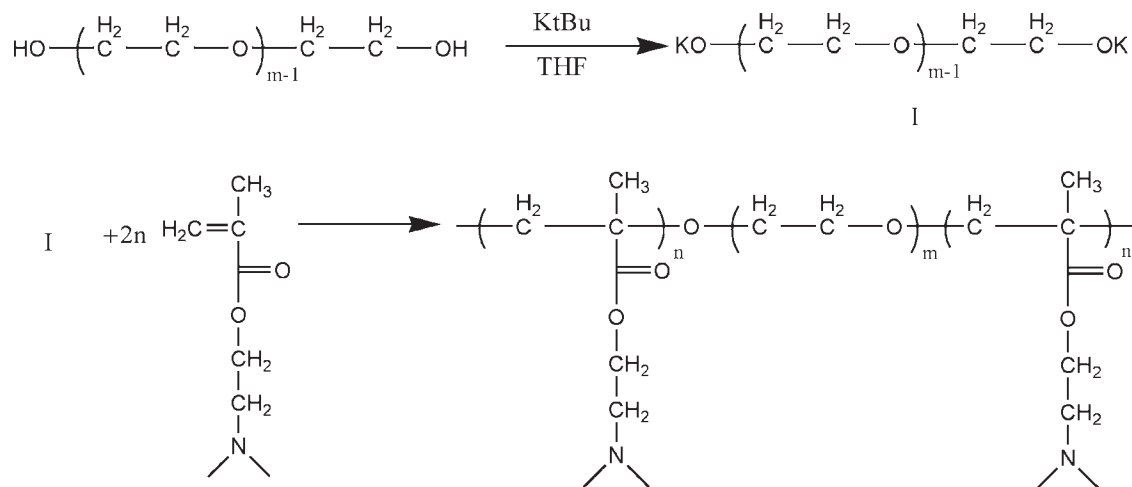
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Scheme 1 Reaction scheme for the synthesis of a PDMAEMA-PEO-PDMAEMA ABA triblock copolymer.

present. The dry THF was then distilled under nitrogen and used immediately. The reactor had an N_2 atmosphere during distillation. The monomer *N,N*-Dimethylamino ethyl methacrylate (DMAEMA; Shanghai Well Done Trade Co., Ltd., Shanghai, China) was first treated with activated Al_2O_3 and then stirred over calcium hydride for at least 24 h before use. The dried monomer was then distilled *in vacuo* immediately before use. PEO (Shanghai Chemical Reagent, Shanghai, China) was heated at $70^\circ C$ for 1 h with a rotary vacuum distillator for the removal of trace water before use. Gel permeation chromatography (GPC) analysis of PEO yielded a number-average molecular weight (M_n) of 2000 and a molecular weight distribution [weight-average molecular weight/number-average molecular weight (M_w/M_n)] of 1.05. All solvents were analytical-grade and were used as received unless stated otherwise.

Block copolymer synthesis

A typical procedure for the synthesis of the PDMAEMA-PEO-PDMAEMA ABA triblock copolymers is shown in Scheme 1. HO-PEO-OH ($M_n = 2000$) was converted into the corresponding potassium alkoxide (KO-PEO-OK) with K *t*-Bu, and then DMAEMA was polymerized with KO-PEO-OK as an initiator to obtain the PDMAEMA-PEO-PDMAEMA ABA triblock copolymer. All glassware was heated at $140^\circ C$ overnight and allowed to cool in a drybox before use. K *t*-Bu was prepared simply by the addition of potassium to excessive *tert*-butyl alcohol. The reaction proceeded at $70^\circ C$ for 1 h, and excessive *tert*-butyl alcohol was distilled. K *t*-Bu was kept in sealed glassware before use. PEO (2 g) was heated at $70^\circ C$ for 1 h with a rotary vacuum distillator for the removal of trace water before use. After this, K *t*-Bu (0.224 g) was added. Half an hour later, the purified monomer (DMAEMA) was added

below $10^\circ C$. After 2 h, the reaction was terminated by the addition of acetic acid to the mixture. The solvent was removed with a rotary vacuum distillator. The copolymer was purified first by repeated precipitation into cold *n*-hexane for the removal of the monomer, and then the trace of PDMAEMA homopolymer precipitation was removed by careful heating of the aqueous solution to around $40^\circ C$. The samples were further purified by dialysis. Dialysis membranes (3500 and 8000–10,000, Huadong Medicine, Hangzhou, China) were heated to $90^\circ C$ before use. The substances were dissolved in distilled water at a concentration of about 10 mg/mL, dialyzed against distilled water with a dialysis membrane (3500 or 8000–10,000) for five or more consecutive cycles, and freeze-dried.

We synthesized PDMAEMA-PEO-PDMAEMA block copolymers with different molecular weights.

GPC measurements

The copolymers were analyzed by GPC with liquid chromatography grade THF as the mobile phase at a flow rate of 1 mL/min with a GMH-HR M column (Viscotek, Houston, TX) and a differential refractive index (RI-101, Shodex, Kawasaki, Japan), and polystyrene standards (Polymer Laboratories, Shropshire, United Kingdom) were used to determine relative molecular weights. The injected volume was 0.1 mL, and the polymer concentration was 5 mg/mL.

NMR spectroscopy

For the three copolymers prepared, 1H -NMR spectra were recorded with a Bruker ARX 500 NMR spectrometer (Wien, Austria) at $25^\circ C$ with $CDCl_3$ as the solvent and tetramethylsilane as the internal standard. M_n of the second block (PDMAEMA) was calculated from the 1H -NMR spectrum of the copolymer

and M_n of the first block (PEO). The $^1\text{H-NMR}$ spectrum of the block copolymer allowed the molar composition to be determined from the relative intensities at 4.13 ($-\text{COOCH}_2-$ of the PDMAEMA block) and 3.66 ppm ($-\text{OCH}_2\text{CH}_2-$ of the PEO block). The aqueous solution properties of the thermosensitive copolymers were also investigated by $^1\text{H-NMR}$ at different temperatures.

DLS

The average copolymer micelle size and size distribution data were obtained with a DLS apparatus (Brookhaven 90 Plus/Bi-MAS multi-angle particle sizing option, Brookhaven Instruments Corp., Holtsville, NY) equipped with a 100-mW argon laser beam operating at a wavelength of 514.5 nm. The intensity of the scattered light was detected at 90° to the incident beam unless otherwise stated. The data were fitted with monomodal cumulant analysis. The block copolymers were first dissolved in THF and dialyzed against water for several days. Samples were filtered through $0.45\text{-}\mu\text{m}$ filters into cells. For each sample, three to five repeated measurements were taken.

TEM

For the TEM measurements, the samples were prepared with one or two drops of an aqueous solution on a copper microgrid coated with a porous carbon film. Then, liquid N_2 was added to freeze the sample quickly. The samples were freeze-dried to remove the water for 5 h and then kept in a desiccator and transferred to a JEM 200CX electron microscope (JEOL, Tokyo, Japan) operating with a 160-kV accelerating voltage under bright-field conditions. Images were recorded on Kodak film at a nominal magnification of up to 30,000. Unless otherwise specified, images were acquired within 24 h of the preparation of the aggregate suspension. We sampled the materials by taking several images of various magnifications over two to three sections per grid to ensure that the analysis was based on a representative region of the sample.

RESULTS AND DISCUSSION

Preparation of the triblock copolymers

One of the advantages of oxyanion-initiated polymerization is that it does not require such strict experimental conditions as anionic polymerization. Another advantage of the technique is that it facilitates the incorporation of polyether blocks with poly(tertiary amine methacrylate)s to produce diblock or triblock copolymers. During the oxyanionic polymerization, the hydroxyl groups, acting as initiators, were transferred to $-\text{O}^-\text{K}^+$ by the use of KH,

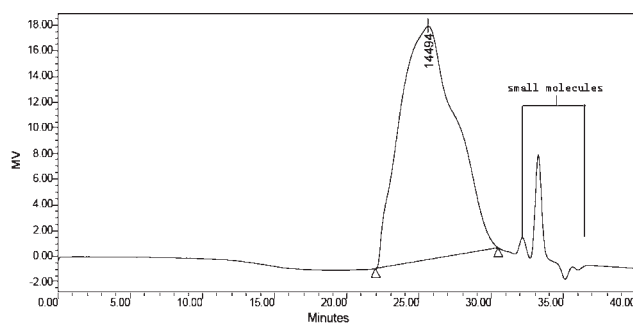


Figure 1 GPC spectra of polymer B after purification. MV, response intensity.

DMSO^-K^+ (where DMSO is dimethyl sulfoxide), potassium naphthalene, or $\text{K } t\text{-Bu}$, and then $-\text{O}^-\text{K}^+$ initiated the polymerization of DMAEMA.^{14,19–22} We tried using $\text{K } t\text{-Bu}$ in its powder state, which is convenient for storage and suitable for quantification. The concentration of PEO in THF was controlled at about 2 g/100 mL. In fact, the homopolymer was unavoidable in anionic copolymerization. We used a little more initiator to ensure that no PEO remained, and because the two hydroxyl groups were equal, no AB copolymer should have been observed. There should have been a little PDMAEMA homopolymer; however, it was not soluble in basic or neutral water at a high temperature, and we removed the trace of the PDMAEMA homopolymer by carefully heating the aqueous solution to around 40°C and filtering out the precipitation. The resulting copolymers were further purified by repeated dialysis. A comparison of the PEO-PDMAEMA diblock copolymers (polydispersity index = 1.2–1.3) showed that the polydispersity index of ABA copolymers was larger (mainly 1.4–1.6). The yield of the ABA copolymers was 85%.

NMR and GPC characterization

Molecular characteristics of the copolymers

The composition of the ABA triblock copolymer was determined from the $^1\text{H-NMR}$ spectrum with the relative intensities at 4.13 ($-\text{COOCH}_2-$ of the PDMAEMA block) and 3.66 ppm ($-\text{OCH}_2\text{CH}_2-$ of the PEO block). Three kinds of different block ratio copolymers were prepared: PDMAEMA₁₀-PEO₄₅-PDMAEMA₁₀ (A), PDMAEMA₄₅-PEO₄₅-PDMAEMA₄₅ (B), and PDMAEMA₉₀-PEO₄₅-PDMAEMA₉₀ (C). GPC showed that they had M_n values of 4830, 16,400, and 28,640 and M_w/M_n values of 1.43, 1.51, and 1.63. All these samples had the same PEO block length with various PDMAEMA block lengths. After the purification, there should have been no homopolymer (PEO or PDMAEMA) left; however, the polydispersity of the prepared copolymer was a little larger, as shown in Figure 1. The molecular weights and polydispersities of the polymers are listed in detail in Table I.

TABLE I
Molecular Characteristics of the ABA Copolymer

Sample	M_n^a	M_w/M_n	Calculated composition				Degree of polymerization ^a	
			By GPC		By ¹ H-NMR		PDMA	PEO
			PDMA	PEO	PDMA	PEO		
Polymer A	4830	1.43	18	45	14	45	16	45
Polymer B	16400	1.51	92	45	68	45	80	45
Polymer C	28640	1.63	170	45	156	45	163	45

^a Calculated from a combination of GPC and ¹H-NMR data. PDMA, unit number of PDMAEMA; PEO, unit number of PEO.

Micellization behavior of different block ratios characterized by ¹H-NMR

¹H-NMR was used earlier by Vamvakaki et al.²³ and recently by Armes et al.²⁴ to characterize the micellization behavior of a Poly(ethylene oxide)-b-poly(die-thylamino ethyl methacrylate) copolymer in an aqueous solution; this allowed the determination of the core-forming block that became insoluble upon micellization, resulting in the disappearance of the corresponding peaks, whereas the second block remained well solvated in the micelle corona, conferring stability to the copolymer micelles.

In this study, ¹H-NMR spectra of polymer **B** were recorded at various temperatures with the aim of a general investigation of the copolymer solution properties with increasing temperature. The ¹H-NMR spectra of polymer **B** with a concentration of 2.0 mg/mL and a pH of about 8.0 are shown in Figure 2. The signals observed at 2.29, 2.69, and 4.13 ppm are due to the protons of the DMAEMA units, whereas the signal at 3.65 corresponds to the protons of PEO chains. The signals of PDMAEMA chains broadened and became much less intense with respect to those of PEO when the solution was heated to 48°C, and this suggested the formation of micelles. The peaks changed slowly from 48 to 58 to 68°C. When the sample was cooled, the same ¹H-NMR spectra could be obtained, showing excellent micellization reversibility with the temperature. For polymer **A**, peaks of the PDMAEMA block did not change obviously, and this indicated that the DMAEMA units were in a good solution. For polymer **C**, the PDMAEMA block was extremely long; at room temperature, broad ¹H-NMR signals belonging to the PDMAEMA block were observed, and as the temperature increased to 45°C, precipitation occurred.

DLS

Yao et al.²⁵ reported the synthesis of amphiphilic ABA-type polyelectrolytes of poly(methyl methacrylate)-b-poly(methacrylic acid)-b-(methyl methacrylate) polymers with different hydrophobic lengths by atom transfer radical polymerization. They proposed that when the polymer was less hydrophobic,

the chains aggregated via an open association process to form multilayer particles (two to three layers), and for a more hydrophobic polymer, a closed association process produced flowerlike or rosette micelles. Under an intermediate hydrophobic condition, P(MMA₃₇-MAA₈₂-MMA₃₇), the flowerlike micelle, coexisted with large aggregates. In PDMAEMA-PEO-PDMAEMA systems, the network structure is supposed to form via an open association process, as shown in Scheme 2. However, at high temperatures, the solubility of water for PEO is poorer than that at room temperature, and some flowerlike structure may form in the network, as PEO chains shrink at high temperatures.

The average count rate represents the intensity of the scattering light in the DLS measurement. Although the average count rate of the solution was below 20 kcps, it suggested no micellization. In this study, we found that all the average count rates of PDMAEMA-PEO-PDMAEMA triblock copolymers under an acidic condition (pH 4.5) were below 20 kcps, and this indicated that no micelle formed. However, at pH 9, all three copolymers had formed

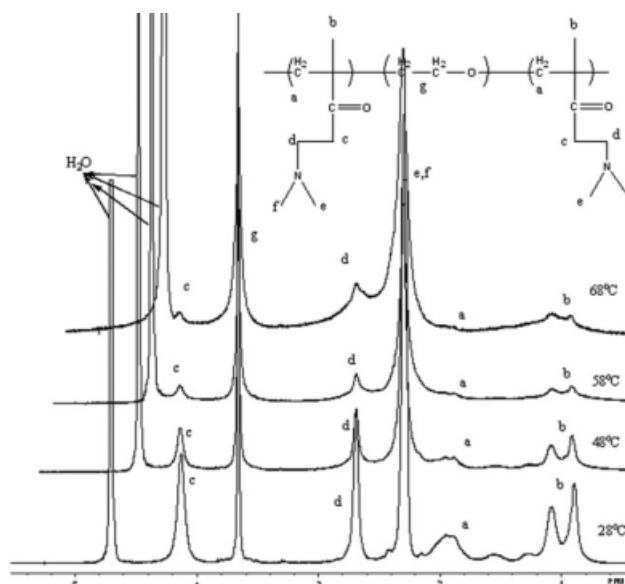
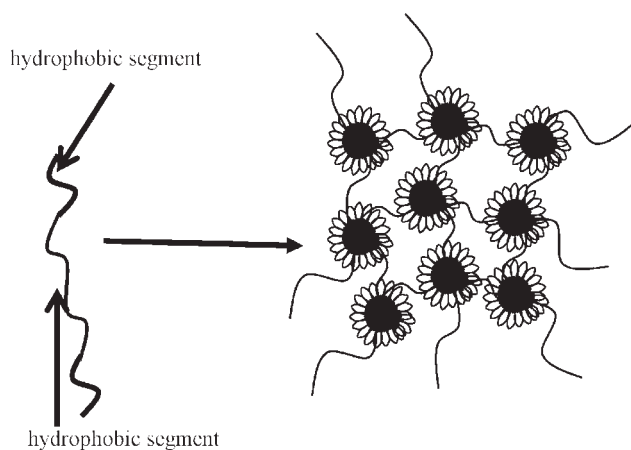


Figure 2 ¹H-NMR spectra of a 2.0 mg/mL polymer **B** solution in D₂O at pH 8 and 28, 48, 58, or 68°C.



Scheme 2 Possible aggregation mechanism for triblock copolymers in an aqueous environment.

micelles, and the difference was that they had different critical micellization temperatures. Hence, the copolymer was pH-sensitive.

The thermosensitivity of the three polymers was also investigated with a concentration of 2.0 mg/mL and a pH of about 8.0. For polymer A, the average count rate increased slowly as the sample was heated, and this indicated no obvious micellization at pH 8.0. For polymer B, below 40°C, the values of the average count rate were nearly the same and were below 20 kcps. As the sample was heated, the average count rate changed very quickly. The critical micellization temperature was 45°C, and the diameters were about 200 nm; afterwards, as the temperature increased, the size decreased. This indicated that the micelles of B were network-like. For polymer C, between 20 and 40°C, the average count rates were very near 290 kcps, and this suggested that, at room temperature, this polymer could form micelles because of the long PDMAEMA chains. The average count rates of polymers A and B varied with the temperature, as shown in Figure 3. The DLS results match the conclusion from the NMR investigations. Figure 4 shows the micelle size as a function of temperature for polymer B at 0.310 mg/mL and pH 8. When each of these samples was cooled back to room temperature, the average count rate was recovered, and this suggested that the micellization processes for these systems were reversible.

For each block copolymer, the critical micelle concentration (cmc) of a neutral environment solution was determined by DLS studies via stepwise dilution of the copolymer solution. For polymer A, at all concentration levels, no micelles were formed according to DLS measurements. ¹H-NMR at 60°C showed no sign of micellization. For polymer B, when the solution was diluted to 0.212 mg/mL, only when the solution was heated to 59°C did micelliza-

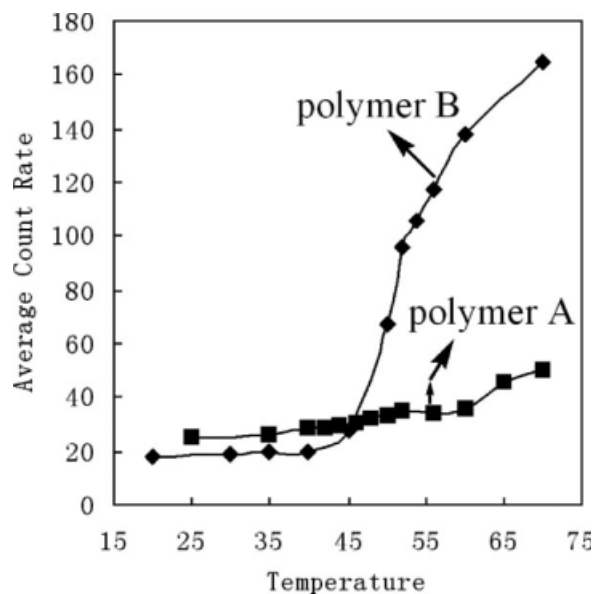


Figure 3 Aqueous solution behavior of ABA triblock copolymers at different temperatures and pH 9: (a) A and (b) B.

tion occur. Further diluted to 0.187 g/mL, the solution remained clear at all temperatures from 25 to 74°C, no blue light was observed, and DLS also showed no micelle formation. Thus, the cmc of polymer B was determined to be between 0.19 and 0.21 mg/mL in a neutral environment. The same methods were also applied to polymer C, and the cmc was between 0.08 and 0.10 mg/mL. By such a simple method, we could easily determine the cmc, although it was a bit rough. Many methods used for

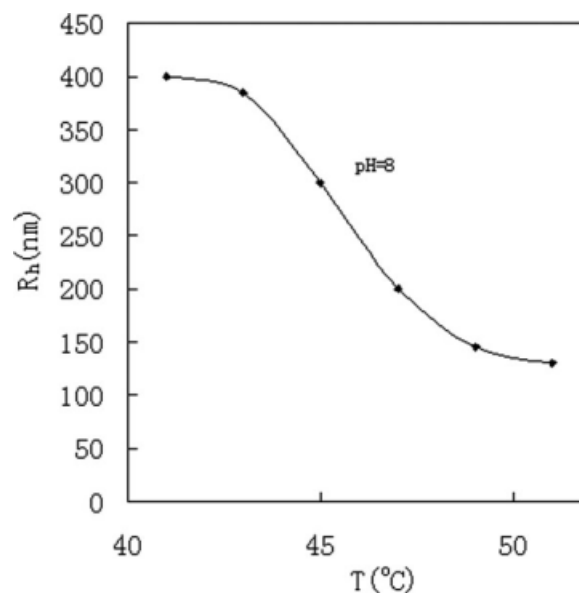


Figure 4 R_h (radius of hydration) as a function of temperature for polymer B (concentration = 0.310 mg/mL) at pH 8.0 (in dilute NaOH).

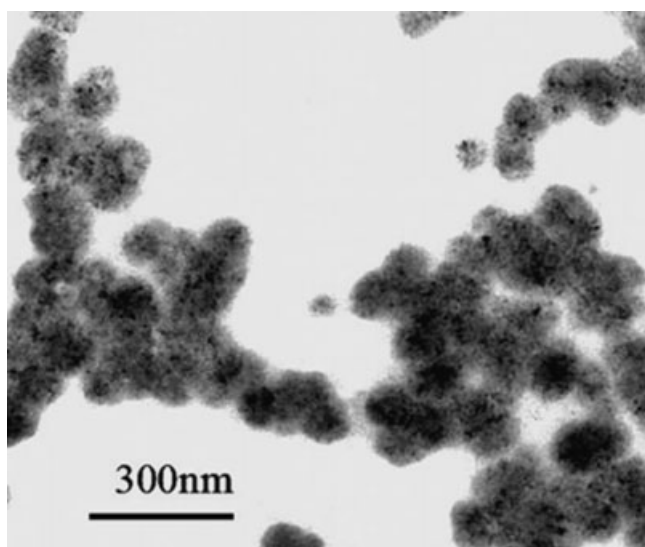


Figure 5 TEM image of polymer **B** made at 55°C and pH 8.

the determination of cmc, including surface tension measurement and fluorescence spectroscopy, can give a more precise value of cmc; however, it seems not necessary here for this block copolymer system.

TEM

Figure 5 shows a TEM image of an ABA triblock copolymer made at 55°C and pH 8. Sphere nanoparticles were observed, and different spheres came into contact with one another. This was more evidence of micellization. The spheres were aggregated because at higher temperatures, the solubility of water for PEO was not as good as at room temperature; hence, PEO chains might be less effective in providing colloidal stabilization to the micelles. Therefore, the spheres could be presented as an interconnected phase in the solution. A single sphere was mainly about 150 nm and was network-like. Many small, dark dots could be observed in the TEM image because in the network-like micelle, the joint points might have had more polymer and could be seen as the dark dots. This is more evidence for network-like micelles.

CONCLUSIONS

Well-defined PDMAEMA-PEO-PDMAEMA was successfully synthesized with different block ratios by oxyanionic polymerization. In comparison with other initiators, *K t*-Bu, used as a powder, did not lose its initial ability for at least 2 months when kept in a sealed vessel and thus provided convenience for oxyanionic polymerization. GPC and NMR were used to determine that the desired copolymers with a single peak were obtained. The self-association

behavior of the ABA triblock PDMAEMA-PEO-PDMAEMA with different lengths in an aqueous medium was determined with $^1\text{H-NMR}$, DLS, and TEM techniques. Under an acidic condition (pH 4.5), no micelle formed for the three copolymers; however, under a basic condition (pH 9), they could form micelles at different critical micellization temperatures. In an almost neutral medium (pH 8), for polymer **C** with the longest PDMAEMA chains, micelles were produced at room temperature; for polymer **A** with the shortest PDMAEMA segments, there was no micellization at any temperature; and for polymer **B** with moderate PDMAEMA segments, micelles formed at 45°C.

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