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Poly(DMAEMA-*co*-PPGMA): Dual-Responsive "Reversible" Micelles

Xian Jun Loh
Melville Laboratory for Polymer Synthesis, Department of Chemistry, University of Cambridge, Cambridge, United Kingdom
Correspondence to: X. J. Loh (E-mail: xianjun_loh@scholars.a-star.edu.sg)

ABSTRACT: A series of copolymers with poly(dimethylaminoethyl methacrylate) (P(DMAEMA)), and poly(propylene glycol methacrylate) (PPGMA) segments were synthesized by atom transfer radical polymerization (ATRP) technique. The composition and structural information of the copolymers were studied by gel permeation chromatography (GPC), ^{1}H-NMR, and TGA. The critical micellization concentrations (CMCs), as well as thermodynamic parameters for micelle formation, of these water-soluble copolymers were determined at different temperatures. The micelles formed were temperature and pH responsive as determined by lower critical solution temperature (LCST) measurements at different pH environments. At elevated temperatures and low pH, micelles will form with the hydrophobic P(PPGMA) core and the hydrophilic P(DMAEMA) corona. When the temperature is lowered and the pH raised, the morphology of the micelles are "reversed." In this situation, micelles will form with the hydrophobic P(DMAEMA) core and the hydrophilic P(PPGMA) corona. Preliminary cytotoxicity studies were carried out and showed that these copolymers were nontoxic to cells. These copolymers thus show significant promise for use as a multiresponsive carrier vehicle for the delivery of drugs and other therapeutics to the body. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: atom transfer radical polymerization (ATRP); biocompatibility; biological applications of polymers; biomaterials

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INTRODUCTION

The self-assembly of amphiphilic block copolymers into particles with different morphologies have attracted extensive research in the past few years. By a precise control of the environmental factors, different micelle morphologies such as rods, spheres, and vesicles can be obtained.[1–5] Polymerization techniques such as atom transfer radical polymerization (ATRP) have been used to prepare stimuli-responsive micelles.[6–9] These micelles have immense potential applications in the different fields, for example, in drug or gene delivery,[10,11] as substrate modifiers,[12,13] in catalysis,[14] and in energy storage.[15] The control of micelle properties can be achieved by using a stimuli-responsive polymer such as poly(N-isopropylacrylamide) (PNIPAAm).[16,17] The water solubility of the copolymers can be adjusted by changing the temperature. In so doing, micelle properties such as internal core hydrophobicity, size, morphology, and dispersion can be controlled. Recent developments have focused on the fabrication of doubly responsive micelles.[18–24] Zhang et al.[18] have prepared PEO-b-P(NIPAAm-*g*-DMAEMA) block copolymers, which showed temperature and pH response. Li et al.[19] have reported dual responsive copolymers with poly(acrylic acid) functioning as the pH responsive component and poly(propylene oxide) functioning as the temperature-responsive component. Jin et al.[20] have also recently reported a temperature and photo-responsive copolymer. In this work, our objective is to synthesize a doubly responsive copolymer based on poly(dimethylaminoethyl methacrylate) (P(DMAEMA)), and poly(poly(propylene glycol methacrylate)) (P(PPGMA)). Such a copolymer can be expected to form micelles in aqueous solutions because of its amphiphilic properties. P(DMAEMA) is able to function as the pH-responsive component as it has a nitrogen moiety, which can be protonated by lowering the pH of the solution.[25,26] On the other hand, poly(propylene glycol) (PPG) is the hydrophobic and temperature responsive part of a very popular triblock copolymer, Pluronics. This copolymer has established biocompatibility and been used in several medical applications.[27–30] This polymer behaves like the temperature-responsive PNIPAAm and has a lower critical solution temperature (LCST) of about 20°C.[30] By synthesizing this copolymer with these two polymeric components, we hope to create a "reversible" micelle. This copolymer is expected to be completely water soluble at low temperatures and low pH. At elevated temperatures and low pH, the PPGMA segment should become hydrophobic and micelles will form with the hydrophobic P(PPGMA) core and the hydrophilic P(DMAEMA) corona. To "reverse" the micelles, environmental stimuli can be used to turn the micelle core inside out. To achieve this, the

temperature has to be kept low and the pH raised. In this situation, the P(DMAEMA) segment should become hydrophobic, and micelles will form with the hydrophobic P(DMAEMA) core and the hydrophilic P(PPGMA) corona. This leads to the formation of "reverse" micelles. The reversible micelle formation is driven by the interactions between the side chains of the copolymer. Such a system bears enormous applicability in the area of drug release where drugs can be incorporated into the two different compartments of the micelle, and the specific drug release can be triggered by activating the specific stimuli.

EXPERIMENTAL

Materials

Ethyl 2-bromoisobutyrate (EBiB; 98%), 2-(di-methylamino)ethyl methacrylate (DMAEMA), poly(propylene glycol) methacrylate (PPGMA) (M_n = 375 g mol^{-1}), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 99%), copper(I) bromide (CuBr, 99%), tetrahydrofuran (THF), and 2-propanol were obtained from Aldrich.

Synthesis of P(DMAEMA-*co*-PPGMA) Copolymers by ATRP

The reaction was performed in a round bottom flask equipped with a magnetic stirrer and under the typical conditions for ATRP. As an example, the synthesis of copolymer R1 is described. DMAEMA (4 g, 25.4 mmol), PPGMA (1 g, 2.7 mmol), EBiB (0.08 g, 0.4 mmol), and HMTETA (0.09 g, 0.4 mmol) were introduced into the flask containing 10 mL of 2-propanol. After DMAEMA and PPGMA had dissolved completely, the reaction mixture was degassed by bubbling with argon for 30 min. Then, copper(I) bromide was added into the mixture under an argon atmosphere. The reaction mixture was purged with argon for another 10 min. The flask was then sealed with a rubber stopper under an argon atmosphere. The polymerization was allowed to proceed under continuous stirring at 50°C for 24 h. The reaction was stopped by diluting with THF. The catalyst complex was removed by passing the blue dilute polymer solution through an aluminum oxide column. A colorless solution was obtained after running the column. THF was removed with a rotary evaporator, and the polymer was obtained in a round-bottom flask. 10 mL of THF was added into the round bottom flask to redissolve the crude product. The copolymer was then precipitated from hexane and followed by reprecipitation in diethyl ether. The copolymers obtained were dried in vacuum overnight at 40°C. Yield for copolymer R1 = 3.81 g (76.2%).

^{1}H-NMR ($CDCl_3$) of P(DMAEMA-*co*-PPGMA) R1: δ (ppm) 0.89–1.02 ((C—CH_3) of the backbone of P(DMAEMA)), 1.15 ((CH_3) of P(PPGMA)), 1.6–2.0 ((C—CH_2) of the backbone of P(DMAEMA)), 2.26 ((N—CH_3) of P(DMAEMA)), 2.56 ((N—CH_2) of the P(DMAEMA)), 3.23–3.94 (—O(CH$_3$)C$\underline{H}$ CH$_2$O— and —O(CH$_3$)CHC$\underline{H_2}$O— of P(PPGMA)), 4.05 (—(CH_2—O—C=O) protons of P(DMAEMA)).

Molecular Characterization

Gel permeation chromatography (GPC) analysis was carried out with a Shimadzu SCL-10A and LC-8A system equipped with two Phenogel 5 μm 50 and 1000 Å columns (size: 300 mm × 4.6 mm) in series and a Shimadzu RID-10A refractive index detector. THF was used as eluent at a flow rate of 0.30 mL min^{-1} at 40°C. Monodispersed poly(ethylene glycol) standards were used to obtain a calibration curve. The ^{1}H-NMR (400 MHz) spectra were recorded on a Bruker AV-400 NMR spectrometer at room temperature. The ^{1}H-NMR measurements were performed with an acquisition time of 3.2 s, a pulse repetition time of 2.0 s, a 30° pulse width, 5208 Hz spectral width, and 32K data points. Chemical shift was referred to the solvent peaks (δ = 7.3 ppm for $CHCl_3$, δ = 4.7 ppm for HOD).

Critical Micellization Concentration (CMC) Determination

Steady-state fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer.[16,17] Excitation spectra were monitored at λ_{em} = 390 nm. Slit widths for both excitation and emission sides were maintained at 3.0 nm. Sample solutions were prepared by dissolving a predetermined amount of copolymer in an aqueous pyrene solution of known concentration, and the solutions were allowed to stand for 1 day for equilibration. The concentration of pyrene was kept at 6.0 × 10^{-7} M. The pH of the solution was kept at 7.4.

LCST Determination

Cloud points were measured with a UV–vis spectrophotometer similar to previous reports.[16,17] Aqueous copolymer solutions (1 wt %) were heated at 2°C min^{-1} while both the transmittance at 650 nm (1 cm path length) and the solution temperature were monitored.

Transmission Electron Microscopy (TEM)

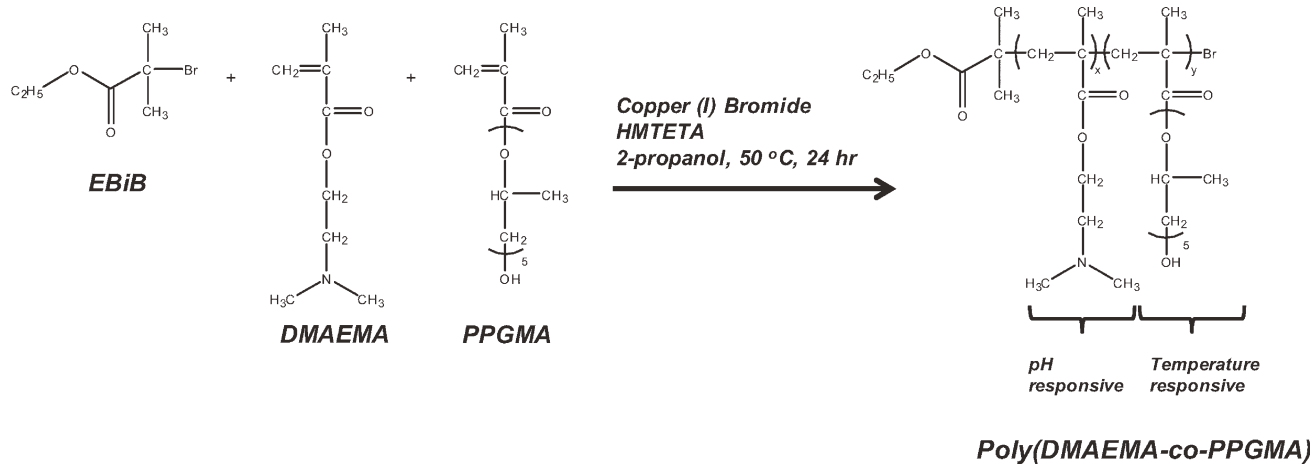
The samples were imaged on a JEOL JEM-2010F FasTEM field emission transmission electron microscope, operated at 100 kV. Samples were prepared at either pH 4 or pH 9. The samples for transmission electron microscopy (TEM) were prepared by directly depositing one drop of sample solution onto copper grids, which were coated in advance with supportive Formvar films and carbon (Agar Scientific). The samples were allowed to dry at either 10°C or 37°C before TEM imaging. A drop of the copolymer aqueous solution (0.5 mg mL^{-1}) containing 0.1 wt % phosphotungstic acid (PTA) was deposited onto a 200 mesh copper grid coated with carbon. Excessive solution was removed with a Kimwipes delicate wipe. The shape of the micelles were directly determined from each transmission electron micrograph.

Cells and Media

L929 mouse fibroblasts were obtained from ATCC and cultivated in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were grown as a monolayer and were passaged upon confluence using trypsin [0.5% w/v in phosphate buffer solution (PBS)]. L929 cells were harvested from culture by incubating in trypsin solution for 10 min. The cells were centrifuged and the supernatant was discarded. Three mL of serum-supplemented DMEM was added to neutralize any residual trypsin. The cells were resuspended in serum-supplemented DMEM at a concentration of 2 × 10^4 cells per milliliter. Cells were cultivated at 37°C and 5% CO_2.

Cell Viability Assay

The cytotoxicity of the copolymers was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)



Scheme 1. Synthesis of poly(DMAEMA-*co*-PPGMA) by ATRP.

assay in L929 cell lines. The cells were cultured in complete DMEM supplemented with 10% FBS at 37°C, 5% CO_2, and 95% relative humidity. The cells were seeded in a 96-well microtiter plate (Nunc, Wiesbaden, Germany) at densities of 3×10^4 cells per well. After 24 h, culture media were replaced with serum-supplemented culture media containing known concentrations of the copolymers, and the cells were incubated for a further 48 h. Then, 10 μL of sterile-filtered MTT stock solution in PBS (5 mg mL^{-1}) was added to each well, reaching a final MTT concentration of 0.5 mg mL^{-1}. After 5 h, unreacted dye was removed by aspiration. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO) (100 microliters per well), and the absorbance was measured using a microplate reader (SpectraPlus, TECAN) at a wavelength of 570 nm. The relative cell viability (%) related to control cells cultured in media without polymers was calculated with $[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ is the absorbance of the wells with polymers and $[A]_{control}$ is the absorbance of the control wells. All experiments were conducted with six repetitions and averaged.

RESULTS AND DISCUSSION

Synthesis of P(DMAEMA-*co*-PPGMA)

P(DMAEMA-*co*-PPGMA) copolymers were synthesized by ATRP of dimethylaminoethyl methacrylate (DMAEMA) and poly(propylene glycol) methacrylate (PPGMA) (Scheme 1). The copolymers were synthesized in 2-propanol at 50°C for 24 h via ATRP of DMAEMA and PPGMA from EBiB initiator. A series of copolymers with different DMAEMA to PPGMA ratios were synthesized by varying the monomer feed. The P(DMAEMA) and P(PPGMA) homopolymers was synthesized for control experiments. The molecular weights of the copolymers are summarized in Table I. Compared with the starting materials, the molecular weight of the products were significantly higher. This indicates that copolymerization of DMAEMA and PPGMA has taken place. After extensive purification of the copolymer by repeated precipitation in hexane and diethylether, the chemical structure of the P(DMAEMA-*co*-PPGMA) copolymer was characterized by ^{1}H-NMR spectroscopy. From ^{1}H-NMR, the composition of the copolymers were calculated and summarized in Table I. No vinyl bonds are detected in the NMR samples, indicating the absence of unreacted monomers. The thermal decomposition profiles under nitrogen atmosphere are shown in Figure 1. P(DMAEMA) homopolymer showed a distinct decomposition step, which occurred at around 340°C, the second decomposition step is observed at about 420°C. On the basis of the percentage weight loss, the first decomposition steps of this curve is attributed to the loss of $-CH_2CH_2N(CH_3)_2$ fragment from the P(DMAEMA). The percentage weight loss from the two decomposition steps agree reasonably well with the calculated values based on the molecular formula of the

Table I. Molecular Characteristics of Poly(DMAEMA-*co*-PPGMA)

	Feed ratio (wt %)		Actual composition (wt %)[b]		Actual composition (wt %)[c]		Copolymer characteristics		
Copolymer[a]	DMAEMA	PPGMA	DMAEMA	PPGMA	DMAEMA	PPGMA	M_n[d] ($\times 10^3$)	M_w/M_n[d]	CMC[e] $\times 10^4$ (g mL^{-1})
R1	80.0	20.0	84.0	16.0	78.0	22.0	9.24	1.11	4.07
R2	60.0	40.0	62.5	37.5	53.0	47.0	7.34	1.24	2.10
R3	20.0	80.0	23.4	76.6	20.1	79.9	8.70	1.38	0.49
R4	0	100.0	0	100.0	0	100.0	7.73	1.42	0.50
R5	100.0	0	100.0	0	100.0	0	6.54	1.16	-

[a]The M_n of PPGMA used for the copolymer synthesis was 375 g mol^{-1}, [b]Calculated from ^{1}H-NMR results, [c]Calculated from TGA results, [d]Determined by GPC, [e]Critical micellization concentration (CMC) in water determined by the dye solubilization technique at 25°C.

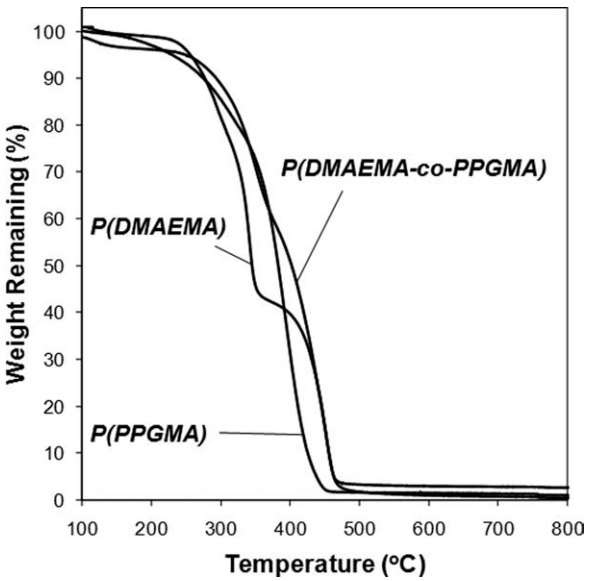


Figure 1. Thermal decomposition profile of the copolymer, R2, in comparison with the homopolymers P(PPGMA) and P(DMAEMA).

homopolymers. On the other hand, P(PPGMA) homopolymer degrades with a one step thermal decomposition profile. With this knowledge in mind, it is possible to calculate the composition of the copolymer based on the thermal decomposition of the copolymer. The calculated values are presented in Table I and they agree well with the composition determined by NMR. Therefore, the TGA and NMR results taken together with the GPC results demonstrate the successful synthesis of the copolymers.

Micelle Properties

NMR spectroscopy is a useful tool to investigate the effect of solvent on the micelle structure.[16,17,27] $CDCl_3$ is a good solvent for both P(PPGMA) and P(DMAEMA), whereas water is a good solvent for P(DMAEMA) but poor for P(PPGMA). In $CDCl_3$, all the peaks because of the PPGMA and P(DMAEMA) segments were sharp and well defined (Figure 2). In an aqueous environment at 37°C, P(DMAEMA) maintains excellent water solubility, and its peak appears sharp and narrow. However, at this temperature, P(PPGMA) has reduced solubility, and its peaks were suppressed. This suggests that the molecular motion of P(PPGMA) is slow in water, indicating a hydrophobic core structure made up of P(PPGMA) with the hydrophilic P(DMAEMA) as the outer corona structure. The P(DMAEMA-*co*-PPGMA) copolymers were soluble in water. Critical micellization concentration (CMC) determination was performed for these copolymers using conventional fluorescence spectroscopy.[16,17] Extensive studies on the micellization of amphiphilic block copolymers have been performed using the fluorescence probe technique. This method relies on the significant changes observed in the emission and excitation spectra of pyrene are observed upon the encapsulation of the dye in the hydrophobic core of the micelles. On encapsulation, a shift of the low-energy band from 334 to 338 nm is observed in the fluorescence excitation spectrum. The ratio of the intensities of the first and third bands in the pyrene fluorescence spectrum, I_{338}/I_{334}, reflects the extent of the encapsulation of the pyrene dye in the core of the micelle. Hence, the CMC values of the P(DMAEMA-*co*-PPGMA) copolymers in aqueous solution were determined using the fluorescence excitation spectra of the pyrene probe, and the results are listed in Table I. The CMC values of the copolymers showed a decreasing trend when the PPGMA content increased due to the increased hydrophobicity of the copolymer. We were unable to obtain any meaningful results using the P(DMAEMA) homopolymer. This is likely because of the fact that this homopolymer does not form micelles readily. To understand the effect of temperature on the micellization of the

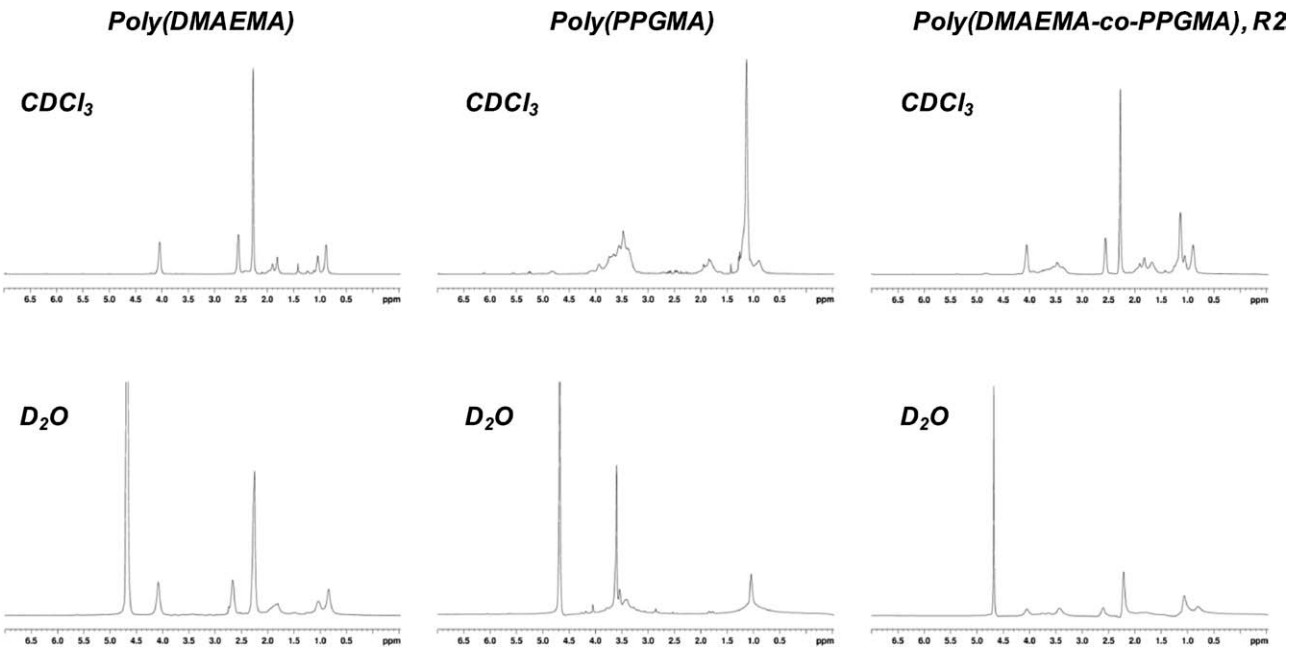


Figure 2. Comparison of the NMR spectra of the homopolymers, poly(DMAEMA), and poly(PPGMA) and the copolymer, R2, in CDCl3 and D2O.

Table II. Thermodynamic Parameters of the Micellization Process of Poly(DMAEMA-*co*-PPGMA)

Copolymer	Temperature (°C)	CMC × 10^4 (g mL^{-1})	ΔG (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	ΔH (kJ mol^{-1})
R1	15	4.37	−33.5	0.320	
	25	4.07	−34.8	0.314	58.7
	35	0.83	−40.0	0.321	
	45	0.56	−42.4	0.318	
R2	15	3.24	−33.6	0.244	
	25	2.10	−35.9	0.243	36.5
	35	1.29	−38.3	0.243	
	45	0.77	−40.9	0.244	
R3	15	0.59	−38.1	0.205	
	25	0.49	−39.9	0.204	20.9
	35	0.37	−41.9	0.204	
	45	0.26	−44.3	0.205	
R4	15	0.56	−37.9	0.169	
	25	0.50	−39.6	0.175	10.8
	35	0.40	−41.5	0.181	
	45	0.38	−42.9	0.187	

copolymer and to investigate the thermodynamics of micelle assembly, the CMC determinations were further performed for these copolymers at 15°C, 25°C, 35°C, and 45°C.[29,30] This allows us to calculate the thermodynamic parameters of micelle formation based on the assumption of a closed association of unimers into micelles. The free energy of micellization ΔG°, can be calculated by

$$\Delta G^\circ = RT \ln(X_{cmc})$$

where R is the gas law constant, T is the temperature in K, and X_{cmc} is the CMC in mole fraction of polymer in the aqueous solution at temperature T. The values of ΔG° are negative, indicating the spontaneity of the micellization process. As the PPGMA content increased, the ΔG° values become more negative, suggesting that PPGMA aids in the micelle formation process. These values also indicate that micelle formation is favored at higher temperatures. The values are tabulated in Table II. The values of the standard enthalpy of micellization, ΔH°, and the standard entropy of micellization, ΔS°, can be extracted from the Arrhenius plot of $\ln(X_{cmc})$ versus T^{-1}.

$$\Delta H^\circ = RT(d \ln X_{cmc}/dT^{-1})$$

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$$

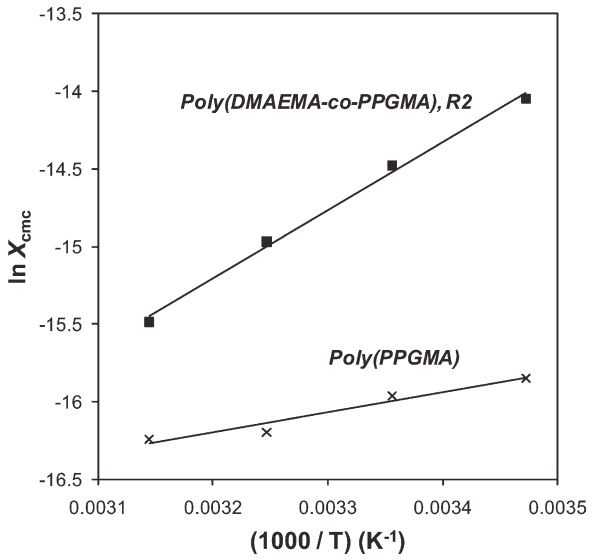


Figure 3. Determination of $\Delta H_{micellization}$ of copolymers by plotting ln X_{cmc} against T^{-1}.

Figure 3 shows the plot of ln X_{cmc} versus T^{-1} for the copolymer R2, compared with P(PPGMA). ΔH° can be calculated from the slope of the linear plot. In all the solutions studied, we observed that the enthalpy of micellization is an endothermic process, similar to aqueous solutions of Pluronic block copolymers. The enthalpy values became less positive (more exothermic) with increasing PPGMA content. This implies that the assembly of the copolymers could be enhanced by increased hydrophobic interactions based on the PPGMA-PPGMA interactions. On the other hand, the entropy contribution is positive with the value becoming less positive with increasing PPGMA content. Overall,

Table III. CMC Values of Copolymer Solutions of Poly(DMAEMA-*co*-PPGMA) at Various pH

	CMC × 10^4 (g mL^{-1})		
Copolymer	pH 4	pH 7	pH 9
R1	20.73	4.07	0.43
R2	12.51	2.10	0.23
R3	2.45	0.49	0.05
R4	2.32	0.50	0.04

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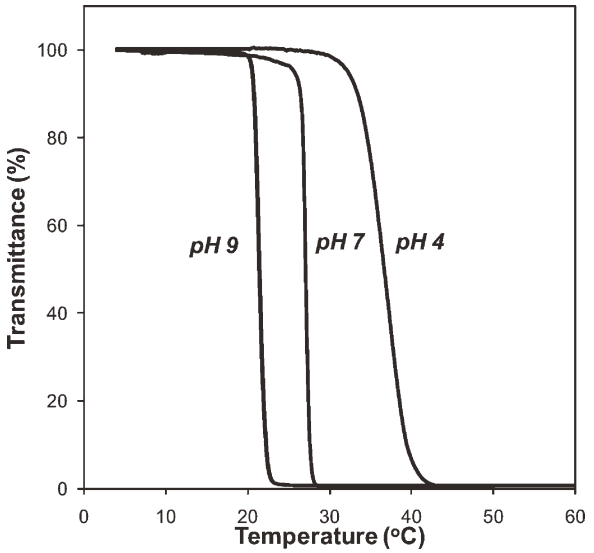


Figure 4. Temperature-induced changes in transmittance of 1 wt % aqueous copolymer solutions of R2 at different pH.

the micellization process is entropy-driven. At low temperatures, when the polymer chains are soluble in water, water molecules interacted closely with the main polymer backbone. On raising the temperature of the solution, the interaction between the water molecules and the polymer backbone becomes less favorable. The result is an expulsion of water molecules from the polymer backbone, leading to an overall entropy gain of the system due to the free water molecules. When the micelles are formed, water is expelled from the PPGMA segments, and the PPGMA segments self assemble to form the hydrophobic core. When the hydrophobic content is increased, the polymer-polymer interaction is increased; however, the polymer-water interaction is reduced. In this case, the entropy gain when the micelle is formed is reduced; hence, we observe a lower ΔS° value.

Furthermore, when the pH is increased, it was observed that the CMC value decreases (Table III). This effect is probably because of the change in the extent of protonation of the P(DMAEMA) segment, which affects the hydrophobicity of the copolymer and its subsequent association into micelles. This is discussed in the next section.

LCST Behavior

Aqueous solutions of these copolymers exhibit a LCST behavior very similar to the PNIPAM solutions. In this work, the point at which there is a sudden drop in transmittance at a certain critical temperature is defined as the LCST. The plot, which illustrates the dependence of the transmittance of the aqueous polymer solution on temperature is shown in Figure 4. The LCST values are tabulated in Table IV. The concentration of the aqueous polymer solution was 1 wt %, which is higher than

Table IV. LCST Values of 1 wt % Copolymer Solutions of Poly(DMAEMA-*co*-PPGMA) at Various pH

	LCST (°C)		
Copolymer	pH 4	pH 7	pH 9
R1	39.1 ± 3.7	29.6 ± 4.4	22.6 ± 1.2
R2	36.8 ± 3.2	27.8 ± 1.5	15.1 ± 2.4
R3	26.9 ± 2.6	23.4 ± 2.7	14.5 ± 2.5
R4	25.7 ± 3.3	15.6 ± 2.8	14.6 ± 2.2
R5	69.4 ± 1.2	44.7 ± 2.2	38.4 ± 2.2

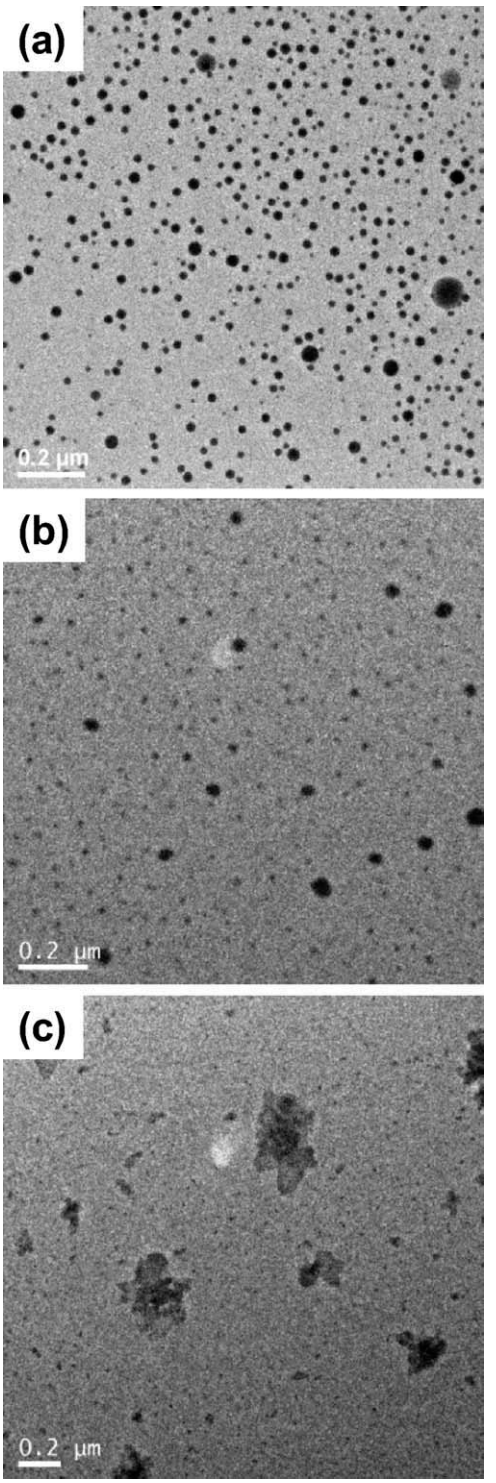
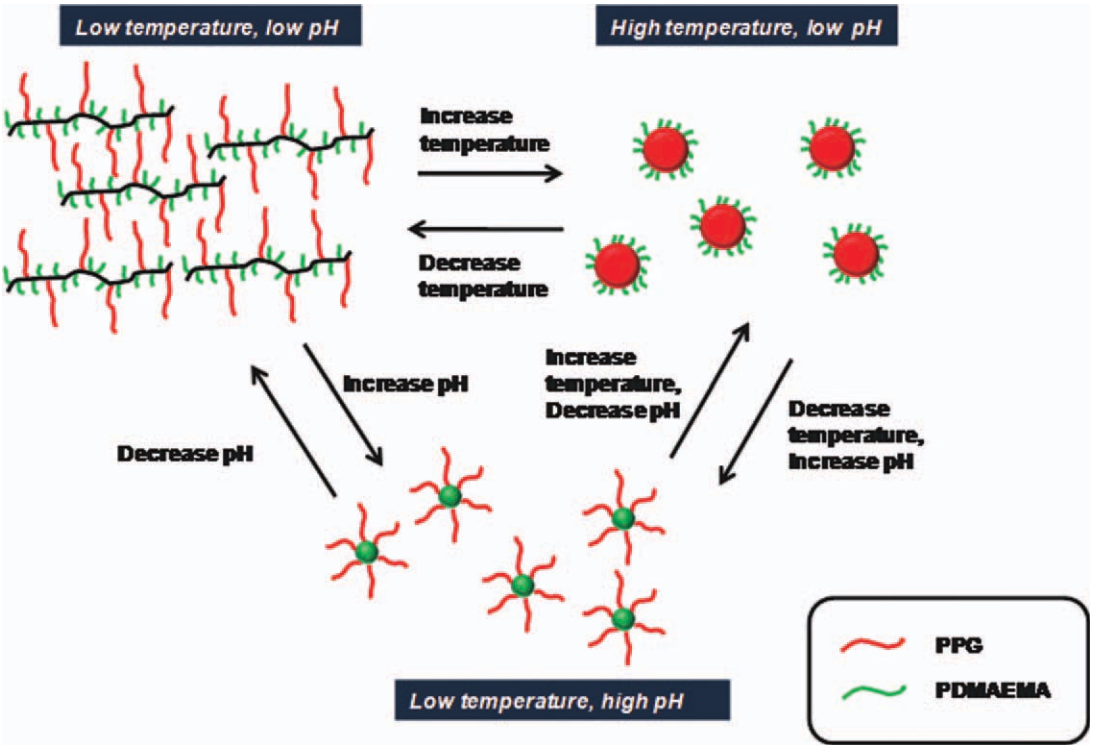


Figure 5. TEM images of the micelles formed under different conditions. (a) pH 4, 37°C, (b) pH 9, 10°C, (c) pH 9, 37°C.



Scheme 2. Diagram to illustrate the "reversible" micelle concept. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the CMC value of the copolymer. For all the copolymers, the solution turned from clear to turbid at a certain temperature, which is reflected as a quantitative measurement as a sudden drop in transmittance by the UV–vis spectrophotometer. The LCST decreases with increasing PPGMA content in the polymer. This is consistent with other studies regarding LCST behavior. In general, it was found that a higher hydrophobic component in the polymer reduced the LCST of the copolymer solution. When the temperature is raised above the LCST, the hydrophobicity of the polymer causes the polymer chains to collapse into each other, leading to the formation of particles. The LCST values obtained at different pHs clearly show that the copolymers are sensitive to pH. A change of pH from pH 4 to pH 7 had resulted in about a 9°C difference in LCST for copolymer, R2. A further increase of the pH from 7 to 9 resulted in a further 12°C reduction in LCST for R2. The effect of pH is due to the various degree of protonation on the basic amine group of DMAEMA in the copolymer. Protonation gives rise to electrostatic repulsion among the side groups of DMAEMA as well as increasing its hydrophilicity, thereby increasing the phase transition temperature. The amine group gets protonated at pH values lower than its pK_a and becomes hydrophilic. The pK_a of P(DMAEMA) has been reported to be 8.4.[31] Therefore, at pH greater than the pK_a; it is nonprotonated and hydrophobic, and precipitates out of solution at a much lower temperature.

Morphology of Micelles

The morphology of copolymer micelles in aqueous solution at different pH was observed by TEM. Figure 5(a–c) show the representative TEM images of the micelles formed by R2 under different conditions. At pH 4 and 37°C (low pH, high temperature), the micelles formed were well-defined spheres with size of about 10–20 nm [Figure 5(a)]. Under this condition, the micelle is supposed to be made of the hydrophilic P(DMAEMA) corona and the hydrophobic PPG core. At pH 9 and 10°C (high pH, low temperature), some well-defined spheres were still observed although the proportion is significantly lesser [Figure 5(b)]. Instead, there are large amounts of lightly stained spheres with size of about 50–60 nm. At this condition, the micelle is supposed to be made of the hydrophilic PPG corona and the hydrophobic P(DMAEMA) core. The lightly stained core could indicate a poor ability of the micelle to entrap the staining agent. Finally, at pH 9 and 37°C (high pH, high temperature), micelle clusters of size about 200 nm can be observed due to the significant hydrophobicity of the polymers, which cause the particles to aggregate [Figure 5(c)]. The proposed pH and temperature-responsive process for aggregation of micelles is presented in Scheme 2. At low pH and low temperatures, the copolymers exist as unimers in solution. When the pH is raised while keeping the temperature low, the P(DMAEMA) segment is deprotonated and forms the hydrophobic core of the micelle, with PPG as the hydrophilic corona. On the other hand, when the temperature is raised while keeping the pH low, the P(DMAEMA) segment remains protonated and forms the hydrophilic corona of the micelle, with PPG forming the hydrophobic core. When both the pH and the temperatures are raised, the copolymer becomes hydrophobic and precipitates out of the solution. This demonstration shows that such dual

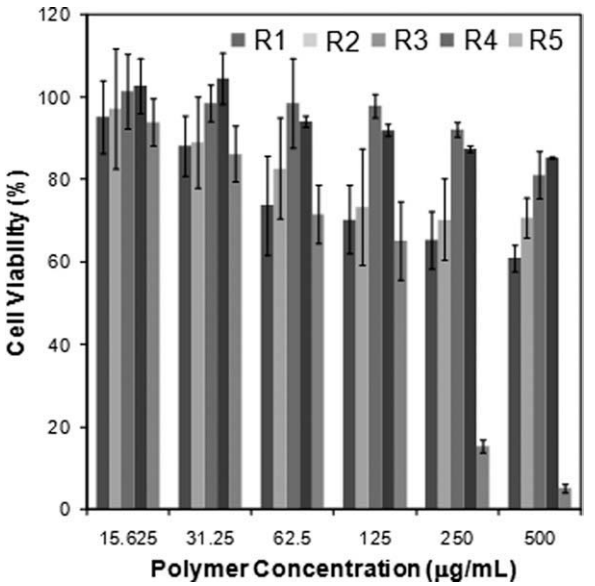


Figure 6. Cell viability of L929 cells incubated with known concentrations of copolymers.

responsive micelles can have the capability to change their morphology when their environmental conditions are changed. Incorporation of two degrees of control over the copolymer allows extended control over the variety of micelle morphologies that can be obtained with these polymers.

Cytotoxicity Study

With an eye toward the application of these copolymers as drug delivery agents, the safety aspects of these copolymers have to be evaluated. Although PPGMA should be safe for applications as it is a part of the Pluronics family of copolymers,[27–30] P(DMAEMA) has been documented to be toxic.[32–34] We evaluated the cytotoxicity of the copolymers by incubating the mouse fibroblast L929 cells with different concentrations of the copolymer solution over a period of 48 h at 37°C. The aim of this experiment is to determine the potential toxicological hazard of the copolymers. Quantification of the cytotoxic response was performed using the MTT assay, as shown in Figure 6. In general, an increase in the P(DMAEMA) content decreases the cell viability, showing that a balance of the P(DMAEMA) content is an important consideration in the design process of the polymers. P(PPGMA) homopolymer does not show significant toxic response, which is encouraging for the future development of temperature-responsive biomaterials comprising PPG segments. The copolymer solutions of R2 and R3 do not show significant cytotoxicity against L929 cells over a solution concentration range of 15–500 μg mL^{-1}. From the MTT assay results of the copolymers, we expect the polymer to be safe for biomedical applications.

CONCLUSIONS

"Reversible" micelles from copolymers with P(DMAEMA) and PPGMA segments were synthesized by ATRP technique. These amphiphilic copolymers formed micelles in solution, and their morphologies can be controlled by temperature and pH of the external environment. The CMCs, as well as thermodynamic parameters for micelle formation, of these water-soluble copolymers were determined at different temperatures. These copolymers showed LCST behavior in solution, and the transition temperature can be tuned by the pH of the solution. At elevated temperatures and low pH, micelles with the hydrophobic P(PPGMA) core and the hydrophilic P(DMAEMA) corona will be formed. To "reverse" the morphology of the micelle, the temperature is lowered and the pH raised, resulting in the formation of micelles with the hydrophobic P(DMAEMA) core and the hydrophilic P(PPGMA) corona. Good biocompatibility was observed when the copolymers were incubated with cells, and these copolymers show great promise for use as a stimuli-triggered drug release vehicle.

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