Aqueous Micellar Gels of Multiresponsive Hydrophilic ABA Linear Triblock Copolymers

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I am submitting herewith a dissertation written by Jeremiah Wallace Woodcock entitled "Aqueous Micellar Gels of Multiresponsive Hydrophilic ABA Linear Triblock Copolymers." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

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Abstract

This dissertation presents the synthesis of a series of well-defined multiresponsive hydrophilic ABA linear triblock copolymers and the study of their aqueous micellar gels. By incorporating a small amount of stimuli-responsive groups into thermosensitive outer blocks of ABA triblock copolymers, the lower critical solution temperatures (LCST) of thermosensitive blocks can be modified by external stimuli. Consequently, the sol-gel transition temperatures \( T_{\text{sol-gel}} \) of their aqueous solutions can be altered.

Chapter 1 describes the synthesis and solution behavior of a series of thermo- and light-sensitive triblock copolymers, poly(ethoxytri(ethylene glycol) acrylate-co-o-nitrobenzyl acrylate)-b-poly(ethylene oxide)-b-poly(ethoxytri(ethylene glycol) acrylate-co-o-nitrobenzyl acrylate), with different contents of light-responsive o-nitrobenzyl groups. Aqueous solutions of these block copolymers with a 10.0 wt% concentration exhibited thermo-induced sol-gel transitions. Upon UV irradiation, the hydrophobic o-nitrobenzyl groups were cleaved, resulting in an increase in the LCST and consequently gel-to-sol transitions. The UV-irradiated solutions again underwent temperature-induced sol-gel transitions but at higher temperatures. The change of \( T_{\text{sol-gel}} \) was, in general, larger for the copolymer with a higher o-nitrobenzyl content after UV irradiation. Chapter 2 presents the synthesis of thermo- and enzyme-responsive ABA triblock copolymers, poly(ethoxydi(ethylene glycol) acrylate-co-4-((dihydroxyphosphoryl)oxy)butyl acrylate)-b-poly(ethylene oxide)-b-poly(ethoxydi(ethylene glycol) acrylate-co-4-((dihydroxyphosphoryl)oxy)butyl acrylate), and the enzyme-induced formation of thermoreversible micellar gels from their moderately concentrated aqueous solutions at 37 °C. The dephosphorylation by acid phosphatase decreased the LCST of
thermosensitive outer blocks from above to below 37 °C. The enzyme-induced gelation of 7.9 wt % aqueous polymer solutions at pH 4.4 was monitored by rheological measurements. The $T_{\text{sol-gel}}$ decreased and the gel strength increased with the increase of reaction time. The gels formed were thermoreversible.

Chapter 3 presents the synthesis of two thermo- and pH-sensitive tertiary amine-containing ABA triblock copolymers and the sol-gel transitions of their aqueous solutions with a 10 wt% concentration at different pH values. Chapter 4 describes the use of reversible addition-fragmentation chain transfer (RAFT) polymerization for the synthesis of well-defined thermosensitive polymethacrylates and polyacrylates. Eight chain transfer agents were synthesized. The RAFT polymerizations of alkoxyoligo(ethylene glycol) (meth)acrylates using these chain transfer agents were well controlled, producing well-defined polymers. A summary of this dissertation research and future work are presented in Chapter 5.
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3.1 (A) Size exclusion chromatography traces of Br-PEO-Br macroinitiator and P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) and (B) \(^1\)H NMR of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA).

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Chapter 1. Multiresponsive Aqueous Micellar Gels from Thermo- and Light-Sensitive Hydrophilic ABA Triblock Copolymers
Abstract

This chapter presents the synthesis of a series of thermo- and light-sensitive hydrophilic ABA linear triblock copolymers, poly(ethoxytri(ethylene glycol) acrylate-co-o-nitrobenzyl acrylate)-b-poly(ethylene oxide)-b-poly(ethoxytri(ethylene glycol) acrylate-co-o-nitrobenzyl acrylate) (P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA)), with various NBA molar contents and the study of sol-gel transitions of their aqueous solutions. The copolymers were prepared by ATRP from a difunctional PEO macroinitiator; the polydispersity indexes of all polymers were < 1.2. We demonstrated that a 10.0 wt % aqueous solution of a triblock copolymer with a NBA content of 9.3 mol % in the thermosensitive blocks underwent a thermo-induced reversible sol-gel transition and a UV-triggered gel-to-sol transition at a constant temperature. The UV-irradiated solution again can undergo a temperature-induced sol-gel transition but at a higher temperature. The thermo-triggered sol-gel transitions stemmed from the formation of a 3-dimensional network with the dehydrated thermosensitive blocks being associated into hydrophobic cores and the central PEO block forming bridges among micelles. Exposure to UV light cleaved the hydrophobic o-nitrobenzyl groups, raised the LCST of the thermosensitive blocks, and thus triggered a gel-to-sol transition at a constant temperature. Rheometry was employed to study the sol-gel transitions and gel characteristics. In addition, we investigated the effects of polymer concentration and NBA content on sol-gel transition temperature ($T_{\text{sol-gel}}$) and gel properties before and after UV irradiation. The $T_{\text{sol-gel}}$ increased with the decrease of polymer concentration; the critical gelation concentration for the triblock copolymer with a NBA content of 9.3 mol % was between 3 and 4 wt % before UV irradiation and between 5 and 6 % after
photocleavage of o-nitrobenzyl groups. The change of $T_{\text{sol-gel}}$ was in general larger for the copolymer with a higher NBA content after UV irradiation.
1.1 Introduction

Stimuli-sensitive hydrophilic AB diblock and ABA linear triblock copolymers, where A represents a responsive polymer whose hydrophilicity can be varied by a stimulus and B represents a permanently water-soluble polymer, can self-assemble in dilute aqueous solutions into micelles with the A block(s) associated into the core and the B block forming the corona upon application of a stimulus.\(^1\)\(^-\)\(^10\) When the polymer concentration is sufficiently high (i.e., above the critical gelation concentration (CGC)), a free-flowing solution can be transformed in situ into a free-standing gel by an external trigger. Although both AB diblock and ABA triblock copolymers can be used to make micellar gels, their gelation mechanisms are different. For diblock copolymers, the sol-to-gel transition occurs when the volume fraction of micelles in solution exceeds a critical value; individual micelles are packed into an ordered structure.\(^1\)\(^-\)\(^3\) This gelation mechanism usually requires a minimum polymer concentration close to 20 wt %. In contrast, for ABA triblock copolymers, a 3-dimensional micellar network is formed with the B block forming bridges among micelles.\(^1\) Consequently, the CGC is significantly lower than that of diblock copolymers. It has been reported that an aqueous solution of a thermosensitive ABA triblock copolymer with a concentration of 7.5 wt % can form a free-standing gel upon heating.\(^11\)

Stimuli-responsive aqueous micellar gels, especially those from ABA linear triblock copolymers, have received growing interest in recent years and have found applications, e.g., in controlled release of drugs and tissue engineering.\(^4\)\(^-\)\(^10\) Compared with chemically crosslinked hydrogels, stimuli-sensitive micellar gels are more advantageous for many applications because the in situ sol-gel transition and the nature of physical crosslinking
allow convenient delivery and removal of polymers. There have been a number of reports on stimuli-sensitive hydrophilic ABA triblock and other multi-block copolymer micellar gels.4-37 For instance, using atom transfer radical polymerization (ATRP), Armes et al. synthesized a series of ABA triblock copolymers that can form gels in water in response to either pH or temperature changes.13-17

Our group has been particularly interested in block copolymer aqueous micellar gels that can respond to multiple stimuli (i.e., the sol-gel transitions can be triggered by more than one external stimulus). Such gels would provide greater design flexibility for many technological applications.4,5,19-30 To date, most of such gels reported in the literature are thermo- and pH-sensitive.19-27 The block copolymers used in these studies were usually prepared by either growing pH-sensitive blocks from or introducing carboxylic acid or other pH-responsive groups to the chain ends of an ABA triblock copolymer that can form thermoreversible gels in water (e.g., PEO-b-PPO-b-PEO).19-26 Besides thermo- and pH-responsive gels, other multi-responsive aqueous micellar gels have also been reported.28-30 For example, Li et al. synthesized thermo- and redox-sensitive ABA triblock copolymers by ATRP using a difunctional initiator that contained a redox-sensitive disulfide bond.28 The sol-gel transitions can be triggered biochemically and thermally.

Light is a very attractive external stimulus for the design of micellar gels as it allows spatial and temporal control. A number of light-responsive gel systems have been reported in the literature.38-41 Our group previously reported the synthesis of thermo- and light-sensitive diblock copolymers, PEO-b-poly(ethoxytri(ethylene glycol) acrylate-co-o-nitrobenzyl acrylate) (PEO-b-P(TEGEA-co-NBA)), which can undergo multiple
micellization and dissociation transitions in water in response to temperature changes and UV irradiation. PTEGEA is a thermosensitive water-soluble polymer with an LCST of 36 °C in water, which belongs to a new class of thermo-responsive water-soluble polymers that contain a short oligo(ethylene glycol) pendant in each repeat unit. o-Nitrobenzyl group is known to undergo a photocleavage reaction when exposed to long wavelength UV light. A characteristics of this type of doubly responsive hydrophilic block copolymers is that the LCST of the thermosensitive block can be modified by a second external stimulus.

In this work, we synthesized a series of thermo- and light-sensitive hydrophilic ABA linear triblock copolymers, P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA), from a difunctional PEO macroinitiator by ATRP of mixtures of NBA and TEGEA with various molar ratios (Scheme 1.1). We demonstrated that a 10.0 wt % aqueous solution of such an ABA triblock copolymer with a NBA molar content of 9.3 % in the thermosensitive blocks can undergo multiple sol-gel/gel-sol transitions in response to temperature changes and UV irradiation. The transitions and gel characteristics before and after the photocleavage of o-nitrobenzyl groups were characterized by rheometry. The concentration effect was studied and the CGCs for the polymer before and after UV irradiation were determined by combining the vial inversion test and rheological measurements. The effect of NBA molar content on the shift of sol-gel transition temperature ($T_{\text{sol-gel}}$) upon UV irradiation was elucidated. Note that very recently Ionov and Diez reported the use of thermo- and light-responsive copolymers of N-isopropyl acrylamide and NBA as photoresist for environmentally responsive photolithography by taking advantage of the LCST change after the photocleavage of o-nitrobenzyl group.
Scheme 1.1 Synthesis of thermo- and light-sensitive ABA linear triblock copolymer, P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA), by ATRP from a difunctional PEO macroinitiator and photocleavage of o-nitrobenzyl groups.
Our multi-responsive aqueous micellar gels could be used for making patterned, erasable hydrogels, which may find applications, e.g., in biomedical fields.

1.2 Experimental Section

1.2.1 Materials

Ethoxytri(ethylene glycol) acrylate (TEGEA) and o-nitrobenzyl acrylate (NBA) were synthesized according to the procedures described in the literature. Poly(ethylene glycol) (HO-PEO-OH, MW = 20,000 g/mol) was obtained from Aldrich. Triethylamine (99 %, Acros) and 2-bromoisoobutyryl bromide (98 %, Acros) were used as received. Dichloromethane and anisole (99 %, Acros) were distilled from calcium hydride and stored in solvent storage flasks prior to use. Toluene was distilled from sodium and benzophenone and used immediately. CuBr (98%, Aldrich) was purified according to the procedure described in the literature and was stored in a desiccator. N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA, Aldrich) was dried with calcium hydride and distilled under reduced pressure. All other chemicals were purchased from either Aldrich or Fisher and used without purification.

1.2.2 General Characterization

Gel permeation chromatography (GPC) was carried out at ambient temperature using PL-GPC 20 (an integrated GPC system from Polymer Laboratories, Inc) with a differential refractive index detector, one PLgel 5 µm guard column (50 × 7.5 mm), and two PLgel 5 µm mixed-C columns (each 300 × 7.5 mm, linear range of molecular weight from 200 to 2,000,000 according to Polymer Laboratories). The data were processed
using Cirrus™ GPC/SEC software (Polymer Laboratories). THF was used as the carrier solvent at a flow rate of 1.0 mL/min. Polystyrene standards (Polymer Laboratories) were used for calibration. The $^1$H (300 MHz) NMR spectra were recorded on a Varian Mercury 300 NMR spectrometer and the residual solvent proton signal was used as the internal standard.

1.2.3 Synthesis of Difunctional PEO Macroinitiator (Br-PEO-Br)

Poly(ethylene oxide) (HO-PEO-OH) with a molecular weight of 20,000 g/mol (15.085 g, 0.754 mmol) was dissolved in toluene (250 mL) in a three-necked flask. After azeotropic distillation of toluene (~ 150 mL) to remove the trace amount of water in HO-PEO-OH, triethylamine (0.760 g, 7.51 mmol) was added and the mixture was stirred at room temperature for 30 min under N$_2$ atmosphere. 2-Bromoisobutyryl bromide (2.010 g, 8.75 mmol) was added dropwise from an addition funnel. The reaction mixture was stirred overnight at ambient temperature and the precipitate was removed by vacuum filtration. The filtrate was concentrated and precipitated in diethyl ether three times. The polymer was dissolved in water with a pH of ~ 8, and was extracted with dichloromethane. The organic extracts were dried over anhydrous sodium sulfate overnight. After the removal of sodium sulfate by filtration, the solution was concentrated and precipitated in diethyl ether two times. The polymer was dried in vacuum and then recrystallized twice in ethanol. After being dried under high vacuum at an elevated temperature for 6 h, the difunctional macroinitiator Br-PEO-Br was obtained as a hard white solid.
1.2.4 Synthesis of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA)

Described below is a procedure for the synthesis of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) by ATRP of a mixture of TEGEA and NBA with a NBA content of 8.6 mol % in the feed. Other ABA triblock copolymers were synthesized by a similar procedure. Copper (I) bromide (10.6 mg, 0.074 mmol), Br-PEO-Br (0.511 g, 2.52 × 10⁻⁵ mol), TEGEA (3.220 g, 13.9 mmol), NBA (0.270 g, 1.30 mmol), anisole (1.52 g), and PMDETA (11.4 mg, 0.066 mmol) were added into a 25 mL two-necked flask. The mixture was degassed by three freeze-pump-thaw cycles and the flask was placed in a 90 °C oil bath. After the polymerization proceeded for 190 min, the flask was removed from the oil bath and the mixture was diluted with THF (10 mL). The copper catalyst was removed by passing the solution through a short neutral aluminum oxide/silica gel column using a mixture of THF and methylene chloride (v/v, 1 : 1) as eluent. The solution was then concentrated and precipitated in a mixture of hexanes and diethyl ether (10 : 1, v/v). The polymer was purified by repetitive precipitation in the same solvent mixture, dried in high vacuum, and then analyzed by GPC and ¹H NMR spectroscopy analysis. GPC analysis results: $M_n,\text{GPC} = 40300$ g/mol; polydispersity index (PDI) = 1.14. The numbers of TEGEA and NBA units in this ABA triblock copolymer were 98 and 10, respectively, calculated from its ¹H NMR spectrum.

1.2.5 Preparation of 10.0 wt % Aqueous Solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) and Photocleavage Experiments

Below is a typical procedure for the preparation of a 10.0 wt % aqueous solution of a thermo- and light-sensitive ABA triblock copolymer P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) with a NBA molar content of 9.3 % in the thermosensitive blocks.
The polymer was added into a 3.7 mL vial and dried at 50 °C under high vacuum for 6 h. The weight of the dried polymer was 0.227 g. Milli-Q water (2.031 g) was added into the vial and the mixture was then sonicated in an ice/water ultrasonic bath (Fisher Scientific Model B200 Ultrasonic Cleaner) to dissolve the ABA triblock copolymer in water. The vial was then stored in a refrigerator (~ 4 °C) overnight to ensure complete dissolution. A clear homogenous polymer solution was obtained and used in the photocleavage experiment.

A 3.7 mL vial that contained 1.280 g of the aforementioned aqueous solution was placed in a 43 °C water bath. The solution turned into a transparent gel, which was then irradiated with the long wavelength (365 nm) UV light from a Spectroline ENF-240C hand-held UV lamp equipped with one 4-watt long wavelength tube filtered at 365 nm and one short wavelength tube filtered at 254 nm. The distance between the vial and the lamp was ~ 2 cm. The degree of photocleavage of o-nitrobenzyl groups in the copolymer was monitored by $^1$H NMR spectroscopy analysis. The cleavage was complete after UV irradiation for 116 h.

1.2.6 Dynamic Light Scattering Study of 0.02 wt % Aqueous Solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) Before and After Photocleavage of o-Nitrobenzyl Groups

The thermo-induced micellization behaviors of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) at a concentration of 0.02 wt % before and after the photocleavage of o-nitrobenzyl groups were studied by dynamic light scattering (DLS). The 0.02 wt % aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) and the polymer obtained after UV irradiation, P(TEGEA-co-acrylic acid)-b-PEO-b-P(TEGEA-co-acrylic
acid) (P(TEGA-co-AA)-b-PEO-b-P(TEGA-co-AA)), were made by diluting the corresponding 10.0 wt % aqueous solution and the irradiated solution with Milli-Q water. DLS measurements were conducted with a Brookhaven Instruments BI-200SM goniometer equipped with a PCI BI-9000AT digital correlator, a temperature controller, and a solid-state laser (model 25-LHP-928-249, $\lambda = 633$ nm) at a scattering angle of 90°. The polymer solutions were filtered into borosilicate glass tubes with an inner diameter of 7.5 mm by the use of 0.2 μm hydrophilic PTFE filters. The glass tubes were then sealed with PE stoppers. The sample was placed into the cell holder of the light scattering instrument and gradually heated. At each temperature, the solution was equilibrated for 20 min prior to data recording. The time correlation functions were analyzed with a Laplace inversion program (CONTIN).

1.2.7 Rheological Measurements

Rheological experiments were conducted using a stress-controlled rheometer (TA Instruments Model TA AR2000). A cone-plate geometry with a cone diameter of 20 mm and an angle of 2 ° (truncation 52 μm) was employed; the temperature was controlled by the bottom Peltier plate. In each measurement, ~ 90 μL of a polymer solution was loaded onto the plate by a micropipette. The solvent trap was filled with water and a solvent trap cover was used to minimize water evaporation. Viscoelastic properties (dynamic storage modulus $G'$ and loss modulus $G''$) of a polymer solution were measured by oscillatory shear experiments performed at a fixed frequency of 1 Hz in a heating ramp at a heating rate of 3 °C/min. The frequency dependences of $G'$ and $G''$ of a polymer solution at selected temperatures were obtained by frequency sweep tests from 0.1 to 100 Hz. A
strain amplitude of $\gamma = 0.2 \%$ was used in all dynamic tests to ensure that the deformation was within the linear viscoelastic regime.

1.3 Results and Discussion

1.3.1 Synthesis of Thermo- and Light-Sensitive ABA Linear Triblock Copolymers P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) with Various NBA Contents

Four thermo- and light-sensitive ABA linear triblock copolymers, P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA)s, were synthesized by ATRP of mixtures of TEGEA and NBA with different molar ratios from a difunctional PEO macroinitiator with a molecular weight of 20,000 g/mol at 90 °C in anisole using CuBr/PMDETA as catalyst (Scheme 1.1). These copolymers were purified by repetitive precipitation, dried in high vacuum at elevated temperatures, and analyzed by GPC and $^1$H NMR spectroscopy analysis. Figure 1.1a shows the GPC traces of macroinitiator Br-PEO-Br and one ABA triblock copolymer synthesized from a mixture of TEGEA and NBA with a NBA content of 8.6 mol % in the feed. Clearly, the peak shifted to the high molecular weight side after the polymerization, though there was a small shoulder peak for this particular polymer. The polydispersity indexes of all four ABA triblock copolymers were < 1.20, demonstrating that the polymerizations were controlled. The numbers of TEGEA and NBA units in the copolymers were determined from the $^1$H NMR spectra using the peaks located at 5.45 ppm (−CH$_2$− of the benzyl group of NBA unit), 4.16 ppm (−CH$_2$OOC− of TEGEA unit), and the peak from 3.2 – 3.9 ppm (-OCH$_2$CH$_2$O- of PEO block and −COOCH$_2$CH$_2$(OCH$_2$CH$_2$)$_2$OCH$_2$CH$_3$ of TEGEA) (see Figure 1.1b). The molar ratio of TEGEA to NBA units in each copolymer was found to be close to that in
Figure 1.1 (a) Gel permeation chromatography traces of macroinitiator Br-PEO-Br and P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3 in Table 1) and (b) $^1$H NMR spectra of ABA-3 before (i) and after (ii) the photocleavage of o-nitrobenzyl groups of NBA units in the copolymer. CDCl$_3$ was used as solvent in $^1$H NMR spectroscopy analysis.
the polymerization feed. For comparison, a thermosensitive ABA triblock copolymer without the incorporation of NBA, PTEGEA-b-PEO-b-PTEGEA, was also prepared and the DP of PTEGEA was determined from its $^1$H NMR spectrum. The characterization data for these five ABA triblock copolymers are summarized in Table 1.1.

1.3.2 Thermo-Induced Sol-Gel Transition of 10.0 wt % Aqueous Solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3).

ABA-3 was used to demonstrate that the aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) can undergo multiple thermo- and light-induced sol-gel/gel-sol transitions. A 10.0 wt % aqueous solution of this triblock copolymer was made using Milli-Q water in a vial that had an inner diameter of 12 mm. The thermo-induced sol-gel/gel-sol transitions were first examined by the vial inversion test. The vial was gradually heated in a water bath. At each temperature, the sample was equilibrated for 10 min and the vial was tilted or inverted to visually examine if the solution was a flowing liquid or an immobile gel. As can be seen from Figure 1.2a, the sample was a free-flowing, clear liquid at 20 °C. Upon heating to ~ 42 °C, the sample turned into a transparent, free-standing gel (Figure 1.2b showing the gel at 43 °C). The sample remained in the gel state and transparent up to the tested temperature limit (65 °C, Figure 1.2c showing the gel at 60 °C). The thermo-induced sol-gel transition was reversible; lowering the temperature converted the gel to a liquid that can flow under its weight when tilted.
Table 1.1 Characterization data for one thermosensitive PTEGEA-b-PEO-b-PTEGEA (ABA-1) and four thermo- and light-sensitive ABA triblock copolymers, P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA)s (ABA-2 to ABA-5).

<table>
<thead>
<tr>
<th></th>
<th>Mₙ,GPC a (g/mol)</th>
<th>PDI a</th>
<th>nTEGEA b</th>
<th>nNBA b</th>
<th>NBA in Feed (mol %)</th>
<th>NBA in polymer (mol %)</th>
</tr>
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<tr>
<td>ABA-1</td>
<td>40400</td>
<td>1.18</td>
<td>101</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>ABA-2</td>
<td>44100</td>
<td>1.11</td>
<td>119</td>
<td>6</td>
<td>4.9 %</td>
<td>4.8 %</td>
</tr>
<tr>
<td>ABA-3</td>
<td>40300</td>
<td>1.14</td>
<td>98</td>
<td>10</td>
<td>8.6 %</td>
<td>9.3 %</td>
</tr>
<tr>
<td>ABA-4</td>
<td>44800</td>
<td>1.11</td>
<td>108</td>
<td>16</td>
<td>13.1 %</td>
<td>12.9 %</td>
</tr>
<tr>
<td>ABA-5</td>
<td>40900</td>
<td>1.10</td>
<td>88</td>
<td>19</td>
<td>17.4 %</td>
<td>17.8 %</td>
</tr>
</tbody>
</table>

a Mₙ,GPC and polydispersity index (PDI) were determined by gel permeation chromatography GPC using polystyrene calibration; b the numbers of TEGEA (nTEGEA) and NBA units (nNBA) in the copolymers were calculated from ¹H NMR spectra.
Figure 1.2 Digital optical pictures of a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3 in Table 1.1) at (a) 20 °C, (b) 43 °C, and (c) 60 °C before UV irradiation and at (d) 43 °C, (e) 52 °C, and (f) 60 °C after UV irradiation. At 43 °C, the solution was irradiated for 116 h with 365 nm UV light from a Spectroline ENF-240C hand-held UV lamp.
To quantitatively study the thermo-induced sol-gel transition and the gel characteristics, we conducted rheological measurements. Figure 1.3a shows the dynamic storage modulus $G'$ and loss modulus $G''$ of this sample as a function of temperature obtained from an oscillatory shear experiment performed at a fixed frequency of 1 Hz in a heating ramp at a heating rate of 3 °C/min. A strain amplitude of $\gamma = 0.2 \%$ was used to ensure that the measurements were taken in the linear viscoelastic regime. Below 27 °C, the values of $G'$ and $G''$ were small and the data points were scattered. In the range of 27 – 41 °C, both $G'$ and $G''$ increased sharply with the increase of temperature and the value of $G''$ was larger than $G'$, indicating that the solution was a viscous liquid. Above 42 °C, $G'$ became greater than $G''$, suggesting that the sample was transformed into a gel. The crossover, $G' = G''$, has been commonly used as an indicator of the sol-gel transition. Thus, the sol-to-gel transition temperature ($T_{\text{sol-gel}}$) of this solution is 41.6 °C, essentially the same as that estimated by the vial inversion test. We also measured the $T_{\text{sol-gel}}$ of a PTEGEA-b-PEO-b-PTEGEA (ABA-1) with a molecular weight similar to that of ABA-3 in a 10.0 wt % aqueous solution. It was 51.1 °C. Thus, the incorporation of 9.3 mol % of NBA into the thermosensitive blocks decreased the $T_{\text{sol-gel}}$ of the triblock copolymer by ~ 10 °C.

Compared with sharp transitions of 20 wt % aqueous solutions of thermosensitive AB diblock copolymers, the sol-gel transitions of ABA triblock copolymers are much broader (see Figure 1.3). This is attributed to the different gelation mechanisms of two types of block copolymers in solutions as mentioned earlier. For AB diblock copolymers, the sol-to-gel transition occurs when discrete, spherical micelles are packed into an
Figure 1.3 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3 in Table 1.1) before (a) and after UV irradiation (b). The data were collected from temperature ramp experiments with a heating rate of 3 °C/min. A strain amplitude of 0.2 % and an oscillation frequency of 1 Hz were used.
ordered structure and the solution undergoes a disorder-to-order transition. For ABA triblock copolymers in moderately concentrated solutions, when the temperature is above the LCST of the thermosensitive outer blocks, a 3-dimensional network is formed with the dehydrated outer blocks associated into the hydrophobic domains and the central PEO blocks forming bridges among micelles. This process is not as sharp as the disorder-to-order transition of spherical micelles of AB diblock copolymers.

The thermo-induced transition from a viscous liquid to an elastic gel can also be seen from the frequency dependencies of $G'$ and $G''$ (Figure 1.4). At 34 °C, where the sample was a clear liquid and can flow when tilted, the storage modulus $G'$ was smaller than $G''$ in the frequency range from 0.1 to 60 Hz. $G'$ and $G''$ exhibited different power law dependencies on frequency $f$ in the low frequency region: $G' \sim f^2$ and $G'' \sim f$. These are the typical rheological behavior of a viscous liquid. At 38 °C, which is close to the sol-gel transition temperature (41.6 °C), $G'$ and $G''$ were of similar magnitudes throughout the frequency range and $G''$ was $\sim f^{0.5}$ in the low frequency zone (Figure 1.4b). This is a signature of the transition between liquid-like and solid-like behaviors. At 60 °C, where the sample was a transparent free-standing gel, $G'$ was significantly greater than $G''$ and was nearly independent of $f$ in the studied frequency range, which is a characteristic of elastic solid-like behavior (Figure 1.4c).

The thermo-induced sol-gel transition stems from the LCST behavior of thermosensitive P(TEGEA-co-NBA) blocks. Upon heating, the P(TEGEA-co-NBA) blocks undergo a hydration-to-dehydration transition and form hydrophobic cores of micelles. To confirm the temperature-induced micellization of P(TEGEA-co-NBA)-b-
**Figure 1.4** Frequency dependences of dynamic storage modulus $G'$ (■) and dynamic loss modulus $G''$ (□) of a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3) at (a) 34, (b) 38, and (c) 60 °C. A strain amplitude of 0.2 % was used in all frequency sweep experiments.
PEO-b-P(TEGEA-co-NBA), we performed a dynamic light scattering (DLS) study of ABA-3 in a 0.02 wt % aqueous solution, which was made by diluting the 10.0 wt % aqueous solution using Milli-Q water. As shown in Figure 1.5a, below 29 °C, the scattering intensity was low and the apparent hydrodynamic size (D_h) obtained from CONTIN analysis was < 10 nm, indicating that the triblock copolymer was dissolved molecularly in water. With the increase of temperature above 30 °C, the solution scattering intensity began to increase; the critical micellization temperature (CMT) was determined to be 30 °C, which was ~ 11 °C lower than the T_{sol-gel} of the 10.0 wt % solution. The apparent D_h of micelles eventually became stabilized at ~ 50 nm. The single-size distribution indicated the absence of the bridge formation among micelles at this concentration.

1.3.3 UV-Induced Gel-to-Sol Transition of 10.0 wt % Aqueous Solution of ABA-3 and Thermo-Induced Reversible Sol-Gel Transition of the Irradiated Solution

Our group reported before that the photocleavage of o-nitrobenzyl groups in a random copolymer P(TEGEA-co-NBA) with a NBA content similar to that in ABA-3 raised the LCST by 11.5 °C due to the conversion of hydrophobic o-nitrobenzyl groups to carboxylic acid groups. For ABA-3, if the T_{sol-gel} increases by a similar magnitude (i.e., the new T_{sol-gel} is ~ 53 °C), the gel at 43 °C shown in Figure 1.2b would be converted to a free-flowing liquid by UV irradiation. Therefore, we irradiated the 10.0 wt % aqueous solution of ABA-3 at 43 °C with 365 nm UV light from a hand-held TLC lamp and used ^1H NMR spectroscopy to monitor the photochemical reaction. After ~ 20 h, a brown-red color, which was from the product of the photocleavage of o-nitrobenzyl groups, appeared and the sample can flow when tilted. ^1H NMR spectroscopy analysis indicated
Figure 1.5 Scattering intensity at a scattering angle of 90° and apparent hydrodynamic size \( D_h \), obtained from CONTIN analysis, as a function of temperature in a dynamic light scattering study of 0.02 wt % aqueous solution of ABA-3 before (a) and after the removal of \( o \)-nitrobenzyl groups (b).
that the photocleavage was complete after 116 h as the characteristic peak located at 5.45 ppm disappeared (Figure 1.1b). As shown in Figure 1.2d, the clear gel was transformed into a dark red but transparent liquid that can flow easily under its own weight when tilted. Upon further heating to ~ 52 °C, the irradiated solution turned into a gel (Figure 1.2e), which remained so at 60 °C (Figure 1.2f). Again, the thermo-induced transition was reversible. Oscillatory shear experiments showed that the $T_{\text{sol-gel}}$ of the irradiated sample was 51.5 °C (Figure 1.3b), which was ~ 10 °C higher than that before UV irradiation (41.6 °C) and was close to the sol-gel transition temperature of a 10.0 wt % aqueous solution of ABA-1 (51.1 °C). A closer examination of rheological data showed that the highest value of $G'$ of the new gel was only 799 Pa, much lower than that of the gel before photocleavage (3281 Pa). Likely, the cleavage of $o$-nitrobenzyl group changed the micellization behavior and the structure of micelles of the triblock copolymer in the solution. The number of bridging chains among micelles was reduced, causing the decrease of dynamic storage modulus $G'$. The frequency sweeps at selected temperatures (41, 48, and 65 °C) showed typical behaviors of viscous liquids and elastic solids, which also suggested the thermo-induced sol-gel transition.

DLS was conducted to investigate the micellization of a 0.02 wt % aqueous solution of the irradiated polymer that was made by diluting the irradiated solution with water (Figure 1.5b). The CMT was 40 °C, which was 10 °C higher than the CMT of ABA-3, consistent with what we expected. The apparent micelle size at elevated temperatures was ~ 50 nm, about the same as that before UV irradiation. Since the UV irradiation converted the polymer to P(TEGEA-co-acrylic acid)-b-PEO-b-P(TEGEA-co-acrylic acid) (P(TEGEA-co-AA)-b-PEO-b-P(TEGEA-co-AA), Scheme 1.1) and the LCST of
P(TEGEA-co-AA) depends on the degree of ionization of carboxylic acid groups, we measured the pH value of the irradiated sample and the 0.02 wt % solution. They were 2.74 and 5.00, respectively. Although there was a difference, we showed before that the LCST of a thermosensitive polymer that contained a small amount of COOH groups varied little with pH when the pH was below 5.0. Thus, it is reasonable that the CMT and \( T_{\text{sol-gel}} \) of ABA-3 increased by a similar magnitude after UV irradiation.

1.3.4 Effect Concentration on Sol-Gel Transition of Aqueous Solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3) Before and After UV Irradiation

Polymer concentration is known to affect sol-gel transition temperature and gel properties.\(^{71,72}\) To investigate the concentration effect and to determine the CGC, we performed rheological measurements on a series of aqueous solutions of ABA-3 and the corresponding irradiated polymer, P(TEGEA-co-AA)-b-PEO-b-P(TEGEA-co-AA), with concentrations ranging from ~ 14 wt % to ~ 3 wt %. These solutions were made by concentrating the 10.0 wt % aqueous solution of ABA-3 and the 9.7 wt % irradiated solution via the evaporation of water or diluting them with water. Figure 1.6 shows temperature ramps of four selected samples: two from non-irradiated solutions with polymer concentrations of 12.0 and 7.9 wt % and two from the irradiated solutions with polymer concentrations of 11.8 and 6.0 wt %. The heating ramps of other solutions can be found in Appendix A.\(^{70}\) The results from rheological measurements are summarized in Figure 1.7, including the sol-gel transition temperatures and the observed highest values of \( G' \) in the studied temperature range. Clearly, for aqueous solutions of both ABA-3 and the corresponding irradiated polymer, the sol-gel transition temperature increased with decrease of polymer concentration. At a similar concentration, the \( T_{\text{sol-gel}} \)
Figure 1.6 Temperature ramps for aqueous solutions of ABA-3 with concentrations of (a) 12.0 and (b) 7.9 wt %, and aqueous solutions of the irradiated polymer, obtained from UV irradiation of ABA-3, with concentrations of (c) 11.8 and (d) 6.0 wt %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure 1.7 (a) Concentration effect on the sol-gel transition temperature ($T_{\text{sol-gel}}$) of aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3, ■) and the corresponding P(TEGEA-co-AA)-b-PEO-b-P(TEGEA-co-AA) (○), obtained from UV irradiation of ABA-3, and (b) the highest value of dynamic storage modulus $G'$ as a function of polymer concentration for ABA-3 (■) and the irradiated polymer (○).
of an irradiated solution was ~ 10 °C higher than that of ABA-3 and the gap became slightly wider with the decrease of concentration (Figure 1.7a). Evidently, a lower polymer concentration requires a higher degree of dehydration of thermosensitive blocks to form a sufficiently mechanically strong 3-dimensional network. For the 3.0 wt % solution of ABA-3 and the 5.0 wt % solution of the irradiated polymer, no free-standing gels were observed by the vial inversion test, suggesting that the CGCs of ABA-3 and the irradiated polymer are between 3 and 4 wt % and between 5 and 6 wt %, respectively. These are significantly lower than the CGCs of diblock copolymers. The slightly higher CGC of the irradiated polymer is likely due to the higher hydrophilicity. Note that the CGC of a hydrophilic ABA triblock copolymer with thermosensitive outer blocks is also dependent on the molecular weight or block length of the thermosensitive blocks.

In addition, for both ABA-3 and the irradiated polymer, the highest gel storage modulus G' increased with the increase of polymer concentration (Figure 1.7b), but not in a linear fashion. A more concentrated solution produces a stronger gel because of the formation of a more extended 3-dimensional network with a greater number of PEO central blocks forming bridges among micelles. Since the number of PEO bridges is not proportional to the polymer concentration, the increase of G' with concentration was slow initially, but became faster when the concentration exceeded a certain value (Figure 1.7b). It is interesting to note that to achieve a similar value of G' in the relatively higher concentration range, the irradiated polymer needs to have a concentration higher by ~ 2 wt % than ABA-3, which is again likely due to the higher hydrophilicity of the thermosensitive blocks in the irradiated polymer.
From Figure 1.6 and the results included in Appendix A, one can find out that when the polymer concentration was above 10 wt %, a plateau was observed for G' with the increase of temperature up to 65 °C and the gel was transparent. For the solution with a concentration lower than 10 wt %, both G' and G” decreased at temperatures above the \( T_{\text{sol-gel}} \) (see Figure 1.6b and the data in Appendix A for the temperature ramps of aqueous solutions of ABA-3 with concentrations of 7.9, 6.0, and 4.0 wt %). The values of G' and G” of the 7.9 wt % solution of ABA-3 dropped sharply at ~ 50 and ~ 60 °C. By gradually heating the sample, we found that the gel turned cloudy at 56 °C (the pictures in Figure 1.6b show the gel at 45 and 65 °C). We also determined the temperatures at which the samples with different polymer concentrations turned cloudy upon heating (see the phase diagram in Appendix A); they were 48 and 52 °C for concentrations of 4.0 and 6.0 wt %, respectively. These temperatures were close to, though not identical to, those temperatures at which G' and G” decreased noticeably in the temperature ramps, suggesting that the phase separation could be the reason for the observation of the decreases of G' and G”. We kept the 3.0 wt % solution of ABA-3 at 65 °C for three days; two layers, the top one clear and the bottom one cloudy, were observed, indicating a macroscopic phase separation. These results are consistent with those theoretical predictions and experimental observations for hydrophobically end-capped PEO polymers. Note that associative polymers in aqueous solutions have been intensively studied in the past decades. For the irradiated solutions, this phenomenon was observed at higher temperatures (Figure 1.6d and Appendix A), likely because the sol-gel transition temperatures of the irradiated solutions were higher. We noticed that the 6.0 wt % aqueous solution of the irradiated polymer turned hazy at 70 and 75 °C (see the picture.
at 75 °C in Figure 1.6d). We believe that at low polymer concentrations, a large number of PEO central blocks formed loops in the corona, resulting in weak bridges among micelles. On the other hand, the solubility of PEO in water is known to become poorer with the increase of temperature,\(^1\) which means that the PEO bridges in the gels undergo slight shrinking at elevated temperatures, causing the phase separation of polymer solutions. The relatively short chain length of thermosensitive blocks (the degree of polymerization for each thermosensitive block of ABA-3 was only 54) may also contribute to this phenomenon as one can imagine that an ABA triblock copolymer with short A blocks could lead to a weaker gel.

1.3.5 Effect of NBA Content on Sol-Gel Transition Temperature and Gel Property

We further studied the effect of NBA content on sol-gel transition temperatures and gel properties of aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) before and after UV irradiation. Aqueous solutions of ABA-2, -4 and -5 with a concentration of 10.0 wt % were made; their sol-gel transition temperatures before and after the removal of o-nitrobenzyl groups were determined by oscillatory shear experiments. \(^1\)H NMR spectroscopy was employed to ensure that the photocleavage for each sample was complete prior to the rheological measurement. The results for these three triblock copolymers along with those for ABA-1 and ABA-3 are summarized in Figure 1.8. With the increase of NBA content, the sol-gel transition temperature decreased continuously in a nearly linear fashion, from 51.1 °C for ABA-1 (no NBA), to 46.3 °C for ABA-2 (4.8 mol % NBA), to 41.6 °C for ABA-3 (9.3 mol % NBA), to 32.4 °C for ABA-4 (12.9 mol % NBA), and 27.5 °C for ABA-5 (17.8 mol % NBA). This is reasonable because o-nitrobenzyl group is hydrophobic and many studies have shown
Figure 1.8 $T_{\text{sol-gel}}$ (a) and the observed highest gel storage modulus $G'$ (b) of 10.0 wt % solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) before (■) and after (●) the photocleavage of $o$-nitrobenzyl groups as a function of NBA molar content.
that the LCST of a thermosensitive water-soluble random copolymer changes essentially linearly with the content of a comonomer.\textsuperscript{49,85} It is interesting to note that the observed highest value of dynamic storage modulus $G'$ increased in a nearly linear fashion with the NBA molar content, from $\sim 1000$ Pa for \textbf{ABA-1} to $\sim 5000$ Pa for \textbf{ABA-5} (Figure 1.8b).

After the photocleavage of \textit{o}-nitrobenzyl groups, the thermosensitive blocks became more hydrophilic and their LCSTs increased accordingly as we have seen from DLS studies for \textbf{ABA-3}. We found that the sol-gel transition temperatures for \textbf{ABA-2} and \textbf{ABA-3} after UV irradiation were almost the same as that of \textbf{ABA-1}, while $T_{\text{sol-gel}}$ for the irradiated \textbf{ABA-4} and \textbf{-5} were lower (Figure 1.8a). Despite this observation, there is a clear trend that the change of $T_{\text{sol-gel}}$ after the removal of \textit{o}-nitrobenzyl groups is larger when the NBA content is higher.

The lower $T_{\text{sol-gel}}$s of the irradiated solutions of \textbf{ABA-4} and \textbf{-5} could be attributed to the following two possible reasons. (i) The polymers after UV irradiation were P(TEGEA-co-AA)-$b$-PEO-$b$-P(TEGEA-co-AA)\textsubscript{s}, which contained carboxylic acid groups in the thermosensitive blocks. The pendant COOH groups can form complexes with the ether linkages of neighboring TEGEA units via intramolecular hydrogen bonding and it has been reported that the acid-ether complexes are less hydrophilic than both components.\textsuperscript{86,87} A higher content of carboxylic acid groups in the thermosensitive blocks would result in more extensive hydrogen bonding with neighboring monomer units and thus a lower LCST and a lower $T_{\text{sol-gel}}$. (ii) The slightly higher $M_{n,\text{GPC}}$ of \textbf{ABA-4} (44800 g/mol) compared with those of \textbf{ABA-3} (40300 g/mol) and \textbf{ABA-5} (40900 g/mol) may explain the lower-than-expected $T_{\text{sol-gel}}$ of the irradiated solution of \textbf{ABA-4}. It is known that a higher molecular weight gives a lower LCST, especially when the
molecular weight of a thermosensitive polymer is in the low to moderate molecular weight range.\textsuperscript{54} In contrast to the observation for the non-irradiated samples, the highest value of $G'$ after the photocleavage of $o$-nitrobenzyl groups changed only slightly with the NBA molar content (Figure 1.8b). It appears that the removal of $o$-nitrobenzyl groups in the thermosensitive blocks changed the structures of micellar gels significantly compared with the non-irradiated gels. In summary, we observed a trend that with the increase of NBA content in the thermosensitive blocks, the shift of $T_{\text{sol-gel}}$ and the change of the highest $G'$ after UV irradiation increased, though the issues were complicated by the formation of acid-ether complexes and the molecular weight effect. We note here that the acid-ether complex formation upon UV irradiation can be eliminated by using a light-sensitive monomer that contains an ethylene glycol or di(ethylene glycol) spacer between the ester group and the $o$-nitrobenzyl group.\textsuperscript{43}

\section*{1.4 Conclusions}

A series of thermosensitive hydrophilic ABA linear triblock copolymers with NBA contents ranging from 0 to 17.8 mol \% in the thermosensitive blocks by ATRP from a difunctional PEO macroinitiator were synthesized.\textsuperscript{88} All polymers had narrow polydispersity indexes. Using a 10.0 wt \% aqueous solution of P(TEGEA-\textit{co}-NBA)-\textit{b}-PEO-\textit{b}-P(TEGEA-\textit{co}-NBA) with a NBA content of 9.3 mol \%, we demonstrated that the solution can undergo multiple sol-gel/gel-sol transitions in response to temperature changes and UV irradiation. Rheometry and DLS studies showed that for this particular copolymer, both $T_{\text{sol-gel}}$ and CMT increased by $\sim 10$ °C after the photocleavage of $o$-nitrobenzyl groups. The removal of hydrophobic $o$-nitrobenzyl groups increased the
hydrophilicity of thermosensitive blocks and thus their LCST. We further studied the effects of polymer concentration and NBA content on $T_{\text{sol-gel}}$ and gel properties before and after UV irradiation. The sol-gel transition temperature increased with the decrease of polymer concentration; the CGC for the polymer with a NBA content of 9.3 mol % was between 3 and 4 wt % before UV irradiation and between 5 and 6 % after the cleavage of o-nitrobenzyl groups. In general, the change of $T_{\text{sol-gel}}$ was greater for the polymer with a higher NBA content after UV irradiation. The work reported here could provide a strategy for the design of multi-responsive aqueous gels for technological applications.
References


70. The data can be found in Appendix A.


73. The relatively long irradiation time is believed to be due to the low intensity of the used UV lamp and the absorption of byproducts from the photochemical reaction (internal filtering effect). See Pillai, V. N. R. *Synthesis* **1980**, *1*, 1-26.


88. The work presented in this Chapter has been published in Soft Matter as an article (Soft Matter 2010, 6, 3325-3336).

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Appendix A

for

Chapter 1. Multiresponsive Aqueous Micellar Gels from Thermo- and Light-Sensitive Hydrophilic ABA Triblock Copolymers
Figure A.1 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for a 10.0 wt % aqueous solution of PTEGEA-*b*-PEO*b*-PTEGEA (ABA-1 in Table 1.1) in a heating ramp. The rheological data were collected using a heating rate of 3 °C/min, a strain amplitude of 0.2 %, and an oscillation frequency of 1 Hz.
Figure A.2 Frequency dependencies of dynamic storage modulus $G'$ (■) and loss modulus $G''$ (□) of a 9.7 wt % aqueous solution of P(TEGEA-co-AA)-b-PEO-b-P(TEGEA-co-AA), obtained from UV irradiation of a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3) at 43 °C for 116 h, at 41 °C. A strain amplitude of 0.2 % was used in the frequency sweep experiments.
Figure A.3 Frequency dependencies of dynamic storage modulus $G'$ (■) and loss modulus $G''$ (□) of a 9.7 wt % aqueous solution of P(TEGEA-co-AA)-b-PEO-b-P(TEGEA-co-AA), obtained from UV irradiation of a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (**ABA-3**) at 43 °C for 116 h, at 48 °C. A strain amplitude of 0.2 % was used in the frequency sweep experiments.
Figure A.4 Frequency dependencies of dynamic storage modulus $G'$ (■) and loss modulus $G''$ (□) of a 9.7 wt % aqueous solution of P(TEGEA-co-AA)-b-PEO-b-P(TEGEA-co-AA), obtained from UV irradiation of a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3) at 43 °C for 116 h, at 65 °C. A strain amplitude of 0.2 % was used in the frequency sweep experiments.
Figure A.5 Temperature ramps for aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEEA-co-NBA) (ABA-3 in Table 1.1) with concentrations of 14.2 wt %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure A.6 Temperature ramps for aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3 in Table 1.1) with concentrations of 10.0 wt %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure A.7 Temperature ramps for aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3 in Table 1.1) with concentrations of 6.0 wt %, The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure A.8 Temperature ramps for aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEOA-co-NBA) (ABA-3 in Table 1.1) with concentrations of 4.0 wt %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure A.9 Temperature ramps for aqueous solutions of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEEA-co-NBA) (ABA-3 in Table 1.1) with concentrations of 3.0 wt %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure A.10 Temperature ramps for aqueous solutions of P(TEGEA-co-AA)-b-PEO-b-P(TEGEEA-co-AA) (obtained from UV irradiation of a 10.0 wt % aqueous solution of ABA-3) with concentrations of 13.8 %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure A.11 Temperature ramps for aqueous solutions of P(TEGEA-co-AA)-b-PEO-b-P(TEGEAA-co-AA) (obtained from UV irradiation of a 10.0 wt % aqueous solution of ABA-3) with concentrations of 7.8 wt %. The rheological data were collected at a constant frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min. The pictures show the states of each solution at four different temperatures.
Figure A.12 Phase diagram of aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-3). ■: Sol-gel transition temperature determined by rheological measurements; ●: temperature at which the gel became cloudy, determined by visual examination; ▲: temperature at which a clear solution turned cloudy, determined by visual examination.
Figure A.13 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-2) before the removal of $o$-nitrobenzyl groups. The rheological data were collected from heating ramps using a heating rate of 3 °C/min, a strain amplitude of 0.2 %, and an oscillation frequency of 1 Hz.
Figure A.14 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (○) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-2) after the removal of $o$-nitrobenzyl groups. The rheological data were collected from heating ramps using a heating rate of 3 °C/min, a strain amplitude of 0.2 %, and an oscillation frequency of 1 Hz.
Figure A.15 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and tan\(\delta\) (●) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-4) before the removal of \textit{o}-nitrobenzyl groups. The rheological data were collected from heating ramps using a heating rate of 3 °C/min, a strain amplitude of 0.2 \%, and an oscillation frequency of 1 Hz.
Figure A.16 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and tan$\delta$ (●) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-4) after the removal of $o$-nitrobenzyl groups. The rheological data were collected from heating ramps using a heating rate of 3 °C/min, a strain amplitude of 0.2 %, and an oscillation frequency of 1 Hz.
Figure A.17 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and tanδ (●) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-5) before the removal of $o$-nitrobenzyl groups. The rheological data were collected from heating ramps using a heating rate of 3 °C/min, a strain amplitude of 0.2 %, and an oscillation frequency of 1 Hz.
Figure A.18 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for a 10.0 wt % aqueous solution of P(TEGEA-co-NBA)-b-PEO-b-P(TEGEA-co-NBA) (ABA-5) after the removal of $o$-nitrobenzyl groups. The rheological data were collected from heating ramps using a heating rate of 3 °C/min, a strain amplitude of 0.2 %, and an oscillation frequency of 1 Hz.
Chapter 2. Enzyme-Induced Formation of Thermoreversible Micellar Gels from Aqueous Solutions of Multiresponsive Hydrophilic ABA Triblock Copolymers
Abstract

This chapter presents the synthesis of thermo- and enzyme-responsive hydrophilic ABA triblock copolymers, poly(ethoxydi(ethylene glycol) acrylate-co-4-((dihydroxyphosphoryl)oxy)butyl acrylate)-b-poly(ethylene oxide)-b-poly(ethoxydi(ethylene glycol) acrylate-co-4-((dihydroxyphosphoryl)oxy)butyl acrylate) (P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA)), and the enzyme-induced formation of thermoreversible micellar gels from their moderately concentrated aqueous solutions at 37 °C. PDEGEA is a thermosensitive water-soluble polymer with a lower critical solution temperature (LCST) at 9 °C in water. The block copolymers were prepared by atom transfer radical polymerization of DEGEA and 4-((di-tert-butoxyphosphoryl)oxy)butyl acrylate and subsequent removal of tert-butyl groups. To seek optimal conditions for enzymatic gelation of aqueous solutions of triblock copolymers, a study of dephosphorylation of a random copolymer P(DEGEA-co-OPBA) by acid phosphatase in water at 37 °C was carried out. The time for the solution to turn cloudy was found to decrease with the decrease of pH from 5.48 to 4.70 and level off from pH 4.39 to 4.23. The cleavage of phosphate groups made the polymer less hydrophilic and decreased the LCST from above to below 37 °C. Therefore, pH 4.4 was selected to conduct the enzyme-induced gelation of 7.9 wt % aqueous solutions of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA). The gelation processes were monitored by rheological measurements; the sol-gel transition temperature decreased and the gel strength increased with the increase of reaction time. The gels formed were thermoreversible; lowering temperature converted the gels to free-flowing liquids. From $^1$H and $^{31}$P NMR spectroscopy analysis, the degree of dephosphorylation was high. The
formation of 3-dimensional micellar network gels stemmed from the thermosensitive properties of the resultant dephosphorylated triblock copolymers, which was confirmed by a dynamic light scattering study. At a slightly higher pH (4.67), the enzyme-induced gelation was significantly slower, consistent with the observation of the effect of pH on dephosphorylation of the random copolymer by acid phosphatase.
2.1 Introduction

Polymer hydrogels have been intensively investigated for biomedical applications including contact lenses, sustained or triggered release of drugs and biomolecules, cell culture, and tissue engineering.\(^1\text{-}^9\) While chemically cross-linked polymer gels are being widely used and continuously evaluated, there is a growing interest in stimuli-responsive block copolymer aqueous micellar gels.\(^10\text{-}^{21}\) These physically crosslinked or jammed micellar gels can be more advantageous for some applications because of the in situ gelation of a liquid precursor induced by environmental changes, allowing, e.g., for minimally invasive administration by injection via syringe and needle. A notable example of injectable gel drug delivery systems, reported by Jeong et al., was based on aqueous solutions of block copolymers that underwent thermo-induced sol-gel transitions.\(^12\) The polymer solutions were loaded with a model drug in the sol state at an elevated temperature. Upon subcutaneous injection at the body temperature, the solutions formed gels instantaneously that subsequently acted as matrices for sustained release of drug molecules.

In general, there are two types of stimuli-responsive block copolymer aqueous micellar gels: 3-dimensional network gels, in which one block, e.g., the central block of an ABA triblock copolymer, forms bridges among micellar cores of other blocks,\(^13\text{-}^{16}\) and physically jammed micellar gels, in which discrete spherical micelles of block copolymers are jammed and packed into an ordered structure.\(^10,11,17\) While the second type of gels typically form at a polymer concentration of ~ 20 wt %, the critical gelation concentration (CGC) of ABA triblock copolymers with stimuli-sensitive outer blocks is significantly lower. For example, Kirkland et al. reported that a 7.5 wt % aqueous
solution of poly(N-isopropylacrylamide)-b-poly(N,N-dimethyacrylamide)-b-poly(N-isopropylacrylamide) formed a free-standing gel upon heating.\textsuperscript{13} We previously reported that the CGC of a thermosensitive ABA triblock copolymer in water was between 3 – 4 wt %.\textsuperscript{14}

Of particular interest to us are block copolymer aqueous micellar gels that can respond to two or more external stimuli. Such gels would offer greater design flexibility and more advantages compared with those that respond to only one external stimulus. There have been a number of reports on multi-responsive aqueous polymer micellar gels.\textsuperscript{18-21} For example, Li et al. reported the synthesis of thermo- and redox-sensitive ABA triblock copolymers and demonstrated the thermally and biochemically induced sol-gel transitions of aqueous solutions of these copolymers.\textsuperscript{18a} Temperature (T)- and pH-sensitive polymer micellar gels are probably the most studied and used multi-responsive gels.\textsuperscript{19,20} The block copolymers used in these studies were usually prepared by either growing pH-sensitive blocks from or introducing carboxylic acid or other pH-responsive groups to the chain ends of an ABA triblock copolymer that could form thermoreversible gels in water (e.g., PEO-\textit{b}-PPO-\textit{b}-PEO).\textsuperscript{19} Other types of multiblock copolymers were also employed.\textsuperscript{20}

We previously reported doubly responsive aqueous micellar gels of ABA triblock copolymers in which a small amount of stimuli-responsive functional groups was incorporated and randomly distributed in the thermosensitive outer blocks.\textsuperscript{14,21} The LCST of the thermosensitive blocks can be modified by applying a second external stimulus. While we have shown that aqueous solutions of such triblock copolymers can undergo multiple sol-gel-sol transitions in response to environmental changes and the phase
transitions can be well controlled, from a biological perspective, it is extremely attractive to use enzymes, a class of highly efficient and specific biocatalysts, to induce the gelation of aqueous solutions or develop enzyme-responsive hydrogels. One can imagine that such enzyme-responsive systems can be advantageous in some situations as they can respond to biological cues, e.g., the overexpression of specific enzymes for certain tissues or diseases. Nature actually uses enzymatic gelation to regulate the self-assembly of biomolecules in cytosol to locally switch part of the cell between the sol and gel states to control cell focal adhesion and migration.\textsuperscript{22,23} Inspired by nature and motivated by biological perspectives, there have been many reports in the literature on enzyme-induced formation of small molecule hydrogels and covalently crosslinked polymer gels as well as enzyme-responsive crosslinked hydrogels.\textsuperscript{23-34} For example, Xu et al. used kinase and phosphatase to control phosphorylation and dephosphorylation of a small molecule hydrogelator and to regulate the formation of supramolecular hydrogels.\textsuperscript{24} Anseth et al. reported human neutrophil elastase-responsive poly(ethylene glycol) hydrogels for controlled release.\textsuperscript{27}

Here we present the synthesis of thermo- and enzyme-responsive hydrophilic ABA triblock copolymers (Scheme 2.1) and the enzyme-induced formation of thermoreversible 3-dimensional micellar network gels from their aqueous solutions at the physiological temperature, 37 °C. We utilize the enzyme-catalyzed dephosphorylation,\textsuperscript{24,35} a common biochemical reaction, in our gel design; a small amount of hydrophilic phosphate groups is incorporated in a random distribution fashion into the thermosensitive outer blocks of ABA triblock copolymers. The enzymatic dephosphorylation decreases the LCST of thermosensitive blocks from above to below the experimental temperature, resulting in
Scheme 2.1 Synthesis of thermo- and enzyme-responsive ABA triblock copolymer

\[
P(\text{DEGEA-co-OPBA})-b-\text{PEO}-b-P(\text{DEGEA-co-OPBA}).
\]
the formation of a thermoreversible 3-dimensional network (Scheme 2.2). The multiresponsive triblock copolymers, poly(ethoxydi(ethylene glycol) acrylate-co-4-((dihydroxyphosphoryl)oxy)butyl acrylate)-b-PEO-b-poly(ethoxydi(ethylene glycol) acrylate-co-4-((dihydroxyphosphoryl)oxy)butyl acrylate) (P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA)), were synthesized by atom transfer radical polymerization (ATRP) of a mixture of DEGEA and 4-((di-tert-butoxyphosphoryl)oxy)butyl acrylate (BPBA) from a difunctional PEO macroinitiator and subsequent removal of tert-butyl groups (Scheme 2.1). PDEGEA is a thermosensitive polymer with a LCST of 9 °C in water, which belongs to a new class of thermosensitive polymers with a short oligo(ethylene glycol) pendant from each repeat unit. We show that 7.9 wt % aqueous solutions of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) undergo gelation in the presence of acid phosphatase, an enzyme that catalyzes dephosphorylation, at a constant temperature, 37 °C, and the gels formed are thermoreversible. To the best of our knowledge, this is the first report on the formation of physically crosslinked micellar gels of block copolymers in water induced by an enzyme at the physiological temperature. Compared with chemically crosslinked hydrogels, thermoreversible micellar gels have many advantages; a notable one is the facile removal.

2.2 Experimental Section

2.2.1 Materials

1,4-Butanediol (99%), di-tert-butyl diisopropylphosphoramidite (95%), tetrazole solution (~0.45 M in acetonitrile), and acid phosphatase (Type II, from potato, lyophilized power, 1.55 units/mg solid) were purchased from Aldrich and used as
Scheme 2.2 Dephosphorylation of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) by acid phosphatase and schematic illustration of enzymatically induced formation of thermoreversible aqueous micellar network gel.
received. Acryloyl chloride (96%, Alfa Aesar) was used as received. N,N,N',N',N''-Pentamethyldiethylenetriamine (PMDETA, Aldrich), ethyl 2-bromoisobutyrate (EBiB, Aldrich), and anisole (99%, Acros) were distilled over calcium hydride under reduced pressure. CuBr (98%, Aldrich) was stirred in glacial acetic acid, filtered, and washed with absolute ethanol and diethyl ether. The purified CuBr was then dried in high vacuum and stored in a desiccator. Poly(ethylene oxide) (HO-PEO-OH, MW = 20,000 g/mol, Aldrich) was end-functionalized via the reaction with 2-bromoisobutyryl bromide as described in a publication,\textsuperscript{14} yielding a difunctional PEO macronitiator, Br-PEO-Br. Trifluoroacetic acid (99%, Acros), H\textsubscript{2}O\textsubscript{2} solution (30%, Fisher Scientific, stabilized with sodium stannate), Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5} (99%, Acros), citric acid (99%, Acros), and citric acid trisodium salt (99%, Acros) were used as received. All other chemicals were purchased from either Aldrich or Fisher/Acros and used without further purification.

2.2.2 General Characterization

Size exclusion chromatography (SEC) was carried out at room temperature using PL-GPC 20 (an integrated GPC system from Polymer Laboratories, Inc.) with a refractive index detector, one PLgel 5 μm guard column (50 × 7.5 mm), and two PLgel 5 μm mixed-C columns (each 300 × 7.5 mm, linear range of molecular weight from 200 to 2,000,000 according to Polymer Laboratories, Inc.). The data were processed using Cirrus\textsuperscript{TM} GPC/SEC software (Polymer Laboratories, Inc.). THF was used as the carrier solvent at a flow rate of 1.0 mL/min. Standard polystyrenes with narrow polydispersity indexes (Polymer Laboratories, Inc.) were used for calibration. The \textsuperscript{1}H (300 MHz), \textsuperscript{13}C (75 MHz), and \textsuperscript{31}P (121 MHz) NMR spectra were recorded on a Varian Mercury 300 NMR spectrometer. The mass spectrometry analysis was performed at the Mass
Spectrometry Center in the Department of Chemistry at the University of Tennessee, Knoxville using a JEOL (Peabody, MA) AccuTOF-D time-of-flight mass spectrometer with a DART (direct analysis in real time) ionization source.

### 2.2.3 Synthesis of 4-Hydroxybutyl Acrylate (OHBA)

1,4-Butanediol (26.9 g, 0.299 mol) and triethylamine (10.9 g, 0.108 mol) were dissolved in dichloromethane (75 mL) in a 250 mL three-necked round bottom flask. The flask was then placed in an ice/water bath and the mixture was stirred with a magnetic stir bar under nitrogen atmosphere. A solution of acryloyl chloride (6.92 g, 0.0765 mol) in dichloromethane (20 mL) was added dropwise into the flask. The mixture was allowed to warm to room temperature and stirred overnight. The precipitate was filtered off and the solvent was then removed by a rotavapor. Diethyl ether was added and the mixture was washed twice with a saturated aqueous solution of sodium bicarbonate and then once with water. The organic layer was dried over anhydrous sodium sulfate overnight. After the removal of sodium sulfate, the solution was concentrated using a rotary evaporator. The crude product was purified by column chromatography using hexanes/ethyl acetate (v/v, 1:1) as eluent. The pure product was obtained as a nearly colorless liquid. $^1$H NMR (CDCl$_3$): $\delta$ (ppm), 6.38 (d, 1H, CHH=CH), 6.09 (dd, 1H, CHH=CH-), 5.80 (d, 1H, CHH=CH-), 4.17 (t, 2H, -COOC$_2$H$_5$), 3.67 (m, 2H, -CH$_2$OH), 1.77-1.60 (m, 4H, -CH$_2$CH$_2$CH$_2$CH$_2$-), 1.46 (s, 1H, -OH); $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 166.30, 130.67, 128.34, 64.30, 62.07, 28.95, 24.99. MS (ESI+): 145.09 ([M + H$^+$]).

### 2.2.4 Synthesis of 4-((di-tert-Butoxyphosphoryl)oxy)butyl Acrylate (BPBA)

4-Hydroxybutyl acrylate (1.13 g, 7.85 mmol) and di-tert-butyl diisopropylphosphoramidite (3.08 g, 0.0111 mol) were dissolved in dichloromethane (50
mL) in a 250 mL three-necked round bottom flask. The flask was placed into an ice/water bath and a solution of tetrazole in acetonitrile (0.45 M, 20 mL, 9.0 mmol) was added dropwise into the flask. The mixture became hazy white in appearance. The reaction mixture was allowed to warm to room temperature and stirred overnight. The flask was again placed in an ice/water bath and a 30 % aqueous solution of hydrogen peroxide (5 mL) was added into the flask in a dropwise fashion. The mixture became clear after the addition of H$_2$O$_2$. The reaction was kept at 0 °C for additional 3 h. A 10 wt % aqueous solution of Na$_2$S$_2$O$_5$ (30 mL) was slowly added into the reaction mixture to quench unreacted hydrogen peroxide (WARNING, noxious fumes). The organic layer was separated, washed with water, and then dried over anhydrous sodium sulfate. The product was isolated by column chromatography using hexanes/ethyl acetate (1:1) as eluent. After drying in vacuum, the pure product was obtained as a colorless liquid (1.72 g, yield: 65.2 %). $^1$H NMR (CDCl$_3$): δ (ppm), 6.37 (d, 1H, CHH=CH-), 6.08 (dd, 1H, CHH=CH-), 5.79 (d, 1H, CHH=CH-), 4.16 (t, 2H, -COOCH$_2$-), 3.96 (m, 2H, -CH$_2$OP-), 1.74 (m, 4H, -CH$_2$CH$_2$CH$_2$CH$_2$-), 1.47 (s, 18H, -OC(CH$_3$)$_3$); $^{13}$C NMR (CDCl$_3$): δ (ppm) 166.13, 130.60, 128.36, 82.07, 81.98, 66.13, 63.92, 30.28, 30.23, 29.81, 29.75, 26.86, 24.94. MS (ESI+): 337.18 ([M + H$^+$]).

2.2.5 Synthesis of P(DEGEA-co-BPBA) by Atom Transfer Radical Polymerization

CuBr (11.3 mg, 0.0788 mmol), ethoxydi(ethylene glycol) acrylate (DEGEA, 2.03 g, 10.8 mmol), 4-((di-tert-butoxyphosphoryl)oxy)butyl acrylate (BPBA, 0.351 g, 1.04 mmol), ethyl 2-bromoisobutyrate (EBiB, 10.6 mg, 0.0543 mmol), anisole (2.43 g), and $N,N,N',N',N''$-pentamethyldiethylenetriamine (29.5 mg, 0.170 mmol) were added into a 25 mL two-necked flask. After the mixture was degassed by three freeze-pump-thaw
cycles, the flask was placed into a 90 °C oil bath. The reaction was monitored by both SEC and $^1$H NMR spectroscopy analysis. After the polymerization proceeded for 175 min, the flask was removed from the oil bath and the solution was diluted with THF. The mixture was passed through an Al$_2$O$_3$/silica gel column using THF as eluent. The solution was concentrated via rotary evaporation and then precipitated three times in hexanes/diethyl ether (v/v = 80 : 20, 100 mL). A nearly colorless polymer was obtained.

SEC analysis results (polystyrene standards): $M_{n,SEC} = 12,000$ g/mol; polydispersity index (PDI) = 1.14. The degree of polymerization (DP) of the polymer was calculated from the monomer conversion and the monomer-to-initiator ratio. The peaks located in the range of 3.9 – 4.4 ppm, which were from –CH$_2$OOC– of DEGEA and BPBA and –CH$_2$OP– from BPBA were used as the internal standard. The conversion was calculated from the integral values of the peaks located at 5.6 – 6.0 ppm (CHH =CH- from both BPBA and DEGEA monomers) at $t = 0$ min and 175 min. The calculated DP was 82. The molar ratio of DEGEA and BPBA units in the copolymer was 100 : 13.3, determined from the $^1$H NMR spectrum using the peaks located at 3.9 – 4.4 ppm, which were from –CH$_2$OOC– of DEGEA and BPBA units and –CH$_2$OP– from BPBA units, and 1.2 ppm, which was the methyl peak of DEGEA units. $^1$H NMR (CDCl$_3$): $\delta$ (ppm), 4.19 (-COOCH$_2$- of DEGEA units), 4.05 (-COOCH$_2$- of BPBA units), 3.98 (-CH$_2$OP of BPBA units), 3.85-3.20 (-COOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGEA units), 2.32 (-CH$_2$CHCOO- of both DEGEA and BPBA units), 2.05-1.36 (-CH$_2$CHCOO- of both DEGEA and BPBA units), 1.70 (-COOCH$_2$CH$_2$CH$_2$CH$_2$OP of BPBA units), 1.47 (s, -C(CH$_3$)$_3$ of BPBA units), and 1.20 (t, -OCH$_2$CH$_3$ of DEGEA units). $^{31}$P NMR (CDCl$_3$): $\delta$ (ppm), -9.71.

2.2.6 Synthesis of P(DEGEA-co-OPBA)
P(DEGEA-co-BPBA) (0.200 g) was dissolved in methylene chloride (5 mL) in a 50 mL round-bottom flask, followed by addition of trifluoroacetic acid (TFA, 2.0 g). After the reaction mixture was stirred at room temperature for 48 h, the volatiles were removed by a rotary evaporator. The residue was dissolved in methylene chloride (25 mL) and the volatiles were evaporated again by a rotavapor. This process was repeated an additional two times to remove as much trifluoroacetic acid as possible. The polymer was purified by precipitation three times in hexanes/diethyl ether (100 : 20, v:v) and then once in hexanes (100 mL). After drying under high vacuum, the polymer was obtained as a clear sticky solid with a yield of 91 % (182.3 mg). The removal of tert-butyl groups of P(DEGEA-co-BPBA) was evidenced by the disappearance of the tert-butyl peak at 1.47 ppm in the $^1$H NMR spectrum and the shift of the $^{31}$P peak from -9.71 to 1.02 ppm in the $^{31}$P NMR spectra. $^1$H NMR (CDCl$_3$): δ (ppm), 4.18 (-COOCH$_2$- of DEGEA units), 4.06 (-COOCH$_2$- and -CH$_2$OP of OPBA units), 3.85-3.20 (-COOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGEA units), 2.32 (-CH$_2$CHCOO- of both DEGEA and OPBA units), 2.05-1.30 (-CH$_2$CHCOO- of both DEGEA and OPBA units), 1.74 (-COOCH$_2$CH$_2$CH$_2$CH$_2$OP of OPBA units), and 1.18 (t, -OCH$_2$CH$_3$ of DEGEA units). $^{31}$P NMR (CDCl$_3$): δ (ppm), 1.02.

2.2.7 Synthesis of P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA)

Below is a typical procedure for the synthesis of P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA). CuBr (14.6 mg, 0.102 mmol), difunctional PEO macroinitiator Br-PEO-Br (0.864 g, 0.043 mol), DEGEA (3.237 g, 17.22 mmol), BPBA (0.610 g, 1.81 mmol), and anisole (3.486 g) were weighed into a 25 mL two-necked flask, followed by the injection of PMDETA (18.8 mg, 0.109 mmol) via a microsyringe. The mixture was
degassed by three freeze-pump-thaw cycles. The polymerization was started by placing the flask into a 90 °C oil bath. After 110 min, the flask was removed from the oil bath and the mixture was diluted with THF. The copper catalyst was removed by passing the solution through a short neutral aluminum oxide/silica gel column with THF as eluent. The polymer solution was then concentrated via rotary evaporation and precipitated in hexanes/ethyl ether (v/v = 80 : 20, 100 mL). Size exclusion chromatography analysis results (polystyrene standards): $M_{n,SEC} = 49800$ g/mol and the polydispersity index (PDI) = 1.12. $^1$H NMR spectroscopy analysis showed that the numbers of DEGEA and BPBA units in the triblock copolymer were 143 and 28, respectively. $^1$H NMR (CDCl$_3$): $\delta$ (ppm), 4.18 (-COOCH$_2$- of DEGEA units), 4.04 (-COOCH$_2$- of BPBA units), 3.97 (-CH$_2$OP of BPBA units), 3.90-3.30 (-CH$_2$CH$_2$O- of PEO block and -COOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGEA units), 2.32 (-CH$_2$CHCOO- of both DEGEA and BPBA units), 2.05-1.36 (-CH$_2$CHCOO- of both DEGEA and BPBA units), 1.70 (-COOCH$_2$CH$_2$CH$_2$CH$_2$OP of BPBA units), 1.47 (s, -C(CH$_3$)$_3$ of BPBA units), and 1.19 (t, -OCH$_2$CH$_3$ of DEGEA units). $^{31}$P NMR (CDCl$_3$): $\delta$ (ppm), -9.71.

2.2.8 Synthesis of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA)

P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA) (0.987 g, $M_{n,SEC} = 49800$ g/mol, PDI = 1.12) was dissolved in dry dichloromethane (10 mL) in a 20 mL scintillation vial, followed by the addition of trifluoroacetic acid (3.813 g). The reaction mixture was stirred at room temperature for 48 h. The volatiles were then removed using a rotavapor. The residue was dissolved in dichloromethane (20 mL) and the volatiles were evaporated again by a rotavapor. This process was repeated an additional two times to remove as much trifluoroacetic acid as possible. The polymer was then dissolved in
THF (5 mL), precipitated in hexanes/diethyl ether (v/v = 80 : 20, 100 mL) three times, and dried in high vacuum. The removal of the tert-butyl groups was confirmed by $^\text{1}H$ NMR spectroscopy analysis; the tert-butyl peak located at 1.47 ppm disappeared. $^\text{1}H$ NMR (CDCl$_3$): δ (ppm), 4.18 (-COOCH$_2$- of DEGEA units), 4.06 (-COOCH$_2$- and -CH$_2$OP of OPBA units), 3.90-3.30 (-CH$_2$CH$_2$O- of PEO block and -COOCH$_2$CH$_2$OCH$_2$CH$_2$CH$_3$ of DEGEA units), 2.32 (-CH$_2$CHCOO- of both DEGEA and OPBA units), 2.05-1.36 (-CH$_2$CHCOO- of both DEGEA and OPBA units), 1.74 (-COOCH$_2$CH$_2$CH$_2$OP of OPBA units), and 1.19 (-OCH$_2$CH$_3$ of DEGEA units).

$^{31}$P NMR (CDCl$_3$): δ (ppm), 1.02.

2.2.9 Preparation of 0.5 wt % Aqueous Solutions of P(DEGEA-co-OPBA) and Study of pH Dependence of Cloud Point of P(DEGEA-co-OPBA) in Water

A 0.5 wt % aqueous solution of P(DEGEA-co-OPBA) was made by dissolving the random copolymer (33 mg, dried in high vacuum) in a 50 mM aqueous citrate buffer. The vial was then placed into the water bath of a Fisher Scientific Isotemp refrigerated circulator. The temperature was gradually increased from 8 °C. At each temperature, the sample was allowed to equilibrate for 20 min. When the solution turned cloudy, the temperature was recorded as the cloud point. The pH of the solution was then adjusted by using either 1.0 M KOH or 1.0 M HCl and the cloud point was determined by visual examination as described above.

2.2.10 Preparation of 0.5 wt % Aqueous Solutions of P(DEGEA-co-OPBA) and Study of pH Effect on Acid Phosphatase-Catalyzed Dephosphorylation

P(DEGEA-co-OPBA) was added into a preweighed 100 mL round bottom flask and dried under high vacuum in a 60 °C oil bath for 3 h. The mass of the dried polymer was
91.5 mg. A 50 mM aqueous citrate buffer with pH of 5.10 (18.235 g) was added into the flask and the mixture was sonicated in an ultrasonic ice/water bath to dissolve the polymer. The resultant homogeneous polymer solution was distributed roughly equally into 6 glass vials with an inner diameter of 12 mm. The solutions were then adjusted to desired pH values by injecting either 1.0 M KOH or 1.0 M HCl aqueous solution via a microsyringe. An aqueous solution of acid phosphatase with a concentration of 1.99 wt% was made by dissolving 14.2 mg of acid phosphatase in deionized water (0.698 g). A calculated amount of the enzyme solution was added into each vial via a microsyringe; the enzyme-to-polymer mass ratio for all solutions was 7.5 : 100. The vials were then placed into a 37 °C water bath. The time at which a solution turned cloudy was recorded as the clouding time. For the samples with pH of 4.23 and 5.48, the polymers were isolated at the clouding times and the $^1$H NMR spectra were recorded. Below is the $^1$H NMR data of the polymer isolated from the experiment at pH = 4.23. $^1$H NMR (CDCl$_3$): δ (ppm), 4.18 (-COOCH$_2$- of both DEGEA and OHBA units), 3.85-3.30 (-COOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGEA units and –CH$_2$CH$_2$OH of OHBA units), 2.32 (-CH$_2$CHCOO- of both DEGEA and OHBA units), 2.08-1.29 (-CH$_2$CHCOO- of both DEGEA and OHBA units, and -COOCH$_2$CH$_2$CH$_2$CH$_2$OH of OHBA units), and 1.19 (-OCH$_2$CH$_3$ of DEGEA units).

2.2.11 Preparation and Enzyme-Induced Gelation of 7.9 wt % Aqueous Solutions of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA)

    Below is a typical procedure for an enzyme-induced gelation experiment. P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) was added into a pre-weighed 3.7 ml vial. The vial was placed into a larger flask and dried under high vacuum in a 60 °C
oil bath for 3 h. The mass of the dried polymer was 0.115 g. The polymer was then dissolved in a 50 mM aqueous citrate buffer with a pH value of 4.90 (1.335 g) and the pH of the solution was adjusted to 4.40 by using a 1.0 M HCl solution. After acid phosphatase (5.60 mg) was added, the vial was capped tightly and placed into a 37 °C water bath ($t = 0$ min). The mixture was stirred with a magnetic stir bar. After the solution became viscous and the stir bar did not move freely, aliquots were begun to take at various time intervals for rheological measurements. Each time, the vial was removed from the 37 °C water bath and placed into an ice/water bath. After a 90 μL aliquot was withdrawn, the vial was sealed and immediately placed back into the 37 °C water bath. The reaction was stopped after the rheological measurements showed no change in the sol-gel transition temperature. The resultant polymer was isolated for $^1$H NMR spectroscopy analysis. $^1$H NMR (CDCl$_3$): $\delta$ (ppm), 4.18 (-COOCH$_2$- of both DEGEA and OHBA units), 3.90-3.30 (-$CH_2$CH$_2$O- of PEO block, -COOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGEA units, and -$CH_2$CH$_2$OH of OHBA units), 2.32 (-$CH_2$CHCOO- of both DEGEA and OHBA units), 2.00-1.34 (-$CH_2$CHCOO- of both DEGEA and OHBA units, and -COOCH$_2$CH$_2$CH$_2$CH$_2$OH of OHBA units), and 1.19 (-OCH$_2$CH$_3$ of DEGEA units).

2.2.12 Rheological Measurements

Rheological experiments were conducted using a rheometer from TA Instruments (Model TA AR2000ex). A cone-plate geometry with a cone diameter of 20 mm and an angle of 2° (truncation 52 μm) was employed; the temperature was controlled by the bottom Peltier plate. In each measurement, 90 μL of a polymer solution was loaded onto the plate by a micropipette. The solvent trap was filled with water and a solvent trap cover was used to minimize water evaporation. Dynamic storage ($G'$) and loss moduli
(G") of a polymer solution were measured by oscillatory shear experiments performed at a frequency of 1 Hz in a heating ramp at a heating rate of 3 °C/min. The frequency dependences of G' and G" of a polymer solution at selected temperatures were obtained by frequency sweep tests. A strain amplitude of \( \gamma = 0.2 \% \) was used in all dynamic tests to ensure that the deformation was within the linear viscoelastic regime.

2.2.13 Dynamic Light Scattering Study of 0.02 wt % Aqueous Solution of P(DEGEA-co-OHBA)-b-PEO-b-P(DEGEA-co-OHBA)

The thermo-induced micellization at a concentration of 0.02 wt % of P(DEGEA-co-OHBA)-b-PEO-b-P(DEGEA-co-OHBA), obtained from the enzymatic dephosphorylation of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA), was studied by dynamic light scattering (DLS). The 0.02 wt % aqueous solution of P(DEGEA-co-OHBA)-b-PEO-b-P(DEGEA-co-OPBA) was prepared by diluting the corresponding 7.9 wt % polymer solution after the enzymatic dephosphorylation with a 50 mM aqueous citrate buffer. The pH value of the solution was maintained at 4.40. The DLS measurement was conducted with a Brookhaven Instruments BI-200SM goniometer equipped with a PCI BI-9000AT digital correlator, a temperature controller, and a solid-state laser (model 25-LHP-928-249, \( \lambda = 633 \text{ nm} \) at a scattering angle of 90°. The polymer solution was filtered into a borosilicate glass tube with an inner diameter of 7.5 mm using a 0.2 \( \mu \text{m} \) hydrophilic PTFE filter. The glass tube was then sealed with a PE stopper. The sample was placed into the cell holder of the light scattering instrument and gradually heated. At each temperature, the solution was equilibrated for 20 min prior to data recording. The time correlation functions were analyzed with a Laplace inversion program (CONTIN).
2.3 Results and Discussion

2.3.1 Synthesis of Monomer 4-((di-tert-Butoxyphosphoryl)oxy)butyl Acrylate (BPBA)

BPBA was synthesized via a two-step procedure as shown in Scheme 2.3. 4-Hydroxybutyl acrylate (OHBA) was prepared first through the reaction of acryloyl chloride with a large excess of 1,4-butanediol. The phosphorylation of OHBA was conducted using 1.4 equiv of di-tert-butyl diisopropylphosphoramidite in the presence of 1.1 equiv of tetrazole, followed by oxidation with a 30 % aqueous solution of hydrogen peroxide. The pure product was isolated by column chromatography using hexanes/ethyl acetate (1:1, v/v) as eluent and the molecular structure was confirmed by $^1$H and $^{13}$C NMR spectroscopy as well as mass spectroscopy analysis.

2.3.2 Preparation of Thermo- and Enzyme-Responsive Hydrophilic ABA Triblock Copolymers and a Random Copolymer

The thermo- and enzyme-responsive hydrophilic ABA triblock copolymers, P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA), were prepared according to the procedure illustrated in Scheme 2.1. The precursor polymers, P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA), were synthesized by ATRP of a mixture of DEGEA and BPBA from a difunctional PEO macroinitiator with a molecular weight of 20000 g/mol at 90 °C using CuBr/PMDETA as catalyst. Figure 2.1A shows the SEC traces of PEO macroinitiator and an ABA triblock copolymer P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA) (ABA-1-P). The peak shifted to the high molecular weight region and remained narrow, though there was a small shoulder peak on the front side, which was likely from the product of the coupling reaction. The number average
Scheme 2.3 Synthesis of 4-((di-tert-butoxyphosphoryl)oxy)butyl acrylate (BPBA)
Figure 2.1 (A) Size exclusion chromatography traces of PEO macroinitiator and an ABA triblock copolymer P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA) (ABA-1-P) and $^1$H NMR spectra of (B) ABA-1-P, (C) ABA-1, and (D) the dephosphorylated triblock copolymer obtained from the enzymatic gelation experiment of ABA-1. CDCl$_3$ was used as solvent in $^1$H NMR spectroscopy. The sharp peak at 1.76 ppm in Figure 2.1B is from water.
molecular weight \(M_\text{n,SEC}\) and polydispersity index (PDI) of ABA-1-P were 49.8 kDa and 1.12, respectively (relative to polystyrene standards). The numbers of DEGEA and BPBA units in the copolymer were determined from \(^1\text{H} \text{NMR}\) spectrum (Figure 2.1B) using the peaks located at 4.36 – 3.90 ppm (-CH\(_2\)OCO of DEGEA units, -CH\(_2\)OCO and -CH\(_2\)OP- of BPBA units), the peak at 2.55 – 2.15 ppm (-CHCOO- of both DEGEA and BPBA units), and the peaks at 1.35 – 1.00 ppm (-CH\(_3\) of DEGEA units). They were 143 and 28, respectively. The \(t\)-butyl groups in the copolymer were then cleaved using trifluoroacetic acid (TFA), yielding the targeted thermo- and enzyme-responsive ABA triblock copolymer P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA). Figure 2.1C shows the \(^1\text{H} \text{NMR}\) spectrum of ABA-1; the removal of \(t\)-butyl groups was evidenced by the disappearance of the \(t\)-butyl peak in the \(^1\text{H} \text{NMR}\) spectrum. TFA is known not to affect other ester bonds.\(^{37f}\) Figure 2.2A and 2.2B display the \(^{31}\text{P}\) \text{NMR}\) spectra of ABA-1-P and ABA-1, respectively. The \(^{31}\text{P}\) peak shifted from -9.7 ppm to 1.0 ppm, also indicating the successful removal of \(t\)-butyl groups.

Two ABA triblock copolymers with slightly different molecular weights and compositions were synthesized and used in this work. The characterization data for these copolymers and their precursors are summarized in Table 2.1. To seek optimal conditions for enzymatic gelation of moderately concentrated aqueous solutions of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA), we synthesized a random copolymer P(DEGEA-co-OPBA) (R-1). Note that all precursor polymers had relatively narrow molecular weight distributions (PDI < 1.15).\(^{38}\)
Figure 2.2 $^{31}$P NMR spectra of (A) P(DEGEA-$co$-BPBA)-$b$-PEO-$b$-P(DEGEA-$co$-BPBA) (ABA-1-P), P(DEGEA-$co$-OPBA)-$b$-PEO-$b$-P(DEGEA-$co$-OPBA) (ABA-1), and (C) the dephosphorylated triblock copolymer obtained from the enzymatic gelation experiment of ABA-1.
Table 2.1 Characterization data for multiresponsive ABA triblock copolymers P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA), random copolymer P(DEGEA-co-OPBA), and their precursor polymers.

<table>
<thead>
<tr>
<th>Polymers a</th>
<th>$M_{n,SEC}$ (g/mol) b</th>
<th>PDI b</th>
<th>Numbers of DEGEA and BPBA (or OPBA) units c</th>
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</thead>
<tbody>
<tr>
<td>ABA-1-P</td>
<td>49800</td>
<td>1.12</td>
<td>143, 28</td>
</tr>
<tr>
<td>ABA-1</td>
<td>NA</td>
<td>NA</td>
<td>143, 28</td>
</tr>
<tr>
<td>ABA-2-P</td>
<td>58900</td>
<td>1.11</td>
<td>187, 45</td>
</tr>
<tr>
<td>ABA-2</td>
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<td>NA</td>
<td>187, 45</td>
</tr>
<tr>
<td>R-1-P</td>
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<td>1.14</td>
<td>72, 10</td>
</tr>
<tr>
<td>R-1</td>
<td>NA</td>
<td>NA</td>
<td>72, 10</td>
</tr>
</tbody>
</table>

a ABA-1 and -2 are thermo- and enzyme-responsive ABA triblock copolymers P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA), which were obtained by the removal of tert-butyl groups of ABA-1-P and ABA-2-P (P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA)), respectively. b The number average molecular weights ($M_{n,SEC}$) and polydispersity indexes (PDI) were measured by SEC using polystyrene calibration. c The numbers of DEGEA and BPBA (or OPBA) units in the copolymers were calculated from $^1$H NMR spectra.
2.3.3 pH Dependence of Cloud Point of P(DEGEA-co-OPBA)

We chose DEGEA as the main component for the thermosensitive blocks to construct thermo- and enzyme-responsive ABA triblock copolymers because the cloud point of PDEGEA in water is 9 °C.\textsuperscript{21b} The cleavage of hydrophilic phosphate groups from thermosensitive blocks would yield hydrophobic 4-hydroxybutyl groups (Scheme 2.2), decrease the LCST of thermosensitive blocks, and induce the gelation of moderately concentrated aqueous polymer solutions at 37 °C, the temperature intended for the enzymatic reaction.

Since phosphoric acid is a weak acid and the cloud point in water of a thermosensitive polymer that contains a small amount of weak acid groups is known to depend on solution pH,\textsuperscript{39} we studied the pH dependence of cloud point of a random copolymer P(DEGEA-co-OPBA) (R-1). The cloud points of aqueous solutions of R-1 in 50 mM citrate buffers at various pH values were measured (Figure 2.3). We found that the cloud point of this polymer at pH = 3.70 was 50 °C. When the pH was ≥ 3.80, no cloud point was observed in the studied temperature range (up to 80 °C). Apparently, the ionization of phosphate groups at pH ≥ 3.80 made the polymer very hydrophilic and caused the LCST to be above 80 °C or disappear. Note that the pK\textsubscript{a1} of H\textsubscript{3}PO\textsubscript{4} is 2.15. With the decrease of pH from 3.70 to 1.49, the cloud point gradually decreased from 50 °C to 14 °C. No change was observed when the pH was further lowered to 1.29.
Figure 2.3 pH dependence of cloud point of P(DEGEA-co-OPBA) (R-1) in a 50 mM citrate buffer at a concentration of 0.5 wt %. The cloud points were determined by visual examination.
2.3.4 Effect of pH on Dephosphorylation of Random Copolymer P(DEGEA-co-OPBA) at 37 °C

The activity of acid phosphatase in the catalysis of dephosphorylation reaction is known to be sensitive to solution pH. To seek optimal conditions for enzymatic gelation of aqueous solutions of ABA triblock copolymers, we studied how the dephosphorylation of R-1 by acid phosphatase was affected by solution pH. Six 0.5 wt % aqueous solutions of R-1 with pH ranging from 4.23 to 5.48 were made in small glass vials using 50 mM citrate buffers, followed by the addition of acid phosphatase (the mass ratio of the enzyme to the copolymer was 7.5 : 100 for all samples). The vials were placed immediately in a 37 °C water bath (t = 0 min). When the solution turned cloudy, the time was recorded as clouding time (t_{clouding}). We found that the clouding time decreased with the decrease of pH, from 385 min at pH 5.48 to 96 min at pH 4.93, to 41.4 min at pH 4.70, and 7.6 min at pH 4.39 (Figure 2.4). Further lowering the pH did not change the clouding time much (t_{clouding} = 5.4 min at pH 4.23). Since the cloud point of the copolymer with a very small amount of uncleaved phosphate groups could depend on the solution pH, we performed $^1$H NMR spectroscopy analysis to look into the degree of the cleavage of phosphate groups at the clouding time. We isolated the polymers from the samples with pH of 4.23 and 5.48 at the clouding times by evaporating water of a small portion of each sample using a stream of nitrogen flow, dried them at 60 °C under high vacuum, and then analyzed them by $^1$H NMR spectroscopy. The two $^1$H NMR spectra appeared to be similar; the peak located at 4.06 ppm, which was from -CH$_2$OCO- and -CH$_2$OP- of OPBA units in the copolymer, mostly disappeared (the peak of -CH$_2$OCO- shifted to ~ 4.2 ppm and overlapped with the peak there while the peak of -CH$_2$OP-
Figure 2.4 Plot of clouding time, the time for a 0.5 wt % solution of P(DEGEA-co-OPBA) (R-1) in a 50 mM aqueous citrate buffer in the presence of acid phosphatase at 37 °C to turn cloudy, as a function of solution pH.
shifted to ~ 3.7 ppm and overlapped with the peaks there). This observation suggested that the degrees of the cleavage of phosphate groups at the two pH values were quite similar, though the clouding times were very different.

2.3.5 Enzyme-Induced Gelation of a 7.9 wt % Aqueous Solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) at pH = 4.40 and 37 °C

In light of the observations shown in Figure 2.4, we chose pH = 4.4 to study the enzymatically induced gelation of a 7.9 wt % aqueous solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) at 37 °C. The 7.9 wt % aqueous solution of ABA-1 was made by using a 50 mM citrate buffer and the pH was adjusted to 4.40. A calculated amount of acid phosphatase was added into the polymer solution (the mass ratio of the enzyme to the thermosensitive blocks was 7.5 : 100, same as that in the enzymatic cleavage experiment of R-1). The vial was capped tightly and placed into a 37 °C water bath (t = 0 min). Note that before the addition of the enzyme, the solution exhibited no change in the viscosity upon heating up to 80 °C.

After 1 h, the solution became viscous and the magnetic stir bar did not move freely. The stirrer stopped moving after 2 h. Figure 2.5 shows the optical pictures of the sample at various times. Evidently, the solution became very viscous after 4.5 h (Figure 2.5D) and was almost a gel at 6.5 h (Figure 2.5E). After the reaction proceeded for 8 h, the sample turned into a gel as it remained immobile when tilted or inverted (Figure 2.5F). The gel was thermoreversible; placing the vial in an ice/water bath transformed the gel into a free-flowing liquid. To quantitatively monitor the gelation process, aliquots were taken at various time intervals (the vial was placed in an ice/water bath to decrease the solution viscosity) and oscillatory shear experiments were conducted at a frequency of 1
Figure 2.5  Digital optical pictures of a 7.9 wt % solution of P(DEGEO-co-OPBA)-b-PEO-b-P(DEGEO-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 before the addition of acid phosphatase (A) and after the enzymatic reaction at 37 °C for 0 min (B), 180 min (C), 270 min (D), 390 min (E), 480 min (F), 593 min (G), and 1314 min (H).
Hz in a heating ramp mode at a heating rate of 3 °C/min. A strain amplitude of $\gamma = 0.2\%$ was used for all aliquots to ensure that the measurements were performed in the linear viscoelastic regime.

Figure 2.6 shows the rheological data for the selected aliquots taken from the sample at 180 min (A), 390 min (B), 480 min (C), and 1314 min (D). Clearly, for all these aliquots, below a certain temperature, the values of dynamic storage modulus $G'$ and loss modulus $G''$ were small and the data points were quite scattered, indicating that they were liquids. With the increase of temperature, $G'$ and $G''$ increased and at a certain point $G'$ became larger than $G''$, suggesting that the aliquots turned into gels. The crossover, $G' = G''$, has been commonly employed as an indicator of the sol-gel transition. Using this simple and convenient method, the sol-to-gel transition temperatures ($T_{\text{sol-gel}}$) of the aliquots were determined and a plot of $T_{\text{sol-gel}}$ versus time was made (Figure 2.7A). At the beginning of the experiment, the $T_{\text{sol-gel}}$ decreased relatively fast, from 53.1 °C at 82 min to 41.0 °C at 180 min. After that, the change became slower; the $T_{\text{sol-gel}}$ decreased by only 8 °C from $t = 180$ to 1740 min. This is reasonable because with the increase of viscosity, the diffusion of acid phosphatase became slower and the access to the remaining phosphate groups on the thermosensitive blocks by the enzyme became more difficult, causing the cleavage reaction to slow down. Note that the $T_{\text{sol-gel}}$ at 390 min was 37.2 °C, in agreement with the visual observation that the sample was almost a gel. The experiment was stopped after 1740 min at which only a very small difference in $T_{\text{sol-gel}}$ from that at 1314 min was observed.
Figure 2.6 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and tanδ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 in the presence of acid phosphatase at 37 °C after 180 min (A), 390 min (B), 480 min (C), and 1314 min (D). The data were collected from temperature ramp experiments performed by using a frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure 2.7 Plot of sol-gel transition temperature ($T_{\text{sol-gel}}$) (A) and the maximum value of $G'$ ($G'_{\text{max}}$) (B) versus reaction time. The values of $T_{\text{sol-gel}}$ and $G'_{\text{max}}$ were obtained from oscillatory shear experiments, which were performed in a heating ramp mode using a frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure 2.7B shows the plot of the maximum value of $G'$ ($G'_{\text{max}}$), obtained from the temperature ramp experiments, versus reaction time. $G'_{\text{max}}$ increased with time, from 151 Pa at $t = 82$ min to 821 Pa at 1740 min. Similar to the trend of $T_{\text{sol-gel}}$, the change in the gel strength was faster at the beginning and became slower with the increase of reaction time. We further characterized the final gel sample by frequency sweep experiments (Figure 2.8). At 25 °C, the solution was a liquid that could flow when tilted. The $G'$ was smaller than $G''$ in the range from 0.1 to 20 Hz and both exhibited power law dependences on frequency $f$ in the low frequency region: $G' \sim f^2$ and $G'' \sim f$ (Figure 2.8A). This is the typical rheological behavior of a viscous liquid.\textsuperscript{41} With the increase of temperature, the frequency dependences of $G'$ and $G''$ evolved (Figure 2.8B), and at 31 °C, which is close to the $T_{\text{sol-gel}}$ (33 °C), $G'$ and $G''$ were of similar magnitudes in the low frequency region and $G''$ scaled with $f^{0.5}$ (Figure 2.8C). This is the signature of the transition between liquid-like and solid-like behavior.\textsuperscript{41} At 40 °C, the sample was a free-standing gel. $G'$ was significantly greater than $G''$ and exhibited a much weaker dependence on frequency (Figure 2.8D), which is a characteristic of solid-like behavior.\textsuperscript{41,42} The plateau modulus $G_N$ of the gel can be obtained from the frequency sweep and it is known that the $G_N$ of a transient gel is a measure of the number density of elastically active polymer chains:

$$G_N = \nu k_B T$$

where $\nu$ is the number density of elastically active polymer chains (number of elastically active bridging chains per unit volume), $k_B$ is Boltzmann constant, and $T$ is the absolute temperature.\textsuperscript{42,43} The $G_N$ is usually evaluated as the $G'$ value at the frequency where $G''$ exhibits the minimum value, because the increase of $G''$ at higher frequencies indicates a
Figure 2.8 Frequency dependences of dynamic storage modulus $G'$ (■) and loss modulus $G''$ (□) at (A) 25, (B) 28, (C) 31, and (D) 40 °C of the final aqueous solution of ABA-1 with pH of 4.40 after the enzymatic reaction at 37 °C for 1740 min. A strain amplitude of 0.2 % was used in all frequency sweep experiments.
fast relaxation process separate from the terminal flow process. This method for the
determination of $G_N$ is well established for the entangled homopolymer melts and has
also been recently used in the study of thermoreversible transient gels. The frequency at
which $G''$ exhibited a minimum value at 40 °C was 12.59 Hz; therefore, the value of $G_N$
was 1103 Pa, close to the modulus in the plateau zone in the heating ramp (821 Pa). If the
central block of every polymer chain is elastically active in the gel, a calculation shows
that the $G_N$ is 3869 Pa at 40 °C. This means that 28.5% of polymer chains formed
effective network strands that could sustain an external stress in the gel at this
temperature. The relatively low percentage of polymer chains acting as bridges among
micellar cores indicated the presence of the defects in the network, e.g., the loops (two
end blocks were located in the same micellar core) and dangling chains (one end block
stayed in water instead of in the micellar cores, see Scheme 2.2).

$^1$H NMR spectroscopy analysis of the polymer isolated after the enzymatic gelation
experiment showed that the peak located at 4.06 ppm, which was from $-CH_2OCO-$ and
$-CH_2OP-$ of OPBA units in the copolymer, almost disappeared (Figure 2.1D). This is the
same as the dephosphorylation of P(DEGEA-co-OPBA) (R-1) by acid phosphatase. The
$^{31}$P NMR spectrum of the dephosphorylated triblock copolymer is presented in Figure
2.2C. Compared with the $^{31}$P NMR spectrum of ABA-1, the peak was nearly invisible.
Thus, both $^1$H and $^{31}$P NMR spectroscopy analysis suggested that the degree of the
cleavage of phosphate groups was high after the enzymatic reaction at 37 °C for 1740
min.

To confirm that the acid phosphatase-induced gelation of the 7.9 wt % aqueous
solution of ABA-1 at 37 °C originated from the LCST behavior of the thermosensitive
blocks formed after the cleavage of phosphate groups (i.e., P(DEGEA-co-OHBA) blocks), we conducted a dynamic light scattering study of the dephosphorylated triblock copolymer in a 50 mM aqueous citrate buffer with a concentration of 0.02 wt % at the same pH value (4.40). Figure 2.9 shows the scattering intensity at scattering angle of 90° and apparent hydrodynamic size, obtained from CONTIN analysis, as a function of temperature. Below 17 °C, the scattering intensity was low and the apparent hydrodynamic size was small (< 10 nm), indicating that the polymer dissolved molecularly in the buffer. When the temperature was raised above 16 °C, the scattering intensity began to increase and above 25 °C the hydrodynamic size was around 60 nm. The critical micellization temperature (CMT) determined from the plot of scattering intensity vs temperature was 17 °C. The thermo-induced micellization was reversible; lowering the temperature dissociated the micelles. Differently, there was very little change in the scattering intensity of 0.02 wt % aqueous solution of ABA-1 in the same temperature range. Clearly, in the gelation experiment, the enzymatic cleavage of hydrophilic phosphate groups made the outer blocks less hydrophilic and decreased the LCST. When the LCST became lower than the experimental temperature (37 °C), the triblock copolymer began to self-assemble into micelles and formed a 3-dimensional network gel with the PEO blocks forming bridges. Note that the $T_{\text{sol-gel}}$ of the final sample was 33 °C, higher than the CMT at the same pH (17 °C). This is understandable because the gelation requires the formation of a 3-dimensional network with a sufficient mechanical strength, while the CMT is the temperature at which the thermosensitive P(DEGEA-co-AA) blocks begin to self-assemble to form micelles in a dilute aqueous solution.
Figure 2.9 Scattering intensity at scattering angle of 90° (A) and apparent hydrodynamic size $D_h$ (B), obtained from CONTIN analysis, as a function of temperature in a dynamic light scattering study of a 0.02 wt% solution of P(DEGEA-co-OHBA)-b-PEO-b-P(DEGEA-co-OHBA) in a 50 mM aqueous citrate buffer with pH of 4.40. The triblock copolymer was obtained after the enzymatic dephosphorylation of ABA-1 in a 7.9 wt % solution proceeded at 37 °C for 1740 min.
2.3.6 Enzyme-Induced Gelation of 7.9 wt % Aqueous Solution of ABA-1 at pH = 4.67

As shown in Figure 2.4, the dephosphorylation of P(DEGEA-co-OPBA) (R-1) by acid phosphatase was heavily affected by the solution pH. For example, the clouding time at pH = 4.70 ($t_{\text{clouding}} = 41.4$ min) was 5.4 times that at pH = 4.39 ($t_{\text{clouding}} = 7.6$ min), though the difference in pH values was only 0.31 pH unit. To gain an insight into the effect of pH on the gelation, we carried out an experiment at a slightly higher pH value (4.67) while other conditions remained the same. The gelation was significantly slower (Figure 2.10A); it took 1560 min for the $T_{\text{sol-gel}}$ to decrease to 37.5 °C, while at pH 4.40 the $T_{\text{sol-gel}}$ reached 37.2 °C in 390 min, which was 4 times faster. Interestingly, the $G'_{\text{max}}$ at the same or similar stage of the gelation process was not heavily dependent on the solution pH. For example, for the aliquot with $T_{\text{sol-gel}} = 37.5$ °C, the $G'_{\text{max}}$ was 542 Pa (Figure 2.10B), essentially identical to that of the aliquot with $T_{\text{sol-gel}}$ of 37.2 °C at pH 4.40 ($G'_{\text{max}} = 524$ Pa, Figure 2.7B). In addition, the highest value of $G'_{\text{max}}$ observed in this gelation experiment (717 Pa) was comparable to that at pH = 4.40 (821 Pa).

2.3.7 Enzyme-Induced Gelation of 7.9 wt % Aqueous Solution of ABA-2 at pH = 4.41

We also studied the gelation of a 7.9 wt % solution of ABA-2 in a 50 mM citrate buffer induced by acid phosphatase under the same conditions (pH = 4.41 and temperature = 37 °C). The amount of acid phosphatase with respect to the mass of the thermosensitive blocks of ABA-2 was also maintained at the same level, that is, 7.5 to 100. Note that ABA-2 had a slightly higher molecular weight (the $M_n,\text{SEC}$ of its precursor ABA-2-P = 58.9 kDa, PDI = 1.11) and a slightly higher OPBA content (see Table 2.1).
Figure 2.10 Plot of sol-gel transition temperature ($T_{\text{sol-gel}}$) (A) and the maximum value of dynamic storage modulus ($G'_{\text{max}}$) (B) versus reaction time for the enzyme-induced gelation of 7.9 wt % aqueous solution of ABA-1 at pH 4.67. The values of $T_{\text{sol-gel}}$ and $G'_{\text{max}}$ were obtained from oscillatory shear experiments, which were performed in a heating ramp mode using a frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Same as ABA-1, the 7.9 wt % aqueous solution of ABA-2 turned into a micellar gel.\textsuperscript{38} Figure 2.11 shows the plots of $T_{\text{sol-gel}}$ and $G'_{\text{max}}$ versus reaction time. Compared with ABA-1, the gelation was slightly faster; the $T_{\text{sol-gel}}$ decreased to 35.8 °C after 331 min. In addition, the $T_{\text{sol-gel}}$ of the final sample was lower, 28.5 °C, and the highest value of $G'_{\text{max}}$ (1036 Pa) was slightly higher (821 Pa for ABA-1). This is likely because the thermosensitive blocks were longer (DP\textsubscript{total} = 232 in comparison to 171 of ABA-1) and the OPBA content was slightly higher. \textsuperscript{38} \textsuperscript{1}H and \textsuperscript{31}P NMR spectroscopy analysis showed that the degree of the cleavage of phosphate groups was similar to that of ABA-1 at the end of the gelation experiment. Thus, one can imagine that after dephosphorylation the thermosensitive blocks of the triblock copolymer were slightly more hydrophobic due to the slightly higher content of hydrophobic 4-hydroxybutyl acrylate units. This was confirmed by a dynamic light scattering study, which showed that the CMT of the dephosphorylated triblock copolymer at the same pH (4.41) was 13 °C,\textsuperscript{38} 4 °C lower than that of the polymer formed from ABA-1.

\textbf{2.4. Conclusions}

By incorporating phosphate groups into thermosensitive blocks, we synthesized two thermo- and enzyme-responsive hydrophilic ABA triblock copolymers, P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA), via ATRP of DEGEA and BPBA from a difunctional PEO macroinitiator and subsequent removal of tert-butyl groups of BPBA units.\textsuperscript{44} A model study using a random copolymer P(DEGEA-co-OPBA) showed that the time for the 0.5 wt% solution to turn cloudy in the presence of acid phosphatase at 37 °C decreased with the decrease of pH from pH 5.48 to 4.39 and leveled off when the pH was
Figure 2.11 Plot of sol-gel transition temperature ($T_{\text{sol-gel}}$) (A) and the maximum value of dynamic storage modulus ($G'_{\text{max}}$) (B) versus reaction time for the enzyme-induced gelation experiment of a 7.9 wt % aqueous solution of ABA-2 at pH = 4.41. The values of $T_{\text{sol-gel}}$ and $G'_{\text{max}}$ were obtained from oscillatory shear experiments, which were performed in a heating ramp mode using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
further lowered to 4.23. Therefore, we chose pH 4.4 to conduct the enzyme-induced gelation experiments. As expected, a 7.9 wt% aqueous solution of ABA-1 in the presence of acid phosphatase at 37 °C turned into a gel. Rheological measurements showed that the $T_{\text{sol-gel}}$ decreased with the increase of reaction time and reached 37.2 °C at 390 min. The frequency sweep study of the final sample indicated that 28.5% of polymer chains formed effective network strands in the gel at 40 °C. $^1$H and $^{31}$P NMR spectroscopy analysis showed that the degree of the cleavage of phosphate groups was high. The enzymatic dephosphorylation of the triblock copolymer yielded hydrophobic 4-hydroxybutyl groups in the thermosensitive outer blocks and decreased the LCST from above to below the experimental temperature (37 °C), resulting in the formation of a 3-dimensional network gel. This was confirmed by a DLS study of the dephosphorylated triblock copolymer showing that the CMT was 17 °C. In contrast, the gelation at pH = 4.67 took a much longer time, which was in line with the effect of pH on the rate of enzymatic cleavage of phosphate groups in a random copolymer. A similar but slightly faster gelation behavior was observed in the study of ABA-2 that had a higher molecular weight and a slightly greater OPBA content. Although the gelation in the present work took > 5 h for both triblock copolymers, we believe that it can be improved, for example, by selecting a thermosensitive water-soluble polymer with a lower LCST, tuning the phosphate content and the block length of thermosensitive blocks, using a larger amount of enzyme, etc. Since the enzyme-catalyzed reactions are highly specific and efficient, the method reported in this article can be used to design biologically induced micellar gels for potential applications in biomedical areas.
References


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38. The data can be found in Appendix B.


44. The work presented in this Chapter has been published in *Macromolecules* as an article (*Macromolecules* **2011**, *44*, 5764-5775).

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Appendix B

for

Chapter 2. Enzyme-Induced Formation of Thermoreversible Micellar Gels from Aqueous Solutions of Multiresponsive Hydrophilic ABA Triblock Copolymers
**Figure B.1** Size exclusion chromatography traces of PEO macroinitiator and ABA triblock copolymer P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA) (ABA-2-P).
Figure B.2 $^1$H NMR spectra of (A) P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA) (ABA-2-P), (B) ABA-2, and the dephosphorylated triblock copolymer obtained from the enzymatic gelation experiment of ABA-2.
Figure B.3 $^{31}$P NMR spectra of (A) P(DEGEA-co-BPBA)-b-PEO-b-P(DEGEA-co-BPBA) (ABA-2-P), (B) ABA-2, and (C) the dephosphorylated triblock copolymer isolated from the enzymatic gelation experiment of ABA-2.
Figure B.4 Size exclusion chromatography trace of a random copolymer P(DEGEA-co-BPBA) (R-1-P).
Figure B.5 $^1$H NMR spectra of (A) R-1-P and (B) R-1. CDCl$_3$ was used as solvent in $^1$H NMR spectroscopy.
Figure B.6 $^{31}$P NMR spectra of (A) P(DEGEA-co-BPBA) (R-1-P) and (B) P(DEGEA-co-OPBA) (R-1). CDCl$_3$ was used as solvent.
Figure B.7 $^1$H NMR spectra of (A) P(DEGEA-co-OPBA) (R-1), (B) the polymer isolated from the 0.5 wt % aqueous solution of R-1 with pH of 4.23 in the presence of acid phosphatase at the clouding time ($t_{\text{clouding}} = 5.4$ min), and (C) the polymer isolated from the 0.5 wt % aqueous solution of R-1 with pH of 5.48 in the presence of acid phosphatase at the clouding time ($t_{\text{clouding}} = 385$ min). CDCl$_3$ was used as solvent in $^1$H NMR spectroscopy.
Figure B.8 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (○) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 in the presence of acid phosphatase at 37 °C after 82 min. The data were collected from a temperature ramp experiment performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.9 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 in the presence of acid phosphatase at 37 °C after 270 min. The data were collected from a temperature ramp experiment performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.10 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (○) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 in the presence of acid phosphatase at 37 °C after 593 min. The data were collected from a temperature ramp experiment performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.11 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (○) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 in the presence of acid phosphatase at 37 °C after 1740 min. The data were collected from a temperature ramp experiment performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.12 Scattering intensity at scattering angle of 90° as a function of temperature in dynamic light scattering studies of a 0.02 wt% solution of P(DEGEO-co-OPBA)-b-PEO-b-P(DEGEO-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.40 (■) and 0.02 wt% solution of P(DEGEO-co-OHBA)-b-PEO-b-P(DEGEO-co-OHBA) in a 50 mM aqueous citrate buffer with pH of 4.40 (□). The triblock copolymer P(DEGEO-co-OHBA)-b-PEO-b-P(DEGEO-co-OHBA) was obtained after the enzymatic dephosphorylation of ABA-1 in a 7.9 wt % solution with pH of 4.40 proceeded at 37 ° for 1740 min).
Figure B.13 Plot of dynamic storage modulus $G'$ ( ■ ), dynamic loss modulus $G''$ ( □ ), and $\tan \delta$ ( ○ ) versus temperature for the aliquots taken from a 7.9 wt% solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.67 in the presence of acid phosphatase at 37 °C after 630 min. The data were collected from temperature ramp experiments performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.14 Plot of dynamic storage modulus G’ (■), dynamic loss modulus G” (□), and \( \tan \delta \) (●) versus temperature for the aliquots taken from a 7.9 wt% solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.67 in the presence of acid phosphatase at 37 °C after 1560 min. The data were collected from temperature ramp experiments performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.15 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and tanδ (♦) versus temperature for the aliquots taken from a 7.9 wt% solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.67 in the presence of acid phosphatase at 37 °C after 3019 min. The data were collected from temperature ramp experiments performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
**Figure B.16** Plot of dynamic storage modulus G' (■), dynamic loss modulus G'' (□), and tanδ (○) versus temperature for the aliquots taken from a 7.9 wt% solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.67 in the presence of acid phosphatase at 37 °C after 4246 min. The data were collected from temperature ramp experiments performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.17 Plot of dynamic storage modulus $G'$ (■), dynamic loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for the aliquots taken from a 7.9 wt% solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-1) in a 50 mM aqueous citrate buffer with pH of 4.67 in the presence of acid phosphatase at 37 °C after 4696 min. The data were collected from temperature ramp experiments performed by using a fixed frequency of 1 Hz, a strain amplitude of 0.2 %, and a heating rate of 3 °C/min.
Figure B.18 Plot of dynamic storage modulus G’ (■), loss modulus G” (□), and tanδ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 205 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
Figure B.19  Plot of dynamic storage modulus $G'$ (■), loss modulus $G''$ (□), and tanδ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 331 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
Figure B.20 Plot of dynamic storage modulus $G'$ (■), loss modulus $G''$ (□), and $\tan \delta$ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 526 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
Figure B.21 Plot of dynamic storage modulus $G'$ (■), loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 1268 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
Figure B.22 Plot of dynamic storage modulus $G'$ (■), loss modulus $G''$ (□), and $\tan\delta$ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 1623 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
Figure B.23 Plot of dynamic storage modulus $G'$ (■), loss modulus $G''$ (□), and tanδ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-b-PEO-b-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 2125 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
Figure B.24 Plot of dynamic storage modulus $G'$ (■), loss modulus $G''$ (□), and tanδ (●) versus temperature for the aliquots taken from a 7.9 wt % solution of P(DEGEA-co-OPBA)-$b$-PEO-$b$-P(DEGEA-co-OPBA) (ABA-2) in a 50 mM aqueous citrate buffer with pH of 4.41 in the presence of acid phosphatase at 37 °C after 2810 min. The data were collected from a temperature ramp experiment using a frequency of 1 Hz, a strain amplitude of 0.2%, and a heating rate of 3 °C/min.
**Figure B.25** Frequency dependences of dynamic storage modulus $G'$ (■) and loss modulus $G''$ (□) at 38 °C of the final aqueous solution of **ABA-2** with pH of 4.41 after the enzymatic reaction at 37 °C for 2810 min. A strain amplitude of 0.2 % was used in all frequency sweep experiments.
**Figure B.26** Scattering intensity at scattering angle of 90° as a function of temperature in a dynamic light scattering study of a 0.02 wt % solution of P(DEGEA-co-OHBA)-b-PEO-b-P(DEGEA-co-OHBA) in a 50 mM aqueous citrate buffer with pH of 4.41. P(DEGEA-co-OHBA)-b-PEO-b-P(DEGEA-co-OHBA) was obtained from the final solution in the enzymatic gelation experiment of **ABA-2** (i.e., after the enzymatic reaction proceeded at 37 ° for 2810 min).
Chapter 3. Thermo- and pH-Responsive Tertiary Amine-Containing
Hydrophilic ABA Triblock Copolymers
Abstract

This chapter describes the synthesis of two well-defined thermo- and pH-sensitive hydrophilic ABA triblock copolymers with thermosensitive outer blocks containing a small amount of tertiary amine groups of different pK\textsubscript{a} values. The two triblock copolymers are poly(ethoxydi(ethylene glycol) methacrylate-co-methoxydi(ethylene glycol) methacrylate-co-N,N-diisopropylaminoethyl methacrylate)-b-poly(ethylene oxide)-b-poly(ethoxydi(ethylene glycol) methacrylate-co-methoxydi(ethylene glycol) methacrylate-co-N,N-diisopropylaminoethyl methacrylate) and poly(ethoxydi(ethylene glycol) methacrylate-co-methoxydi(ethylene glycol) methacrylate-co-N,N-dibutylaminoethyl methacrylate)-b-poly(ethylene oxide)-b-poly(ethoxydi(ethylene glycol) methacrylate-co-methoxydi(ethylene glycol) methacrylate-co-N,N-dibutylaminoethyl methacrylate). The incorporation of tertiary amine groups into thermosensitive blocks allowed the LCST to be tuned by changing solution pH. The well-defined triblock copolymers were made by atom transfer radical polymerization from a difunctional PEO macroinitiator and their polydispersity indexes were below 1.15. Rheological measurements showed that the sol-gel transition temperature changed with the change of solution pH.
3.1 Introduction

Thermosensitive hydrophilic ABA triblock copolymers, composed of thermosensitive outer blocks and a hydrophilic central block, are known to form gels in water via the formation of a three-dimensional network with the central block bridging micellar cores. In order to develop thermosensitive injectable hydrogels that can respond to acidic diseased tissues for triggered release of drug molecules, our group previously synthesized a thermo- and pH-sensitive, tertiary amine-containing ABA triblock copolymer, poly(ethoxydi(ethylene glycol) methacrylate-co-methoxydi(ethylene glycol)-co-N,N-diethylaminoethyl methacrylate)-b-PEO-b-poly(ethoxydi(ethylene glycol) methacrylate-co-methoxydi(ethylene glycol)-co-N,N-diethylaminoethyl methacrylate) (P(DEGEMA-co-DEGMMA-co-DEAEMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DEAEMA)). The $pK_a$ value of the conjugate acid of poly(DEAEMA) in water was reported to be 7.4. It was anticipated that with the decrease of pH, the tertiary amine groups would be protonated, causing an increase in the LCST of thermosensitive blocks. Consequently, the dissolution of the gel and the release of drug molecules loaded in the gel would be accelerated. Although a 10 wt % aqueous solution of P(DEGEMA-co-DEGMMA-co-DEAEMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DEAEMA) formed a clear gel at room temperature when the pH was 9.65, no sol-gel transition was observed in the temperature range of 0 – 100 °C at pH of 8.60. This is likely because of the relatively high $pK_a$ value of PDEAEMA.

The goal of the work described in this chapter is to synthesize thermo- and pH-sensitive hydrophilic linear ABA triblock copolymers by incorporating different tertiary amine groups into thermosensitive outer blocks. Two monomers, $N,N-$
diisopropylaminoethyl methacrylate (DPMA) and N,N-dibutylaminoethyl methacrylate (DBMA) are used. The pK<sub>a</sub> values of the conjugate acids of poly(DPMA) and poly(DBMA) are 6.3 and 5.1, respectively. The triblock copolymers are synthesized by atom transfer radical polymerization from a PEO macrominitiator with a molecular weight of 20000 g/mol (Scheme 3.1). The obtained polymers are characterized by size exclusion chromatography and 1H NMR spectroscopy.

3.2 Experimental Section

3.2.1 Materials

Methoxydi(ethylene glycol) methacrylate was purchased from TCI and purified by vacuum distillation. Triethylamine (99 %, Acros) and 2-bromoisobutyryl bromide (98 %, Acros) were used as received. Ethoxydi(ethylene glycol) methacrylate (DEGEMA) was synthesized using a procedure established in our group. N,N-Diisopropylethylaminoethyl methacrylate (DPMA, 97%, Aldrich) was purchased from Aldrich and the inhibitor was removed by passing it through an alumina column. N,N-Dibutylaminoethyl methacrylate (DBMA) was synthesized from N,N-dibutylaminoethanol (99%, Aldrich) and methacryloyl chloride (97%, Aldrich) using a similar procedure to that for ethoxydi(ethylene glycol) methacrylate and the molecular structure was confirmed by 1H and 13C NMR spectroscopy analysis. Poly(ethylene glycol) (HO-PEO-OH, MW = 20,000 g/mol) was obtained from Aldrich. The difunctional PEO macroinitiator (Br-PEO-Br) was synthesized according to a procedure described previously. Dichloromethane and anisole (99 %, Acros) were distilled from calcium hydride and stored in solvent storage flasks prior to use. Toluene was distilled from sodium and benzophenone and
Scheme 3.1 Synthesis of thermo-and pH-responsive linear ABA triblock copolymers

\[ \text{P(DEGEMA-co-DEGMMA-co-DPMA)} - b - \text{PEO} - b - \text{P(DEGEMA-co-DEGMMA-co-DPMA)} \quad (R = \text{isopropyl}) \]

\[ \text{P(DEGEMA-co-DEGMMA-co-DBMA)} - b - \text{PEO} - b - \text{P(DEGEMA-co-DEGMMA-co-DBMA)} \quad (R = n\text{-butyl}) \]

\[ \text{P(DEGEMA-co-DEGMMA-co-DPMA)} - b - \text{PEO} - b - \text{P(DEGEMA-co-DEGMMA-co-DPMA)} \]

\[ \text{and} \]

\[ \text{P(DEGEMA-co-DEGMMA-co-DBMA)} - b - \text{PEO} - b - \text{P(DEGEMA-co-DEGMMA-co-DBMA)}. \]
immediately. CuBr (98%, Aldrich) was purified according to the procedure described in the literature\(^5\) and was stored in a desiccator. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA) was purchased from Aldrich and used as received. All other chemicals were purchased from either Aldrich or Fisher and used without purification.

### 3.2.2 General Characterization

Size exclusion chromatography (SEC) was carried out at ambient temperature using PL-GPC 50 Plus (an integrated GPC/SEC system from Polymer Laboratories, Inc) with a differential refractive index detector, one PSS GRAL guard column (50 × 8 mm, Polymer Standards Service-USA, Inc.), and two PSS GRAL linear columns (each 300 × 8 mm, molecular weight range from 500 to 1,000,000 according to Polymer Standards Service-USA, Inc.). The data were processed using Cirrus\(^\text{TM}\) GPC/SEC software (Polymer Laboratories, Inc.). \(N,N\)-Dimethylformamide (DMF) was used as the carrier solvent at a flow rate of 1.0 mL/min. Standard monodisperse polystyrenes (Polymer Laboratories, Inc.) were used for calibration. \(^1\)H NMR (300 MHz) spectra were recorded on a Varian Mercury 300 NMR spectrometer and the residual solvent proton signal was used as the internal standard.

### 3.2.3 Synthesis of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA)

Copper (I) bromide (4.7 mg, 0.033 mmol), Br-PEO-Br (0.509 g, 0.025 mmol), DEGEMA (1.570 g, 7.78 mmol), DEGMMA (1.277 g, 6.79 mmol), anisole (4.418 g), DPMA (0.351 g, 1.65 mmol), and HMTETA (6.6 mg, 0.029 mmol) were added into a two-necked round bottom flask equipped with a stir bar and a gas flow adapter. After the
polymerization mixture was degassed by three freeze-pump-thaw cycles, the flask was placed into a 60 °C oil bath. The polymerization was allowed to proceed for 51 min. The flask was then removed from the oil bath and the mixture was diluted with THF. The copper catalyst was removed by passing the solution through a short neutral aluminum oxide/silica gel column using a mixture of THF and methylene chloride (v/v, 1 : 1) as eluent. The solution was then concentrated and precipitated in a mixture of hexanes and diethyl ether (10 : 1, v/v). The polymer was purified by repetitive precipitation in the same solvent mixture, dried in high vacuum, and then analyzed by SEC and 1H NMR spectroscopy analysis. SEC analysis results: $M_{n,SEC} = 72500$ g/mol; polydispersity index (PDI) = 1.12. The numbers of DEGEMA, DEGMMA, and DPMA units in this ABA triblock copolymer were 94 and 86, and 20 respectively, calculated from its 1H NMR spectrum.

3.2.4 Synthesis of P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA)

Copper (I) bromide (5.1 mg, 0.035 mmol), Br-PEO-Br (0.508 g, 0.025 mmol), DEGEMA (1.569 g, 7.77 mmol), DEGMMA (1.184 g, 6.30 mmol), anisole (4.418 g), DBMA (0.374 g, 1.55 mmol), and HMTETA (6.2 mg, 0.027 mmol) were added into a two-necked round bottom flask equipped with a stir bar and a gas flow adapter. The flask was sealed with a rubber septum. After the reaction mixture was degassed by three freeze-pump-thaw cycles, the flask was placed into a 60 °C oil bath. After 165 min, the flask was removed from the oil bath and the mixture was diluted with THF. The copper catalyst was removed by passing the solution through a short neutral aluminum oxide/silica gel column using a mixture of THF and methylene chloride (v/v, 1 : 1) as
eluent. The solution was then concentrated and precipitated in a mixture of hexanes and diethyl ether (10 : 1, v/v). The polymer was purified by repetitive precipitation in the same solvent mixture, dried in high vacuum, and then analyzed by SEC and $^1$H NMR spectroscopy analysis. GPC analysis results: $M_{n,SEC} = 60200$ g/mol; polydispersity index (PDI) = 1.10. The numbers of DEGEMA, DEGMMA, and DBMA units in this ABA triblock copolymer were 66, 59, and 6 respectively, calculated from its $^1$H NMR spectrum.

3.2.5 Preparation of a 10 wt % aqueous solution of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA)

P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) was added into a 3.7 mL vial and dried at 50 °C under high vacuum for 3 h. The weight of the dried polymer was 0.253 g. Milli-Q water (2.288 g) was added into the vial and the mixture was then sonicated in an ice/water ultrasonic bath (Fisher Scientific Model B200 Ultrasonic Cleaner) to dissolve the polymer in water. The vial was then stored in a refrigerator (~ 4 °C) overnight to ensure complete dissolution. The pH of the polymer solution was adjusted at 0 °C using either 1.0 N KOH or 1.0 N HCl to the desired value before rheological experiments.

3.2.6 Preparation of a 10 wt% aqueous solution of P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA)

P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA) was added into a 3.7 mL vial and dried at 50 °C under high vacuum for 3 h. The weight of the dried polymer was 0.198 g. Milli-Q water (1.773 g) was added into the vial and the mixture was then sonicated in an ice/water ultrasonic bath (Fisher Scientific...
Model B200 Ultrasonic Cleaner) to dissolve the triblock copolymer in water. The vial was then stored in a refrigerator (~ 4 °C) overnight to ensure complete dissolution. For rheological measurements, the pH of the polymer solution was adjusted at 0 °C by using either 1.0 N KOH or 1.0 N HCl.

3.2.7 Rheological Experiments

Rheological experiments were conducted using a rheometer from TA Instruments (Model TA AR2000ex). A cone-plate geometry with a cone diameter of 20 mm and an angle of 2 ° (truncation 52 μm) was employed; the temperature was controlled by the bottom Peltier plate. In each measurement, ~ 90 μL of a polymer solution was loaded onto the plate by a micropipette. The solvent trap was filled with water and a solvent trap cover was used to minimize water evaporation. Dynamic viscoelastic properties (dynamic storage modulus $G'$ and loss modulus $G''$) of a polymer solution were measured by oscillatory shear experiments performed at a fixed frequency of 1 Hz in a heating ramp at a heating rate of 3 °C/min. A strain amplitude of $\gamma = 0.2 \%$ was used to ensure that the deformation was within the linear viscoelastic regime.

3.3 Results and Discussion

3.3.1 Synthesis of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA)

Thermo- and pH-sensitive hydrophilic ABA triblock copolymer P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) was synthesized by ATRP of a mixture of DEGEMA, DEGMMA, and DPMA with a molar ratio of 53 : 47 : 11 from a difunctional PEO macroinitiator with a molecular weight of
20,000 g/mol at 60 °C in anisole using CuBr/HMTETA as catalyst (Scheme 3.1). The polymer was purified by repetitive precipitation, dried at 40 °C under high vacuum, and then analyzed by SEC and 1H NMR spectroscopy analysis. Figure 3.1A shows the SEC traces of both PEO macroinitiator and the triblock copolymer. The number average molecular weight and the polydispersity index of the obtained triblock copolymer were 72.5 kDa and 1.12, respectively. The numbers of DEGEMA, DEGMMA, and DPMA units were 94, 86, and 20, respectively, using the peaks located at 1.2 ppm (-OCH₂CH₃ of DEGEMA), 3.0 ppm (-OCH₂CH₂N- of DBMA units), 3.4 ppm (-OCH₃ of DEGMMA), and 3.50 – 3.75 ppm (-CH₂CH₂O- of PEO block, -COOCH₂CH₂OCH₂CH₂OCH₃ of DEGMMA units, and –COOCH₂CH₂OCH₂CH₂OCH₂CH₃ of DEGEMA units) (See Figure 3.1B).

### 3.3.2 Synthesis of P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA)

P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA) was synthesized by ATRP of a mixture of DEGEMA, DEGMMA, and DBMA with a molar ratio of 55 : 45 : 11 from a difunctional PEO macroinitiator under a condition similar to that for the synthesis of another triblock copolymer (Scheme 3.1). Figure 3.2A shows the SEC traces of PEO macroinitiator and the copolymer. The Mₙ,SEC and PDI were 60.2 kDa and 1.10, respectively. The number of DEGEMA, DEGMMA, and DBMA units were 66, 59, and 6, respectively, which were calculated from the 1H NMR spectrum using the peaks located at 1.2 ppm (-OCH₂CH₃ of DEGEMA), 2.6 ppm (-OCH₂CH₂N- of DBMA units), 3.4 ppm (-OCH₃ of DEGMMA), and 3.50 – 3.75 ppm (-
Figure 3.1 (A) Size exclusion chromatography traces of Br-PEO-Br macroinitiator and P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) and (B) $^1\text{H}$ NMR of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA).
Figure 3.2 (A) Size exclusion chromatography traces of Br-PEO-Br macroinitiator and P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA) and (B) $^1$H NMR of P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA).
CH$_2$CH$_2$O- of PEO block, -COOCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGMMMA units, and –
COOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_3$ of DEGEMA units) (See Figure 3.2B).

### 3.3.3 Thermo-Induced Sol-Gel Transitions of 10 wt % Aqueous Solutions of

P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) and P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA)

Aqueous solutions of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) and P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA) with a concentration 10 wt % were made by using Milli-Q water. The pH values of the solutions were adjusted by using either 1.0 N KOH or 1.0 N HCl at 0 °C. Their sol-gel transitions were studied by rheological measurements. Figures 3.3 and 3.4 show the plots of dynamic storage moduli G’ and dynamic loss moduli G'' of these samples as a function of temperature obtained from oscillatory shear experiments performed at a strain amplitude of $\gamma = 0.2 \%$ and a fixed frequency of 1 Hz in a heating ramp at a heating rate of 3 °C/min. The temperatures at which G’ = G'' were taken as the values of $T_{\text{sol-gel}}$. For the 10 wt % aqueous solution of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA), the $T_{\text{sol-gel}}$ was found to be 11.6 °C at pH = 9.07 and 19.7 °C at pH = 8.50. For the 10 wt % aqueous solution of P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA), the $T_{\text{sol-gel}}$ was found to be 30.5 °C at pH = 7.30 and 40.4 °C at pH = 6.60. With the decrease of pH, the tertiary amine groups are protonated, making the thermosensitive blocks more hydrophilic. As a result, the LCST of thermosensitive blocks increased, causing an increase in the sol-gel transition
Figure 3.3 Plots of dynamic storage modulus $G'$ (■) and dynamic loss modulus $G''$ (□) as a function of temperature for a 10 wt % aqueous solution of P(DEGEMA-co-DEGMMA-co-DPMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DPMA) with pH of (A) 9.07 and (B) 8.50.
Figure 3.4 Plots of dynamic storage modulus $G'$ (■) and dynamic loss modulus $G''$ (□) as a function of temperature for (a) 10 wt % aqueous solution of P(DEGEMA-co-DEGMMA-co-DBMA)-b-PEO-b-P(DEGEMA-co-DEGMMA-co-DBMA) with pH of (A) 7.30 and (B) 6.60.
temperature. Thus, these experiments indicated that the sol-gel transition temperatures of aqueous solutions of these two triblock copolymers can be tuned in a convenient pH range.

3.4 Conclusions

Two well-defined thermo- and pH-sensitive hydrophilic ABA triblock copolymers were successfully synthesized by ATRP from a difunctional PEO macroinitiator with a molecular weight of 20.0 kDa by the incorporation of a small amount of different tertiary amine groups into thermosensitive outer blocks. Rheological measurements showed that the sol-gel transition temperatures of 10 wt % aqueous solutions of two triblock copolymers changed with the change of solution pH. These polymers will be used for applications in triggered release of substances from aqueous micellar gels.
References


Chapter 4. Reversible Addition Fragmentation Chain Transfer (RAFT)

Polymerization for the Synthesis of Thermosensitive Water-Soluble Polymers of Alkoxyoligo(ethylene glycol) (Meth)acrylates
Abstract

In an effort to explore the use of reversible addition-fragmentation chain transfer (RAFT) polymerization for the synthesis of thermosensitive hydrophilic block copolymers in our group, I synthesized a total of 8 different chain transfer agents; they are benzyl dithiobenzoate (CTA-1), 1-phenylethyl dithiobenzoate (CTA-2), 1,4-bis(thiobenzoylthiomethyl)benzene (CTA-3), 4-cyanopentanoic acid dithiobenzoate (CTA-4), methoxypoly(ethylene glycol) 4-cyano-4-(phenylcarbonothioyl)pentanoate (CTA-5), 4,4’-ethane-1,2-diyl bis(4-cyano-4-((phenylcarbonothioyl)thio)pentanoate) (CTA-6), ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (CTA-7), and 2,2’-(thiocarbonylbis(sulfanediyl))bis(2-methyl-propanoic acid) (CTA-8). Their molecular structures were confirmed by $^1$H and $^{13}$C NMR spectroscopy analysis. RAFT polymerizations of alkoxyoligo(ethylene glycol) (meth)acrylates were tested using CTA-1, -2, -3, -5, -6, and -7. The polymerizations were well controlled, producing polymers with relatively narrow molecular weight distributions. The work presented in this Chapter has set a foundation for our group to further explore the use of RAFT polymerization for the synthesis of well-defined multi-responsive hydrophilic block copolymers.
4.1 Introduction

Thermosensitive water-soluble polymers have been intensely studied in the past decades.\textsuperscript{1-3} These polymers exhibit a phase transition in water from a water-soluble state to a water-insoluble state when the temperature is higher than a critical point (lower critical solution temperature, LCST) or lower than a critical point (upper critical solution temperature, UCST).\textsuperscript{1-3} Of particular interest to us are those thermosensitive polymers that exhibit LCST-type phase transitions in water. They have found a wide range of applications, including delivery of substances,\textsuperscript{4} fabrication of stimuli-responsive (smart) surfaces,\textsuperscript{5,6} catalysis,\textsuperscript{7} and responsive Pickering emulsions.\textsuperscript{8} A representative example of such polymers is poly(N-isopropylacrylamide) which displays an LCST in H\textsubscript{2}O at 32 °C.\textsuperscript{2}

More recently, a new family of LCST-type thermosensitive water-soluble polymers, including poly(vinyl ether)s,\textsuperscript{9-11} polymethacrylates,\textsuperscript{12-14} polystyrenics,\textsuperscript{15,16} and polyacrylates,\textsuperscript{17,18} has been developed. A common feature of these new thermosensitive polymers is that every repeat unit contains a short oligo(ethylene glycol) pendant. The cloud point can be easily tuned by varying the polymer backbone, the length and end group of the oligo(ethylene glycol) side chain.\textsuperscript{9-19} While well-defined thermosensitive polymers of vinyl ethers and alkoxyoligo(ethylene glycol) methacrylates have been successfully prepared by living cationic and anionic polymerizations, respectively,\textsuperscript{9-12} the process of purifying monomers and solvents is tedious and the reactions are conducted in the total absence of oxygen (air) and water. In contrast, controlled/“living” radical polymerization is more tolerant to impurities and requires less stringent polymerization conditions.\textsuperscript{20-25} Among three most widely used “living”/controlled radical polymerization techniques, atom transfer radical polymerization (ATRP),\textsuperscript{20} nitrooxide-mediated radical
polymerization (NMRP),\textsuperscript{21-23} and reversible addition fragmentation chain transfer polymerization (RAFT),\textsuperscript{24,25} ATRP requires the use of a metal catalyst, most commonly a copper ligand complex, which could present problems in some applications.\textsuperscript{20} Although NMRP has been applied to the polymerization of styrenics, acrylates, and acrylamides through the use of the universal initiator,\textsuperscript{21-23} this technique is intrinsically more suited for the polymerization of styrenic monomers.\textsuperscript{21} In addition, the rather high temperature for the polymerization might be a concern in some situations.

RAFT has been proven to be a very versatile “living”/controlled radical polymerization technique;\textsuperscript{24-26} it does not require the addition of a catalyst and can be applied to a variety of monomer types, e.g., acrylates, methacrylates, styrenics, acrylamides, and acrylonitrile. This is accomplished by its unique mechanism (Scheme 4.1), which contains a sequence of addition-fragmentation equilibria.\textsuperscript{25} Initiation and radical-radical termination occur as in conventional free radical polymerization. The addition of a propagating radical ($P_n^\cdot$) to the initial RAFT chain transfer agent (CTA) and the fragmentation of the formed intermediate provide a polymeric RAFT agent and a new radical ($R^\cdot$). The fragmented radical can then reinitiate a new propagating chain. The rapid equilibrium between the dormant species and the active propagating radicals allows the polymer chains to have equal probability to grow, resulting in narrow molecular weight distributions. Proper selection of CTA-monomer pairs is necessary to achieve a well-controlled polymerization. This is governed by the R and Z substituents of CTAs (See CTA in Scheme 4.1). More specifically, the stability of the intermediate is dictated by the Z substituent and the fragmentation is governed by the R group, which also must be stable towards the initiation of monomer during the early stages of the polymerization.
Scheme 4.1 Mechanism for reversible addition fragmentation chain transfer (RAFT) polymerization. Note that initiation takes place as in conventional radical polymerization as well as the possibility of radical-radical termination.
The efficiency of the fragmented moiety to initiate a new propagating chain can be connected with a delay or slowing of the polymerization in the early time period, also known as an induction period, with a lower efficiency displaying a longer induction period or even resulting in a dead polymerization. The penultimate group of the fragmenting moiety has been shown to affect reinitiation.\textsuperscript{27} More substituted moieties such as tertiary cyanoalkyls tend to favor methacrylates while aromatic groups favor the more activated acrylates. A summary of guidelines for selection of RAFT agents is presented in Scheme 4.2.

RAFT has been shown to be particularly suitable for controlled polymerization of polar monomers with functional groups.\textsuperscript{26,28} This Chapter details my effort in bringing the RAFT polymerization technique to our research laboratory and my contribution to the preparation of well-defined thermosensitive water-soluble polymers, in particular, polyacrylates and polymethacrylates, by RAFT. A number of RAFT chain transfer agents have been synthesized with the purpose of developing a foundation for the preparation of well-defined thermosensitive hydrophilic block copolymers of alkoxyoligo(ethylene glycol) acrylates and methacrylates for future work with RAFT in our research laboratory. This has already shown promise in a recent paper published in \textit{Macromolecules} from our laboratory.\textsuperscript{29}

A summary of CTAs can be found in Scheme 4.3. Benzyl dithiobenzoate (\textbf{CTA-1}) and 1-phenylethyl dithiobenzoate (\textbf{CTA-2}) are suitable for polymerization of acrylates and styrenics. The aromatic penultimate group has been shown to be effective at reinitiating acrylates while the resonance with the benzene ring stabilizes the intermediates. Their application as CTAs in the RAFT polymerization of
**Scheme 4.2** Guidelines for the selection of a RAFT agent by Z and R groups, where the dotted arrows indicate some capacity for polymerization and the solid lines indicate good ability to control the polymerization. MMA: methyl methacrylates; MAM: methyl acrylamides; AM: acrylamides; AN: acrylonitrile; VAc: vinyl acetates; NVP: \(N\)-vinylpyrrolidone; NVC: \(N\)-vinylcarbazole.\(^{25}\)
Scheme 4.3 Chemical structures of RAFT chain transfer agents presented in this Chapter: benzyl dithiobenzoate (CTA-1), 1-phenylethyl dithiobenzoate (CTA-2), 1,4-bis(thiobenzoylthiomethyl)benzene (CTA-3), 4-cyanopentanoic acid dithiobenzoate (CTA-4), methoxypoly(ethylene glycol) 4-cyano-4-(phenylcarbonothioyl)pentanoate (CTA-5), 4,4’-ethane-1,2-diyl bis(4-cyano-4-((phenylcarbonothioyl)thio)pentanoate) (CTA-6), ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (CTA-7), and 2,2’-(thiocarbonylbis(sulfanediyl))bis(2-methyl-propanoic acid) (CTA-8).
methoxydi(ethylene glycol) acrylate (DEGMA), will be presented. Note that the cloud point of poly(DEGMA) (PDEGMA) in water is 38 °C, respectively.\textsuperscript{17,19} 1,4-Bis(thiobenzoylthiomethyl)benzene (CTA-3) was used in the literature for the synthesis of polyacrylamides.\textsuperscript{30} This difunctional CTA has the same R and Z groups as the monofunctional benzoate CTA-1, and is also tested here for the polymerization of DEGMA. 4-Cyanopentanoic acid dithiobenzoate (CTA-4) contains a tertiary cyanoalkyl moiety that is proficient with the reinitiation of methacrylates while the intermediate species is also stabilized by a benzene ring.\textsuperscript{28,31} In addition, the carboxylic acid group allows for further functionalization. For example, CTA-5, a macro-CTA, was prepared by the reaction of CTA-4 with a poly(ethylene oxide) (PEO) monomethyl ether. This PEO macro-CTA can be used to prepare well-defined block copolymers, e.g., PEO-b-poly(methoxydi(ethylene glycol) methacrylate) (PEO-b-PDEGMMA) as shown in this Chapter. CTA-6, 4,4’-ethane-1,2-diyl bis(4-cyano-4-((phenylcarbonothioyl)thio)pentanoate), is a new difunctional RAFT that has not been reported in the literature; it was synthesized by coupling ethylene glycol with CTA-4. Trithiocarbonates have also been shown to be effective in the RAFT polymerization of both methacrylates and acrylates.\textsuperscript{25} Ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (CTA-7) has a tertiary alkyl fragmentation moiety, which is best suited for methacrylates but also shows a capability in controlling the polymerization of acrylates. The use of CTA-7 in the synthesis of PDEGMA is presented. 2,2’-(Thiocarbonylbis(sulfanediyl))bis(2-methyl-propanoic acid) (CTA-8) is a difunctional trithiocarbonate with the same fragmentation moiety as CTA-7; however, this RAFT
agent is unique because of the absence of a defined Z substituent. This RAFT agent can be used for polymerization of both acrylates and methacrylates.25

4.2 Experimental Section

4.2.1 Materials

Methoxydi(ethylene glycol) acrylate (DEGMA, Aldrich, 98%), methoxydi(ethylene glycol) methacrylate (or di(ethylene glycol) methyl ether methacrylate, DEGMMA, Aldrich, 95%), and ethoxydi(ethylene glycol) acrylate (or di(ethylene glycol) monoethyl ether acrylate, DEGEA, 90+%, Aldrich) were dried over calcium hydride and distilled under reduced pressure. Methylene chloride was dried over CaH2 and distilled. Tetrahydrofuran (THF) was dried by refluxing over sodium followed by distillation. Carbon disulfide (99%) and benzyl bromide (99%) were purchased from Alfa Aesar and used as received. Poly(ethylene glycol) monomethyl ether (5000 g/mol) was obtained from Aldrich. Phenylmagnesium bromide (2.8 M in diethyl ether), sodium methoxide (30%, Acros), sulfur (sublimed, 99.5%), potassium ferricyanide (98%, Acros), N,N′-dicyclohexylcarboimide (99%, Acros), 4-dimethylaminopyridine (99%, Acros), 1-dodecane thiol (98.5%, Acros), aliquat 336 (Acros), oxalyl chloride (99%, Fisher Sci.), and ethanol (200 proof, Decon Labs) were used as received. 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%, Aldrich) was purified by recrystallization from ethanol. 4,4′-Azobis(4-cyanopentanoic acid) (98%, Aldrich) was purchased from Aldrich and used without purification.

4.2.2 General Characterization
Size exclusion chromatography (SEC) was carried out at room temperature using PL-GPC 20 (an integrated GPC system from Polymer Laboratories, Inc.) with a refractive index detector, one PLgel 5 µm guard column (50 × 7.5 mm), and two PLgel 5 µm mixed-C columns (each 300 × 7.5 mm, linear range of molecular weight from 200 to 2,000,000 according to Polymer Laboratories, Inc.). The data were processed using Cirrus™ GPC/SEC software (Polymer Laboratories, Inc.). THF was used as the carrier solvent at a flow rate of 1.0 mL/min. Standard polystyrenes with narrow polydispersity indexes (Polymer Laboratories, Inc.) were used for calibration. The $^1$H (300 MHz) and $^{13}$C (75 MHz) NMR spectra were recorded on a Varian Mercury 300 NMR spectrometer.

4.2.3 Synthesis of benzyl dithiobenzoate (CTA-1)

CTA-1 was synthesized by a procedure modified from the literature.²⁸ Phenylmagnesium bromide (2.8 M in diethyl ether, 7.0 mL, 20 mmol) and dry tetrahydrofuran (40 mL) were injected into a 250 mL three-necked round bottom flask and the solution was heated to 40 °C. Carbon disulfide (5.512 g, 72.4 mmol) was added dropwise into the flask over a period of 15 min. The reaction mixture turned a bright red color. Benzyl bromide (2.229 g, 22.5 mmol) was then added in a dropwise fashion, after which the temperature was raised to 50 °C and the mixture was stirred overnight. Cold water (~ 0 °C, 100 mL) was then added into the flask; the slurry was partitioned with diethyl ether. The organic layer was separated, washed with water (50 mL) three times, and then dried over anhydrous sodium sulfate. The product was purified using column chromatography with methylene chloride/hexanes as eluent (1/5, v/v). The product benzyl dithiobenzoate was obtained as a red oil (3.412 g, 71% yield). $^1$H NMR (CDCl$_3$):
δ (ppm), 4.60 (s, 2H, CH$_2$-Ph), 7.30-7.60 (m, 8H aromatic), 8.02 (m, 2H, aromatic). $^{13}$C NMR (CDCl$_3$): δ (ppm), 144.7, 140.0, 132.4, 126.9-129.3, and 42.3.

4.2.4 Synthesis of 1-phenylethyl dithiobenzoate (CTA-2)

CTA-2 was synthesized by a modified protocol from that reported in the literature. Phenylmagnesium bromide (2.8 M in diethyl ether, 7.9 mL, 22 mmol) was dissolved in THF (60 mL) in a 250 mL three-necked round bottom flask and the solution was heated to 40 °C. Carbon disulfide (5.130 g, 67.4 mmol) was added dropwise and the mixture was allowed to react for 1 h. (1-Bromoethyl)benzene (4.016 g, 22.0 mmol) was added dropwise and the reaction temperature was raised to 50 °C. After the mixture was stirred overnight, cold water (~ 0 °C, 100 mL) was added and the slurry was partitioned with diethyl ether (150 mL). The organic layer was separated, washed three times with water (75 mL), and then dried over anhydrous sodium sulfate. The product was purified by column chromatography using methylene chloride and hexanes (1/6, v/v) as eluent. After drying under high vacuum, the pure product 1-phenylethyl benzodithioate was obtained as a red oil in a yield of 45% (2.571 g). $^1$H NMR (CDCl$_3$): δ (ppm), 7.34-7.62 (m, 8H, aromatic), 8.08 (d, 2H aromatic), 1.92 (d, 3H, CH$_3$), 5.39 (q, 1H, CH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$): δ (ppm), 144.9, 141.3, 132.3, 126.9, and 50.3, 20.8.

4.2.5 Synthesis of 1,4-bis(thiobenzoylthiomethyl)benzene (CTA-3)

CTA-3 was synthesized as reported in the literature. Phenylmagnesium bromide (2.8 M in ethyl ether, 6.8 mL, 19 mmol) was dissolved in THF (60 mL) in a 250 mL three-necked round bottom flask. The flask was then placed in a 40 °C oil bath. Carbon disulfide (3.617 g, 47.5 mmol) was added dropwise into the flask. The reaction mixture took on a deep red color after the addition of carbon disulfide. After 1 h, a solution of
1,4-bis(bromomethyl)benzene (2.021 g, 7.65 mmol) in THF (10 mL) was added dropwise into the flask and the temperature was raised to 60 °C. After the reaction mixture was stirred overnight, cold water (~ 0 °C, 100 mL) was added to the flask and the slurry was partitioned with diethyl ether (100 mL). The organic layer was separated, washed three times with water (50 mL), and dried over anhydrous sodium sulfate. The crude product was purified using column chromatography with hexanes and methylene chloride as eluent (3/2, v/v). \( ^1 \text{H NMR (CDCl}_3 \) \( \delta \) (ppm), 8.0 (d, 2H aromatic), 7.57 (m, 8H aromatic), 7.4 (m, 2H aromatic), 4.6 (s, 4H \( \text{CH}_2\text{-S}) \). \( ^{13} \text{C NMR (CDCl}_3 \) : \( \delta \) (ppm), 228.1, 145.1, 135.0, 132.8, 129.9, 128.7, 127.2, 41.7.

4.2.6 Synthesis of 4-cyanopentanoic acid dithiobenzoate (CTA-4)

CTA-4 was synthesized according to the literature.\(^{33}\) A 30% sodium methoxide solution in methanol (10.249 g sodium methoxide, 190.0 mmol), elemental sulfur (5.137 g, 160 mmol), and dry methanol (40 mL) were weighed into a 250 mL round bottom flask. After the mixture was stirred for 1 h, benzyl chloride (9.874 g, 78.0 mmol) was added in a dropwise fashion. The flask was then placed into a 75 °C oil bath and the mixture was refluxed for 18 h. The reaction was then quenched in an ice/water bath and the mixture was concentrated via rotary evaporation. The crude product was dissolved in deionized water (150 mL). The solution was made acidic with 1.0 M HCl solution (30 mL) and the product was extracted with anhydrous diethyl ether. After the organic layer was separated, a 1.0 M NaOH aqueous solution (40 mL) was added and the product was extracted back into distilled water. This washing procedure was repeated an additional two times and sodium benzyl dithioate was ended in the aqueous phase.
Potassium ferricyanide (30.823 g, 93.6 mmol) dissolved in distilled water (150 mL) was added dropwise into the aqueous solution of sodium benzyl dithioate obtained above. A red precipitant was formed, collected using vacuum filtration, and washed thoroughly with deionized water. The product was then dissolved in methylene chloride and dried over anhydrous sodium sulfate overnight. After the removal of the solvent, di(thiobenzoyl)disulfide was obtained as a sticky purple solid which was purified by recrystallization from benzene.

Di(thiobenzoyl)disulfide (3.505 g, 12.5 mmol) and 4,4’-azobis(4-cyanopentanoic acid) (3.945 g, 12.9 mmol) were dissolved in dry ethyl acetate (50 mL) in a 250 mL three-necked flask. The solution was then degassed by two freeze-pump-thaw cycles. The flask was then placed into a 70 °C oil bath and the mixture was refluxed for 20 h. The reaction solution was concentrated using rotary evaporation. The product was purified by column chromatography using ethyl acetate and hexanes (2/1, v/v) with 0.1% by volume acetic acid as eluent. The acetic acid was removed by azeotropic distillation with toluene. The product was further purified by recrystallization twice using toluene and hexanes (1/1, v/v). 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTA 4) was isolated as a red crystalline solid (2.466 g, 70.6% overall yield). $^1$H NMR (CDCl$_3$): δ (ppm), 1.93(s, 3H CH$_3$), 2.34-2.47 (m, 2H, CH$_2$-C=O), 2.49-2.76 (mm, 2H, CH$_2$-CH$_2$C=O), 7.38 (m, 2H, aromatic), 7.56 (m, 1H, aromatic), 7.89 (m, 2H, aromatic). $^{13}$C NMR (CDCl$_3$): δ (ppm), 222.8, 177.29, 144.71, 133.3, 128.8, 126.9, 118.6, 45.8, 33.3, 29.7, and 24.1.

4.2.7 Synthesis of methoxypoly(ethylene glycol) 4-cyano-4-(phenylcarbonothioylthio)pentanoate (CTA-5)
Poly(ethylene glycol) monomethyl ether (MW 5000 g/mol, 0.485 g, 0.097 mmol) was dried first by azeotropic distillation with toluene (75 mL) and then under high vacuum at 60 °C for 2 h. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (66.3 mg, 0.237 mmol), poly(ethylene glycol) monomethyl ether (0.485 g, 0.097 mmol), and dimethylaminopyridine (7.4 mg, 0.061 mmol) were dissolved in dry methylene chloride (25 mL) in a 100 mL three-necked round bottom flask. The flask was placed into an ice/water bath and the solution was stirred for 1.5 h, followed by the addition of dicyclohexyl carbodiimide (74.0 mg, 0.359 mmol). The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature and proceed for 17 h.

The reaction was filtered and the filtrate was concentrated via rotary evaporation. The product was then precipitated in diethyl ether (100 mL) three times. After drying, the product was recrystallized from ethanol (50 mL) two times. The PEO macro-CTA was isolated as a light red powder (287 mg, 0.054 mmol) with a 55.7% yield. 1H NMR (CDCl₃): δ (ppm), 1.92 (s, 3H CH₃-C), 2.41-2.47 (m, 2H, CH₂-C=O), 2.56-2.76 (mm, 2H, CH₂-CH₂C=O), 3.62 (m, 456H, O-CH₂CH₂O-), 4.25 (t, 2H, CH₂-O-C=O), 7.37 (m, 2H, aromatic), 7.55 (m, 1H, aromatic), 7.89 (m, 2H, aromatic).

4.2.8 Synthesis of 4,4’-ethane-1,2-diyl bis(4-cyano-4-((phenylcarbonothioyl)thio) pentanoate) (CTA-6)

4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (0.597 g, 2.14 mmol), dimethylaminopyridine (29.1 mg, 0.238 mmol), and ethylene glycol (56.0 mg, 0.902 mmol) were dissolved in dry methylene chloride (20 mL) in a 100 mL three-necked round bottom flask. The flask was placed into an ice/water bath. After the mixture was stirred for 30 min, dicyclohexyl carbodiimide (0.544 g, 2.64 mmol) was added. The
solution was then stirred at 0 °C for 2 h and allowed to warm to room temperature and proceed for 20 h. The precipitant was removed by filtration and the filtrate was concentrated using a rotary evaporator. The product was purified by column chromatography using ethyl acetate and hexanes (1/4, v/v) and obtained as a red oil after drying under high vacuum (0.213 g, 0.364 mmol, 34.3% yield). $^1$H NMR (CDCl$_3$): $\delta$ (ppm), 1.92 (S, 6H, CH$_3$-CCN), 2.37-2.47 (m, 4H, CH$_2$-C=O), 2.57-2.77 (m, 4H, CH$_2$-C-CH$_3$CN), 4.32 (t, 4H, O-CH$_2$CH$_2$-O), 7.37 (t, 4H, aromatic), 7.55 (m, 2H, aromatic), 7.89 (m, 4H, aromatic). $^{13}$C NMR (CDCl$_3$): $\delta$ 171.3, 144.5, 133.1, 128.6, 126.7, 118.4, 62.6, 45.7, 33.3, 29.7, and 24.2.

4.2.9 Synthesis of ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (CTA-7)

CTA-7 was synthesized via previously reported in the literature.$^{34}$ 1-Dodecane thiol (4.040 g, 20.0 mmol), acetone (9.64 g, 0.166 mol), and Aliquat 336 (0.396 g, 0.98 mmol) were weighed into a three-necked flask. The flask was placed in a 10 °C water bath and a 50 wt% sodium hydroxide solution (1.64 g, 20.5 mmol) was added dropwise. After stirring for 30 min, the mixture became a white slurry. Carbon disulfide (1.54 g, 20.2 mmol) was then added, followed by a second portion of acetone (2.30 g, 39.6 mmol). After 30 min, a second portion of a 50 wt% sodium hydroxide aqueous solution (7.9 g, 98.8 mmol) was added dropwise into the flask. The reaction was allowed to proceed at 10 °C for 15 h. The mixture was filtered by vacuum filtration, and the obtained yellow solid was stirred in 2-propanol for 1 h. The solution was then filtered and the solvent was removed. The resultant solid was recrystallized from hexanes, yielding 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoic acid as a bright yellow solid.
2-(((Dodecylthio)carbonothioyl)thio)-2-methylpropanoic acid (0.469 g, 1.28 mmol) was dissolved in THF (20 mL) and the solution was cooled to 0 °C under a nitrogen atmosphere. Oxalyl chloride (0.276 g, 2.17 mmol) was added via a syringe and the solution was stirred for 2 h. A solution of ethanol (0.060 g, 1.30 mmol) in THF (5 mL) was added dropwise into the flask. The reaction mixture was allowed to warm to room temperature and stirred overnight. The volatiles were removed and the product was purified by column chromatography using hexanes and ethyl acetate (2/1, v/v) as eluent. The pure product ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate was obtained as a yellow oil. $^1$H NMR (DMSO): $\delta$ (ppm), 0.86 (t, 3H, $CH_3CH_2$), 1.29 (m, 18H, -$CH_2CH_2$-), 1.29 (m, 3H, $CH_3CH_2OC=O$), 1.60 (s, 6H, (CH3)$_2$-C-), 1.60, (m,$CH_2CH_2S$), 3.25 (t, 2H, $CH_2S-C=S$), 4.14 (q, 2H, $CH_2OC=O$). $^{13}$C NMR (DMSO), $\delta$ (ppm), 172.9, 62.0, 56.0, 37.0, 29.5, 29.0, 28.0, 25.5, 22.8, 14.2.

4.2.10 Synthesis of 2,2'-(thiocarbonylbis(sulfanediyl))bis(2-methyl-propanoic acid) (CTA-8)

CTA-8 was synthesized via a procedure reported in the literature. Carbon disulfide (5.378 g, 70.64 mmol), chloroform (21.558 g, 0.180 mol), acetone (9.536 g, 0.180 mol), and Aliquatte 336 (1.101 g, 2.72 mmol) were dissolved in hexanes (50 mL) in a 250 mL round bottom flask under a nitrogen atmosphere. The flask was placed in an ice/water bath. After the solution was cooled in an ice water bath for 20 min, a 50% sodium hydroxide aqueous solution (18.540 g, 0.231 mol) was added dropwise into the flask. The mixture turned a deep yellow color after ~ 15 min and then a brownish red color after 1 h. The reaction was allowed to proceed at 0 °C overnight.
Deionized water (100 mL) was added into the flask, followed by dropwise addition of a concentrated HCl solution (30 mL) until the solution was acidic. The organic layer was separated and bubbled with nitrogen to remove the volatiles. The resultant slurry was filtered by vacuum filtration, and the collected solid was stirred in toluene/hexanes (v/v: 50/50). 2,2’-(Thiocarbonylbis(sulfanediyl))bis(2-methylpropanoic acid) was obtained as a yellow powder. 1H NMR (CDCl₃): δ (ppm), 1.67 (12H CH₃). 13C NMR (CDCl₃): δ (ppm), 180.0, 55.8, 25.14.

4.2.11 Synthesis of poly(methoxydi(ethylene glycol) acrylate) using CTA-1

Benzyl dithiobenzoate (16.4 mg, 0.067 mmol), 2,2’-azobis(2-methylpropionitrile) (AIBN, 0.5 mg, 0.0031 mmol), methoxy(diethylene glycol) acrylate (DEGMA, 1.756 g, 10.09 mmol), and anisole (1.189 g) were added into a 25 mL two-necked flask fitted with a gas flow adaptor. The flask was sealed with a rubber septum, and the reaction mixture was degassed by three freeze-pump-thaw cycles. The flask was then placed into a 70 °C oil bath. After 313 min, a sample was taken for 1H NMR spectroscopy analysis and the polymerization was stopped by removing the flask from the oil bath, opening it to air, and diluting with THF. The degree of polymerization of the polymer was 76, calculated from the monomer conversion which was determined by 1H NMR spectroscopy analysis. Size exclusion chromatography using polystyrene calibration showed that the value of Mn,SEC was 10.7 kDa and the PDI was 1.14.

4.2.12 Synthesis of Poly(methoxydi(ethylene glycol) acrylate) using CTA-2

1-Phenylethyl benzodithioate (21.7 mg, 0.084 mmol), AIBN (2.5 mg, 0.015 mmol), DEGMA (2.318 g, 13.3 mmol), and anisole (2.067 g) were added into a 25 mL two-necked round bottom flask fitted with a gas flow adaptor. The flask was sealed with a
rubber septum and the mixture was degassed by three freeze-pump-thaw cycles. The flask was placed into a 70 °C oil bath. After 218 min, the flask was removed from the oil bath, opened to air, and diluted with THF. The degree of polymerization of the polymer was 73, calculated from the monomer conversion which was determined by $^1$H NMR spectroscopy analysis. The polymer was precipitated in hexanes three times and obtained as a clear sticky solid after drying under high vacuum. Size exclusion chromatography showed that the value of $M_{n,SEC}$ was 10.6 kDa and the PDI was 1.11 (relative to polystyrene calibration).

4.2.13 Synthesis of poly(methoxydi(ethylene glycol) acrylate) using CTA-3

CTA-3 (26.5 mg, 0.064 mmol), DEGMA (2.020, 11.6 mmol), AIBN (1.8 mg, 0.011 mmol), and anisole (1.946 g) were weighed into a two-necked round bottom flask. After the mixture was degassed by three freeze-pump-thaw cycles, the flask was placed into a 70 °C oil bath. The polymerization was stopped after 332 min by removing the flask from the oil bath, opening it to air, and diluted with THF. The mixture was precipitated in hexanes. The obtained polymer PDEGMA was further purified by precipitation of its THF solution in hexanes two times and obtained as a clear sticky solid after drying in vacuum. The DP of the polymer was 62, calculated from the monomer-to-CTA ratio and the monomer conversion which was determined by $^1$H NMR spectroscopy analysis. Size exclusion chromatography showed that the value of $M_{n,SEC}$ was 7000 g/mol and the PDI was 1.21 (relative to polystyrene calibration).

4.2.14 Synthesis of poly(ethylene oxide)-$b$-poly(methoxydi(ethylene glycol) methacrylate) using PEO macro-CTA (CTA-5)
PEO macro-CTA (CTA-5, 0.194 g, 0.037 mmol), AIBN (0.6 mg, 0.0037 mmol), and DEGMA (1.014 g, 5.39 mmol) were dissolved in anisole (1.022 g) in a 25 mL two-necked flask equipped with a gas flow adapter. The solution was degassed by three freeze-pump-thaw cycles and the flask was placed into a 70 °C oil bath. After 210 min, the polymerization was halted by removing the flask from the oil bath, opening it to air, and diluting with THF. The polymer was precipitated in hexanes and diethyl ether (v/v, 80/20) and purified by repetitive precipitation. Size exclusion chromatography showed that the value of $M_{n,SEC}$ was 11400 g/mol and the PDI was 1.10 (relative to polystyrene calibration).

4.2.15 Synthesis of poly(methoxydi(ethylene glycol) methacrylate) with CTA-6

CTA-6 (8.4 mg, 0.014 mmol), DEGMA (2.012 g, 10.7 mmol), and AIBN (0.4 mg, 0.0024 mmol) were dissolved in anisole (3.06 mg) in a 25 mL two-necked round bottom flask. The flask was fitted with a gas flow adaptor and sealed with a rubber septum. Three freeze-pump-thaw cycles were performed to degas the solution. The flask was then placed into a 70 °C oil bath. After 207 min, the polymerization was halted by removing the flask from the oil bath, opening it to air, and diluting with THF. The solution was precipitated in hexanes and the obtained polymer was further purified by precipitation in hexanes (100 mL) two times. The DP of the polymer was 252, calculated from the monomer-to-CTA ratio and the monomer conversion which was determined by $^1$H NMR spectroscopy. SEC results: $M_{n,SEC} = 30,600$ g/mol; PDI = 1.16.

4.2.16 Synthesis of poly(methoxydi(ethylene glycol) acrylate) with CTA-7

CTA-7 (28.9 mg, 0.076 mmol), DEGMA (1.991 g, 11.42 mmol), and AIBN (2.43 mg, 0.014 mmol) were dissolved in anisole (1.945 g) in a 25 mL two-necked round
bottom flask. The solution was degassed by three freeze-pump-thaw cycles. The flask was placed into a 70 °C oil bath to start the polymerization. After 172 min, the reaction was stopped by removing the flask from the oil bath, opening it to air, and diluting it with THF. The mixture was precipitated in hexanes and purified by repetitive precipitation. The polymer PDEGMA was obtained as a yellow sticky solid after drying under high vacuum. The DP of the polymer was 148, calculated from the monomer conversion and the monomer-to-CTA ratio. SEC results: $M_{n,SEC} = 19700$ g/mol; PDI = 1.16.

4.3 Results and Discussion

4.3.1 Synthesis of CTA-1, -2, and -3

CTA-1, -2, and -3 have the same Z group (-Ph) and their R groups are benzyl, 1-phenylethyl, and benzyl, respectively, which make them suitable for controlled radical polymerization of acrylates and styrenics by RAFT (Scheme 4.2). The synthetic routes for these CTAs are similar and illustrated in Scheme 4.4. Phenylmagnesium bromide, a Grignard reagent, reacted with carbon disulfide at a moderate temperature, producing $\text{C}_6\text{H}_5\text{CSS}^-$, which was then reacted with benzyl bromide or 1-phenylethyl bromide or 1,4-bis(bromomethyl)benzene. The products were thoroughly purified by column chromatography and their molecular structures were confirmed by $^1\text{H}$ and $^{13}\text{C}$ NMR spectroscopy analysis.

4.3.2 RAFT polymerization of alkoxyoligo(ethylene glycol) acrylates using CTA-1, -2, and -3 as chain transfer agents

CTA-1, -2, and -3 were used as chain transfer agents for RAFT polymerization of methoxydi(ethylene glycol) acrylate (DEGMA) as shown in Scheme 4.5. Poly(DEGMA)
Scheme 4.4 Synthesis of benzyl dithiobenzoate (CTA-1), 1-phenylethyl dithiobenzoate (CTA-2), and 1,4-bis(thiobenzoylthiomethyl)benzene (CTA-3).
Scheme 4.5 Reversible addition-fragmentation chain transfer polymerization of methoxydi(ethylene glycol) acrylate using CTA-1, -2, and -3.
(PDEGMA) is a thermosensitive water-soluble polymer exhibiting a LCST transition in water at 38 °C. The polymerizations were carried out at 70 °C using AIBN as initiator and stopped after ~ 4.5 – 6 h. Figure 4.1 shows the size exclusion chromatography (SEC) traces of the three polymers of DEGMA. The peaks were narrow and symmetric, suggesting that the polymerizations were well-controlled. Using polystyrene calibration, the polydispersity indexes (PDIs) of the three polymers prepared by using CTA-1, -2, and -3 were 1.14, 1.11, and 1.21, respectively. The PDI of the polymer prepared from CTA-3 was slightly higher than those of the other two polymers. Since the R and Z groups of this difunctional RAFT agent are the same as those of CTA-1, the broader molecular weight distribution could be a result of its difunctional nature.

The monomer conversions were calculated from $^1$H NMR spectroscopy analysis using the integral values of the peak at $\delta = 5.78$ ppm ($HCH=CH$) at $t = 0$ min and at the time at which the polymerization was stopped; the peaks at 4.36 – 3.90 ppm were employed as internal standard (-COOCH$_2$ of both monomer DEGMA and PDEGMA; the sum was a constant). The monomer conversions were reasonably good, ranging from 30% to 50%. This observation indicated that the induction period was quite short for all three polymerizations, though no kinetic studies were performed. The DPs were calculated from the monomer conversions and the monomer-to-CTA ratios. The results are summarized in Table 4.1 along with the polymerization conditions.

The polymers prepared by RAFT polymerization can be used as macro-CTAs to synthesize block copolymers. For example, using CTA-1, our group synthesized a doubly thermosensitive hydrophilic diblock copolymer, poly(methoxytri(ethylene glycol) acrylate-co-tert-butyl acrylate)-$b$-poly(ethoxydi(ethylene glycol) acrylate) (P(TEGMA-
Figure 4.1 Size exclusion chromatography traces for three polymers of methoxydi(ethylene glycol) acrylate (DEGMA) synthesized by RAFT polymerization using CTA-1 (A), CTA-2 (B), and CTA-3 (C).
Table 4.1 Synthesis of poly(methoxydi(ethylene glycol) acrylate) (PDEGMA) by RAFT polymerization using CTA-1, -2, and -3 as chain transfer agent and the characterization data for the three polymers of DEGMA.

<table>
<thead>
<tr>
<th>No.</th>
<th>CTA</th>
<th>[M]:[CTA]:[I]₀</th>
<th>Time (min)</th>
<th>Monomer Conv.</th>
<th>DP</th>
<th>Mₙ,SEC (g/mol)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CTA-1</td>
<td>151:1:0.05</td>
<td>313</td>
<td>50%</td>
<td>76</td>
<td>10700</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>CTA-2</td>
<td>158:1:0.07</td>
<td>218</td>
<td>46%</td>
<td>73</td>
<td>10600</td>
<td>1.11</td>
</tr>
<tr>
<td>3</td>
<td>CTA-3</td>
<td>181:1:0.02</td>
<td>332</td>
<td>34%</td>
<td>62</td>
<td>7000</td>
<td>1.21</td>
</tr>
</tbody>
</table>

a CTA stands for chain transfer agent. b the initial molar ratio of monomer (M) to chain transfer agent (CTA) and initiator [I]. c The polymerization time. d The monomer conversion was determined by ¹H NMR spectroscopy analysis. e DP: degree of polymerization, calculated from the monomer-to-CTA ratio and the monomer conversion. The values of number-average molecular weight (Mₙ,SEC) and polydispersity index (PDI) were obtained by size exclusion chromatography using polystyrene standards for calibration.
co-tBA)-b-PDEGNA (see Figure 4.2 for SEC traces).\textsuperscript{29} The removal of tert-butyl groups using trifluoroacetic acid yielded multi-responsive block copolymer, P(TEGMA-co-acrylic acid)-b-PDEGNA. The 20 wt % aqueous solution of this polymer exhibited multiple transitions upon heating, from a free-flowing clear liquid to a clear gel, to a hot clear liquid, and to a cloudy liquid, and the gel-to-sol transition temperature can be tuned by changing the solution pH.

4.3.3 Synthesis of CTA-4, -5, and -6.

CTA-4, -5, and -6 represent a different type of chain transfer agents in that the R groups in these three CTAs are a tertiary carbon with two alkyl groups and one electron-withdrawing cyano group, which makes them suitable for controlled polymerization of both methacrylates and acrylates. CTA-4 was synthesized according to a procedure reported in the literature (Scheme 4.6). Sodium benzyl dithioate, prepared from benzyl chloride and elemental sulfur in the presence of sodium methoxide, was oxidized to di(thiobenzoyl)disulfide by potassium ferricyanide. The desired product was obtained from the reaction between di(thiobenzoyl)disulfide and 4,4’ azobis(4-cyanopentanoic acid) and purified by column chromatography using ethyl acetate and hexanes (2/1, v/v) containing 0.1% by volume acetic acid as eluent. The molecular structure was confirmed by \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy analysis. The PEO macro-CTA was prepared by a DCC coupling reaction of CTA-4 with poly(ethylene glycol) monomethyl ether (Scheme 4.6). CTA-6 is a difunctional chain transfer agent synthesized by the DCC coupling reaction of CTA-4 with ethylene glycol (Scheme 4.6). The product was isolated by column chromatography using ethyl acetate and hexanes (1/4, v/v) as eluent and the molecular structure was confirmed by \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy analysis. CTA-5
Figure 4.2 (a) Size exclusion chromatography traces of macro-CTA poly(methoxytri(ethylene glycol) acrylate-co-tert-butyl acrylate) (P(TEGMA-co-tBA)) and diblock copolymer P(TEGMA-co-tBA)-b-poly(ethoxydi(ethylene glycol) acrylate) (P(TEGMA-co-tBA)-b-PDEGEA), and $^1$H NMR spectra of P(TEGMA-co-tBA)-b-PDEGEA (b) and P(TEGMA-co-acrylic acid)-b-PDEGEA (c).$^{29}$ P(TEGMA-co-acrylic acid)-b-PDEGEA was prepared from P(TEGMA-co-tBA)-b-PDEGEA using trifluoroacetic acid to remove the $t$-butyl groups. $n_{\text{TEGMA}}$, $n_{\text{tBA}}$, and $n_{\text{DEGEA}}$ are the number of TEGMA, $t$BA, and DEGEA units in the polymer, respectively. CDCl$_3$ was used as solvent in $^1$H NMR spectroscopy analysis.
Scheme 4.6 Synthesis of 4-cyanopentanoic acid dithiobenzoate (CTA-4), methoxypoly(ethylene glycol) 4-cyano-4-(phenylcarbonothioyl)pentanoate (CTA-5), and 4,4’-ethane-1,2-diyl bis(4-cyano-4-((phenylcarbonothioyl)thio)pentanoate) (CTA-6).
and -6 were used as chain transfer agents in the RAFT polymerization of methoxydi(ethylene glycol) methacrylate detailed below.

4.3.4 Preparation of thermosensitive block copolymer PEO-b-poly(methoxydi(ethylene glycol) methacrylate) (PEO-b-PDEGMMA) by RAFT polymerization using CTA-5

CTA-5, a PEO macro-CTA, can be used to prepare well-defined block copolymers by RAFT polymerization (Scheme 4.7). As an example, the synthesis of thermosensitive diblock copolymer PEO-b-poly(methoxydi(ethylene glycol) methacrylate) (PEO-b-PDEGMMA) was performed. The polymerization was carried out in anisole at 70 °C using AIBN as initiator. The molar ratios of [DEG_MMA]₀ : [CTA-5]₀ : [I]₀ = 146 : 1 : 0.1. The reaction was stopped after 210 min. Figure 4.3 shows the SEC chromatograms of macro-CTA and the block copolymer. The peak shifted to the high molecular weight side and remained symmetrical, indicating that the polymerization was controlled. The \( M_{n,SEC} \) was 11400 g/mol and the PDI was 1.10 (relative to polystyrene standards). Using the fact that the DP of the PEO block was 113, the DP of PDEGMMA was calculated to be 40 from the \(^1\)H NMR spectrum of the block copolymer.

4.3.5 Preparation of poly(methoxydi(ethylene glycol) methacrylate) (PDEGMMA) by RAFT polymerization using CTA-6.

CTA-6 was used as chain transfer agent for the RAFT polymerization of methoxydi(ethylene glycol) methacrylate (Scheme 4.7). The reaction was carried out at 70 °C in anisole using AIBN as initiator. The molar ratios of [DEG_MMA]₀ : [CTA-6]₀ : [I]₀ were 764 : 1 : 0.17. The polymerization was stopped after 207 min. Figure 4.4 shows the SEC trace of the obtained polymer. The \( M_{n,SEC} \) was 30600 g/mol and the PDI was
Scheme 4.7 Synthesis of PEO-\(b\)-PDEGMMA using CTA-5, PDEGMMA using CTA-6, and PDEGMA using CTA-7 by RAFT polymerization.
Figure 4.3 Size exclusion chromatography traces of PEO macro-CTA (CTA-5) and a diblock copolymer, PEO-\(b\)-poly(methoxydi(ethylene glycol) methacrylate) (PEO-\(b\)-PDEGMMA).
Figure 4.4 Size exclusion chromatography trace of poly(methoxydi(ethylene glycol) methacrylate) synthesized by RAFT polymerization using CTA-6.
1.16 (relative to polystyrene standards). The monomer conversion was 33\%, calculated from $^1$H NMR spectra of the polymerization mixture at $t = 0$ min and 207 min. Thus, the DP of PDEGMMA was 252.

4.3.6 Preparation of trithiocarbonate chain transfer agents, CTA-7 and CTA-8

CTA-7 and -8 are trithiocarbonates suitable for controlled radical polymerization of both methacrylates and acrylates. These two chain transfer agents were prepared according to Scheme 4.8 following the procedures described in the literature. For CTA-7, 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoic acid was prepared first, followed by the esterification reaction with ethanol. We converted the carboxylic acid group into the ester group because the polymer with an acid group at the chain end could interact with the packing materials of the SEC column. The product was purified by column chromatography and the molecular structure was verified by $^1$H and $^{13}$C NMR spectroscopy analysis. CTA-8 was synthesized using a similar reaction.

4.3.7 RAFT polymerization of methoxydi(ethylene glycol) acrylate (DEGMA) using CTA-7

A polymerization of methoxydi(ethylene glycol) acrylate using CTA-7 as chain transfer agent and AIBN as initiator was conducted in anisole at 70 °C (Scheme 4.7). The molar ratios of [DEGMA]$_o$ : [CTA-7]$_o$ : [I]$_o$ = 152 : 1 : 0.19. The reaction was stopped after 172 min. SEC analysis that the polydispersity index was 1.16, indicating that the polymerization was controlled (Figure 4.5). The $M_{n,SEC}$ was 19700 g/mol.

CTA-8 is a difunctional chain transfer agent. The use of this CTA in the RAFT polymerization of alkoxyoligo(ethylene glycol) (meth)acrylates has not been tested because of the presence of two carboxylic acid that could cause a problem in the SEC
Scheme 4.8 Synthesis of ethyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (CTA-7), and 2,2’-(thiocarbonylbis(sulfanediyl))bis(2-methyl-propanoic acid) (CTA-8).
Figure 4.5 Size exclusion chromatography trace of PDEGMA synthesized by RAFT polymerization using CTA-7.
analysis. The carboxylic acid groups can be converted to ester groups and its application in the RAFT polymerization will be explored by other group members.

4.4 Conclusions

A total of 8 RAFT chain transfer agents were synthesized and purified. Their molecular structures were confirmed by $^1$H and $^{13}$C NMR spectroscopy analysis. Preliminary studies using dithiobenzoates (CTA-1, -2, and -3) showed that they can be used as CTAs for RAFT polymerization of alkoxyoligo(ethylene glycol) acrylates, producing well-defined polymers. A well-defined diblock copolymer PEO-$b$-PDEGMMA was prepared by using a PEO macro-CTA (CTA-5). The suitability of CTA-6 in the controlled polymerization of DEGMMA was tested and a well-defined polymer was obtained. CTA-7 was tested for the polymerization of DEGMA. The work presented in this Chapter has set a foundation for our group to explore the synthesis of multi-responsive hydrophilic block copolymers by RAFT.
References


Chapter 5. Summary and Future Work
5.1 Summary

A series of well-defined multi-responsive hydrophilic ABA linear triblock copolymers were prepared via the incorporation of a small amount of stimuli-responsive groups into the thermosensitive outer blocks. A characteristic feature of these block copolymers is that the lower critical solution temperature of the outer thermosensitive blocks can be modified by applying an external stimulus, allowing us to manipulate the critical micellization temperature of the block copolymer in dilute aqueous solutions and the sol-gel transition temperature in moderately concentrated aqueous solutions.\textsuperscript{1,2}

The thermo- and light-sensitive poly(ethoxytri(ethylene glycol) acrylate-\textit{co-o-}nitrobenzyl acrylate)-\textit{b-}poly(ethylene oxide)-\textit{b-}poly(ethoxytri(ethylene glycol) acrylate-\textit{co-o-}nitrobenzyl acrylate) (P(TEGEA-\textit{co-NBA})-\textit{b-}PEO-\textit{b-}P(TEGEA-\textit{co-NBA})) with various NBA contents were made by atom transfer radical polymerization (ATRP) of TEGEA and NBA with different feed ratios from a difunctional PEO macroinitiator with a molecular weight of 20000 g/mol.\textsuperscript{1} The triblock copolymers were well-defined as indicated by their relatively low polydispersity indices. A 10.0 wt % aqueous solution of a triblock copolymer with a NBA content of 9.3 mol % was shown to undergo multiple sol-gel/gel-sol transitions in response to temperature changes and UV irradiation. The thermo-triggered sol-gel transitions stemmed from the formation of a 3-dimensional network with the dehydrated thermosensitive blocks associated into hydrophobic cores and the central PEO block forming bridges among micelles. The exposure to 365 nm UV light cleaved the hydrophobic o-nitrobenzyl groups,\textsuperscript{3} resulting in an increase in the LCST and consequently a gel-to-sol transition. The effects of polymer concentration and NBA content on sol-gel transition temperature ($T_{sol-gel}$) and gel properties before and after UV
irradiation were investigated. The $T_{\text{sol-gel}}$ increased with the decrease of polymer concentration. The change of $T_{\text{sol-gel}}$ was in general larger for the copolymer with a higher NBA content after UV irradiation.

The advantage of our design of multi-responsive block copolymers lies in the random incorporation of stimuli-responsive groups into the thermosensitive outer blocks of ABA triblock copolymers; the mechanisms of sol-gel/gel-sol transitions are clear and defined. We further explored this strategy by incorporating enzyme-responsive phosphate groups into the thermosensitive outer blocks of ABA triblock copolymers and demonstrated enzyme-triggered gelation of their moderately concentrated aqueous solutions.\(^2\) The thermo- and enzyme-responsive block polymers, poly(ethoxydi(ethylene glycol) acrylate-\(co\)-4-((dihydroxyphosphoryl)oxy)butyl acrylate)-\(b\)-poly(ethylene oxide)-\(b\)-poly(ethoxydi(ethylene glycol) acrylate-\(co\)-4-((dihydroxyphosphoryl)oxy)butyl acrylate) (P(DEGEA-\(co\)-OPBA)-\(b\)-PEO-\(b\)-P(DEGEA-\(co\)-OPBA)), were made by ATRP and the post-polymerization modification. We found that the cleavage of phosphate groups by acid phosphatase was highly dependent on the solution pH and pH 4.4 was selected to conduct the enzyme-induced gelation of 7.9 wt % aqueous solutions of P(DEGEA-\(co\)-OPBA)-\(b\)-PEO-\(b\)-P(DEGEA-\(co\)-OPBA). Although the gelation process was slow due to the decreased mobility of both polymer chains and enzyme with the increase of the viscosity of medium, thermoreversible gels were obtained. The dephosphorylation decreased the LCST of thermosensitive blocks from above to below 37 °C, resulting in the formation of 3-dimensional micellar network gels. It is possible to change the OPBA content and molecular weight of thermosensitive blocks as well as the enzyme/polymer ratio to accelerate the gelation process.
Diseased tissues are known to exhibit acidic pH values. In an effort to make aqueous micellar gels that can release the substances loaded in the gels at a greater rate in an acidic environment, two well-defined tertiary amine-containing thermosensitive ABA triblock copolymers were synthesized. The sol-gel transition temperatures of 10 wt % aqueous solutions of the two polymers changed with the change of solution pH. In a separate effort, I explored in our group the RAFT polymerization for the synthesis of well-defined thermosensitive water-soluble polymethacrylates and polyacrylates. A number of chain transfer agents were prepared and thermosensitive polymers with narrow molecular weight distributions were obtained. This effort has paved the way for our group to use RAFT to make multi-responsive block copolymers as RAFT is well-suited for the controlled polymerization of hydrophilic functional monomers.4

5.2 Future Work

One possible project along this line of research is the development of hybrid multi-responsive aqueous micellar gels of ABA triblock copolymers embedded with other stimuli-sensitive, for example, light-sensitive AB diblock copolymer micelles for programmable release of substances for practical applications. One can envision that a hydrophobic substance can be loaded into the hydrophobic core of light-responsive AB diblock copolymer micelles and a hydrophilic substance can be incorporated into the interstitial space of ABA network gels. Thus, changing the condition can trigger the gel-to-sol transition of the ABA network gel,5 the dissolution of the ABA triblock copolymer, and the release of AB diblock copolymer micelles and the hydrophilic substance. The AB diblock copolymer micelles can be induced to dissociate to release the hydrophobic
substance under different conditions. These hybrid gels possess multiple functions and could be suitable for biomedical applications.
References


Vita

Jeremiah Woodcock was born in Wichita Falls, Texas. He joined the armed forces after graduating from high school in 1994. After leaving active service in 2000, he married Leigh Woodcock and began his chemistry career at Middle Tennessee State University in 2001. After September 11, 2001, he was deployed to Fort Bragg. At the end of his deployment, he finished his B.S. degree in chemistry in the spring of 2006. In August, 2006, Jeremiah enrolled as a graduate student in the Department of Chemistry at the University of Tennessee, Knoxville. He joined Dr. Bin Zhao’s group in December, 2006, where he worked on multiresponsive hydrophilic block copolymers. Jeremiah Woodcock received a Doctor of Philosophy Degree in Chemistry from the University of Tennessee in the fall of 2011.