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# Biocompatible Thermoresponsive Polymers with Pendent Oligo(ethylene glycol) Chains and Cyclic Ortho Ester Groups

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ABSTRACT: Two acrylate monomers with six-member cyclic ortho ester groups, i.e., 2-(1,3-dioxan-2-yl-oxy)ethyl acrylate (DEA) and 2-(5,5-dimethyl-1,3-dioxan-2-yloxy) ethyl acrylate (DMDEA), were synthesized. These two monomers were copolymerized with an oligo(ethylene glycol) acrylate (OEGA) under atom transfer radical polymerization conditions to afford two series of thermoresponsive copolymers, poly(DEA-*co*-OEGA)s and poly(DMDEA-*co*-OEGA)s. All the copolymers were soluble in water to form transparent solutions at low temperature, however, some of them exhibited association behaviors below the cloud point (CP) as evidenced by $^1$H NMR, dynamic light scattering (DLS) and fluorescence method. The aggregation tendency of the copolymers depends on their composition as well as the structure of the ortho ester units. With a similar composition, poly(DMDEA-*co*-OEGA) showed a stronger aggregation tendency than poly(DEA-*co*-OEGA). In addition, increasing molar content of the ortho ester units in a copolymer promoted its aggregation in water. Thermally induced phase transitions of these copolymers were studied by various methods including turbidimetry, temperature-dependent $^1$H NMR, DLS, and microscopy. The results indicate that CP of the copolymers increased with increasing the content of OEGA units, but the changing behaviors of CP were rather different for two types of copolymers, which can be ascribed to the difference in hydrophobicity of the ortho ester units. Little hysteresis was observed for the copolymers with more OEGA units while those with more ortho ester units showed significant hysteresis probably due to the hydrophobic character of the ortho ester. The formation of coacervate droplets above CP reveals that the copolymers underwent a liquid−liquid phase separation upon heating. pH-dependent hydrolyses of the copolymers were studied by turbidimetry and $^1$H NMR methods. The hydrolysis rate depends greatly on pH and the hydrophilic/hydrophobic balance of the copolymers: lower pH and more hydrophilic character resulted in a faster hydrolysis rate. Finally, this type of acid-labile thermoresponsive copolymers and the acid-catalyzed hydrolysis products have low cytotoxicity.

## Introduction

In the past 2 decades, stimuli-responsive polymers have attracted great interest in both scientific and applied research areas.[1−5] Among them, thermoresponsive polymers with lower critical solution temperature (LCST) have been extensively studied and widely used, alone or combined together with other materials, in various fields such as catalysis, intelligent drug/gene delivery, tissue engineering, etc.[6−9] Generally speaking, there are two categories of thermoresponsive polymers with LCST. Poly(*N*-isopropylacrylamide) (PNIPAM) is a representative example whose phase transition is accompanied by the formation of inter- and/or intrachain hydrogen bonds (H-bonds) above its LCST.[10] Another type of thermoresponsive polymers, exemplified by poly(*N*,*N*-diethylacrylamide), poly(vinyl ether)s, or poly(2-oxazoline)s, undergo the thermally induced phase transition/separation without forming inter- and/or intrachain H-bond in the polymer-rich phase.[11−13]

Poly(ethylene glycol) (PEG) is a neutral and water-soluble polymer, which has been approved by FDA for biomedical and pharmaceutical applications due to its excellent biocompatibility. PEG and oligo(ethylene glycol) (OEG) are thermoresponsive but with LCSTs higher than 100 °C, depending on molecular weight and pressure.[14,15] However, combination of PEG or OEG with other structural motifs will afford water-soluble thermoresponsive (co)polymers with the LCSTs lower than 100 °C if an appropriate hydrophilic/hydrophobic balance is achieved. For examples, Ishizone and colleagues have prepared thermoresponsive polymethacrylates with OEG methyl ether side chains by living anionic polymerization. Cloud point (CP) of the polymers was dependent on the length of OEG as well as the stereoregularity of the polymer backbone.[16] Lutz et al. prepared thermoresponsive copolymers with a similar structure by atom transfer radical copolymerization of different OEG methyl ether methacrylates, and studied in detail their aqueous solution properties, biocompatibility, and potential for fabricating various stimuli-responsive materials.[17−20] Various other LCST-type polymers with OEG side chains including polystyrenics and polyacrylates,[21−23] poly(vinyl ether)s,[24] poly(norbornenyl esters),[25] and copolymers of methacrylates were also reported.[26−28] Recently, OEG motif as a side chain or a segment in the backbone has been used to synthesize degradable,[29−33] dendritic/hyperbranched,[34,35] or dendronized thermoresponsive polymers.[36] A unique feature of these OEG-containing water-soluble polymers is that most of them show a sharp and reversible phase transition with little hysteresis.

Multistimuli responsive polymers that undergo sequential and predictable changes in morphology and properties under different stimuli are expected to play an important role in building various smart systems.[37] Thermo/pH or thermo/photo doubly responsive polymers with OEG side chains have also been reported.[38−43] However, a majority of the thermo/pH responsive polymers were prepared by the incorporation of reversibly ionizable groups such as carboxylic or amino groups. In recent

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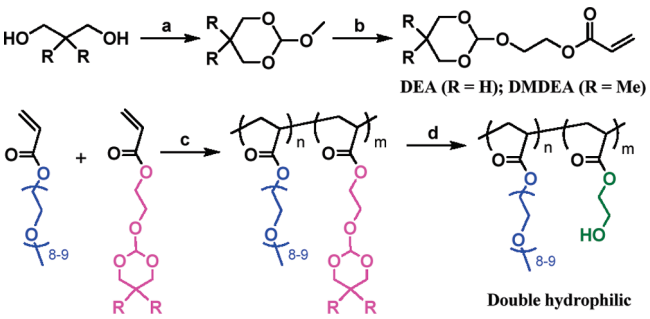
**Scheme 1. Synthetic Route of Monomers DEA and DMDEA and Their Copolymers with OEGA[a]**




[a] Key: (a) trimethyl orthoformate, TsOH·Py in $CH_2Cl_2$, room temperature, 12 h. (b) 2-hydroxyethyl acrylate, TsOH·Py in toluene, 120 °C, 20 h. (c) [EBiB]/[CuBr]/[$Me_6$TREN] = 1.0/1.0/1.0 molar ratio in anisole, 50 °C, 17 h. (d) $H_2O/H^+$.

years, another strategy has been used to prepare thermo/acid doubly responsive polymers by introducing acid-labile linkages in the pendent groups or the backbone.[44−47] We have reported a family of acid-liable thermoresponsive poly(meth)acrylamides with pendent ortho ester moieties. The phase transition temperature of these polymers can be manipulated by tuning the hydrolysis of the ortho ester groups.[48] Although these poly(meth)acrylamides have potential for constructing intracellular drug delivery systems, their long-term biocompatibility has not been clearly demonstrated. In this article, we report the synthesis and aqueous solution properties of thermoresponsive polyacrylates with pendent OEG chains and cyclic ortho ester groups (Scheme 1). Since the acid-triggered hydrolysis products are the copolymers of OEG acrylate and 2-hydroxyethyl acrylate, this type of OEG-containing thermoresponsive polymers may have better biocompatibility compared to the poly(meth)acrylamides as previously reported. In addition, hydrolysis rate of the ortho ester is much faster than that of the acetal linkage, making the present acid-labile polymers sensitive to a milder acidic environment than that in literature,[46] which is an important factor for intracellular drug delivery. Finally, these OEG-containing polymers have no hydrogen donor and there is no strong intra- and/or interchain H-bond between the polymer chains, and therefore they would show different thermally induced phase transition behaviors compared to the amide-containing polymers as we reported previously.[48]

## Experimental Section

**Materials.** 1,3-Propanediol, 4-methoxyphenol (Acros), trimethyl orthoformate (Alfa Aesar), 2-hydroxyethyl acrylate (HEA), copper(I) bromide (CuBr), and 2-bromoisobutyrate (EBiB) (Aldrich) were used as received. 2-Methoxy-1,3-dioxane and 2-methoxy-5,5-dimethyl-1,3-dioxane were prepared according to the reported procedure.[49,50] Oligo(ethylene glycol) monomethyl ether acrylate (OEGA, $M_n$ = 454 g/mol) (Aldrich) was passed through a basic $Al_2O_3$ column prior to use. Dichloromethane ($CH_2Cl_2$) and tetrahydrofuran (THF) were distilled over $CaH_2$ and sodium prior to use, respectively. Anisole and toluene were washed sequentially with $H_2SO_4$ (98%), water, 5% $NaHCO_3$ aqueous solution, and dried with anhydrous $K_2CO_3$. Then, toluene was distilled over sodium, and anisole was distilled under reduced pressure. $CDCl_3$ was treated with anhydrous $K_2CO_3$ before NMR measurements of the ortho ester-containing samples. Tris(2-dimethylaminoethyl)amine ($Me_6$TREN) was synthesized according to the literature's method with minor modification.[51] Pyrene (Acros) was recrystallized from ethanol twice. Other solvents and reagents were purchased from Beijing Chemical Reagent Co. and used as received.

**Synthesis of Monomers.** 2-(1,3-Dioxan-2-yloxy)ethyl acrylate (**DEA**): 2-methoxy-1,3-dioxane (5.9 g, 0.05 mol), 2-hydroxyethyl acrylate (7.6 g, 0.065 mol), pyridinium-*p*-toluenesulfonate (0.25 g, 1 mmol), and 4-methoxyphenol (0.1 g) were dissolved in 50 mL of toluene, and the reaction mixture was stirred at 120 °C for 20 h. The solution was washed thrice with 10% NaOH aqueous solution and dried with anhydrous $K_2CO_3$. After removing the solvent on a rotary evaporator, the residue was purified through a basic $Al_2O_3$ column using petroleum ether/ethyl acetate (5:2 v/v) as the eluent. The product is a colorless oil (4.2 g, 42%). $^1$H NMR (400 MHz, TMS, $CDCl_3$, ppm, Figure S1a): 6.42−6.46 (q, 1H, C$H_2$=CH−), 6.13−6.20 (q, 1H, $CH_2$=C$H$−), 5.83−5.86 (q, 1H, C$H_2$=CH−), 5.36 (s, 1H, C$HO_3$), 4.34−4.37 (t, 2H, $J$ = 4.8 Hz, −$CO_2CH_2CH_2O$−), 4.15−4.20 (m, 2H, −$OCH_2CH_2CH_2O$−), 3.88−3.90 (t, 2H, $J$ = 4.8 Hz, −$CO_2CH_2CH_2O$−), 3.79−3.87 (m, 2H, −$OCH_2CH_2CH_2O$−), 1.72−1.78 (m, 2H, −$OCHH_2CH_2$-$CH_2O$−). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm, Figure S1b): 166.1 (−C=O), 131.1 ($CH_2$=C−), 128.3 ($CH_2$=C−), 109.6 (−$CHO_3$), 63.6 (−$CO_2CH_2CH_2O$−), 62.9 (−$CO_2CH_2CH_2O$−), 62.3 (−$OCH_2CH_2CH_2O$−), 24.8 (−$OCH_2CH_2CH_2O$−). Anal. Calcd ($C_9H_{14}O_5$): C, 53.46; H, 6.98. Found: C, 53.42; H, 7.07.

2-(5,5-Dimethyl-1,3-dioxan-2-yloxy)ethyl acrylate (DMDEA) was synthesized by a similar procedure as for DEA, to afford a colorless oil with 53% yield. DMDEA. $^1$H NMR (300 MHz, TMS, $CDCl_3$, ppm, Figure S2a): 6.42−6.49 (q, 1H, C$H_2$=CH−), 6.13−6.22 (q, 1H, $CH_2$=C$H$−), 5.84−5.88 (q, 1H, C$H_2$=CH−), 5.37 (s, 1H, C$HO_3$), 4.34−4.38 (t, 2H, $J$ = 5.0 Hz, −$CO_2CH_2$-$CH_2O$−), 3.88−3.91 (t, 2H, $J$ = 5.0 Hz, −$CO_2CH_2CH_2O$−), 3.75−3.79 (d, 2H, $J$ = 11.2 Hz, −$OCH_2CH_2CH_2O$−), 3.39−3.43 (d, 2H, $J$ = 11.2 Hz, −$OCH_2CH_2CH_2O$−), 0.99 (s, 6H, −$C(CH_3)_2$). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm, Figure S2b): 166.1 (−C=O), 131.0 ($CH_2$=C−), 128.3 ($CH_2$=C−), 109.2 (−$CHO_3$), 72.1 (-$OCH_2CH_2CH_2O$−), 63.6 (−$CO_2CH_2CH_2O$−), 62.8 (−$CO_2$-$CH_2CH_2O$−), 29.7 (−$OCH_2CH_2CH_2O$−), 22.7, 22.1 (-C($CH_3$)$_2$). Anal. Calcd ($C_{11}H_{18}O_5$): C, 57.38; H, 7.88. Found: C, 57.29; H, 7.80.

**Polymerization.** Homopolymers of DEA or DMDEA, and their copolymers with OEGA were prepared by the atom transfer radical polymerization (ATRP). Take the synthesis of copolymer A3 in Table 1 as an example, DEA (0.4 g, 2.0 mmol), OEGA (0.23 g, 0.5 mmol), EBiB (4.9 mg, 0.025 mmol), and $Me_6$TREN (5.8 mg, 0.025 mmol) were charged into a polymerization tube, to which anisole (1.0 g) was added to dissolve the monomers and initiator. After three cycles of freeze−pump−thaw to thoroughly remove oxygen, CuBr (3.6 mg, 0.025 mmol) was added into the tube under a nitrogen atmosphere, and the tube was sealed in vacuo. The polymerization was carried out at 50 °C for 17 h. Then, the reaction mixture was diluted with THF and passed through a basic $Al_2O_3$ column to remove the catalyst. The polymer was precipitated from petroleum ether twice and dried in vacuo to afford a white sticky solid. Other (co)polymers with different compositions were prepared by the similar procedure; they are all white sticky solids. Compositions of the copolymers were determined by $^1$H NMR spectra, using peaks at ~5.4 ppm and ~3.4 ppm. For the copolymers of DEA and OEGA (A series), molar percent content (F) of the ortho ester unit was equal to $3I_5/(3I_5 + I_{12}) \times 100$, where $I_5$ and $I_{12}$ denote the integration intensity of peak 5 (~5.4 ppm) and peak 12 (~3.4 ppm), respectively (Figure 1a). For the copolymers of DMDEA and OEGA (B series), intensity of proton 6 has to be subtracted because the signals of proton 6 and proton 12 overlapped. Molar percent content ($F$) of the ortho ester unit was calculated to be $3I_5/(I_5 + I_{6,12}) \times 100$, where $I_5$ and $I_{6,12}$ denote the integration intensity of peak 5 (~5.4 ppm) and the peaks at ~3.4 ppm, respectively (Figure 2a).

**NMR Spectroscopy.** $^1$H NMR spectra (400 MHz) of the monomers and copolymers in $CDCl_3$ and $D_2O$ were recorded on a Bruker ARX 400 MHz spectrometer. $^{13}$C NMR spectra (75 MHz) of monomers were measured on the Varian Mercury Plus 300 MHz NMR spectrometer. $^1$H NMR spectra of the homopolymers and copolymers with varying temperature or

**Table 1. Characterization and Properties of the Polymers [a]**

| polymer | $M_1/M_2/I$[b] | yield (%) | F[c] (%) | $M_n$[d] | $M_w/M_n$[d] | CP[e] (°C) heating | CP[e] (°C) cooling | temperature range (°C)[f] |
|---|---|---|---|---|---|---|---|---|
| PDEA | 100:0:1 | 64 | 100 | 14 400 | 1.19 | | | |
| PDMDEA | 100:0:1 | 69 | 100 | 17 600 | 1.13 | | | |
| **A1** | 90:10:1 | 54 | 86 | 21 700 | 1.23 | 12.9 | | 4.1 |
| **A2** | 85:15:1 | 67 | 81 | 26 700 | 1.15 | 24.4 | 24.3 | 1.8 |
| **A3** | 80:20:1 | 72 | 77 | 31 700 | 1.11 | 27.2 | 26.8 | 1.8 |
| **A4** | 75:25:1 | 58 | 73 | 21 500 | 1.10 | 30.8 | 30.6 | 1.7 |
| **A5** | 85:15:2 | 68 | 80 | 16 000 | 1.16 | 25.0 | 25.1 | 2.9 |
| **A6** | 80:20:2 | 64 | 76 | 14 900 | 1.16 | 27.5 | 27.3 | 2.2 |
| **B1** | 80:20:1 | 73 | 78 | 27 300 | 1.14 | 22.1 | | 7.5 |
| **B2** | 75:25:1 | 79 | 74 | 28 400 | 1.13 | 22.6 | 19.2 | 1.3 |
| **B3** | 70:30:1 | 75 | 70 | 26 300 | 1.11 | 29.8 | 29.2 | 1.7 |
| **B4** | 65:35:1 | 66 | 66 | 25 800 | 1.11 | 35.8 | 35.5 | 2.1 |
| **B5** | 75:25:1.3 | 70 | 73 | 20 300 | 1.19 | 22.7 | 20.0 | 2.1 |
| **B6** | 75:25:2 | 78 | 75 | 15 600 | 1.16 | 23.1 | 20.3 | 2.7 |
| **B7** | 75:25:3 | 67 | 75 | 10 100 | 1.14 | 23.1 | 21.3 | 2.9 |
| **B8** | 75:25:4 | 58 | 76 | 5900 | 1.24 | 23.2 | 21.5 | 4.2 |

[a] Experimental conditions: 17 h, 50 °C in anisole (monomer/anisole = 1:1.5 (w/w)); [EBiB]/[CuBr]/[$Me_6$TREN]) = 1/1/1 in molar ratio. [b] $M_1$, $M_2$, and I denote the ortho ester monomer (DEA for A series and DMDEA for B series), OEGA, and EBiB, respectively. [c] Molar percent content of the ortho ester unit in the copolymers determined by $^1$H NMR. A and B series denote the copolymers derived from DEA and DMDEA, respectively. [d] Measured by GPC in THF, PSt standards. [e] Defined as the temperature where 50% transmittance change occurs during the heating or cooling process, for aqueous solutions with a polymer concentration of 1.0 mg/mL. [f] Temperature difference between 90 and 10% transmittance during the heating process.

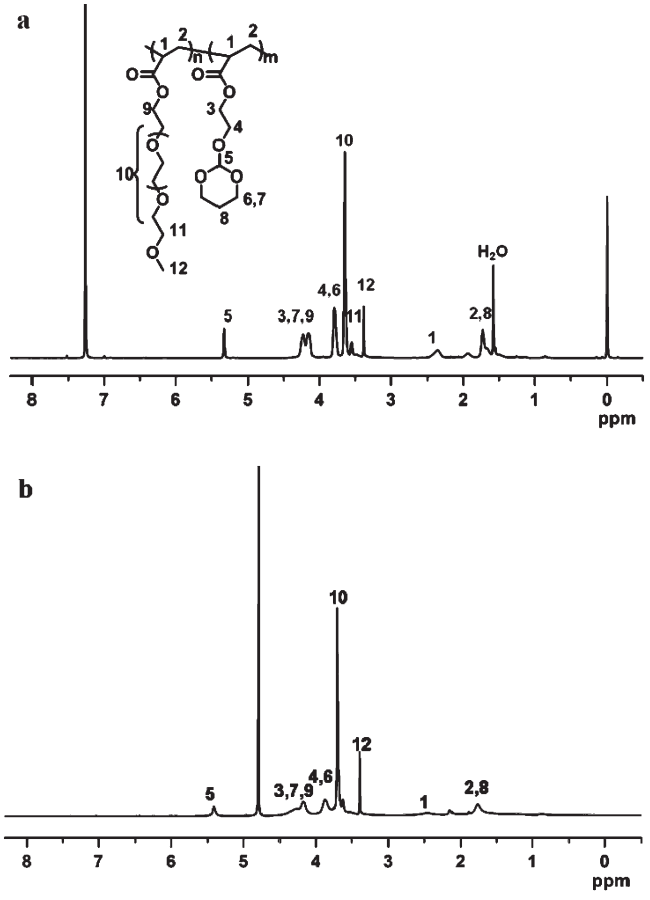



**Figure 1.** $^1$H NMR spectra of copolymer A3 recorded in $CDCl_3$ (a) and $D_2O$ (b) at 25 °C. Polymer concentration: 5.0 mg/mL.

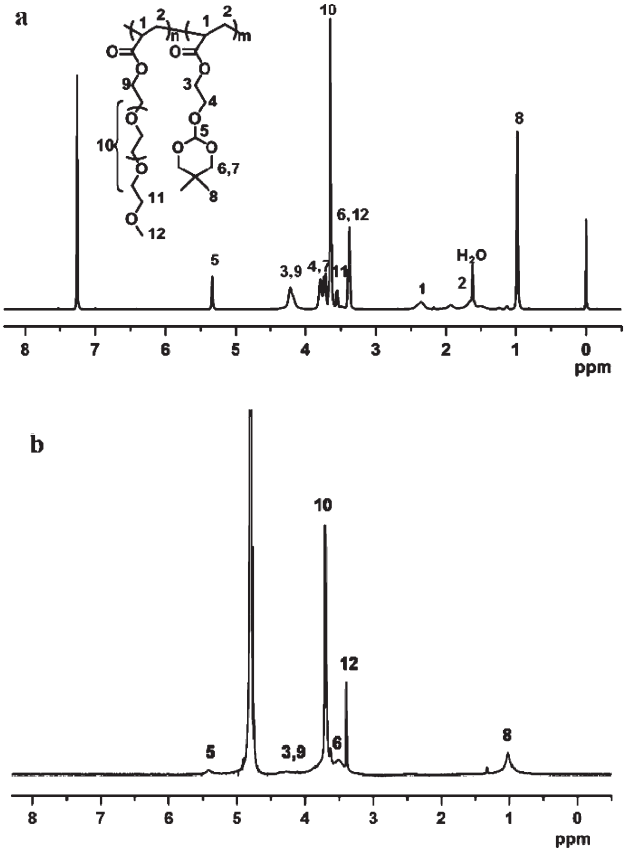



**Figure 2.** $^1$H NMR spectra of copolymer B3 recorded in $CDCl_3$ (a) and $D_2O$ (b) at 25 °C. Polymer concentration: 5.0 mg/mL.

time-dependent hydrolysis in the deuterated buffers were recorded on the Varian Mercury Plus 300 MHz NMR spectrometer.

**Gel Permeation Chromatography (GPC) Measurement.** Molecular weights and molecular weight distributions were determined by GPC performed at 35 °C in THF with a flow rate of 1.0 mL/min. The GPC equipment consists of a Waters 1525 binary HPLC pump, a Waters 2414 refractive index detector, and three Waters Styragel columns (HT2, HT3, and HT4). For calibration, a family of narrow dispersed polystyrenes was used as the standards.

**Cloud Point (CP) Measurement.** Turbidimetric measurements of the polymers in 10 mM phosphate buffer (PB, pH 8.0) solutions were carried out on a Shimadzu 2101 UV−vis spectrometer in a 1 cm quartz cell at 500 nm. The same buffer without polymer was used as a reference. During the heating or cooling process, the solutions were equilibrated for 10 min at each temperature prior to detection unless otherwise mentioned.

CP was defined as the temperature when the transmittance was 50%. The PB of pH 8.0 was used to avoid possible hydrolysis of the ortho ester groups during the measurements.

**Dynamic Light Scattering (DLS).** DLS measurements of the copolymer aqueous solutions (1.0 mg/mL, pH 8.0, 10 mM PB) were performed on a particle size analyzer (ZETA PALS, Brookhaven Instruments Corporation, BIC) equipped with a temperature controller and a 35 mW He−Ne solid state laser ($\lambda$ = 660 nm, detection angle: 90 °). The data were analyzed with a BIC particle sizing software (9kpsdw32, ver. 2.3) according to the Stokes−Einstein equation. The solutions were filtered through a Millipore 0.45 $\mu$m PVDF filter into a dust-free vial prior to measurements. In the heating/cooling processes, the solutions were equilibrated for 10 min at each temperature unless otherwise mentioned.

**pH-Dependent Hydrolysis.** Hydrolysis behaviors of the copolymers were monitored by turbidimetric method and $^1$H NMR measurement, respectively. In the former case, the polymer solution (1.0 mg/mL, in 10 mM PB, pH 8.0) prepared at low temperature and incubated at 4 °C overnight was heated to 37 °C and maintained at this temperature for 10 min, affording a transmittance of 0% using the polymer-free PB as a reference (100% transmittance). Then, the polymer solution was lowered to pH 4.0 by adding 5.0 M pH 4.0 acetate buffer (as 0 time point), and the time-dependent transmittance of the solution was measured at 37 °C. For hydrolyses at other pHs, 5.0 M acetate buffers with different pHs (4.6, 5.0, 5.4) were used. For the hydrolysis at pD 4.6 monitored by $^1$H NMR measurement, the polymer solution in $D_2O$ (~5 mg/mL) was first maintained at 37 °C for 10 min, and the $^1$H NMR spectrum was measured and used as that for 0 time point. After addition of the acetate buffer (pD 4.6, 5.0 M), the polymer solution was mixed quickly and incubated at 37 °C, and the $^1$H NMR spectra were recorded at specific time points.

**Cytotoxicity Measurement.** Copolymer B2 was dissolved in 5.0 acetate buffer (10 mM) and incubated for 24 h at ambient temperature. During the incubation period, the turbid polymer solution (1.5 mg/mL) gradually became clear due to the acid-catalyzed hydrolysis of the ortho ester groups. This solution was denoted as "B2-hydrolyzed". Copolymer B2 was dissolved in pH 8.0 phosphate buffer (10 mM) with a polymer concentration of 1.5 mg/mL and incubated at ~4 °C for 24 h. This solution was used as the intact copolymer B2. MTT assay was applied to evaluate the cytotoxicity of the two samples in Hela cells. PEG 5000 and branched polyethylenimine ($M_w$ 25 KDa) were used as the negative and positive controls, respectively. Hela cells seeded in a 96-well plate were cultured at 37 °C in 5% $CO_2$ humidified atmosphere for 24 h. Polymer solutions with different polymer concentrations (10 $\mu$L) were added to each well, and the cells were subjected to MTT assay after being incubated for another 24 h. The absorbance of the solution was measured on a Bio-Rad model 550 microplate reader at 570 nm. Cell viability (%) was equal to $(A_{sample}/A_{control}) \times 100$, where $A_{sample}$ and $A_{control}$ denote absorbance of the sample well and control well (without polymer), respectively. Experiments were performed in triplicate.

## Results and Discussion

**Synthesis and Characterization of Monomers and Polymers.** Monomers DEA and DMDEA were synthesized according to the route as shown in Scheme 1. NMR spectra and the elemental analysis data confirmed their structure and purity. PDEA and PDMDEA were synthesized by ATRP at 50 °C in anisole using EBiB as an initiator and CuBr/$Me_6$TREN as a catalyst. Characterization of these two homopolymers is shown in Table 1 and Figure S3. PDEA was slightly soluble in 10 mM phosphate buffer (< 1 mg/mL) at low temperature (~4 °C), but gradually precipitated from the aqueous solution at ambient temperature. PDMDEA was more hydrophobic, and therefore it was insoluble in water even at 4 °C. In order to get water-soluble polymers, OEGA was copolymerized with DEA or DMDEA under ATRP conditions. By varying the feed molar ratio of the two monomers or changing the ratio of comonomers to initiator, we prepared two series of copolymers (A or B) with different compositions or molecular weights. All of the (co)polymers have narrow molecular weight distributions (Table 1). Compositions of the copolymers were determined by their $^1$H NMR spectra as described in the experimental part. The contents of the ortho ester unit in the copolymers are very close to those in monomer feed, implying the random distribution of the two monomer units in the copolymer chains.

**Aqueous Solution Properties below LCST.** At low temperatures, all the copolymers were soluble in water due to the presence of the hydrophilic OEG chains, and the polymer solutions were visually transparent. Upon increasing temperature, the aqueous solutions of these copolymers showed obvious thermoresponsive properties, becoming cloudy at a specific temperature, i.e.,CP. A key factor for the improved water solubility of these copolymers is believed to be the formation of H-bonds between the ether oxygen atoms of OEG side chains and water molecules.[52,53] However, this favorable H-bond effect may be counterbalanced by the relatively apolar backbone and the pendent ortho ester groups, and microenvironmental heterogeneity in the polymer aqueous solutions below their individual CP may exist. In order to demonstrate the solution properties of these copolymers, $^1$H NMR spectra of copolymers A3 and B3 in $D_2O$ at 25 °C (below their CPs) were compared with those in $CDCl_3$ (Figure 1 and Figure 2). When the $^1$H NMR spectrum of copolymer A3 was recorded in $CDCl_3$, all the proton signals were clear and sharp, and the integration ratios of different signals fitted well to the theoretical values, revealing that the polymer chains were uniformly dissolved at a molecular level. However, in $D_2O$, the proton signals of OEG side chains (peak 10 at ~3.6 ppm and peak 12 at ~3.4 ppm) remained sharp and intense, but the proton signals of the backbone and ortho ester units were significantly broadened and reduced in intensity. These results indicate that copolymer A3 was not homogeneously hydrated even below its CP. The pendent OEG chains were well hydrated and flexible while the ortho ester groups tend to adopt a relatively compact conformation. For copolymer B3, similar phenomenon was observed, but the intensity reduction of the ortho ester signals was more pronounced as compared to copolymer A3, indicating that copolymer B3 probably adopted a more compact conformation (Figure 2).

The solution properties of the copolymers below their CPs were further investigated by DLS. Although DLS intensity size distribution showed the coexistence of two or three components with different diameters for each of the copolymers (Figure S4), weight fractions of the larger components should be very small and negligible.[18] Indeed, when the volume distribution was used, most of the copolymer solutions demonstrated only one component except for copolymers A2 and B1 (Figure 3). It can be seen that the average diameters of copolymers A3, A4 and B4 were smaller than 10 nm, revealing that these copolymers likely adopted a single chain coiled conformation. For copolymer A2, polymer associates of ca. 20 nm in diameter could be detected besides the coiled single chains. In the case of copolymers B1, B2, and B3, the average diameters were larger than 10 nm and increased in the order of B3, B2 < B1, which indicates that these copolymers were mostly dissolved in water in the forms of associates. The DLS results can be rationally explained by the hydrophobic effect of the ortho ester units. With increasing the molar contents of the ortho

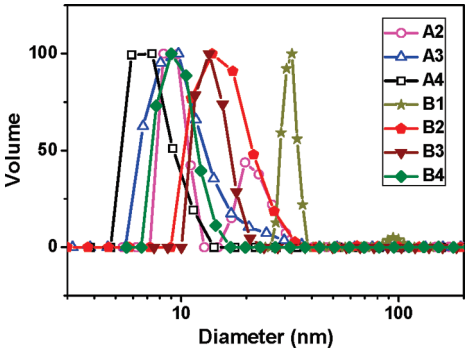



**Figure 3.** Volume size distribution of the copolymer aqueous solutions below CPs measured by DLS. Polymer concentration: 1.0 mg/mL. Temperature: 20 °C for copolymers A3, A4, B3, and B4; 17 °C for copolymers A2 and B2; 15 °C for copolymer B1.

ester units in the copolymers, aggregation tendency of the copolymer chains in water are expected to increase due to the hydrophobicity of the ortho ester units. When comparing copolymer A3 and copolymer B1, both having similar molar contents of the ortho ester units, we found that the latter has a much stronger tendency to aggregate, which is definitely attributed to the more hydrophobic nature of the ortho ester units with two additional methyl groups in copolymer B1 chains. The aggregation behavior of copolymer B1 below its CP was further proved by fluorescence method using pyrene as a probe (Figure S5). Copolymer B1 showed a critical association concentration (CAC) of ~0.05 mg/mL while copolymer A3 did not exhibit CAC up to 2 mg/mL. The aggregation or association phenomenon below LCST has also been reported for other thermoresponsive (co)polymers with hydrophobic units in the polymer chains.[54−56]

**Thermoresponsive Properties of the Copolymers.** The thermoresponsive properties of these copolymers were first studied by turbidimetry. Figure 4 shows optical transmittances of the polymer aqueous solutions as a function of temperature in both heating and cooling processes at 500 nm. In the heating process, the solution transmittance decreased sharply at a specific temperature for most of the polymers except copolymer B1, whose solution underwent a relatively smooth falling in transmittance. Upon cooling, obvious hysteresis was observed only for the copolymers with more ortho ester units, i.e., copolymers A1, B1, and B2. CP of each copolymer solution is defined as the temperature when the transmittance is 50%. The CP values of all the polymer solutions are listed in Table 1.

It is generally considered that an appropriate hydrophilic/hydrophobic balance is essential for a (co)polymer to be thermoresponsive. CPs of the thermoresponsive polymers can be easily tuned by changing the hydrophilic/hydrophobic balance: the more the hydrophilic unit in the copolymer, the higher the CP.[57−61] As expected, CPs of copolymer A and copolymer B also varied with their compositions (Figure 5), increasing the molar content of the OEGA unit in the copolymers enhanced the CPs. Almost linear relationships between their CPs and compositions were observed for the copolymers containing more OEGA units (A2 to A4 and B2 to B4). This result is in agreement with that for other thermoresponsive copolymers.[62−67] However, these two series of copolymers displayed rather different behaviors. DMDEA units in copolymer B exerted a more significant effect on CPs of the copolymers. For example, in copolymer B, a 4% increase in molar content of ortho ester unit in the copolymer resulted in ca. 6 °C decrease of CP, while only ca. 3 °C decrease of CP for copolymer A was observed. More interestingly, CPs did not decrease linearly with further

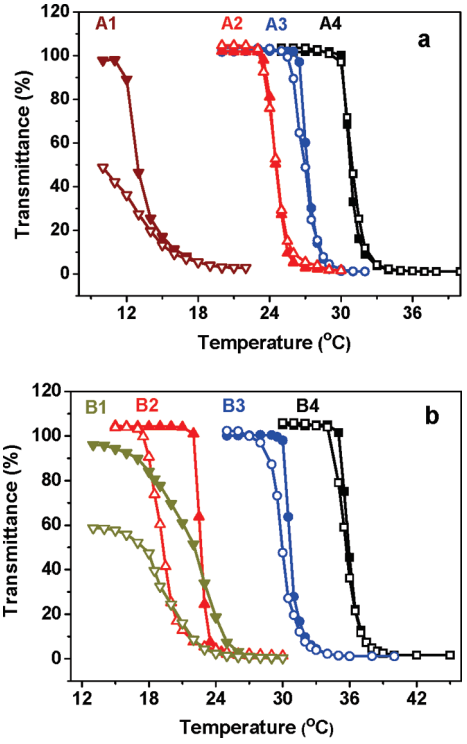



**Figure 4.** Transmittance versus temperature plots of A series copolymers (a) and B series copolymers (b) in 10 mM PB (1.0 mg/mL, pH 8.0) upon heating (solid symbols) and cooling (empty symbols) processes, $\lambda$ = 500 nm. Heating/cooling rate: a temperature point/10 min. For copolymers B1, B2, and A1, the cooling rate is a temperature point/20 min.

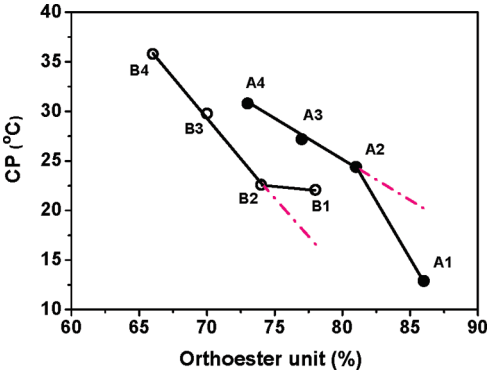
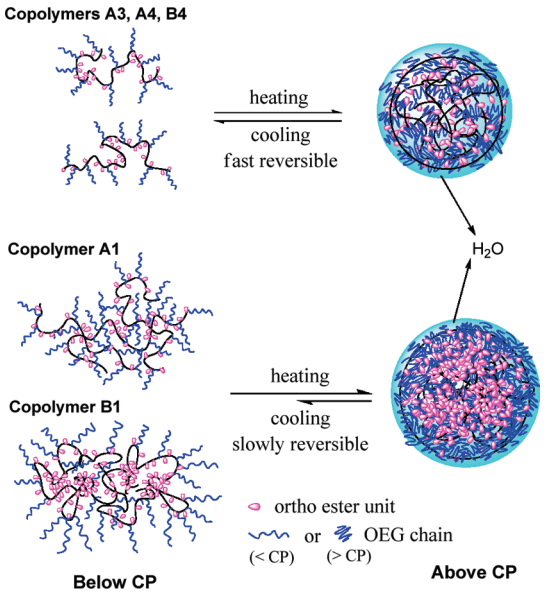



**Figure 5.** Plots of CP as a function of percent molar content of the ortho ester units for A series copolymers (solid symbols) and B series copolymers (empty symbols).

increasing the content of the ortho ester units in the copolymers (copolymers A1 and B1). From A2 to A1, an increase of 5% molar content of the DEA units caused a 11.5 °C drop in CP, by contrast, from B2 to B1, 4% increase in molar content of DMDEA units did not obviously affect the CP of the copolymers, with only a decrease of ca. 0.5 °C (Table 1 and Figure 5). These irregular changes of CPs at high contents of the ortho ester units can be explained by their different aggregation behaviors in water below their individual CP (Scheme 2). Copolymer A1 is speculated to form loose associates below its CP, which can be supported by the pyrene fluorescence result (Figure S5). In this kind of associates, most of the ortho ester groups remained in contact with water and could lead to the decrease of CP as for other A series copolymers. On the other hand, the association of the polymer chains resulted in a locally increased density of OEG

**Scheme 2. Schematic Illustration for Aqueous Solution Behavior of the Copolymers below and above CPs**




side chains, which would result in a decrease of hydration extent of the OEG chains[53] and induce their dehydration at a lower temperature. A similar phenomenon was also reported by other researchers for the peripherally isobutyramide-modified dendrimers[68] or flower micelles formed by the hydrophobically modified telechelic PNIPAM.[69] In the case of B series copolymers, as aforementioned, copolymer B1 is expected to form nanosized associates with a relatively compact conformation below its CP, due to the more hydrophobic nature of DMDEA units compared to DEA units. In these associates, the hydrophobic ortho ester groups preferred to form phase-separated microdomains that were surrounded by the OEG-rich hydrophilic shell (Scheme 2). The hydrophobic domains would exert little influence on CP because they were mostly isolated from the aqueous media. This result is consistent with that of other hydrophobically modified thermoresponsive polymers.[55,70]

Another interesting result observed from Figure 4 is the strong hysteresis displayed by copolymers A1, B1, and B2. For B2, a hysteresis of ~4 °C was observed in a heating/cooling cycle. In the case of copolymers A1 and B1, the transmittances could not go back to 100% but reached only ~50−60% in the time range of measurements upon cooling, even a much slower cooling rate (a temperature point/20 min for cooling vs a temperature point/10 min for heating) was used. The hysteresis of copolymer B1 was further proved by DLS results (Figure 6). In the heating process, sizes of the polymer aggregates gradually increased and only large ones more than 400 nm in diameter could be detected when the temperature reached 27 °C or above. This gradual increase in size of the aggregates is consistent with the observation of a gradual fall in transmittance as detected by the turbidimetric approach. Upon cooling from 31 °C (above CP), the aggregate diameters did not go back to less than 100 nm, but became larger. When the solution temperature was cooled back to 15 °C (~3 h from 31 to 15 °C), the aggregates were still very large with a bimodal distribution (~700 nm and ~4000 nm). Small sized aggregates were obtained only after incubation of the solution at low temperature for a long time (Figure 6c). By contrast, no obvious hysteresis was observed for copolymer B3 which has more hydrophilic OEG side chains (Figure S6). Regarding the reversibility of thermo-

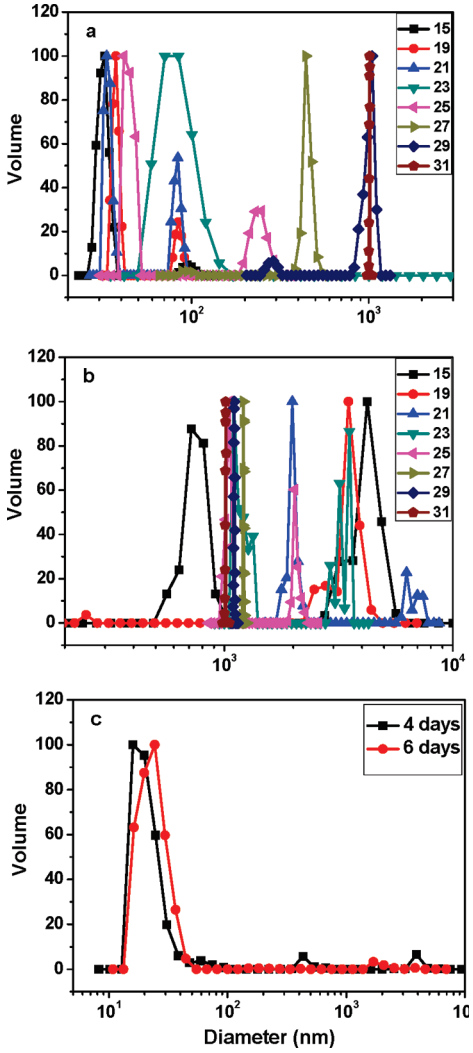



**Figure 6.** Volume size distribution of copolymer B1 aqueous solution at different temperatures (°C) during heating (a) and cooling (b) processes. Polymer concentration: 1.0 mg/mL. Heating/cooling rate: a temperature point/20 min. After a heating/cooling cycle, the sample was incubated at 4 °C for 4 and 6 days, respectively, and remeasured at 15 °C. The DLS results are shown in part c.

responsive polymers, hysteresis in the heating/cooling cycle generally occurs only for the polymers which can form intra- and/or interchain H-bonds in the collapsed polymer aggregates above their LCSTs.[42,71−74] Recently, Sedlak et al. prepared stable nanoparticles by simply heating the aqueous solution of poly(ethylacrylic acid). Hydrogen bonds between $CO_2H$ and $CO_2^-$ are thought to be the main force to stabilize the nanoparticles even at low temperature.[75] By contrast, there is no obvious hysteresis for those thermoresponsive polymers without H-bond above their LCSTs.[12,16,17,31,36,76]

In the present work, no or little hysteresis was detected for the copolymers with more OEG units, which can be reasonably explained by the lack of interchain hydrogen bonding in the aggregates of copolymers above their LCSTs. However, hysteresis became stronger as the content of the hydrophobic ortho ester units in the copolymers was increased, which can not be clearly clarified at present stage. We speculate that, above the CP, the more hydrophobic character of copolymer B1 or A1 leads to the formation of more stable aggregates, in which larger hydrophobic domains and possible chain entanglements kinetically prevent redispersion/redissolvation of the copolymers upon cooling (Scheme 2). Similar phenomena

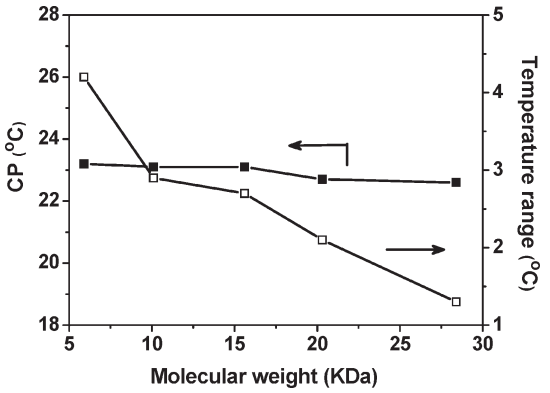



**Figure 7.** Plots of CP (solid symbols) and the temperature range of phase transition (empty symbols) as a function of molecular weight of B series copolymers. The molar ratios of the copolymers (B2, B5−B8 in Table 1) are ca. 75:25.

were reported for the amphiphilic block copolymer of *N*-acryloyl pyrrolidone and butyl acrylate[77] or small amphiphilic molecules containing OEG segment.[78]

Figure 7 shows the effect of molecular weight (MW) on CP and the transition temperature range of the B series copolymers with similar compositions. It is seen that MW exerted little influence on CP but did significantly affect the phase transition temperature range of the copolymers. The phase transition temperature range expanded from 1.3 to 4.2 °C as MW of the copolymers decreased from ~30000 to ~6000 (Table 1 and Figure S7). Lutz and colleagues also reported that MW of copolymers P($MEO_2$MA-*co*-OEGMA) had very little influence on their CPs but a broader transition was observed for the shorter copolymers.[19,79]

In order to deeply understand the mechanism of the thermally induced phase transition or separation of these copolymers, temperature-dependent $^1$H NMR spectra of copolymers A3 and B3 were measured in buffered $D_2O$ (pD 8.0) (Figure 8). It can be seen that for both copolymers at low temperatures, the proton signals (3.3 and 3.6 ppm) of the pendent OEG chains were sharp and clear, while the signals of the backbone or the ortho ester units were very weak, in particular for copolymer B3, indicating that the backbone and the ortho ester groups were not well solvated. As the temperature was increased across the CPs (~27 °C for copolymer A3 and ~30 °C for copolymer B3), a drastic drop of the peak intensity of the OEG proton signals was detected. Upon further increasing the temperature, OEG proton signals decreased gradually but still could be clearly observed at 42 °C. By contrast, in the measured temperature range, little change of proton signals was detected for the backbone or the ortho ester units. These results indicate that the thermally induced dehydration of the OEG side chains is the main driving force for phase transition or separation of the copolymers. In addition, the apparent peaks (10 and 12) of the OEG side chains are clearly observable even at the temperature more than 10 °C above the CPs, suggesting that the copolymers underwent a liquid−liquid phase separation in the heating process, which is further proved by the microcalorimetric measurements (dada not shown) and microscopic observations (Figure S8).

**pH-Dependent Hydrolysis and Cytotoxicity.** One unique feature of these thermoresponsive copolymers is the acid-labile property which is afforded by the pendent ortho ester groups. The pH-dependent hydrolysis behaviors of the copolymers were first studied by turbidimetric method. Figure 9 shows the turbidimetric changes as a function of time of copolymers A2 and B2 at 37 °C (above CPs) in

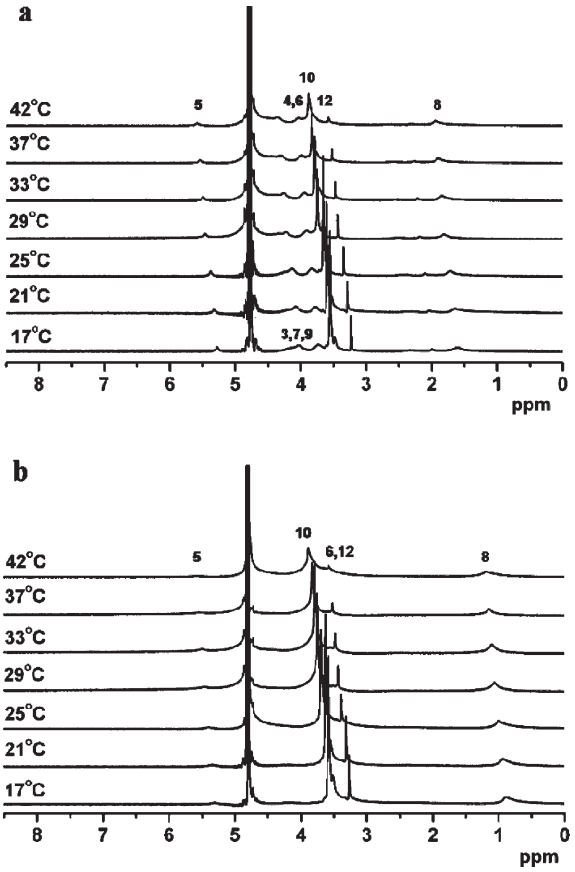



**Figure 8.** Temperature-dependent $^1$H NMR spectra of copolymer A3 (a) and copolymer B3 (b) in $D_2O$ (~5 mg/mL). The numbers denote the same proton signals as shown in Figure 1 and 2, respectively.

aqueous buffer solutions of different pHs. It was seen that the turbid solutions gradually became transparent with time due to the hydrolysis of the pendent ortho ester groups. The hydrolysis rate was dependent on pH: a lower pH resulted in a faster rate. At the same pH, the time for copolymer A2 solution to become transparent was much shorter than that for copolymer B2 solution. Since the molar content of the ortho ester units in copolymer B2 is less than in copolymer A2, the slower hydrolysis rate of copolymer B2 is definitely due to the more hydrophobic character of DMDEA unit as compared to DEA unit. In addition, the hydrolysis rate of the copolymers in the same series became faster as the content of the hydrophobic ortho ester groups was reduced (Figure S9).

To obtain detailed information on both hydrolysis kinetics and products, we measured $^1$H NMR spectra of copolymers A3 and B3 in the deuterated buffer (pD 4.6) at 37 °C at different incubation times (Figure 10). At beginning, the proton signals of the backbone and ortho ester units were very weak, especially for copolymer B3. Although the signals of the OEG side chains were clearly observable, the peak intensities were greatly reduced than that at lower temperatures (Figure 8). As hydrolysis time increased, the proton signals of the ortho ester groups (peak a for A3 and peaks a, d for B3) gradually disappeared. At the same time, the proton signals (a′, b′, and d′) assigned to the hydrolysis products became apparent (Figure 10 and Scheme 3). It can be seen that copolymer A3 was completely hydrolyzed within 45 min, while it took ~180 min for copolymer B3 to become totally hydrolyzed. These results are consistent with that obtained by the turbidimetric method as aforementioned, which further

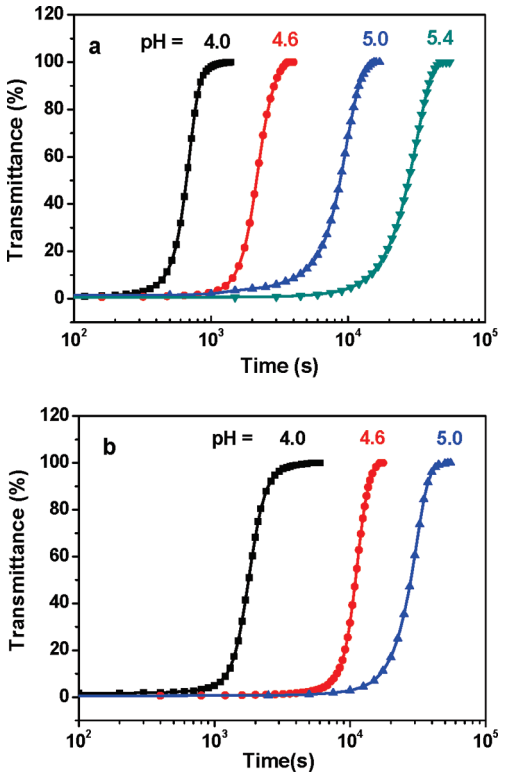



**Figure 9.** Transmittance versus hydrolysis time plots of copolymer A2 (a) and copolymer B2 (b) in acetate buffer solutions of various pHs at 37 °C, $\lambda$ = 500 nm, 1.0 mg/mL.

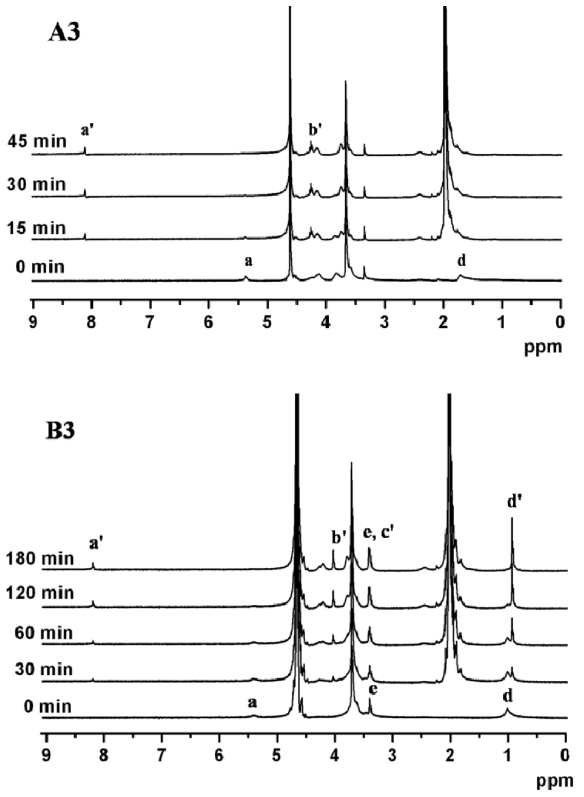



**Figure 10.** $^1$H NMR spectra of copolymer A3 (top) and copolymer B3 (bottom) in the deuterated acetate buffer (pH 4.6) at different hydrolysis times (37 °C).

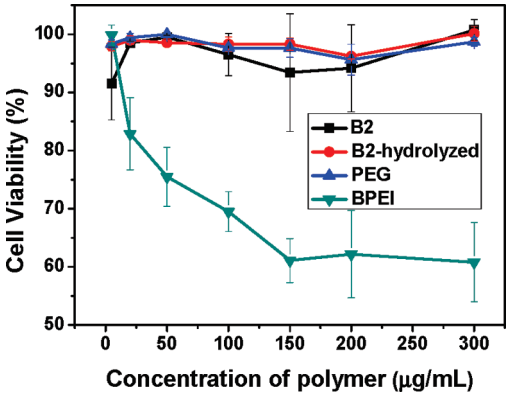


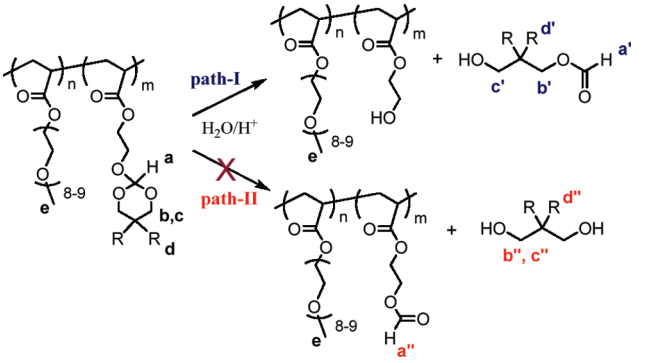

**Figure 11.** Hela cell viability detected with MTT assay. Results are presented as the mean ± SD in triplicate. The cells were incubated with the polymers for 24 h.

confirms that hydrophobicity is a key factor to control the hydrolysis rate. Regarding the hydrolytic products, there are two possible paths for the hydrolysis of ortho ester (Scheme 3). In path-I, cleavage of the exocyclic alkyloxy group of the ortho ester takes place first, producing the small molecular weight alkanediol monoformates. By contrast, through path II, the endocyclic ring cleavage would take place first, affording the alkanediols. From the $^1$H NMR spectra of the completely hydrolyzed samples, we can see that only one sharp peak appeared at ~8.0 ppm for both copolymers A3 and B3. This sharp peak is assigned to the formate proton a′, which can be supported by the appearance of the sharp peaks b′ and d′. Furthermore, acid-catalyzed hydrolysis of monomer DEA produced only HEA and 1,3-propanediol monoformate (Figure S10).

In order to clarify whether the ester bonds have been hydrolyzed during the acid-triggered hydrolysis of the ortho esters, both POEGA (homopolymer of OEGA) and copolymer B3 were treated by incubation in acetate buffer (pH 4.6) at 37 °C for 6 h. After dialysis and lyophilization, the purified polymers were characterized by $^1$H NMR measurements (Figure S11). It is seen that POEGA remains almost intact and hydrolysis of the ester bonds has not been observed after incubation in the weakly acidic buffer. This is also true for copolymer B3, we only observed the hydrolysis of ortho ester groups in the mildly acidic medium, affording a copolymer of OEGA and HEA with a similar molar ratio of the comonomers as for the parent copolymer B3. These NMR results reveal that both copolymers A3 and B3 are hydrolyzed through path-I, which is consistent with the acid-catalyzed hydrolysis mechanism of other six-member ortho ester and partially due to the stereoelectronic effect.[80]

A potential application of the present copolymers is for intelligent drug delivery systems. In order to evaluate the biocompatibility of this type of acid-labile copolymers,

**Scheme 3. Hydrolysis of the Ortho Ester Groups**



cytotoxicity of copolymer B2 and its hydrolyzed products, i.e., the copolymer of OEGA and HEA, as well as the alkanediol monoformates, was measured in Hela cells using MTT assay. PEG (MW 5K) and branched polyethylenimine (BPEI, MW 25K) were used as the negative and positive controls, respectively. As shown in Figure 11, the cell viability of both copolymer B2 and its hydrolyzed products were similar to that of PEG, indicating they had very low cytotoxicity within the tested concentration range.

## Conclusion

Acid-labile thermoresponsive polyacrylates with pendent OEG chains and cyclic ortho ester groups have been synthesized by copolymerization of OEGA with DEA or DMDEA. These copolymers are water-soluble at low temperature, but upon heating, undergo a liquid−liquid phase separation caused by thermally induced dehydration of the OEG side chains. Aqueous solution properties below LCST, thermally induced phase transition, as well as the pH-dependent hydrolysis behaviors of these copolymers are greatly influenced by the structure of the ortho ester units and composition of the copolymers. The copolymers with more hydrophobic ortho ester units have a stronger tendency to form associates below LCST and show obvious hysteresis during phase separation. All the copolymers are subject to mildly acidic media, resulting in the formation of OEGA and HEA copolymer. This type of acid-labile copolymers and their polymeric hydrolysis products have low cytotoxicity comparable to that of PEG; therefore, they may find applications as polymer carriers for tumor tissue and intracellular drug delivery.

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**Supporting Information Available:** Text giving experimental details and figures showing NMR spectra of the monomers and their homopolymers, more DLS results, micrographs, results of fluorescence probe, and more results of turbidimetric measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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