

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/258032260>

Toward Tertiary Amine-Modulated Acid-Triggered Hydrolysis of Copolymers Containing Pendent Ortho Ester Groups

ARTICLE in *MACROMOLECULES* · FEBRUARY 2013

Impact Factor: 5.8 · DOI: 10.1021/ma301964n

CITATIONS

11

READS

31

6 AUTHORS, INCLUDING:



Jing Cheng

Northwestern University

6 PUBLICATIONS 95 CITATIONS

SEE PROFILE



Fu-Sheng Du

Peking University

98 PUBLICATIONS 1,939 CITATIONS

SEE PROFILE



Zichen Li

Peking University

161 PUBLICATIONS 3,507 CITATIONS

SEE PROFILE

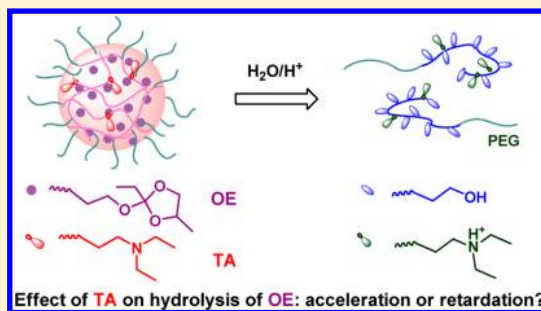
Toward Tertiary Amine-Modulated Acid-Triggered Hydrolysis of Copolymers Containing Pendent Ortho Ester Groups

Cheng-Cheng Song,[†] Cui-Cui Su,[†] Jing Cheng, Fu-Sheng Du,^{*} De-Hai Liang,^{*} and Zi-Chen Li^{*}

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, People's Republic of China

Supporting Information

ABSTRACT: We describe an efficient approach to modulate the hydrolysis of the ortho ester-containing polymers by incorporating different amount of tertiary amino (TA) groups. The block copolymers (NE0–NE3) contain a hydrophilic poly(ethylene glycol) (PEG) segment and hydrophobic chains constituted by random methacrylate copolymers with pendent acid-labile cyclic ortho ester and TA groups and were synthesized by a two-step approach. First, atom transfer radical copolymerization of 2-hydroxyethyl methacrylate and 2-(diethylamino)-ethyl methacrylate using mPEG₄₅-Br as a macroinitiator afforded the block copolymer precursors. Then, reaction of the precursors with 2-ethylidene-4-methyl-1,3-dioxolane transformed the pendent hydroxyl groups into cyclic ortho ester groups. The hydrophobic chains are similar in degree of polymerization, but the percent molar content of TA increases from 0% for NE0 to ~15% for NE3. In phosphate buffer at pH 8.4, all the four amphiphilic block copolymers can self-assemble into stable nanoparticles with a monomodal distribution, which have a similar hydrophilic/hydrophobic balance as revealed by using pyrene and Nile red (NR) as fluorescent probes. Kinetic measurements of the ortho ester hydrolysis in the copolymer nanoparticles were studied at different pH values by ¹H NMR spectroscopy, NR fluorescence probe, and light scattering. The results indicated that all the copolymer nanoparticles exhibit the pH-dependent hydrolysis behaviors with the half-life times ranging from hundreds of minutes at pH 5.4 to several days at neutral pH. More importantly, we found that the TA units have an amphoteric effect on the hydrolysis kinetics of the surrounding pendent ortho esters in acidic media. When compared with copolymer nanoparticles of NE0 with no TA unit, the NE1 nanoparticles with a small amount of TA unit hydrolyzed much slower, whereas a faster hydrolysis was observed for the NE3 nanoparticles containing a higher amount of TA unit.



INTRODUCTION

The degradation or hydrolysis rate of a biodegradable polymer is a key factor for its use in biomedical applications. For example, the performance of a DNA-based vaccine formulation or the immunological pathway of a protein-based vaccine system was closely related to the release profile of the bioactive payload (DNA or protein), which was mainly controlled by degradation rate of the polymeric carriers used.^{1,2} In addition, the degradation profile (surface vs bulk) of the polymeric scaffolds may influence their performance for tissue engineering of bone.³ Various factors such as polymer structure and composition, molecular weight and its distribution, crystallinity, hydrophobicity, specimen size and shape, and so forth affect the degradation kinetics of the degradable polymers.^{4–9}

The pH-sensitive polymers, including those with ionizable weak acid or base and those with acid-cleavable linkage, represent an important type of intelligent polymers which have great potential for applications in many fields.^{10–13} Among various pH-sensitive polymers, the ortho ester- or acetal-containing polymeric materials are usually neutral and may have better biocompatibility.^{2,14–16} The backbone-degradable poly(ortho ester)s (POEs) have been studied extensively over the

past several decades, showing promising properties as drug vehicles because of their unique features such as tunable degradation times, various physical forms, ease of manufacture, and good biocompatibility.^{17,18} Furthermore, polyacetals (or polyketals) have attracted great interests as drug carriers, in particular for curing the acute inflammatory diseases, partly due to their neutral degradation products.^{14,19,20} Besides these backbone-type POEs and polyacetals, various acid-labile polymers with pendent ortho ester or acetal groups have been reported recently.^{15,21–27}

There are many factors that influence degradation or hydrolysis of the acid-labile polymers containing ortho ester or acetal groups. While the acid-triggered hydrolysis rates of ortho esters or acetals depend mainly on their chemical structures,^{28,29} the degradation behaviors of the polymers can be modulated by tuning other parameters such as hydrophilic/hydrophobic balance, addition of acidic or basic excipients, and so forth.^{30–33} Heller and co-workers have developed a family of

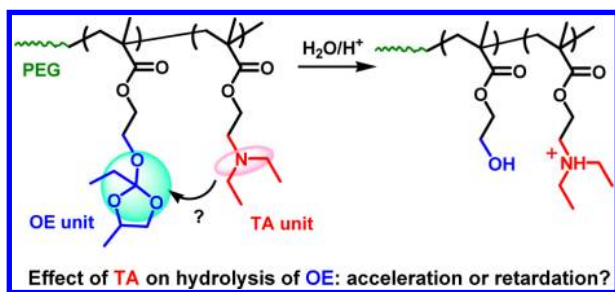
Received: September 19, 2012

Revised: January 23, 2013

Published: February 1, 2013

autocatalyzed POEs by incorporating a short segment of latent acids (oligomers of lactic acid or glycolic acid) into the backbone. The erosion rates of the polymers can be finely tuned in a wide range by controlling the content of the latent acids as well as the polymer hydrophilicity. Compared to the physical addition of acidic excipients, these autocatalyzed POEs have the advantages of reproducible production and easy control of mechanical or thermal properties.¹⁶ Recently, Almutairi et al. reported a type of logic gate nanoparticles formed from a poly(β -amino ester) containing a ketal moiety in the backbone. They found that the hydrophilicity of the particle increased due to the protonation of the tertiary amine as the buffer pH decreased from 7.4 to 5.0, which greatly accelerated the ketal hydrolysis.³⁴ Although some POEs containing tertiary amino groups in the backbone were prepared for drug or gene delivery, the effect of the amines on hydrolytic degradation of the POEs has not been studied in detail.^{1,35} In recent years, we have developed a series of acid-labile polymers with pendent cyclic ortho esters and found that the chemical structure and stereo configuration of the pendent ortho esters as well as the hydrophobic/hydrophilic balance of the polymers affect the hydrolysis profiles of the ortho esters.^{29,36,37} In this work, we intend to report another approach to modulate the ortho ester hydrolysis by covalently incorporating tertiary amino (TA) groups into the ortho ester-containing polymers (Scheme 1).

Scheme 1. Effect of Tertiary Amine (TA) on the Hydrolysis of Ortho Ester



Our purpose is to demonstrate how the TA content in the polymers and the buffer pH influence the ortho ester hydrolysis in a combined and modulated fashion. We found that, at neutral pH, the TA unit showed little effect on the hydrolysis of the ortho ester groups. In acidic media, the TA unit accelerated the ortho ester hydrolysis for the copolymer with a high TA content. Unexpectedly, a hindering effect was observed when the content of the TA unit decreased to some magnitude.

These results are helpful for designing pH-sensitive polymers that may have potential application for intracellular anticancer drug delivery or polymer-based vaccine.^{2,15,26}

EXPERIMENTAL SECTION

Materials. 2-Hydroxyethyl methacrylate (HEMA, Aldrich) and 2-(diethylamino)ethyl methacrylate (DEAEMA, Aldrich) were passed through a short basic Al_2O_3 column prior to use. Macroinitiator mPEG₄₅-Br and 2-ethylidene-4-methyl-1,3-dioxolane (EMD) were synthesized by using the reported procedures.^{29,38} CuBr was sequentially washed with acetic acid, methanol, and ether. DMSO was dried over CaH_2 overnight, followed by distillation under reduced pressure. THF was distilled over sodium prior to use. Triethylamine (TEA) and CDCl_3 were dried over KOH and K_2CO_3 , respectively, prior to use. The deuterated phosphate buffers with different pHs were prepared from NaOD (40% in D_2O , Alfa) and deuterated phosphoric acid (85% in D_2O) by changing their ratios. Nile red (NR, Aldrich), pyrene (Py, Acros), 2,2'-bipyridine (Alfa), methanol (HPLC grade, Tianjin Siyou Co.), and d_4 -MeOD (Cambridge Isotope Laboratories, Inc.) were used as received.

Polymerization. Copolymers of HEMA and DEAEMA were synthesized using mPEG₄₅-Br as a macroinitiator by the atom transfer radical polymerization (ATRP). Take the synthesis of copolymer N3 in Table 1 as an example. HEMA (2.00 g, 15.4 mmol), DEAEMA (0.43 g, 2.31 mmol), mPEG₄₅-Br (0.51 g, 0.25 mmol), and 2,2'-bipyridine (80 mg, 0.51 mmol) were charged into an ampule tube, to which methanol (2.0 g) was added to dissolve the monomer and initiator. After two cycles of freeze–pump–thaw to thoroughly remove oxygen, CuBr (36 mg, 0.26 mmol) was added into the tube under a nitrogen atmosphere, and the tube was sealed in vacuo. The polymerization was carried out at 25 °C for 2 h and quenched by quickly cooling in liquid nitrogen. After warming to room temperature, the solution was diluted with methanol and passed through a short basic Al_2O_3 column to remove the catalyst. The polymer was precipitated from a mixed solvent of THF and diethyl ether (1:2, v/v) at ~ 0 °C and dried in vacuo to afford a white solid in 62% yield. The other block copolymers (N0, N1, and N2) were prepared in a similar way in 60–80% yields by changing the feed molar ratios of the two monomers. Compositions of the copolymers were determined by ^1H NMR measurement. Molecular weights of the copolymers were measured by gel permeation chromatography (GPC) using DMF as eluent and poly(methyl methacrylate)s as standards (see the Supporting Information).

Synthesis of Block Copolymers with Pendent Ortho Ester.

Four block copolymers (NE0 to NE3) with pendent ortho ester groups were prepared by modifying their precursors (N0 to N3) with EMD. Take copolymer NE3 as an example. Block copolymer N3 (0.30 g, ~ 1.5 mmol of hydroxyl groups) was thoroughly dissolved in dried DMSO (2.5 g) at 30 °C. After sequential addition of 0.70 g of EMD (6.1 mmol in 0.7 g of THF) and 150 μL of *p*-toluenesulfonic acid (TsOH)–THF solution (10 mg/mL), the mixture was stirred at 30 °C

Table 1. Characterization of the Copolymers of N Series and NE Series

N series ^a	(<i>m</i> + <i>n</i>): <i>p</i> ^b	<i>C</i> _{amine} ^c (%)	<i>M</i> _{n,NMR} ^d	<i>M</i> _n ^e	PDI ^e	NE series	<i>m</i> : <i>n</i> : <i>p</i> ^f	DS ^g (%)	CAC ^h (mg/L)	<i>I</i> ₁ / <i>I</i> ₃ ^h	λ_{max} ⁱ (nm)
N0	66:0	0	10 700	17 000	1.64	NE0	61:5:0	92	0.51	1.42	607
N1	56:3	5.1	9900	15 000	1.66	NE1	53:3:3	94	0.63	1.43	608
N2	50:6	10.7	9700	14 500	1.63	NE2	46:4:6	92	0.61	1.44	606
N3	54:9	14.3	10 800	14 700	1.54	NE3	50:4:9	93	0.64	1.43	607

^aExperimental condition: 2 h, 25 °C in methanol, mPEG₄₅-Br as the macroinitiator, CuBr/bipyridine as the catalyst. ^bMolar ratio of HEMA unit to DEAEMA unit in the N series copolymers calculated by ^1H NMR spectroscopy. ^cPercent molar content of DEAEMA unit in the N series copolymers as calculated by $p/(m + n + p)$. ^dMolecular weight of N series copolymers calculated by ^1H NMR spectroscopy. ^eNumber-averaged molecular weight and the polydispersity index of the N series copolymers determined by GPC using PMMA standards in DMF. ^fMolar ratio of the units of ortho ester, HEMA and DEAEMA in NE series copolymers calculated by ^1H NMR spectroscopy. ^gDegree of substitution of the ortho ester groups to the total hydroxyl groups as calculated by $m/(m + n)$. ^hCritical aggregation concentration of the polymers and I_1/I_3 ratio of the nanoparticles (0.1 mg/mL) were measured by using pyrene as a fluorescence probe at 25 °C. ⁱMaximum wavelength of the fluorescence spectra of Nile red loaded in the particles of NE series copolymers.

for 5 h, followed by adding ~0.2 mL of TEA to quench the reaction. The crude product was obtained by precipitation from diethyl ether and purified by three cycles of dissolving in CH_2Cl_2 and precipitating from a mixed solvent of diethyl ether and hexane containing 1% TEA. After drying in vacuum at ambient temperature, colorless viscous product was obtained in 80% yield. Other copolymers (NE0, NE1, and NE2) were prepared by a similar procedure in 70–80% yields. The degree of substitution (DS) of the ortho ester to the hydroxyl group of HEMA unit was determined by ^1H NMR spectroscopy.

NMR Measurement. ^1H NMR spectra of the polymers in d_4 -MeOH or CDCl_3 were recorded on a Bruker ARX 400 MHz spectrometer at ambient temperature. ^1H NMR spectra of the polymers upon pH-dependent hydrolysis in the deuterated phosphate buffer (PB) were measured on the Varian Mercury Plus 300 MHz spectrometer at 37 °C.

Critical Aggregation Concentration (CAC). The aggregate dispersions (1.0 mg/mL) of NE copolymers in PB were prepared by a solvent-displacement method. Briefly, the copolymer (5.0 mg) was dissolved in DMSO (1.0 mL) containing 10 μL of TEA. This solution was added dropwise into 3 mL of pH 8.4 PB (10 mM) under ultrasound at ambient temperature in 5 min. After an additional 10 min of incubation with sonication, the obtained dispersion was dialyzed against PB (pH 8.4, 10 mM) for 4 h and adjusted to a volume of 5.0 mL by using the same buffer. This stock dispersion (1.0 mg/mL) was used to prepare samples of different polymer concentrations. During the dialysis, a tubing with MWCO 14 kDa was used, and the buffer was changed 3-fold. For the samples of fluorescence measurements, 100 μL of pyrene (Py) in acetone (0.06 mM) was added into a 10 mL volumetric flask, and the acetone was evaporated at 45 °C (~2 h). To the flask containing Py, various volumes of the stock dispersion were added and diluted to 10 mL. After sonication for 30 min and incubation overnight in a shaker, the excitation spectra of the polymer dispersions were recorded from 300 to 360 nm on a Hitachi F-4500 fluorescence spectrometer equipped with a temperature controller, with an emission wavelength of 390 nm and a scanning rate of 240 nm/min at 25 °C. Both excitation and emission slit widths were 2.5 nm. I_{338}/I_{333} was defined as the ratio of emission intensity at 338 nm to that at 333 nm. For emission spectra of pyrene, an excitation wavelength of 339 nm was used. I_1/I_3 was defined as the intensity ratio of the first to the third bands of the emission spectrum.

pH-Dependent Hydrolysis Monitored by ^1H NMR Spectroscopy. Aggregate dispersions of the block copolymers (6.0 mg/mL) in deuterated PB (pH 8.4, 1.0 mM) were prepared by a solvent evaporation method. Take NE3 as an example; 18 mg of the polymer was dissolved in 0.25 mL of deuterated acetone, followed by slowly adding 3 mL of deuterated PB (pH 8.4, 1.0 mM) under stirring. During this procedure, the transparent solution gradually became opalescent. After removal of the acetone on a rotary evaporator under reduced pressure, the aggregate dispersion was sonicated for 30 min and stirred in air for an additional 0.5 h at room temperature. Then, the polymer concentration of the aggregate stock dispersion was adjusted to 6.0 mg/mL by adding deuterated PB. For ^1H NMR measurements, 0.5 mL of NE3 stock dispersion was charged into a NMR tube, which was air-sealed by using Parafilm. After incubation at 37 °C for 10 min, the ^1H NMR spectrum was collected and used as that of 0 time point. Then, 50 μL of deuterated PB (0.5 M, pH 5.2) was added to adjust the polymer dispersion to pH 5.4, and the time-dependent ^1H NMR spectra were recorded at specific times at 37 °C. For the hydrolysis experiments at pH 7.6 and 6.5, the deuterated PBs (50 μL , 0.5 M) with pH ~7.4 and ~6.2 were added, respectively. For other polymers, the same experimental procedure was applied. The half-life time ($t_{1/2}$) of each sample was estimated from its kinetics curve.

pH-Dependent Hydrolysis Monitored by NR Fluorescence. Aggregate dispersions of the block copolymers (0.1 mg/mL) in PB (pH 8.4, 10 mM) were prepared by a similar procedure as for the samples of CAC measurements but using acetone instead of DMSO to dissolve the polymers. To 10 mL of the aggregate dispersion, 10 μL of NR in ethanol (1.0 mM) was added, and the mixed solution was sonicated for 30 min at ambient temperature. After incubation for an

additional 10 min at 37 °C, the fluorescence spectrum of the dispersion was collected and used as that for 0 time point. Then, the dispersion was adjusted to pH 5.5 by adding a concentrated acetate buffer (pH ~5.2, 1.0 M); the time-dependent emission spectra were recorded at specific time points at 37 °C. For hydrolyses at pH 6.1, 6.7, and 7.5, the concentrated phosphate buffers (0.5 M) with different pHs were applied. Both the excitation and emission slit widths were 5.0 nm. The emission spectra were collected from 560 to 700 nm at an excitation wavelength of 545 nm.

pH-Dependent Hydrolysis Monitored by Light Scattering.

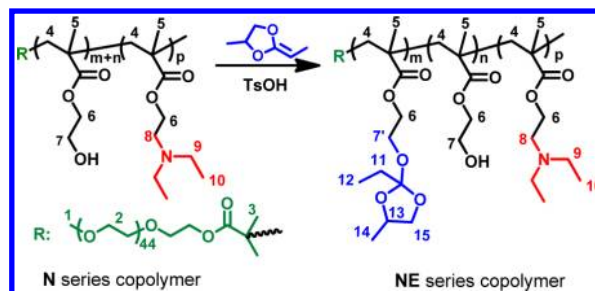
Aggregate dispersions of the block copolymers (0.1 mg/mL) in PB (pH 8.4, 10 mM) were prepared by the same procedure as for the samples of CAC measurements. The dispersion was filtered through a 0.45 μm PES filter into a dust-free vial which was maintained at 37 °C for 30 min prior to the LLS measurement. Hydrolysis of the samples was initiated by adding a low-pH buffer with high concentration. For example, prefiltered acetate buffer (5.0 M, pH 5.2) was added in the aggregate dispersion to tune the pH to 5.5. The moment of mixing was set as the 0 time point. The mixed solution was quickly vortexed at 700 rpm for 20 s right before the measurement of LLS at 37 °C. A commercialized spectrometer from Brookhaven Instruments Corporation (BI-200SM Goniometer, Holtsville, NY) was used to perform both static light scattering (SLS) and dynamic light scattering (DLS) over a scattering angular range of 20°–150°. A vertically polarized, 100 mW solid-state laser (GXC-III, CNI, Changchun, China) operating at 532 nm was used as the light source, and a BI-TurboCo Digital Correlator (Brookhaven Instruments Corporation) was used to collect and process data. For details, please refer to the Supporting Information.

RESULTS AND DISCUSSION

Synthesis and Characterization of Block Copolymers.

Four block copolymers (N0–N3) were prepared by the atom transfer radical copolymerization of HEMA and DEAEMA with different feed ratios, using mPEG₄₅-Br as a macroinitiator. These copolymers are designed to have a similar HEMA content but an increased amount of DEAEMA unit from copolymers N0 to N3 (Scheme 2 and Table S1). By comparing

Scheme 2. Modification of N Series Copolymers by EMD to Afford NE Series Copolymers



the integration intensities of peak 7 (~3.8 ppm), peak 8 (~2.8 ppm), and peak 1 (~3.4 ppm) in the ^1H NMR spectra of the N series copolymers, the degree of polymerization (DP, $m + n + p$) and the percent molar contents of DEAEMA unit were determined (Figure 1 and Figures S1–S4). From copolymers N0 to N3, the percent molar content of DEAEMA unit increases from 0 to ~15% while they all have similar chain lengths as indicated by the ^1H NMR and GPC results (Table 1 and Figure S5).

Cyclic ketene acetal (CKA) quickly and efficiently reacts with primary or secondary hydroxyl groups to afford cyclic ortho esters under very mild conditions.²⁹ Here, the amphiphilic block copolymers (NE0–NE3) having the pendent ortho ester

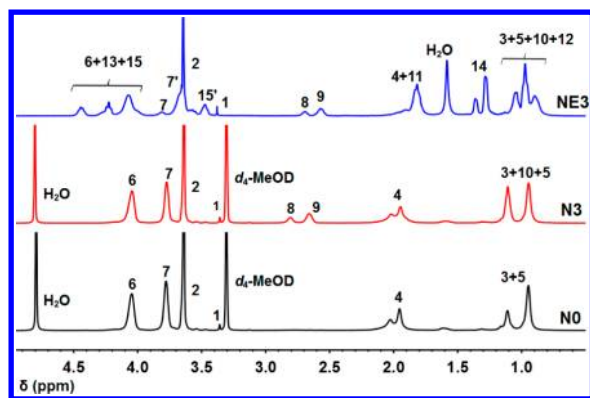


Figure 1. ^1H NMR spectra of copolymers of N0 and N3 in d_4 -MeOD and NE3 in CDCl_3 .

groups were easily prepared by modifying the N series copolymers with EMD (Scheme 2). The degree of substitution (DS) of the ortho ester groups to the total hydroxyl groups, defined as $m/(m+n)$, was determined by comparing the integration intensities of peak 7 (~ 3.8 ppm) and peak 14 (~ 1.3 ppm) in the ^1H NMR spectra (Figure 1 and Figures S1–S4). It is seen that all the four copolymers had similar DSs (Table 1).

Characterization of the Copolymer Aggregates. The diblock copolymers are amphiphilic in nature with PEG as the hydrophilic segment and the ortho ester containing copolymer being hydrophobic. Therefore, all the four copolymers self-assembled into monomodal distributed nanoparticles in pH 8.4 phosphate buffer as revealed by DLS and TEM. The average dynamic radii (R_h s) of the particles at zero angle range from 30 to 60 nm, with the size of NE0 particle being the largest (Figure 2, Figure S6, Table S2). Critical aggregation concentrations

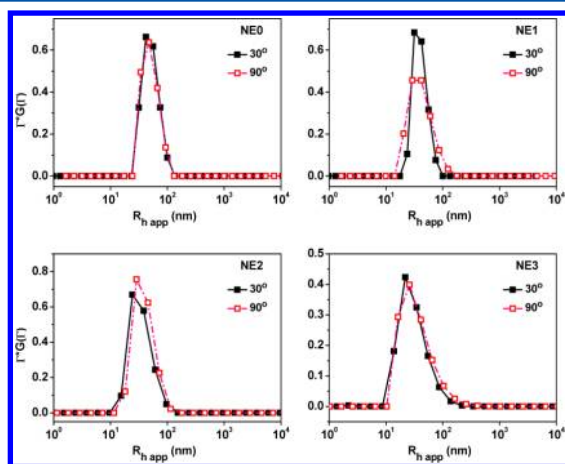


Figure 2. Size distribution of the copolymer nanoparticles in PB (pH 8.4, 10 mM). Polymer concentration: 0.1 mg/mL.

(CACs) of the block copolymers were then determined by using pyrene as a fluorescence probe, and the data are only slightly different, ranging from 0.51 mg/L for NE0 to 0.64 mg/L for NE3 (Table 1 and Figure S7). It means that incorporating TA unit only slightly influences the CACs of the amphiphilic copolymers. In addition, we measured the fluorescence spectra of NR and pyrene loaded in the block copolymer nanoparticles and found that the maximum wavelengths of NR (Figure S8) or the I_1/I_3 ratios of pyrene (Table 1) were almost the same for all the copolymer particles. Collectively, these results reveal that

the copolymer nanoparticles may have a similar hydrophobicity in their hydrophobic microdomains.

pH-Dependent Hydrolysis Monitored by ^1H NMR and Fluorescence Probe. The pH-dependent hydrolysis behaviors of the copolymer nanoparticles were first studied by ^1H NMR measurements at 37°C . The representative time-dependent ^1H NMR spectra of NE2 nanoparticles at pH 5.4 are shown in Figure 3. Initially, proton signals of the

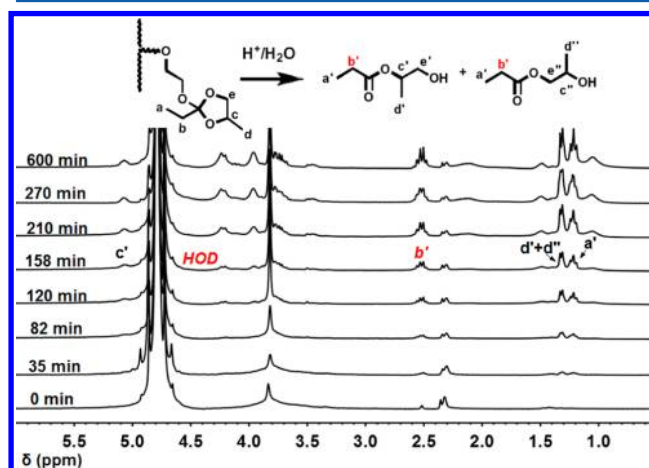


Figure 3. ^1H NMR spectra of copolymer NE2 (6 mg/mL) in deuterated PB (pH 5.4, 50 mM) at different hydrolysis times. Relative amount of the hydrolyzed ortho ester is calculated by the formula $(I_t - I_0)/(I_\infty - I_0)$, where I_0 , I_t , and I_∞ denote the peak intensities of proton b' (~ 2.5 ppm) at 0 min, t min, and of the completely hydrolyzed sample (at 600 min). Proton peak of HOD (~ 4.8 ppm) is used as the internal standard.

hydrophobic block were almost invisible; unexpectedly, the signal of PEG protons was also very weak, though it gradually enhanced with time. We speculate that the nanoparticles did not have the typical micellar morphology, and some PEG chains may be trapped within the core. This is well consistent with their R_g/R_h ratios, being ~ 1.0 (Table S2). After NE2 particles were completely hydrolyzed, all the proton signals of the hydrolysis products were clearly observed.²⁹ Hydrolysis degree of the ortho ester groups was calculated by monitoring the intensity increase of the peak at ~ 2.5 ppm (proton b') using the peak at ~ 4.8 ppm (HOD) as a standard. For other copolymer particles or at different pHs, the data were obtained in the same way. The hydrolysis kinetic curves are shown in Figure 4, and the half-life times ($t_{1/2}$) are summarized in Table 2. It was found that all the copolymer particles demonstrated pH-dependent hydrolysis profiles with half-life times ranging from hundreds of minutes at pH 5.4 to several days at neutral pH. This can be rationally explained by the extremely pH-sensitive nature of the pendent ortho ester.

At the same pH, hydrolysis rates of the ortho esters followed a general order of $\text{NE3} > \text{NE0} \approx \text{NE2} > \text{NE1}$. However, the differences between $t_{1/2}$ values of various samples depended greatly on the medium pH. (1) At pH 7.4, the hydrolysis rates of the four copolymer nanoparticles were only slightly different, with the $t_{1/2}$ ratio being 0.84:1.0:1.1:1.2 ($\text{NE3}:\text{NE0}:\text{NE2}:\text{NE1}$). (2) At pH 5.4, however, the differences of $t_{1/2}$ values became much more pronounced, and the ratio changed to 0.47:1.0:1.1:1.9 ($\text{NE3}:\text{NE0}:\text{NE2}:\text{NE1}$). In other words, as compared to NE0 nanoparticle with no TA unit, hydrolysis of the NE3 sample with more TA was faster, whereas the NE1

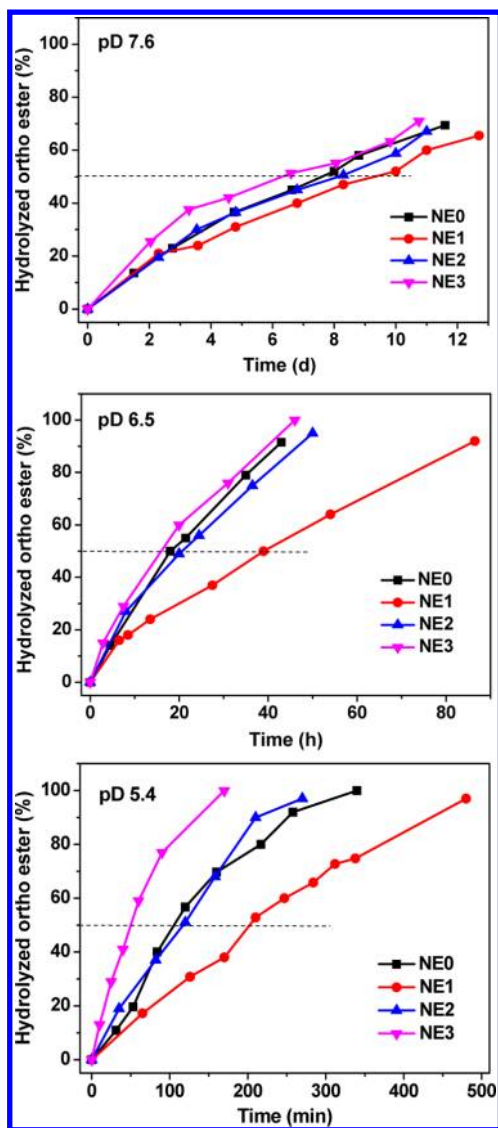


Figure 4. Hydrolysis kinetics of NE series copolymers at different pHs. Hydrolysis condition: 50 mM deuterated PB, 37 °C.

Table 2. Half-Life Times ($t_{1/2}$) of the Hydrolyses of NE Nanoparticles at Different pHs^a

polymer	$t_{1/2}$		
	pH = 7.4 (days)	pH = 6.5 (h)	pH = 5.4 (min)
NE0	7.7 (1)	18.0 (1)	106 (1)
NE1	9.4 (1.2)	39.0 (2.2)	203 (1.9)
NE2	8.2 (1.1)	20.6 (1.1)	117 (1.1)
NE3	6.5 (0.84)	16.0 (0.89)	50.0 (0.47)

^aThe values of $t_{1/2}$ were obtained from the kinetic curves shown in Figure 4. The $t_{1/2}$ ratios of NE x ($x = 1-3$) to NE0 are shown in parentheses.

nanoparticle with less TA hydrolyzed even much slower. (3) At pH 6.5 (mildly acidic medium), hydrolysis of NE0 nanoparticle was still much faster than that of NE1, whereas the $t_{1/2}$ difference between NE3 and NE0 samples became less distinguishable. These results can be elucidated by the amphoteric effect of the TA unit on hydrolysis of the pendent ortho ester.

Like other polyesters,^{7,8,39} degradation or hydrolysis rates of the ortho ester-containing acid-sensitive polymers are significantly influenced by their hydrophilic/hydrophobic balance.^{29,40} Furthermore, incorporation of acidic components³² or latent acids^{33,41} greatly accelerates the degradation of poly(ortho ester)s while the basic ingredients such as Mg(OH)₂ or Na₂CO₃ exert a contrary effect.^{32,42} In our case, TA units may exert two opposite but competitive effects on the hydrolysis of the ortho ester, depending on both TA percent content and pH of the buffer used. Protonation of the TA unit in acidic medium results in an increase in their hydrophilicity, enhancing the uptake of water molecules and hydronium ions into the nanoparticles.^{34,43,44} This will speed up hydrolysis of the ortho ester moieties. On the other hand, protonation of the TA unit also consumes the hydronium ions, which may temporarily increase the local pH of the microdomains around the TA units. This buffering effect generally slows down the degradation of poly(ortho ester)s. In the present system, these two opposite effects work in different ways, resulting in different hydrolysis behaviors of the nanoparticles. (1) At pH 5.4, partially protonation of the TA unit of NE3 nanoparticles can generate a number of interconnected hydrophilic channels which are essential for water molecules or hydronium ions to diffuse efficiently into the core of the particles. Possible buffering effect of the TA groups can be quickly counterbalanced by the rapid uptake of hydronium ions from outside. In contrast, NE1 copolymer contains fewer TA groups and is much more hydrophobic at pH 5.4 than NE3. Therefore, less amounts of hydrophilic channels are formed, being probably not well interconnected each other. This results in difficulty for NE1 particle adsorbing water molecules and hydronium ions. In such a case, the buffering effect might dominate, making NE1 hydrolyze even slower than NE0. (2) At pH 6.5, the buffering effect of the TA unit that slows down the ortho ester hydrolysis still plays a dominating role for NE1 particle. In the case of NE3 particle, however, the accelerating effect caused by TA protonation at pH 6.5 was greatly reduced as compared to that at pH 5.4 because the protonation degree of the TA units is expected much lower at pH 6.5 than at pH 5.4. When the PB buffer concentration was increased from 50 to 100 mM, which is helpful for increasing the degree of TA protonation, the accelerating effect of the TA unit reoccurred (Figure S11A). (3) In neutral buffer, the TA units were little protonated because the buffer pH (~7.6) was slightly higher than the pK_a (~7.0–7.4) of poly(2-(diethylamino)ethyl methacrylate).^{44,45} Thus, the TA units exerted little effect on the ortho ester hydrolysis.

Nile red (NR) is a highly polarity-dependent fluorescence probe; it displays very weak fluorescence in aqueous solution but is highly fluorescent in hydrophobic environment.⁴⁶ We also used NR as a probe to monitor the hydrolysis profiles of the NE series particles. As expected, the fluorescence intensity of NR in all of the particles gradually decreased with incubation time due to the hydrophilicity increase caused by the hydrolysis of the ortho esters (Figure 5 and Figure S12). A lower pH resulted in a faster hydrolysis rate (Figure S13). When the buffer pH was identical, the hydrolysis behaviors detected by using NR probe were in general consistent with the ¹H NMR results, following the hydrolysis rate order of NE3 > NE0 (>NE2) > NE1 in acidic media. At pH 7.4, there was no distinguishable difference for NE0 to NE3 nanoparticles, implying little effect of the TA unit on the ortho ester hydrolysis.

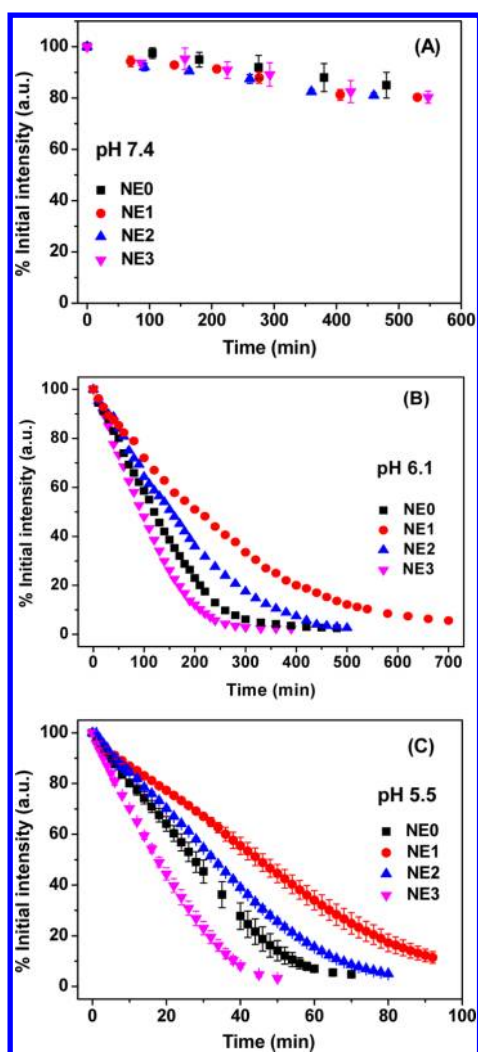


Figure 5. Time-dependent change of the normalized intensity of Nile red (NR) fluorescence in NE nanoparticles at different pHs (7.4, 6.1, and 5.5). Concentrations of the polymer, NR, and buffer are 0.1 mg/mL, 1.0×10^{-6} M, and 100 mM, respectively. $\lambda_{\text{ex}} = 545$ nm, 37 °C. The measurements at pH 7.4 (A) and 5.5 (C) were conducted in triplicate.

Hydrolysis Monitored by Light Scattering. The hydrolysis profiles of the NE nanoparticles were further studied by light scattering measurements. These particles were stable at pH 8.4; no prominent changes in either size or scattered intensity were observed in 30 h, suggesting that the hydrolysis of the pendent ortho ester was extremely slow (Figure S14). However, the hydrolysis was significantly promoted with decreasing pH to 5.5. Figure 6 shows the evolutionary changes of the excess scattered intensity and hydrodynamic radius of the particles during the hydrolysis at pH 5.5. For a better comparison, the scattered intensity was normalized by intensity of the dispersion at the same polymer concentration but with pH 8.4.

Clearly, NE0 particles underwent a two-stage hydrolysis: dissociation and reaggregation. The scattered intensity of NE0 dispersion sharply decreased in the first 50 min, with no change of the apparent hydrodynamic radius ($R_{\text{h,app}}$), suggesting that the density of the particle decreased. After 50 min, both the scattered intensity and $R_{\text{h,app}}$ increased dramatically, which can be attributed to the reaggregation of the completely or partially

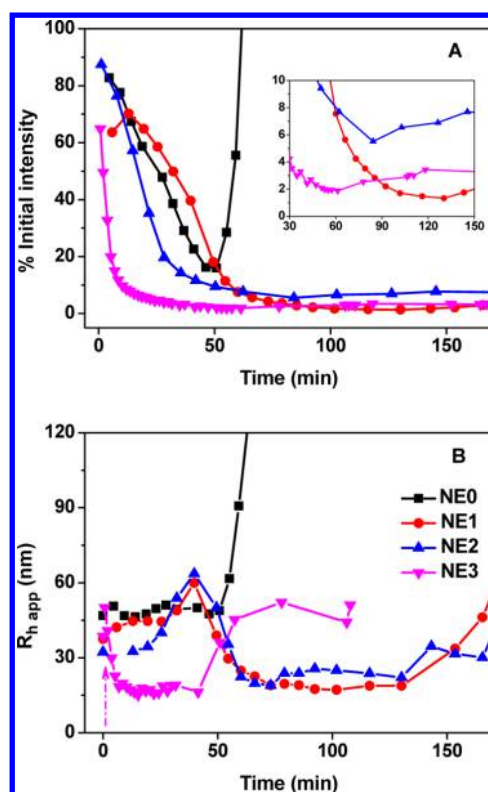


Figure 6. Time dependence of the scattered intensity (A) and hydrodynamic radius of the particles during hydrolysis at pH = 5.5. The inset in panel A magnifies the intensity of NE1 and NE3 in the time range of 50–180 min. The lines are used to guide the eyes. The initial concentrations for all the polymers are 0.1 mg/mL.

hydrolyzed NE0, that is, the PEG-*b*-PHEMA block copolymer or its derivatives with some pendent ortho ester groups. It was well-known that PHEMA with a DP more than 20 was not well soluble in water at 37 °C, partly due to the presence of H-bond between hydroxyl groups on the polymer chains.⁴⁷ In the case of TA-containing copolymers (NE1–NE3), again, we observed the amphoteric effect of TA unit on the ortho ester hydrolysis. The decreasing rate in scattered intensity followed an order of NE3 > NE2 > NE0 > NE1. The scattered intensity of NE3 dropped ~80% in 5 min, while it took ca. 45 min for the intensity of NE0 to decrease to the same level. On the contrary, the hydrolysis of NE1 was a little slower than that of NE0. Furthermore, the hydrolyzed polymer of NE1 to NE3, at least in the middle stage, was smaller in size and lower in molecular weight (demonstrated as the extremely low scattered intensity) compared to that of NE0. For NE1–NE3, the minimum scattered intensity during hydrolysis was lower than 6% of the original value, and the minimum $R_{\text{h,app}}$ was below 20 nm. This can be rationally ascribed to the hydrophilicity of the protonated TA unit as well as the electrostatic repulsion between them. The increase in size or in scattered intensity at ~50 min for NE3, ~80 min for NE2, and ~120 min for NE1 might be attributed to the loose polymeric associates formed by the hydrolyzed polymers.

It should be noticed that the TA-containing particles swelled in the early stage of hydrolysis. In particular, swelling of NE3 particles was very fast, the initial particle size of NE3 was ca. 10 nm larger than that at pH 8.4 (indicated by the arrow in Figure 6B). This also indicated the rapid uptake of water molecules as well as hydronium ions due to the TA protonation, which is

well consistent with the ^1H NMR results. The swelling of NE1 particles was much slower as compared to NE3. As shown in Figure 6B, it took ~ 45 min for the $R_{h,app}$ to increase from 38 to ~ 60 nm, indicating a relatively slow uptake of water molecules and hydronium ions. Thus, the buffering effect of the TA unit dominates over its accelerating effect caused by the increase in hydrophilicity.

CONCLUSION

We have prepared a series of acid-labile amphiphilic block copolymers containing pendent ortho ester and tertiary amino (TA) groups using sequentially ATRP and the postmodification method. These copolymers could self-assemble in pH 8.4 PB to form stable spherical nanoparticles and exhibited pH-dependent hydrolysis behaviors. Our results demonstrated that the TA groups exert two opposite but competitive effects on the hydrolysis of the surrounding ortho ester groups, that is, buffering effect to slow down the hydrolysis and protonation-induced hydrophilicity increase to speed up the hydrolysis. The effects were dependent on both the TA contents in the copolymers and the buffer pH of the medium used. In mildly acidic media, a low TA content ($\sim 5\%$) in the copolymer retarded the hydrolysis of the ortho ester, while a higher TA content ($\sim 14\%$) accelerated the ortho ester hydrolysis. This work provides a facile method to tune the hydrolysis of the ortho ester-containing polymers by covalently introducing different amounts of TA unit.

ASSOCIATED CONTENT

Supporting Information

Additional ^1H NMR spectra, NR emission spectra, GPC curves, more light scattering results, and CAC measurements. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Tel +86-10-62757155; Fax +86-10-62751708; e-mail fsdu@pku.edu.cn (F.D.); dliang@pku.edu.cn (D. L.); zcli@pku.edu.cn (Z.L.).

Author Contributions

† These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (21090351, 21174002, and 21225416). We thank Prof. Xuefeng Fu and Mr. Yaguang Zhao for their help in GPC measurements.

REFERENCES

- (1) Wang, C.; Ge, Q.; Ting, D.; Nguyen, D.; Shen, H. R.; Chen, J.; Eisen, H. N.; Heller, J.; Langer, R.; Putnam, D. *Nat. Mater.* **2004**, *3*, 190–196.
- (2) Broaders, K. E.; Cohen, J. A.; Beaudette, T. T.; Bachelder, E. M.; Fréchet, J. M. J. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 5497–5502.
- (3) Andriano, K.; Tabata, Y.; Ikada, Y.; Heller, J. *J. Biomed. Mater. Res.* **1999**, *48*, 602–612.
- (4) Lu, L.; Garcia, C. A.; Mikos, A. G. *J. Biomed. Mater. Res.* **1999**, *46*, 236–244.
- (5) Eglin, D.; Mortisen, D.; Alini, M. *Soft Matter* **2009**, *5*, 938–947.

- (6) Shah, S.; Wertheim, J.; Wang, C.; Pitt, C. J. *Controlled Release* **1997**, *45*, 95–101.
- (7) Zhu, X.; Fryd, M.; Tran, B. D.; Ilies, M. A.; Wayland, B. B. *Macromolecules* **2012**, *45*, 660–665.
- (8) Andersson, S. R.; Hakkarainen, M.; Albertsson, A. C. *Biomacromolecules* **2010**, *11*, 3617–3623.
- (9) Antheunis, H.; van der Meer, J. C.; de Geus, M.; Kingma, W.; Koning, C. E. *Macromolecules* **2009**, *42*, 2462–2471.
- (10) Gillies, E. R.; Fréchet, J. M. J. *Pure Appl. Chem.* **2004**, *76*, 1295–1308.
- (11) Esser-Kahn, A. P.; Odom, S. A.; Sottos, N. R.; White, S. R.; Moore, J. S. *Macromolecules* **2011**, *44*, 5539–5553.
- (12) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Prog. Polym. Sci.* **2010**, *35*, 278–301.
- (13) Du, F. S.; Wang, Y.; Zhang, R.; Li, Z. C. *Soft Matter* **2010**, *6*, 835–848.
- (14) Sy, J. C.; Seshadri, G.; Yang, S. C.; Brown, M.; Oh, T.; Dikalov, S.; Murthy, N.; Davis, M. E. *Nat. Mater.* **2008**, *7*, 863–869.
- (15) Tang, R.; Ji, W.; Panus, D.; Palumbo, R. N.; Wang, C. J. *Controlled Release* **2011**, *151*, 18–27.
- (16) Heller, J.; Barr, J.; Ng, S. Y.; Abdellauoi, K. S.; Gurny, R. *Adv. Drug Delivery Rev.* **2002**, *54*, 1015–1039.
- (17) Heller, J.; Barr, J. *Expert Opin. Drug Delivery* **2005**, *2*, 169–183.
- (18) Heller, J. *Adv. Drug Delivery Rev.* **2005**, *57*, 2053–2062.
- (19) Fiore, V. F.; Lofton, M. C.; Roser-Page, S.; Yang, S. C.; Roman, J.; Murthy, N.; Barker, T. H. *Biomaterials* **2010**, *31*, 810–817.
- (20) Paramonov, S. E.; Bachelder, E. M.; Beaudette, T. T.; Standley, S. M.; Lee, C. C.; Dashe, J.; Fréchet, J. M. J. *Bioconjugate Chem.* **2008**, *19*, 911–919.
- (21) Huang, X. N.; Du, F. S.; Cheng, J.; Dong, Y. Q.; Liang, D. H.; Ji, S. P.; Lin, S. S.; Li, Z. C. *Macromolecules* **2009**, *42*, 783–790.
- (22) Morinaga, H.; Morikawa, H.; Wang, Y.; Sudo, A.; Endo, T. *Macromolecules* **2009**, *42*, 2229–2235.
- (23) Chen, W.; Meng, F.; Li, F.; Ji, S. J.; Zhong, Z. *Biomacromolecules* **2009**, *10*, 1727–1735.
- (24) Zhang, D.; Zhang, H.; Nie, J.; Yang, J. *Polym. Int.* **2010**, *59*, 967–974.
- (25) Liu, Z.; Tang, Y.; Li, N.; Lu, L.; Deng, J.; Cai, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 495–508.
- (26) Zubris, K. A. V.; Colson, Y. L.; Grinstaff, M. W. *Mol. Pharmaceutics* **2012**, *9*, 196–200.
- (27) Lu, J.; Li, N.; Xu, Q.; Ge, J.; Lu, J.; Xia, X. *Polymer* **2010**, *51*, 1709–1715.
- (28) Cordes, E. H.; Bull, H. G. *Chem. Rev.* **1974**, *74*, 581–603.
- (29) Cheng, J.; Ji, R.; Gao, S. J.; Du, F. S.; Li, Z. C. *Biomacromolecules* **2012**, *13*, 173–179.
- (30) Chatterjee, S.; Ramakrishnan, S. *Macromolecules* **2011**, *44*, 4658–4664.
- (31) Mahmoud, E.; Sankaranarayanan, J.; Morachis, J. M.; Kim, G.; Almutairi, A. *Bioconjugate Chem.* **2011**, *22*, 1416–1421.
- (32) Merkli, A.; Heller, J.; Tabatabay, C.; Gurny, R. *J. Controlled Release* **1995**, *33*, 415–421.
- (33) Sparer, R. V.; Shih, C.; Ringeisen, C. D.; Himmelstein, K. J. *J. Controlled Release* **1984**, *1*, 23–32.
- (34) Sankaranarayanan, J.; Mahmoud, E. A.; Kim, G.; Morachis, J. M.; Almutairi, A. *ACS Nano* **2010**, *4*, 5930–5936.
- (35) Yu, J.; Lu, Z.; Zheng, H.; Zhuo, R. *Eur. Polym. J.* **2002**, *38*, 971–975.
- (36) Du, F. S.; Huang, X. N.; Chen, G. T.; Lin, S. S.; Liang, D. H.; Li, Z. C. *Macromolecules* **2010**, *43*, 2474–2483.
- (37) Qiao, Z. Y.; Du, F. S.; Zhang, R.; Liang, D. H.; Li, Z. C. *Macromolecules* **2010**, *43*, 6485–6494.
- (38) Jankova, K.; Chen, X.; Kops, J.; Batsberg, W. *Macromolecules* **1998**, *31*, 538–541.
- (39) Pitt, C. G.; Shah, S. S. *J. Controlled Release* **1995**, *33*, 397–403.
- (40) Ng, S.; Shen, H.; Lopez, E.; Zhrebina, Y.; Barr, J.; Schacht, E.; Heller, J. *J. Controlled Release* **2000**, *65*, 367–374.
- (41) Ng, S.; Vandamme, T.; Taylor, M.; Heller, J. *Macromolecules* **1997**, *30*, 770–772.

- (42) Heller, J.; Penhale, D.; Helwing, R.; Fritzing, B. *Polym. Eng. Sci.* **1981**, *21*, 727–731.
- (43) Shen, Y.; Zhan, Y.; Tang, J.; Xu, P.; Johnson, P. A.; Radosz, M.; Van Kirk, E. A.; Murdoch, W. J. *AIChE J.* **2008**, *54*, 2979–2989.
- (44) Zhou, K.; Wang, Y.; Huang, X.; Luby-Phelps, K.; Sumer, B. D.; Gao, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 6109–6114.
- (45) Tamura, A.; Oishi, M.; Nagasaki, Y. *J. Controlled Release* **2010**, *146*, 378–387.
- (46) Kucherak, O. A.; Oncul, S.; Darwich, Z.; Yushchenko, D. A.; Arntz, Y.; Didier, P.; Mély, Y.; Klymchenko, A. S. *J. Am. Chem. Soc.* **2010**, *132*, 4907–4916.
- (47) Longenecker, R.; Mu, T.; Hanna, M.; Burke, N. A. D.; Stöver, H. D. H. *Macromolecules* **2011**, *44*, 8962–8971.