Thermal- and pH-Responsive Degradable Polymers

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The structures and properties of stimuli-responsive polymers can change with environmental conditions such as temperature, pH, and ionic strength; therefore, these smart polymers can be exploited for various biomedical applications.[1−3] Further multistimuli-responsive polymers are more interesting due to integration of the more controllable properties and functions, such as thermo- and pH-responsive polymers.[1a,3] One of the most interesting and investigated stimuli-responsive polymers is poly(*N*-isopropylacrylamide) (PNIPAAm). PNIPAAm has a lower critical solution temperature (LCST) of 32 °C which can be feasibly tuned to be around biological temperature, 37 °C.[4] PNIPAAm-based smart micelles, hydrogels, and bioconjugates have been exploited for various applications, e.g., drug delivery and tissue engineering.[1,2a,3−5] Nevertheless, nonbiodegradability renders unavoidable hurdles to their applications. Instead of radical polymerization, here NIPAAm units are grafted to poly(amino ester)s containing secondary amines in the backbones, and thermo- and pH-responsive degradable polymers, NIPAAm-*g*-poly(amino ester)s, are obtained. NIPAAm-*g*-poly(amino ester)s show thermo-responsive behaviors similar to PNIPAAm but with a relatively lower density of NIPAAm unit along the hydrophilic backbones, and the poly(amino ester) backbones are degradable, pH-responsive, and feasible for further modification.[6]

As described in Scheme 1, first the linear poly(amino ester)s containing secondary amines in their backbones were prepared via Michael addition polymerization of diacrylates with equimolar trifunctional amines because the formed secondary amines remain intact due to their much lower reactivity as reported in our previous works.[6d,7] For example, Michael addition polymerization of 1,4-butanediol diacrylate (BDA) and an equimolar amount of 1-(2-aminoethyl)piperazine (AEPZ) was carried out in dimethyl sulfone (DMSO) at ambient temperature to produce linear poly(BDA−AEPZ), containing secondary amines in the backbone (Supporting Information, Figure S1). Then, NIPAAm was added and grafted to the secondary amines in the backbone via Michael addition reaction. The reactions were performed at 80 °C, and only NIPAAm-*g*-poly(BDA−AEPZ) were produced (Supporting Information, Figures S2−S4). NIPAAm grafting degree, i.e., the molar ratio of the NIPAAm unit to the repeating unit of poly(BDA−AEPZ), and molecular weights of the products were determined using ^{1}H NMR (Figure S2 and eq S1 of Supporting Information) and GPC, respectively (Supporting Information, Table S1).

Figure 1A shows the temperature dependence of transmittance of 1% (w/v) aqueous solution of NIPAAm-*g*-poly(BDA−AEPZ) with a NIPAAm grafting degree of 0.6 (NIPAAm$_{0.6}$-*g*-poly(BDA−AEPZ)) at 500 nm. At pH 7, the LCST of NIPAAm$_{0.6}$-*g*-poly(BDA−AEPZ) is ca. 30.5 °C. NIPAAm$_{0.6}$-*g*-poly(BDA−AEPZ) is highly soluble in water forming a transparent solution when the temperature is below 30.5 °C; the solution becomes turbid when the temperature is higher than 30.5 °C. The thermo-responsive behavior is pH dependent. The transmittance of 1% (w/v) aqueous solution of NIPAAm$_{0.6}$-*g*- poly(BDA−AEPZ) is reduced by only 10% up to 40 °C at pH 5 or 3 as reflected in Figure 1A. This is due to the increased hydrophilic nature of the backbone induced by the higher degrees of protonation of the amino groups, as indicated by further shifting downfield of these protons adjacent to amines in ^{1}H NMR spectra (Supporting Information Figure S5).

The LCST of NIPAAm-*g*-poly(amino ester)s can also be adjusted via a change in the chemistry of the backbones. Substitution of BDA unit with poly(ethylene glycol) diacrylate (PEGDA) (M_n = 258) (Supporting Information Figure S6 and Table S1) increases the hydrophilic nature of the backbone; the LCST of NIPAAm-*g*-poly(PEGDA−AEPZ) with a grafting degree of 1.0 (NIPAAm$_{1.0}$-*g*-poly(PEGDA−AEPZ)) (Supporting Information eq S2) is elevated to ca. 33.0 °C as indicated in Figure 1B. When a more hydrophilic PEGDA (M_n = 575) was used instead, no thermal response was observed even though the NIPAAm grafting degree was 1.0. Moreover, the LCST can be tuned via adjustment of the NIPAAm grafting degree. The NIPAAm grafting degree could be feasibly controlled via the grafting reaction time and the molar ratio of the feed. A lower NIPAAm grafting degree results in a higher LCST. Figure 1B shows that the LCST of NIPAAm-*g*-poly(PEGDA−AEPZ) is elevated to 36.0 °C when the NIPAAm grafting degree is reduced to 0.46 (Supporting Information Figure S6B and Table S1). But a further decrease of the NIPAAm grafting degree to 0.15 leads to loss of thermal response.

It was reported that the thermo-responsive property of the copolymers of NIPAAm and hydrophilic monomers such as acrylic acid (AAc) disappears when the content of AAc is higher than 20%.[8] Note here that these poly(amino ester)s backbones are hydrophilic as indicated by their good solubility in water,[7a] and the poly(amino ester)s units, especially poly(PEGDA−AEPZ), are much longer than the ethylene units in poly(NIPAAm-*co*-AAc). However, the thermo-responsive property still can be observed. Therefore, a relatively low density of NIPAAm units along the hydrophilic backbones can still render NIPAAm-*g*-poly(amino ester)s the thermal responsive property. This implies that the function of NIPAAm units in the thermo-responsive behavior of NIPAAm-*g*-poly(amino ester)s is different from those in PNIPAAm. Since NIPAAm is isomeric with leucine, the thermo-responsive behavior of NIPAAm-*g*-poly(amino ester)s is comparable to that of elastin-like polypeptides caused by the hydrophobic assembly.[9]

Furthermore, thermo- and pH-responsive amphiphilic copolymers were prepared by grafting cholesteryl (CE) to NIPAAm$_{0.46}$-*g*-poly(PEGDA−AEPZ) via the reaction of the remaining secondary amines with cholesteryl chloroformate to produce CE-*g*-NIPAAm-*g*-poly(PEGDA−AEPZ) (Scheme 1). Amphiphilic CE-*g*-NIPAAm-g-poly(PEGDA−AEPZ) can form micelles in aqueous solution via self-assembly. When the CE grafting degree was ca. 0.48 (Supporting Information Figure S7A, Table S1, and eq S3), CE$_{0.48}$-*g*-NIPAAm$_{0.46}$-*g*-poly(PEGDA−AEPZ) has a critical micelle concentration (cmc) of 3.1 mg L^{-1} in aqueous solution (Supporting Information Figure S8). In comparison with the ^{1}H NMR spectrum of CE$_{0.48}$-*g*-NIPAAm$_{0.46}$-*g*-poly(PEGDA−AEPZ) in a good solvent, $CDCl_3$ (Supporting Information Figure S7A), the peaks ascribed to CE totally disappear but those ascribed to

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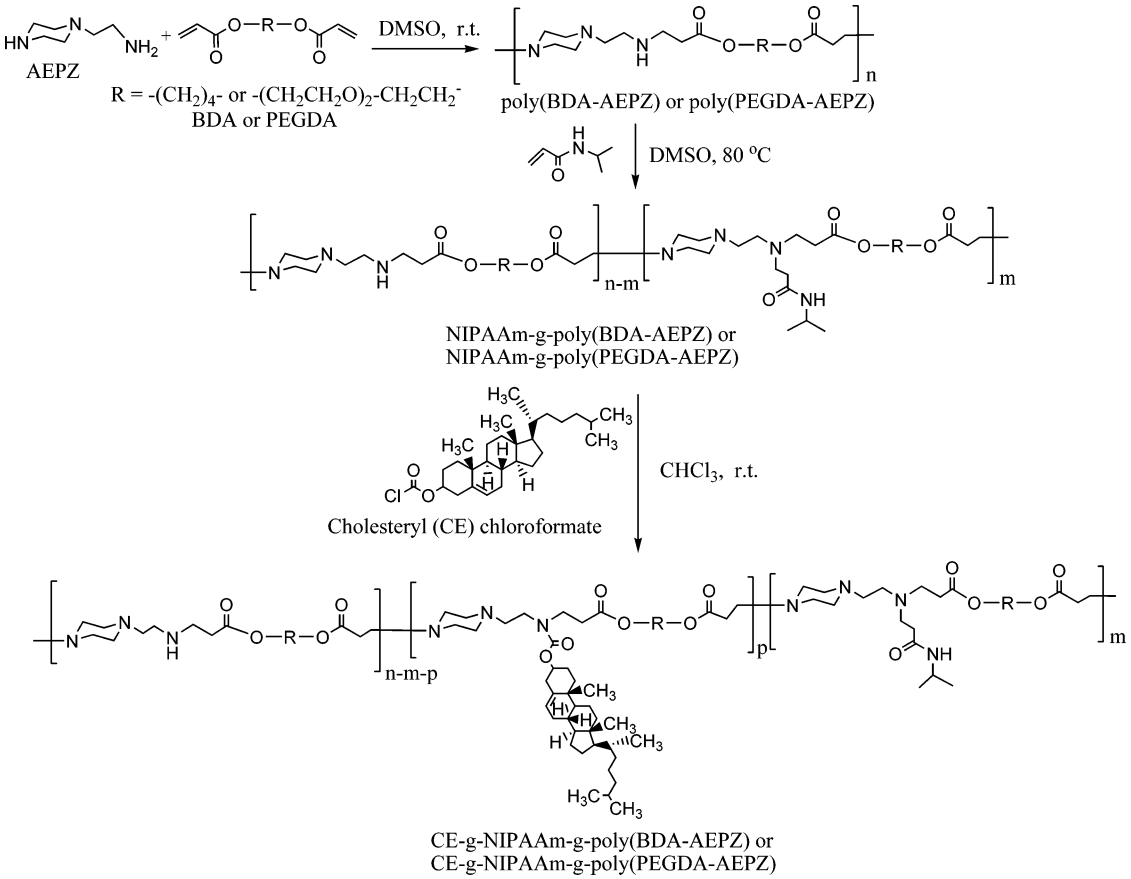
Scheme 1. Synthesis of Thermo- and pH-Responsive Polymers



$NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ) still exist in the ^1H NMR spectrum recorded in D_2O (Supporting Information Figure S7B). So the micelles formed in aqueous solution have cores containing CE and shells composed of $NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ). The plot a in Figure 2 shows that the aqueous solution of the micelles has a LCST of ca. 36.5 °C close to that of $NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ) shown in Figure 1B. This is reasonable because the shells of the micelles, i.e., $NIPAAm_{0.46}$-*g*-poly(PEGDA− AEPZ), determine the thermal-responsive behavior. When the temperature is below 36.5 °C, the dynamic radius (R_h) of the micelles, determined using dynamic layer light scattering (DLLS), is ca. 10 nm. However, the R_h of the aggregates increased remarkably to 161 nm at 42 °C. This is caused by the increased hydrophobic nature of the thermo-responsive shells. Moreover, the larger aggregates produced at the elevated temperature are still pH responsive. When the solution was titrated to pH 5, the R_h of the aggregates was reduced to 57 nm (plot b of Figure 2). This should be caused by the higher degree of protonation of the amines in the backbones at pH 5. This thermo- and pH-responsive biocompatible material should be more promising for targeted drug delivery in hyperthermia therapy compared to those amphiphilic thermo-responsive polymers from PNIPAAm.[10]

It has been well demonstrated that degradation of poly(amino ester)s can be readily realized via hydrolysis of the ester bonds in aqueous solution,[6,11] and the degradation profile is affected by pH[6a,b,11] and polymer topology.[6d−f] The grafting of NIPAAm does not alter the hydrolysis property. For example, the hydrolysis of $NIPAAm_{0.51}$-*g*-poly(BDA−AEPZ) occurred readily as reflected by the reducing relative integral intensity of the

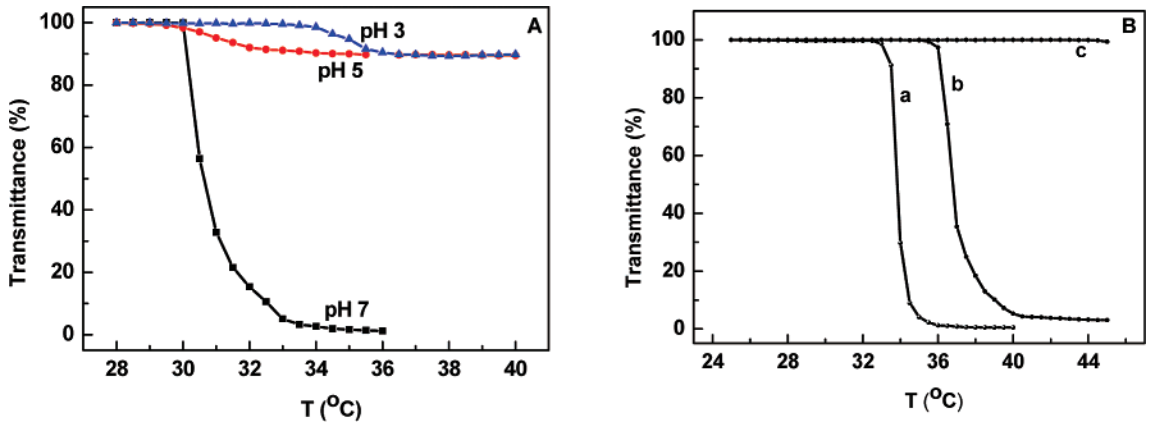


Figure 1. (A) Temperature and pH dependencies of the transmittance of 1% (w/v) aqueous solution of $NIPAAm_{0.6}$-*g*-poly(BDA-AEPZ). (B) Temperature dependence of the transmittance of 1% (w/v) aqueous solution of NIPAAm-*g*-poly(PEGDA−AEPZ) with a NIPAAm grafting degree of (a) 100, (b) 46, and (c) 15%.

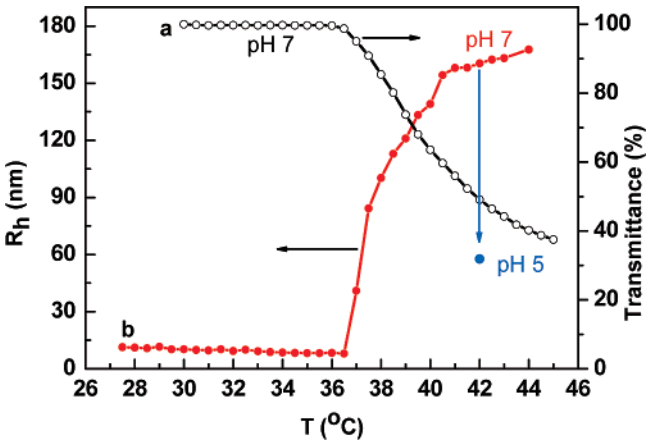


Figure 2. (a) Temperature dependence of the transmittance of 1% (w/v) aqueous solution of micelles formed from $CE_{0.48}$-*g*-$NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ). (b) Effect of temperature and pH on R_h of the micelles formed in 0.05% (w/v) aqueous solution of $CE_{0.48}$-*g*-$NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ).

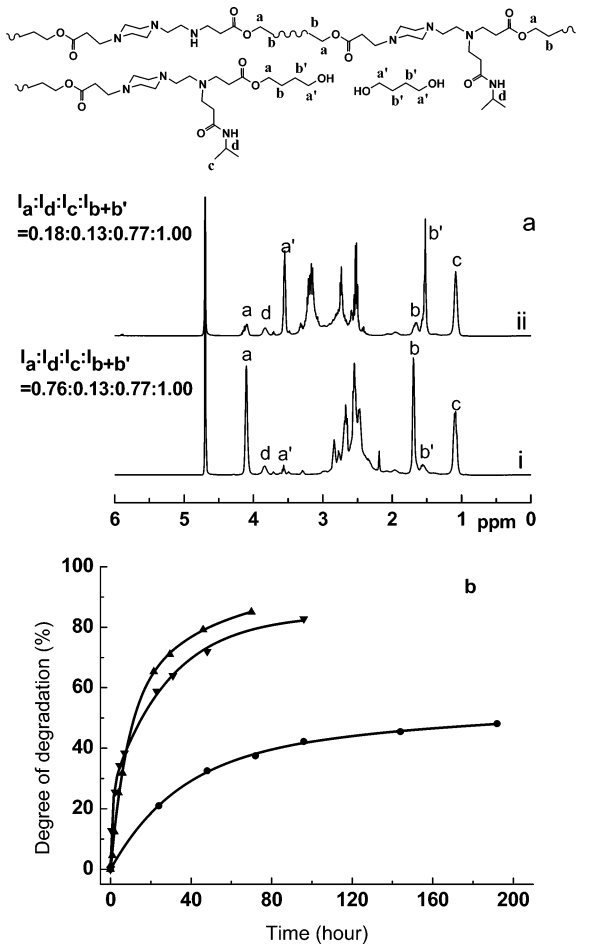


Figure 3. (a) ^{1}H NMR spectra of $NIPAAm_{0.51}$-*g*-poly(BDA−AEPZ) after being kept in D_2O for (i) 2 h and (ii) 96 h. (b) Comparison of the hydrolysis profile of (▼) $NIPAAm_{0.51}$-*g*-poly(BDA−AEPZ), (▲) $NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ), and (●) $CE_{0.48}$−$NIPAAm_{0.46}$-*g*-poly(PEGDA−AEPZ).

peaks ascribed to the protons in the ester groups, such as the reducing ratio of I_a to I_c shown in Figure 3a. The hydrolysis profiles of different NIPAAm-*g*-poly(amino ester)s were obtained by monitoring the processes using ^{1}H NMR (Supporting Information Figures S7C and S9−S11 and eqs S4−S6). The grafting of NIPAAm shows insignificant effects on the hydrolysis rate compared to the related polymers (Supporting Information Figures S10 and S11). Figure 3b reflects that the polymer from BDA has a degradation rate comparable to that containing PEG. However, CE grafting reduces the hydrolysis rate remarkably due to less accessibility of the hydrophobic cores of the micelles to water molecules.

In conclusion, thermo- and pH-responsive degradable NIPAAm-*g*-poly(amino ester)s are developed with thermo-responsive properties similar to elastin-like polypeptides. The chemistry of the poly(amino ester)s backbones and the NIPAAm grafting degree can be adjusted to tune the thermo- and pH-responsive properties. Further reactions can be carried out such as with the amine groups. Hence NIPAAm-*g*-poly(amino ester)s are promising platform materials for many applications, such as fabrication of biodegradable thermo- and pH-responsive micelles and gels for drug delivery and tissue engineering and modification of proteins and DNA.

Supporting Information Available: Experimental protocols, materials preparation procedures, NMR spectra, hydrolysis profiles, critical micelle concentration, and LCST determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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