Lower Critical Solution Temperature Behavior of Amphiphilic Copolymers Based on Polyaspartamide Derivatives

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ABSTRACT: Novel copolymers consisting of poly(*N*-isopropylaminoethyl-*co*-6-hydroxyhexyl aspartamide) and poly(*N*-isopropylaminoethyl-*co*-hexyl aspartamide) were prepared from polysuccinimide, which was the thermal polycondensation product of L-aspartic acid, via a ring-opening reaction with 6-amino-L-hexanol (AH) or hexylamine (HA) and *N*-isopropylethylenediamine at different ratios. The copolymers, containing 75–90 mol % of AH and 35–45 mol % of HA, produced thermoresponsive polymers through their lower critical solution temperatures (LCSTs) in aqueous solution. We could control the LCST could be controlled by modifying the hydrophobic–hydrophilic balance by changing the content of AH or HA. The pH dependencies of the LCST were opposite in these two different copolymer systems. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 509–513, 2008

Key words: graft copolymers; hydrophilic polymers; solution properties; stimuli-sensitive polymers

INTRODUCTION

Nowadays, water-soluble stimulus-responsive polymers are becoming increasingly attractive for use in biotechnology and medicine. Among them, thermoresponsive polymers that show a lower critical solution temperature (LCST) have widely been investigated because of their potential applications in such areas as controlled drug delivery, biomimetic actuators, chromatographic separations, gene-transfection agents, and immobilized biocatalysts. Different researchers have explained the LCST phenomenon of polymers in water.[1–5] This behavior was ascribed to the greater entropy in the two-phase system than in a homogeneous solution,[2] the formation of hydrogen bonds between the polymer and water,[3] the hydrophobic interactions between the polymer side chain groups,[4] and the disruption of specific hydrogen-bonded cyclic structures in alkyl amide units and hydroxyl functions of water.[5] Materials that show this behavior are of great interest for medical and biomaterial applications.[6]

The most studied synthetic responsive polymer is poly(*N*-isopropylacrylamide), which undergoes a sharp coil–globule transition in water at 32°C, changing from a hydrophilic state below this temperature to a hydrophobic state above it.[7–10] The temperature at which this occurs is the LCST. Besides poly(*N*-isopropylacrylamide), various thermoresponsive polymers, typically poly(vinyl methyl ether), poly(2-isopropyl-2-oxazoline), poly(*N*-vinylalkylamide)s, and poly(phosphazene)s, have been developed. However, most of them are nonbiodegradable; this limits their use in biomedical fields.

Amino acid based polymers have been widely studied because of their wide potential application as biocompatible and biodegradable materials. Polyaspartate, a poly(amino acid) with a carboxylate side chain, which is synthesized by the thermal polymerization of aspartic acid followed by alkaline hydrolysis, has received much attention as a new and useful class of biodegradable, water-soluble polymeric material. Because of its potential applications in biomedical fields, on the basis of its reversible phase-transition behavior, many strategies have been reported for modifying the properties of polyaspartate.[11] For example, Tachibana et al.[12] attached amino alcohols to produce poly(*N*-substituted α/β asparagines) that showed a sharp LCST at a temperature of around 20–40°C. Also, Yoshimura et al.[13] studied superabsorbent hydrogels based on polyaspartate with sodium sulfonate pendant groups.

In this study, we prepared copolymers consisting of poly(*N*-isopropylaminoethyl-*co*-6-hydroxyhexyl

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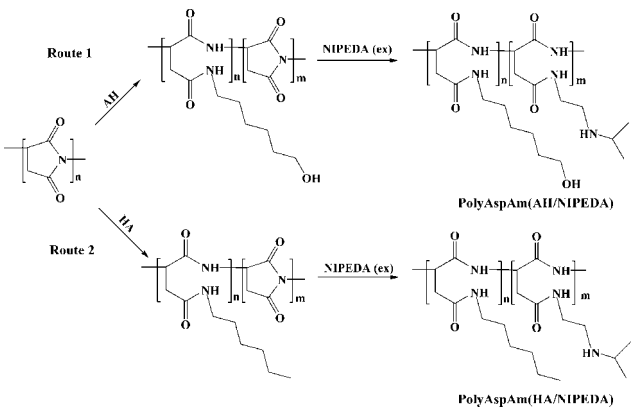


Scheme 1 Synthesis of the amphiphilic polyaspartamide derivatives.

aspartamide) [PolyAspAm(AH/NIPEDA)] and poly (*N*-isopropylaminoethyl-*co*-hexyl aspartamide) [PolyAspAm(HA/NIPEDA)], which showed both pH- and thermal-responsive properties in aqueous solution.

EXPERIMENTAL

Materials and measurements

L-Aspartic acid (L-Asp; >98%), *o*-phosphoric acid (98%), *N*,*N*′-dimethylformamide (DMF; anhydrous 99.8%), 6-amino-1-hexanol (AH; 97%), hexylamine (HA; 99%), *N*-isopropylethylenediamine (NIPEDA; 98%) were all purchased from Sigma-Aldrich and were used without further purification. ^{1}H-NMR spectra were recorded on a Bruker AMX-500 spectrometer (Karlsruhe, Germany) with D_2O and dimethyl sulfoxide (DMSO)-d_6 as the solvents. Fourier transform infrared (FTIR) spectra were obtained on a PerkinElmer FTIR spectrometer (model SPECTRUM 2000) (Norwalk, CT). The LCST of the polymer in phosphate buffer solution (PBS; pH 7.4) or distilled water was measured by an ultraviolet–visible spectrometer (Biochrom LibraS22) (Cambridge, UK) equipped with a cell holder and a temperature controller at 1°C/min. The change in transmittance as a function of temperature was observed from a visible source at 500 nm, and the polymer concentration was 1 wt %.

Preparation of polysuccinimide (PSI)

L-Asp (20 g) and *o*-phosphoric acid (20 g) were blended and mixed at a low temperature. The mixture was put into a one-necked flask, which was immersed in an oil bath with a heat controller and connected to a rotary evaporator rotating at a speed of about 90–100 rpm. We carried out the reaction by increasing the temperature from 25 to 200°C and slowly decreasing the pressure to full vacuum. About 5 h after the maximum temperature was reached, the reaction was completed. DMF was added to dissolve the brown polymer (PSI). The solution was precipitated with 1.5 L of methanol and washed with a large amount of deionized water until it was neutral (pH 6–7, as measured by a pH meter). The solid residue was dried at 60°C for 2 days in a vacuum. The prepared PSI had a reduced viscosity of 0.45 dL/g in DMF. The molecular weight was estimated to be approximately 132,000 Da, as calculated from an empirical equation relating the solution viscosity to the molecular weight.[4]

Synthesis of PolyAspAm(AH/NIPEDA)

PSI (0.5 g) and different amounts of AH were dissolved in DMF before they were put in a three-necked flask, which was placed in a water bath at 60°C, and subjected to continuous stirring for 24 h. Then, an excess amount of NIPEDA was added, and the solution was stirred for another 24 h. The product was collected after it was precipitated in a cosolvent containing acetone and hexane, washed with acetone, and dried *in vacuo* at 25°C for 1 day (yield = 70–75%). The composition of the prepared copolymer was analyzed by ^{1}H-NMR spectroscopy.

Synthesis of PolyAspAm(HA/NIPEDA)

PSI (0.5 g) and different amounts of HA were dissolved in DMF before they were put in a three-necked flask, which was placed in a water bath at 70°C, and subjected to continuous stirring for 6 h. Then, an excess amount of NIPEDA was added, and the solution was stirred for 24 h. The product was collected after it was precipitated in 200 mL of diethyl ether and dried in a vacuum at 25°C for 1 day (yield = 72–80%). The composition of the

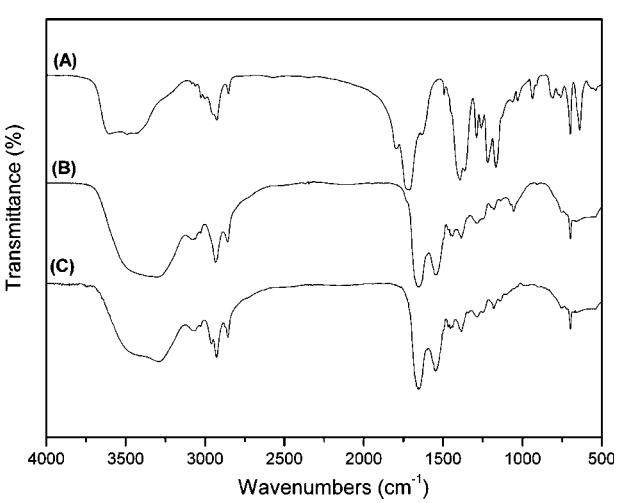


Figure 1 FTIR spectra of (A) PSI, (B) PolyAspAm(AH/NIPEDA), and (C) PolyAspAm (HA/NIPEDA).

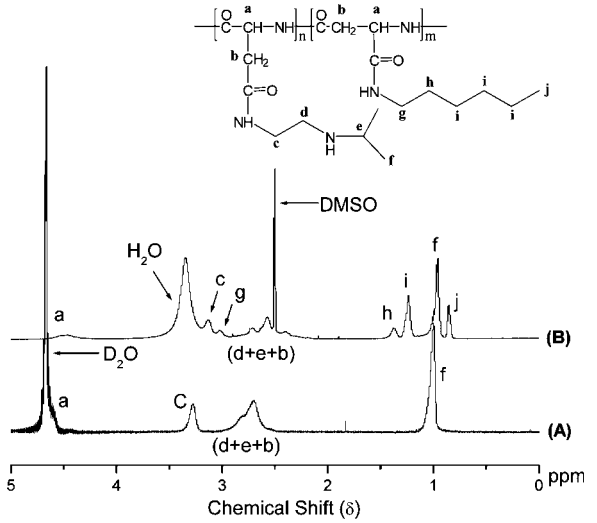


Figure 2 ^{1}H-NMR spectra of (A) PolyAspAm(NIPEDA) and (B) PolyAspAm(HA/NIPEDA).

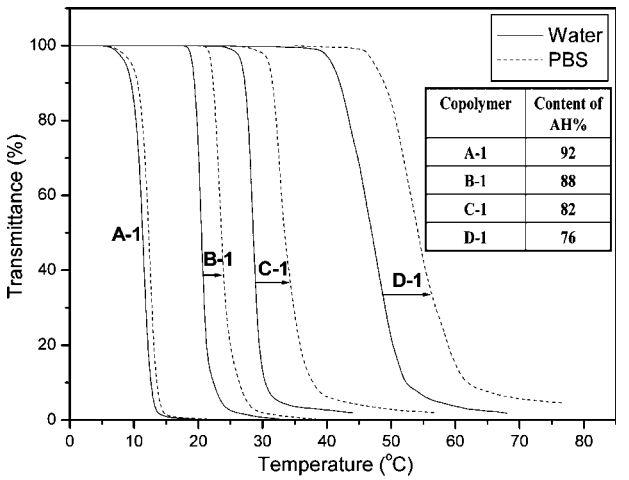
prepared copolymer was analyzed by ^{1}H-NMR spectroscopy.

RESULTS AND DISCUSSION

Preparation and characterization of amphiphilic polyaspartamide copolymers

Novel amphiphilic polyaspartamide derivatives with *N*-isopropylamine pendant groups were synthesized from PSI via a nucleophilic ring-opening reaction with either AH or HA and NIPEDA, as shown in Scheme 1. The compositions of the copolymers were analyzed by both ^{1}H-NMR and FTIR spectroscopy.

Figure 1 shows the FTIR spectra of (A) PSI, (B) PolyAspAm(AH/NIPEDA), and (C) PolyAspAm(HA/NIPEDA). Both spectra B and C show characteristically strong bands at 1649 cm^{-1} (amide I) and 1545 cm^{-1} (amide II) and in the range 3500–3305 cm^{-1} (—NH—) corresponding to the aspartamide backbone structure; the peak at 2905 cm^{-1}, corresponding to CH_2 stretching, appeared after the aminolysis reaction between PSI and NIPEDA. The band in the range 3500–3305 cm^{-1} in copolymer C looked sharper than that in copolymer B, which contained a hydroxyl group and had the same effective range with the secondary amine group.



Copolymer	Content of AH%
A-1	92
B-1	88
C-1	82
D-1	76

Figure 4 LCST curves of a 1 wt % aqueous solution of PolyAspAm(AH/NIPEDA).

Figures 2 and 3 show typical ^{1}H-NMR spectra of two different copolymer systems, where the proton peaks C, D, E, and F were assigned to the NIPEDA pendant groups. As shown in Figure 2, the G, H, I, and J peaks were related to the methylene and terminal methyl protons of the HA pendant groups. In Figure 3, the G, H, and I peaks were characterized as the methylene protons of the AH pendant groups.

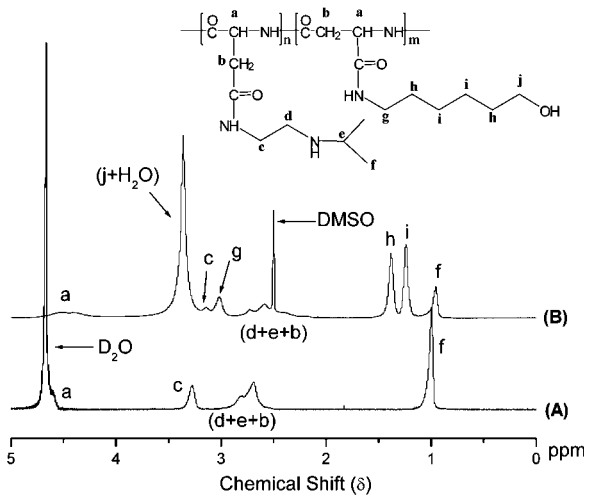
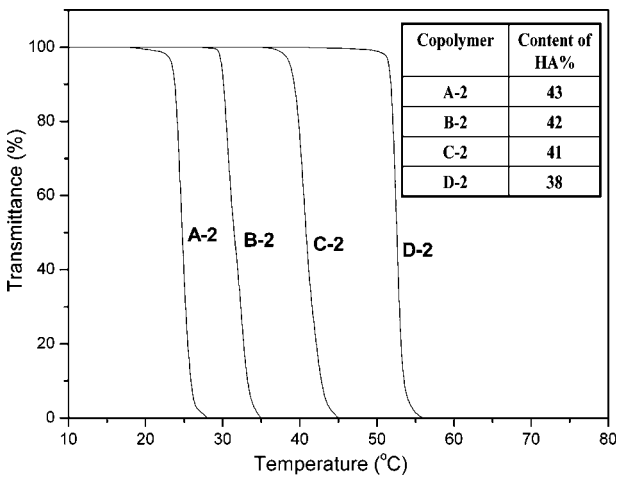


Figure 3 ^{1}H-NMR spectra of (A) PolyAspAm(NIPEDA) and (B) PolyAspAm(AH/NIPEDA).



Copolymer	Content of HA%
A-2	43
B-2	42
C-2	41
D-2	38

Figure 5 LCST curves of a 1 wt % aqueous solution of PolyAspAm(HA/NIPEDA).

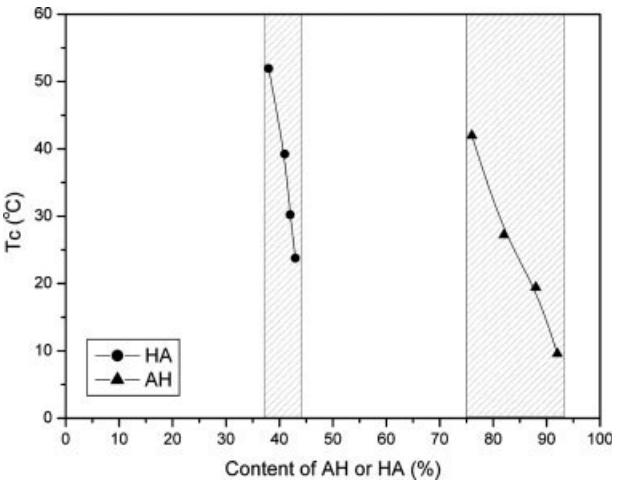


Figure 6 Plot of LCST versus the chemical composition of PolyAspAm(HA/NIPEDA) and PolyAspAm(AH/NIPEDA) aqueous solutions. (T_c: temperature at 90% transmittance of light).

LCST BEHAVIORS OF AMPHIPHILIC POLYASPARTAMIDE COPOLYMERS

The temperature dependences of the light transmittance of 1 wt % PolyAspAm(AH/NIPEDA) and PolyAspAm(HA/NIPEDA) in aqueous solution are shown in Figures 4 and 5, respectively. The variation in transmittance with temperature was observed from a visible source at 500 nm with a cell holder and a temperature controller at 1°C/min. The higher the AH or HA content was, the lower the LCST was, which suggested that there was an overall increase in the hydrophobicity of the copolymer caused by the increase of these relatively hydrophobic components. The LCSTs were observed and measured at HA contents in the range 38–42 mol % in the PolyAspAm(HA/NIPEDA) system and were found to be quite sensitive to a small change in their composition. On the other hand, the LCSTs of the PolyAspAm (AH/NIPEDA) system were observed at a higher AH content in the range 75–92%, and the variation with composition was less pronounced, as shown in Figure 6. The structural difference between these two systems was limited to the presence of a terminal hydroxyl group on the AH moiety instead of hydrogen. The polar —OH group should have helped to reduce the overall hydrophobicity of the copolymer system, so that the LCSTs were observed at much higher contents (AH = 75–92 mol %) because of the modified hydrophilic/hydrophobic balance. On the other hand, the LCSTs of PolyAspAm(HA/NIPEDA) in both pure water and PBS (pH 7.4) were almost the same with a slight shift in their curves. In the case of PolyAspAm(AH/NIPEDA), however, the LCSTs in PBS were shifted to a higher temperature, probably because of the presence of the hydroxyl group in the AH pendant and the effect of intramolecular and intermolecular

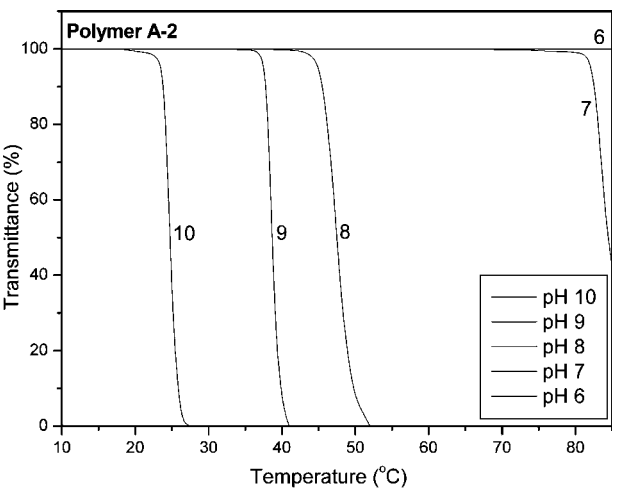


Figure 7 Effect of pH on the LCST behavior in aqueous solutions of PolyAspAm(HA/NIPEDA) (sample: polymer B-2).

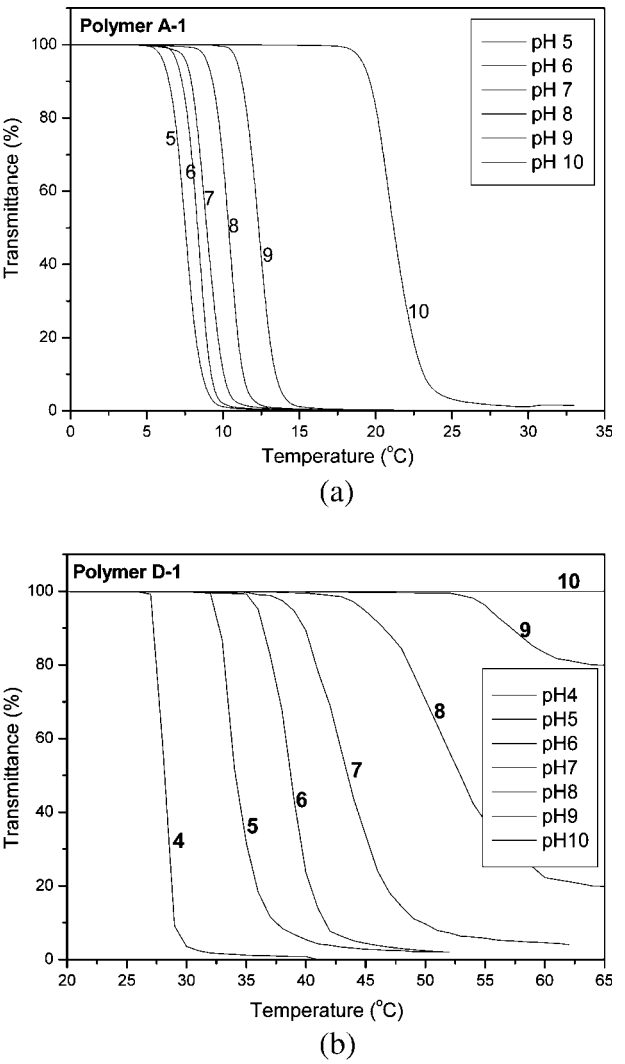


Figure 8 Effect of pH on the LCST behavior in aqueous solutions of PolyAspAm(AH/NIPEDA).

hydrogen bonding. The extent of this shift increased as the NIPEDA content was increased.

Figures 7 and 8 show the pH sensitivity of the polyaspartamide derivatives, as determined by light transmittance with the procedure described previously, in different pH buffer solutions. As shown in Figure 7, the LCST of PolyAspAm(HA/NIPEDA) increased with decreasing pH value and no phase transition occurred at pH 6, which was explained by the basic properties of the secondary amine groups in the NIPEDA pendant. Interestingly, the opposite results were observed in the case of PolyAspAm(AH/NIPEDA); that is, the higher the pH value was, the higher the LCST was. The results from two typical samples, one with a high AH content and the other with a low AH content, are shown in Figure 8(A) and 8(B), respectively. The intramolecular hydrogen bonding between the hydroxyl groups (in AH) and secondary amine groups (in NIPEDA) may have played an important role in this system; that is, this physical link could have made the copolymer more soluble in a high pH environment. On the contrary, it would have been partially broken at a low pH value so as to release the long alkyl chains because the basic amine pendant would be protonized, and this might have had the effect of raising the overall hydrophobicity of the copolymer, which resulted in a decrease in LCST. A detailed investigation of this issue and the formation of nanoparticles from this amphiphilic copolymer system in aqueous solution are currently under way.

CONCLUSIONS

New pH-responsive and thermoresponsive polyaspartamides containing AH or HA and NIPEDA as pendant groups were synthesized, and their solution properties were characterized. We could control LCST in aqueous solution by modifying the hydrophobic–hydrophilic balance by varying the content of AH or HA. LCST varied depending on the pH of the solution in a manner that was different in the two systems. The solution properties of the multifunctional system, PolyAspAm(AH/NIPEDA), seemed to be more complex than those of PolyAspAm(HA/NIPEDA), which were similar to those reported in recent studies.[14,15] These novel amphiphilic copolymers have the potential to be used for various biomedical applications, such as drug delivery and bioconjugation.

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