# Preparation of Biodegradable Thermo-responsive Polyaspartamides with *N*-Isopropylamine Pendent Groups (I)

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Novel amphiphilic, thermo-responsive polyaspartamides which showed both LCST (lower critical solution temperature), and sol-gel transition were prepared and characterized. The polyaspartamide derivatives were synthesized from polysuccinimide, the polycondensate of aspartic acid monomer, via successive nucleophilic ring-opening reaction by using dodecylamine and *N*-isopropylethylenediamine (NIPEDA). At the intermediate composition ranges, the dilute aqueous solution exhibited a thermally responsive phase separation due to the presence of LCST. The phase transition temperature was controllable by changing the content of pendent groups. In addition, a physical gelation, *i.e.* the sol-gel transition was observed from the concentrated solutions, which was elucidated by dynamic viscoelastic measurements. These novel injectable and thermo-responsive hydrogels have potential for various biomedical applications such as tissue engineering and current drug delivery system.

**Key Words :** Thermo-responsive polymer, Polyaspartamide, Amphiphilic, LCST, Sol-gel transition

## Introduction

Stimuli-responsive polymers have attracted great interest in the field of novel drug delivery, cell encapsulation, and tissue engineering due to their promising potential. Among them, temperature and pH responsive systems have widely been investigated.[1,2] Poly(*N*-isopropyl acrylamide), PNIPAAm, is one of the most typical thermosensitive polymers, which undergoes a rapid and reversible hydration-dehydration change through the LCST(lower critical solution temperature). PNIPAAm, NIPAAm-containing copolymers, and the hydrogels have been widely applied to controlled drug delivery and various bio-related applications.

Recently injectable polymeric hydrogels are becoming very attractive in their applications such as novel drug delivery and tissue engineering field. The polymers are loaded with bioactive molecules or cells in an aqueous solution and *in-situ* turn into physically crosslinked hydrogels by external stimuli such as temperature, pH, and light. Among them, thermo-responsive hydrogels have been extensively investigated which can undergo rapid transformation from a liquid form to a gel state at body temperature without any additives. Most of the reported injectable hydrogels, however, are non-biodegradable, and this may limit their use in biomedical field.

Polypeptides and related synthetic poly(amino acid)s have become important because of their desirable properties such as biocompatibility and biodegradability, which are useful for various bio-related industries. Poly(aspartic acid), PASP, is one of water-soluble and biodegradable polyamide, which can be produced from the hydrolysis of polysuccinimide (PSI), the polycondensate of L-aspartic acid monomer. Poly(*N*-2-hydroxyethyl-DL-aspartamide), PHEA, is another derivative polymer, obtained by coupling PSI with ethanolamine, which has been proposed as a potential plasma extender and material for drug delivery, such as macromolecular prodrugs, polymeric micelles, and nanoparticles. The attachment and chemical modification of pendent groups either by the aminolysis reaction to PSI or by the secondary reaction through hydroxyl or carboxylic groups of the PASP and PHEA can provide a variety of biodegradable functional polymers with specific properties. Very extensive studies on this class of material have been carried out by Giammona *et al.* and other groups.[3-8] Also, Watanabe et al reported some thermosensitive systems based on polyaspartamide derivatives recently.[9,10]

In this work, we prepared novel amphiphilic graft copolymers based on polyaspartamides containing *N*-isopropylamine moiety to identify materials which showed both LCST behavior and sol-gel transition in aqueous solution.

## Experimental

**Materials.** L-aspartic acid (98+%), *o*-phosphoric acid (98%), *N*-isopropylethylenediamine (NIPEDA, 98%), dodecylamine (laurylamine, LA 98%), *N,N*-dimethylformamide (DMF, anhydrous 99.8%) were purchased from Aldrich Chemical Co. and used as received. Diethylether (99%) was obtained from DaeJung Chemical Co. (Korea). All of the other chemicals purchased were of high quality and used without purification.

**Measurements.** $^1$H-NMR spectra were recorded on a Bruker AMX-500 spectrometer using $D_2O$ and DMSO-$d_6$ as the solvent. The FT-IR spectra were obtained on a Perkin-Elmer FT-IR spectrometer (Model SPECTRUM 2000). The LCST of the polymer in phosphate buffer solution (PBS, pH 7.4) or distilled water was measured by a UV-visible spectrometer (Biochrom Libras22) equipped with a cell holder and

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%. A dynamic light scattering instrument (DLS, Brookhaven, BI-2000AT, USA) equipped with a temperature controller and Ne-He laser was used to measure the average diameter and particle size distribution of polymeric spheres in distilled water (concentration 1 wt%). The polymer product was magnetically dispersed in distilled water and then filtrated using 0.45 $\mu$m pore-sized filter paper to remove oversized materials. The light intensities scattered from the polymeric spheres were measured at the angle of 90 °C. The viscoelasticity of the polymer solutions were measured using a stress-controlled rheometer (AR2000 TA-Instrument, USA) with a parallel plate with a diameter 40 mm at a heating ratio of 1 °C/min and a frequency of 1.0 Hz.

**Synthesis of Polysuccinimide (PSI).** L-aspartic acid (20 g) and *o*-phosphoric acid (20 g) were charged into a round-bottom flask and stirred under reduced pressure at 200 °C for 5 h. The reaction mixture was then cooled and DMF was added to dissolve the product. The resulting solution was precipitated in excess water and the precipitate was washed several times with water to remove the residual phosphoric acid. The final product was dried at 80 °C under vacuum. The prepared PSI had a reduced viscosity of 0.52 dL/g in DMF. The molecular weight was estimated to be approximately 160,000 Da, as calculated from an empirical equation relating the solution viscosity to the molecular weight.[11]

**Synthesis of PolyAspAm(NIPEDA).** 0.5 g of PSI was dissolved in 5 mL DMF in a three-neck round flask at 25 °C, and an equimolar amount of NIPEDA was then added dropwise at 0 °C. This mixture was stirred at room temperature for 24 h, and then the solution was precipitated into 10-fold ethylether. The filtered precipitate of the PolyAspAm (NIPEDA) was washed with flash ethylether for several time and dried at 25 °C in vacuum (yield 95%).

$^1$H-NMR (500 MHz, $D_2O$): $\delta$ 2.5-3 (m, 5H, CH-$CH_2$-CO-NH-$CH_2$-$CH_2$-NH-$CH$-$(CH_3)_2$), 4.56-4.7 (m, 1H, NH-$CH$-CO-$CH_2$), 3.2-3.4 (br, 2H, NH-$CH_2$-$CH_2$-NH-CH-$(CH_3)_2$), 0.9-1.2 (br, 6H, NH-$CH_2$-$CH_2$-NH-CH-$(CH_3)_2$), Figure 1a.

**Synthesis of Amphiphilic PolyAspAm(LA/NIPEDA).** A typical procedure to prepare amphiphilic copolymer is described as follows: 0.5 g of PSI was dissolved in 5 ml DMF in a three-neck round flask equipped with a nitrogen inlet and outlet. 45 mol% (based on succinimide) of LA was then added dropwise at 0 °C. Subsequently, the reaction flask was placed in a water bath controlled at 70 °C and stirrer for 6 h. 55 mol% of NIPEDA was then slowly added to the solution and stirred at 30 °C for 24 h. The final solution was then precipitated into a 10-fold ethylether. The filtered precipitate of the PolyAspAm(LA/NIPEDA) was dried at 25 °C in vacuum (yield 74%).

$^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ 2.5-3 (m, 5H, CH-$CH_2$-CO-NH-$CH_2$-$CH_2$-NH-$CH$-$(CH_3)_2$), 4.5-4.7 (m, 1H, NH-$CH$-CO-$CH_2$), 3.2-3.4 (br, 2H, NH-$CH_2$-$CH_2$-NH-CH-$(CH_3)_2$), 0.92-1.12 (br, 6H, NH-$CH_2$-$CH_2$-NH-CH-$(CH_3)_2$), 1.32-1.47 (br, 2H, NH-$CH_2$-$(CH_2)_{10}$-$CH_3$), 1.11-1.27 (br, 10H, NH-$CH_2$-$(CH_2)_{10}$-$CH_3$), 0.8-0.76 (br, 3H, NH-$CH_2$-$(CH_2)_{10}$-$CH_3$). Figure 1b.

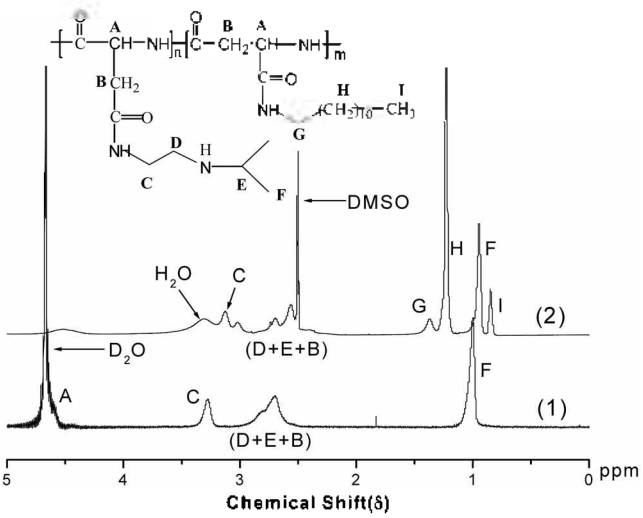
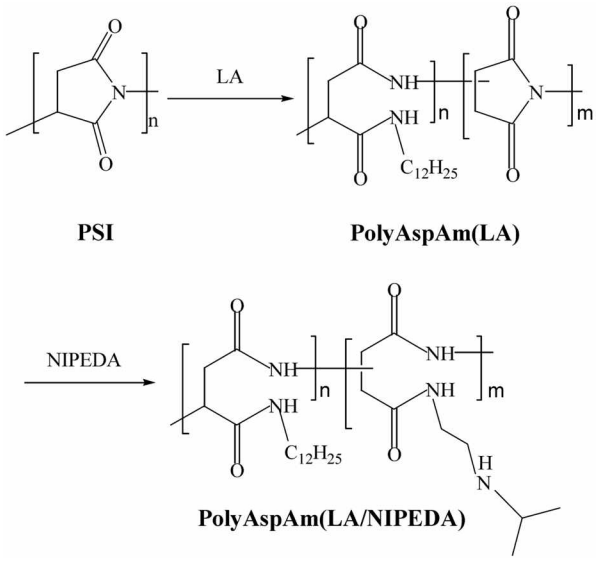



**Figure 1.** $^1$H-NMR spectra of PolyAspAm(NIPEDA) (**1**) and PolyAspAm(LA/NIPEDA) (**2**).

## Results and Discussion

The preparation of polysuccinimide, the precursor polymer, has been well described in literature.[11,12] Novel amphiphilic polyaspartamide derivatives with *N*-isopropylamine pendant were synthesized from PSI via successive nucleophilic ring-opening reaction by using laurylamine (LA) and *N*-isopropylethylenediamine (NIPEDA).




The reaction was carried out in anhydrous DMF in which the succinimide group of PSI was quantitatively reacted to form N-substituted aspartamide unit. The composition of the prepared copolymer was analyzed by $^1$H-NMR spectroscopy. Figure 1 shows the $^1$H-NMR spectra of the PolyAspAm (NIPEDA) (**1**) and PolyAspAm(LA/NIPEDA) (**2**) copolymer. As shown in Figure 1, the proton peaks C and F were

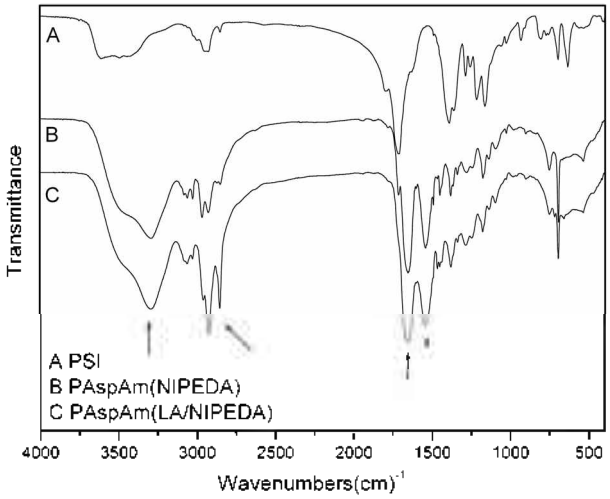



**Figure 2**. FT-IR spectra of PSI (A), PolyAspAm(NIPEDA) (B), and PolyAspAm (LA/NIPEDA) (C).

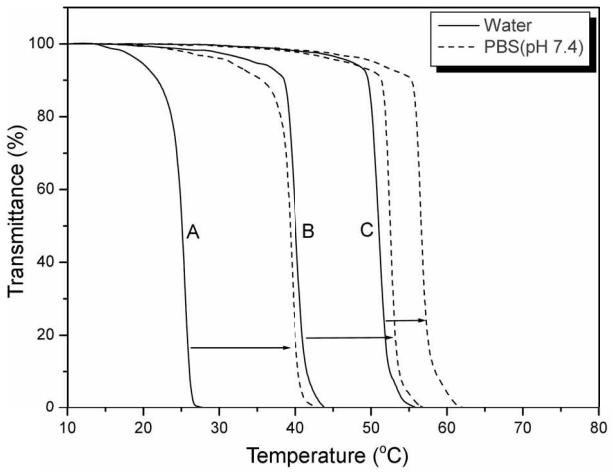



**Figure 3**. LCST behavior of amphiphilic copolyaspartamides. (LA content in mol%: A = 41; B = 39; C = 36)

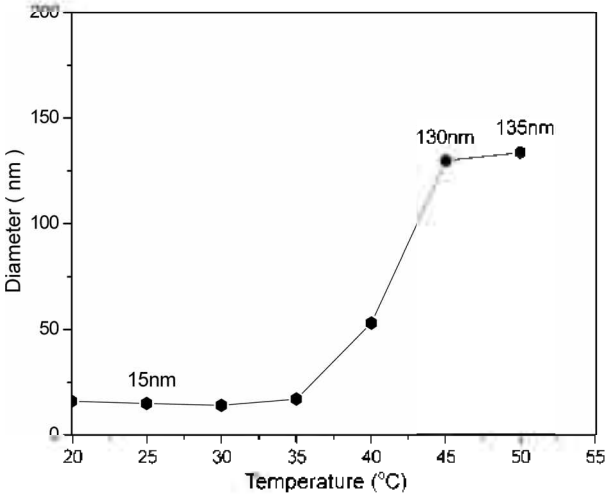



**Figure 4**. Temperature dependence of particle size of polymer B in water.

assigned to the NIPEDA pendants, and the G, H, and I peaks were related to methylene and terminal methyl protons of the LA pendants. The composition of each group in the polyaspartamide copolymer was determined from the integration ratio between peak F and I.

Figure 2 shows the FT-IR spectra of PSI (A), PolyAspAm (NIPEDA) (B), and PolyAspAm (LA/NIPEDA) copolymer (C). Both spectrum B and C show characteristic strong bands at 1649 cm$^{-1}$ (amide I), 1545 cm$^{-1}$ (amide II) and 3305 cm$^{-1}$ (-NH-) corresponding to the aspartamide backbone structure, and the band at 2950 cm$^{-1}$ corresponding to the $CH_2$ stretching appeared after the aminolysis reaction between PSI and NIPEDA. On the other hand, Spectrum C shows stronger alkylene absorption band at 2950 cm$^{-1}$ due to the introduction of LA moiety. FT-IR and $^1$H-NMR analyses indicated that the polyaspartamide derivative polymers had been prepared successfully from the aminolysis reaction of PSI.

Thermal analysis of the PolyAspAm(NIPEDA) and PolyAspAm(LA/NIPEDA) under nitrogen showed Tg's of the polymer at around 40 °C, as determined by the mid-point of the baseline change in DSC. These polymers were stable up to *ca.* 200 °C without any weight loss as determined by the TGA in nitrogen.

At the intermediate composition ranges of PolyAspAm (LA/NIPEDA) copolymer, the dilute aqueous solution exhibited a thermally responsive phase separation. The temperature dependence of light transmittance of 1 wt% aqueous solution at 500 nm is shown in Figure 3. Polymer A-C exhibited a relatively sharp phase separation in the temperature range from 25 to 45 °C due to the presence of LCST in this particular system. As the content of hydrophobic pendant (LA) increased, the transition temperature decreased linearly. At the LA content over 45 mol%, the polymers were found to be insoluble in water at room temperature. In phosphate buffer saline (PBS, pH 7.4) solution the transition temperature was shifted to a higher temperature with different degrees as shown in the Figure 3.

Figure 4 shows a representative temperature dependence of the particle size of the polymer B in water, as determined by dynamic light scattering. The diameter began to increase at 35 °C and grew to about 130 nm at 45 °C, which suggest the phase separation occurred near LCST, yielding of larger sized nanoparticle. In addition, a physical gelation, *i.e.* the sol-gel transition was observed from the concentrated solutions of the same copolymer system. To elucidate the thermo-responsive sol-gel transition, the dynamic viscoelastic properties of the 10 wt% aqueous solutions of polymer were investigated. Figure 5 shows a typical curve on the temperature dependence of storage modulus (G') and loss modulus (G") for the polymer B with 39% LA content. At around 30 °C, both G' and G" values increased sharply and the two values became equal at around 45 °C (G'=G", $\tan\delta$ = 1), suggesting the phase transition from sol-like to gel-like viscoelastic state. Above 45 °C the G' value remained higher than G" up to 70 °C. Figure 6 shows the visual demonstration of this sol-gel transition described above.

In summary, we prepared new thermo-responsive poly-

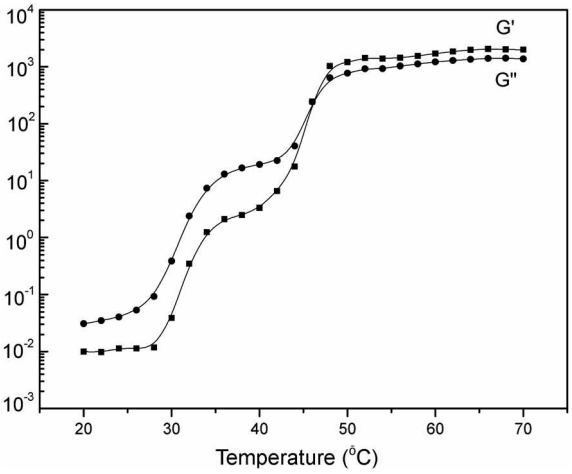



**Figure 5**. Temperature dependence of storage and loss modulus (G' and G") for 10 wt% aqueous solution of polymer B.

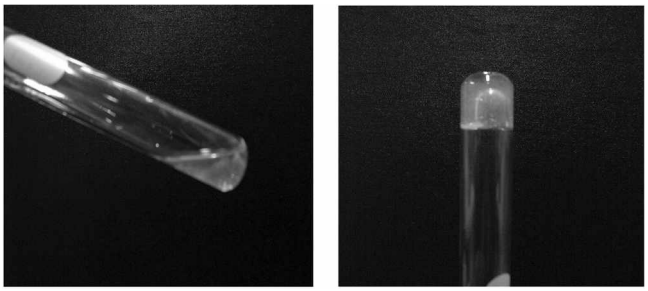



**Figure 6**. Photographs of sol-gel transition of amphiphilic Poly-AspAm (LA/NIPEDA).

aspartamide derivatives containing *N*-isopropylamine groups. which showed both LCST and sol-gel transition. The phase transition temperature was controllable by changing the content of pendent groups. A combination of different hydrophilic groups and hydrophobic alkyl groups in the side chains can provide thermo-responsive property as the similar results are reported recently.[6,9,10] This novel injectable hydrogel system has potential for biomedical applications including tissue engineering and drug delivery system.

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