

Unusually large hysteresis in temperature-responsive phase-transition behavior of aqueous solutions of isotactic copolymers comprising *N*-ethylacrylamide and *N*-isopropylacrylamide

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Abstract

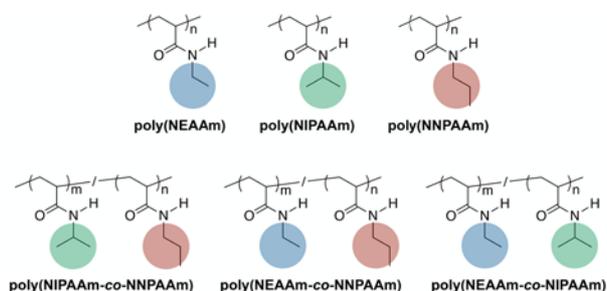
Radical copolymerizations of *N*-ethylacrylamide (NEAAm) and *N*-isopropylacrylamide (NIPAAm) at various ratios were conducted in *N*-ethylacetamide at -40°C to prepare isotactic copolymers with *mm* triad contents of 48.7 % - 50.8 %. The temperature-induced phase transition behaviors of their aqueous solutions were investigated using light transmittance (500 nm) and dynamic light scattering. Reversible and sharp phase transitions were observed in the transmittance of aqueous solutions (1.0 wt%) of copolymers with NIPAAm compositions of ≤ 9.1 mol%. The phase transition temperatures in both the heating and cooling processes gradually decreased with increasing hydrophobic NIPAAm composition. However, copolymers with NIPAAm compositions of 13.9 to 28.0 mol% exhibited large hystereses (*ca.* 40°C) in which the phase transition temperature in the cooling process dramatically decreased. Furthermore, the copolymer with NIPAAm composition of 37.8 mol% was insoluble in water. Syndiotactic poly(NEAAm-*co*-NIPAAm)s were soluble in water and showed reversible and sharp phase transitions regardless of the NIPAAm composition, which indicates that stereostructure plays an important role in the induction of unusually large hysteresis. Dynamic light scattering analysis suggested that the dehydrated polymers were swollen in the cooling process, even below the phase transition temperature in the heating process,

likely because of the cross-linking domain formed by isotactic NIPAAm segments with hydrophobic interaction in the dehydrated state.

Keywords: Stereoregular *N*-alkylacrylamide copolymers; Chemical composition; Phase-transition behavior

1. Introduction

Poly(*N*-isopropylacrylamide) [poly(NIPAAm)] (Scheme 1) has been extensively studied as a temperature-sensitive polymer material [1-4]. Their aqueous solutions show a soluble-to-insoluble transition, termed the coil-globule transition, at ca. 32 °C. This transition occurs rapidly and reversibly in response to temperature change and is well-known as lower critical solution temperature (LCST) phase behavior.



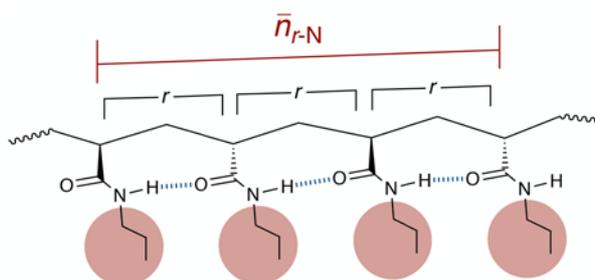
Scheme 1 Chemical structures of (co)polymers of acrylamides described in this paper

Stereoregularity of poly(NIPAAm) significantly influences phase transition behavior. For example, an increase in *meso* (*m*) diad broadens the phase transition and decreases the phase transition temperature [5]. Furthermore, increased *m* diad content increases the hysteresis, which is a retardation in the cooling process compared with the heating process [6].

We have reported stereospecific radical polymerizations of *N*-alkylacrylamides, in which isotactic [7], syndiotactic [8, 9] and heterotactic polymers [10] were obtained *via* radical mechanism. Accordingly, we examined the effect of poly(NIPAAm) stereoregularity on the phase transition behavior in more detail [10]. For example, increased *racemo* (*r*) diad enhanced the sharpness of the phase transition and slightly increased the phase transition temperature [9, 10]. Moreover, increased *r* diad content significantly decreased the hysteresis.

Poly(*N*-*n*-propylacrylamide) [poly(NNPAAm)] (Scheme 1) also exhibits a temperature-responsive behavior [11-14]. The phase transition occurs at ca. 25 °C because the *n*-propyl groups on the nitrogen atom are more hydrophobic than isopropyl groups in the NIPAAm monomeric units. Increased *r* diad content of poly(NNPAAm) also sharpened the phase transition at slightly higher temperatures in the heating process. However, the phase-transition temperature in the cooling process decreases significantly,

which results in enlarged hystereses ($> 15\text{ }^{\circ}\text{C}$) [15]. Infrared (IR) spectra of poly(NNPAAm)s dissolved in D_2O revealed that increased r diad content enhanced the relative intensity of the amide I band resulting from intra/intermolecular hydrogen-bonded C=O at temperatures higher than their phase-transition temperatures [16]. We therefore concluded that intramolecular hydrogen bonding between the amide groups in the contiguous NNPAAm units in syndiotactic stereosequence in the dehydrated state induces the large hysteresis (Scheme 2). The formation of the cooperative hydrogen bonding in the dehydrated state would induce the large hysteresis *via* the similar mechanism to that proposed for isotactic poly(NIPAAm)s, in which the liquid-liquid phase separation and sol-gel transition coexist [17, 18].



Scheme 2. Proposed structure for intramolecular hydrogen bonding between the amide groups in the contiguous NNPAAm units in syndiotactic stereosequence in the dehydrated state.

Similar large hystereses were observed even for the aqueous solutions of syndiotactic copolymers (Scheme 1), poly(NIPAAm-*co*-NNPAAm) [19] and poly(*N*-ethylacrylamide-*co*-*N*-*n*-propylacrylamide) [poly(NEAAm-*co*-NNPAAm)] [20]. The phase transition temperature in the cooling process strongly depends on the chemical composition. The large hystereses did not occur unless the average lengths of *r* diad with contiguous NNPAAm units (\bar{n}_{r-N} ; Scheme 2) exceeded *ca.* 2.8. Notably, the critical length was scarcely influenced, even when NEAAm, which is considerably more hydrophilic than NIPAAm and NNPAAm [11, 21, 22], was employed as a comonomer.

Isotactic poly(NIPAAm) and poly(NNPAAm) become insoluble in water even at low temperatures [5, 10, 23, 24]. Therefore, the molecular-level mechanism on the phase-transition behavior of isotactic poly(*N*-alkylacrylamide)s had been little investigated in detail, except for oligomer-level mechanistic insight [25, 26]. The introduction of hydrophilic NEAAm as a component enabled us to investigate the effect of chemical composition on the phase transition behavior of aqueous solutions of isotactic copolymers. Isotactic copolymers with the average lengths of *m* diad with contiguous NNPAAm units (\bar{n}_{m-N}) of 1.42–2.01 exhibited large hystereses (> 20 °C) [20]. Furthermore, isotactic copolymers with $\bar{n}_{m-N} > 2.01$ were insoluble. Assuming that the

significant change in the solubility originated *via* a similar mechanism to that described for syndiotactic copolymers (Scheme 2), isotactic stereosequences may favor the formation of intramolecular hydrogen bonding more than syndiotactic ones [25, 26].

In the above systems, the most hydrophobic NNPAAm unit was key to large hysteresis induction. Accordingly, it is expected that the copolymers comprising NEAAm and NIPAAm, which are more hydrophilic than NNPAAm, exhibit no clear hystereses. Therefore, we prepared poly(NEAAm-*co*-NIPAAm)s (Scheme 1) to confirm the above hypothesis. However, unusually large hystereses (> 40 °C) were observed for the isotactic poly(NEAAm-*co*-NIPAAm)s. To the best of our knowledge, the hysteresis is the largest among those for the LCST-type thermosensitive polymers so far reported [27-31]. A mechanism for large hysteresis induction was proposed based on the results obtained using dynamic light scattering (DLS) analyses.

2. Experimental

2.1. Materials

NEAAm and NNPAAm were prepared according to the literature [15, 20, 22]. Dimethyl 2,2'-azobisisobutyrate (MAIB) (supplied by Otsuka Chemical, Osaka, Japan) was recrystallized from methanol. Toluene (Kanto Chemical, Tokyo, Japan) was purified by

fractional distillation. *N*-ethylacetamide and 3-methyl-3-pentanol (3Me3PenOH; Tokyo Chemical Industry, Tokyo, Japan) were used without further purification.

2.2. Copolymerization

A typical copolymerization procedure was as follows. NEAAm (0.421 g, 4.25 mmol), NIPAAm (0.085 g, 0.75 mmol), and MAIB (5.8 mg, 2.52×10^{-2} mmol) were dissolved in *N*-ethylacetamide to prepare 5 mL of solution. Four millilitres of the solution were transferred to a glass ampoule, which was degassed under a vacuum and filled with nitrogen six times at -50 °C. The mixture was irradiated at a distance of *ca.* 5 cm using a UV-LED lamp (LED-41UV375N100VF, $\lambda = 375$ nm, 410 mW, Optocode Co., Tokyo, Japan) to initiate copolymerization. After 75 min, the reaction mixture was poured into diethyl ether (200 mL). The precipitate was collected by centrifugation and dried *in vacuo*. The centrifuged product was then dialyzed against water (Spectra/Por 3, molecular mass cutoff 3.5 kDa, Spectrum Laboratories Inc., Shiga, Japan), and freeze-dried to obtain the copolymer. The copolymer yield was determined gravimetrically.

2.3. Measurements

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with an ECZ-400S

or ECA-500 spectrometer (JEOL Ltd., Tokyo, Japan). The chemical composition of the copolymers obtained was determined from the integral intensities of ^1H NMR signals due to the N-**CH₂** groups (**c**) in the NEAAm unit (3.1 ppm) and N-**CH** groups (**f**) in the NIPAAm unit (3.8 ppm), measured in deuterated dimethyl sulfoxide (DMSO-*d*₆) at 150°C (Fig. 1). The diad tacticity of the copolymers was determined from the ^1H NMR signals of the main-chain **CH** groups (**b** and **b'**) and one of the signals of the main-chain **CH₂** groups (**a** and **a'**) with *m* configuration (1.7 ppm; Fig. 1). The triad tacticity was determined from the ^{13}C NMR signals due to the **CH** groups (**b** and **b'**) in the main chain, measured in a mixed solvent (DMSO-*d*₆ : D₂O : 2,2,3,3,4,4,5,5-octafluoro-1-pentanol (PenOH-F₈) = 75 : 10 : 15 wt%) at 100 °C (Fig. 2) [10]. The ^1H chemical shifts were referenced to the signal of the methyl group of tetramethylsilane (0 ppm), which was used as an internal reference. The ^{13}C chemical shifts were referenced to the signal of OCH₂ in PenOH-F₈ (59.06 ppm), which was used as a component of the mixed solvent.

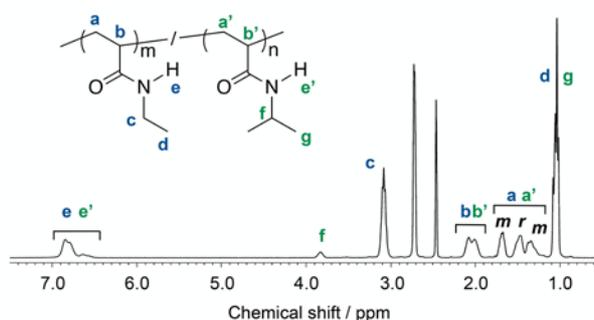


Fig. 1 ^1H NMR spectrum of isotactic poly(NEAAm-*co*-NIPAAm) with NIPAAm content

of 13.9 mol% (DMSO- d_6 , 150 °C).

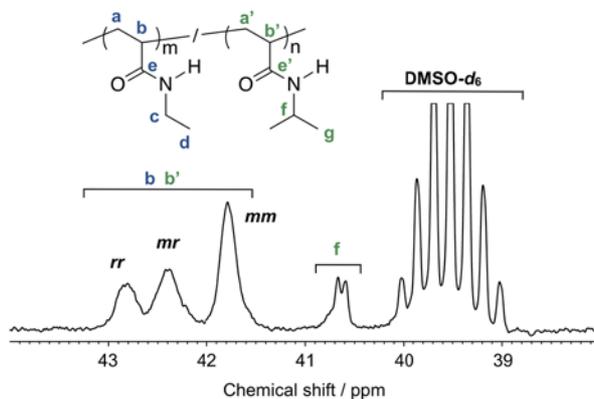


Fig. 2 Expanded ^{13}C NMR spectrum of isotactic poly(NEAAm-co-NIPAAm) with NIPAAm content of 13.9 mol% (DMSO- d_6 : D $_2$ O : PenOH-F $_8$ = 75 : 10 : 15 wt%, 100 °C).

The molecular weights and molecular weight distributions of the copolymers were determined using size exclusion chromatography using poly(methyl methacrylate) (PMMA) samples as molecular weight standards. Size exclusion chromatography was performed with a PU-4185 pump (JASCO, Tokyo, Japan) connected to refractive index detector (RI-4035, JASCO) and column oven (380-B, Chemco Plus Scientific Co., Ltd., Osaka, Japan) equipped with TSK gel columns [SuperHM-M (150 × 6.5 mm, i.d) and SuperHM-H (150 × 6.5 mm, i.d)] (Tosoh Corp.). Dimethylformamide containing LiBr (10 mmol/L) was used as the eluent at 40 °C with flow rate 0.35 mL/min. The initial polymer concentration was set at 3.0 mg/mL.

The transmittance of the aqueous solutions of copolymers was monitored at 500

nm as a function of temperature with a UV/VIS spectrophotometer (V-550, JASCO, Tokyo, Japan) equipped with a Peltier thermostated single cell holder (ETC-505, JASCO). The temperature was changed at 0.5 °C/min from 2 °C to 90 °C, held at 90 °C for ca. 15 min, then changed at 0.5 °C/min from 90 °C to 2 °C. The cloud point (T_c) in the heating and cooling processes was taken as the temperature at which transmittance was 50%. When some transparency remained even above the phase-transition temperature, T_c was defined as the temperature at which the average transmittance before and after the phase transition was observed.

DLS measurements were conducted on a Malvern Zetasizer NanoZSP instrument (Malvern Panalytical Ltd., Malvern, UK) equipped with a Ne-Ne Laser (10 mW) of 633 nm wavelength. The scattering angle was fixed at 173 °. The distributions of intensity-average hydrodynamic diameters (D_{HS}) of the copolymers and/or dispersions were obtained using DLS with the non-negative least-squares fitting algorithm. Glass cuvettes were used and the solutions were filtered through 0.5- μ m membrane filters to remove dust prior to measurement. The temperature was changed from 10 °C to 90 °C, held at 90 °C for 8 min, then changed from 90 °C to 10 °C. The measurements were conducted at an interval of 5 °C after 8 min of equilibration time. Because each measurement data was saved ca. every 12 min, the heating and cooling rates to collect the

distributions of D_{HS} were equivalent to 0.42 °C/min, which almost corresponds to the rates in the transmittance experiments.

3. Results and Discussion

3.1. Synthesis of isotactic poly(NEAAm-co-NIPAAm)s

We have reported that isotactic random copolymers can be prepared by radical copolymerization of NEAAm and NNPAAm in *N*-ethylacetamide at -40 °C [20]. Therefore, radical copolymerization of NEAAm and NIPAAm was conducted in *N*-ethylacetamide at -40 °C (Table 1). The chemical compositions of NIPAAm units in the copolymers agreed well with the NIPAAm fractions in feed, although the copolymerizations were quenched before the monomers were completely consumed. This result confirms that the radical copolymerizations of NEAAm and NIPAAm proceeded in an almost random manner as well as those of NEAAm and NNPAAm [20]. The diad tacticities determined from ^1H NMR spectra were almost the same (m diad = 64.1 % - 64.8 %), regardless of the chemical compositions. This result also confirmed that the isotactic-specificity induced in *N*-ethylacetamide did not depend on the monomer ratio in feed.

Table 1 Radical copolymerization of NEAAm and NIPAAm in *N*-ethylacetamide at -40 °C for 75 min and T_c in the heating and cooling processes of the polymers obtained^a

Run	NIPAAm / mol%		Yield %	Diad ^b / %		Triad ^c / %		\bar{n}_{m-1} ^d	M_n^e $\times 10^{-4}$	M_w/M_n^e	T_c^f / °C	
	in feed	in copolymer		<i>m</i>	<i>mm</i>	<i>mr</i>	<i>rr</i>				heating	Cooling
1	0.0	0.0	45	64.1	-	-	-	-	12.1	2.4	78.5	78.1
2	5.0	4.6	73	64.4	50.7	29.9	19.4	1.04	16.4	1.7	75.3	74.7
3	10.0	9.1	74	64.5	49.2	32.3	18.5	1.07	17.1	1.7	70.4	70.0
4	14.8	13.9	25	64.2	48.7	31.0	20.3	1.12	19.0	2.2	65.5	23.8
5	19.9	18.3	27	64.1	49.2	29.5	21.3	1.16	18.2	2.2	62.0	22.3
6	30.1	28.0	69	64.1	49.1	32.0	18.9	1.27	16.0	1.7	62.2	21.7
7	39.5	37.8	66	64.8	50.7	29.0	20.3	1.42	10.1	2.0	-	-

a. $[\text{NEAAm+NIPAAm}]_0 = 1.0$ mol/L, $[\text{MAIB}]_0 = 0.5 \times 10^{-2}$ mol/L. b. Determined by ^1H NMR signals of the main-chain methine and methylene groups. c. Determined by ^{13}C NMR signals of the main-chain methine groups. d. Average length of *m* diad with contiguous NIPAAm units. e. Determined using size exclusion chromatography (PMMA standards). f. 0.5 °C/min, 1.0 wt %.

The *m* diad fractions can be also calculated using the following equation: $m = mm + mr/2$, in which triad tacticities determined from ^{13}C NMR spectra were used. The calculated values agreed well with those determined from ^1H NMR spectra. This means that the triad tacticities were determined with a satisfactory level of accuracy. Therefore, the average lengths of *m* diad with contiguous NIPAAm units (\bar{n}_{m-1}) were estimated using the triad tacticities, on the assumption that the monomer sequence distribution obeys Bernoullian statistics, according to the method described in the previous paper in detail [19].

3.2. Phase-transition behaviors of aqueous solutions of isotactic poly(NEAAm-co-NIPAAm)s

We have reported phase transition behaviors of stereoregular (co)polymers under a concentration of 0.1 wt% with a scanning rate of 0.5 °C/min [9, 10, 15, 19, 20]. Therefore, the phase transition behavior of aqueous poly(NEAAm-*co*-NIPAAm)s was also examined under the same conditions (Fig. S1). However, the transmittance showed little change even after heating to 90 °C. A similar tendency was observed for isotactic poly(NEAAm-*co*-NNPAAm)s [20]. The tendency was enhanced for poly(NEAAm-*co*-NIPAAm), probably because the NIPAAm units are more hydrophilic than the NNPAAm units. The higher concentration significantly improved the transmittance change due to phase transition. The aqueous solution at 1.0 wt% exhibited a sharp transmittance change. Accordingly, we set the concentration at 1.0 wt%.

Fig. 3 shows temperature dependences of the transmittance of aqueous solutions of isotactic poly(NEAAm-*co*-NIPAAm)s in the heating and cooling processes. The phase-transition temperature in the heating process ($T_{c, \text{heating}}$) decreased gradually with increasing NIPAAm composition from 0.0 to 18.3 mol% (Fig. 3a). Therefore, increasing hydrophobicity by the NIPAAm units is likely responsible for the decrease in the phase-transition temperature like the poly(NIPAAm-*co*-NNPAAm) system [19, 32]. Further increases in the NIPAAm composition significantly affected copolymer solubility. For instance, the phase-transition behavior of the copolymer with the 28.0 mol%

NIPAAm unit broadened compared with those with lower NIPAAm compositions. Taking into account that the copolymer with the NIPAAm unit of 37.8 mol% was insoluble in water at 1.0 wt% concentration, increased NIPAAm units would assemble each other with hydrophobic interaction [33].

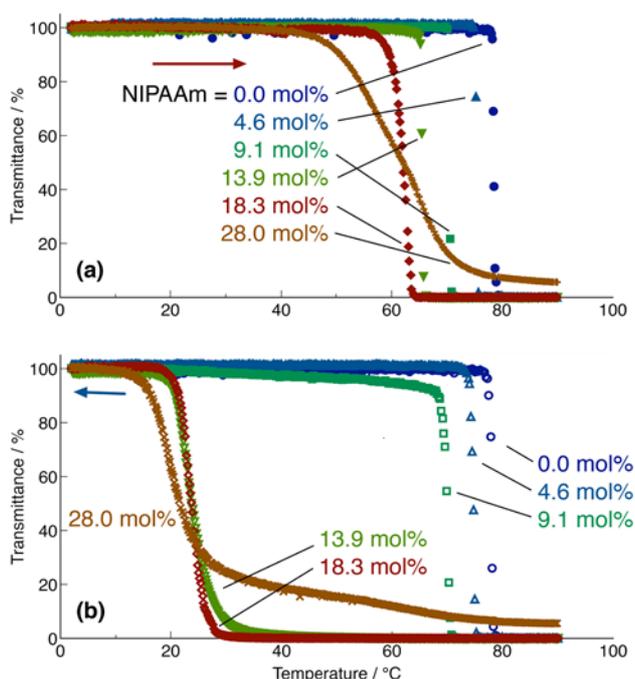


Fig. 3 Effect of chemical composition on temperature dependence of light transmittance of isotactic poly(NEAAm-co-NIPAAm)s in the (a) heating and (b) cooling processes; 1.0 wt%, heating and cooling rates = 0.5 °C/min.

The phase-transition temperature in the cooling process ($T_{c, \text{cooling}}$) also decreased gradually with increasing NIPAAm composition for copolymers with lower NIPAAm compositions (Fig. 3b). However, large decreases in the phase-transition

temperature were observed for the copolymers with 13.9, 18.3 and 28.0 mol% of NIPAAm units with slight broadening of the phase transition. Notably, such a large decrease in the phase transition temperature was repeatedly observed. Furthermore, the phase transition temperatures in the cooling process correspond to those of isotactic-rich NIPAAm homopolymers [5, 10].

To examine the effect of stereochemistry on phase transition, syndiotactic copolymers with the NIPAAm unit of 20.2, 49.5 and 79.4 mol% were prepared by radical copolymerization of NEAAm and NIPAAm in toluene at -40°C for 20 min in the presence of 3Me3PenOH (Table 2) [9]. The copolymerizations also proceeded in an almost random manner, and tacticities were scarcely affected by the monomer ratio in feed [19, 20]. Fig. 4 shows the temperature dependences of the transmittance of aqueous solutions of syndiotactic poly(NEAAm-*co*-NIPAAm)s in heating and cooling processes. The phase transition occurred sharply in both processes and hysteresis was scarcely observed, regardless of the chemical composition in the copolymers.

Table 2 Radical copolymerization of NEAAm and NIPAAm in toluene at -40°C for 20 min in the presence of 3Me3PenOH and T_c in the heating and cooling processes of the polymers obtained^a

Run	NIPAAm / mol%		Yield %	Diad ^b / %		\bar{n}_{r-1} ^c	M_n^d $\times 10^{-4}$	M_w/M_n^d	T_c^e / $^{\circ}\text{C}$	
	in feed	in copolymer		<i>m</i>	<i>r</i>				heating	cooling
1	19.6	20.2	66	34.5	65.5	1.15	9.7	2.0	64.7	64.3
2	49.7	49.5	65	33.5	66.5	1.49	13.6	1.9	51.4	50.6
3	79.3	79.4	60	32.5	67.5	2.15	13.5	1.9	41.0	40.5

a. $[\text{NEAAm}+\text{NNPAAm}]_0=0.5$ mol/L, $[\text{MAIB}]_0=0.5\times 10^{-2}$ mol/L, $[\text{3Me3PenOH}]_0=2.0$ mol/L. b. Determined by ^1H NMR signals of the main-chain methylene groups. c. Average length of *r* diad with contiguous NIPAAm units. d. Determined using size exclusion chromatography (PMMA standards). e. $0.5^{\circ}\text{C}/\text{min}$, 1.0 wt %.

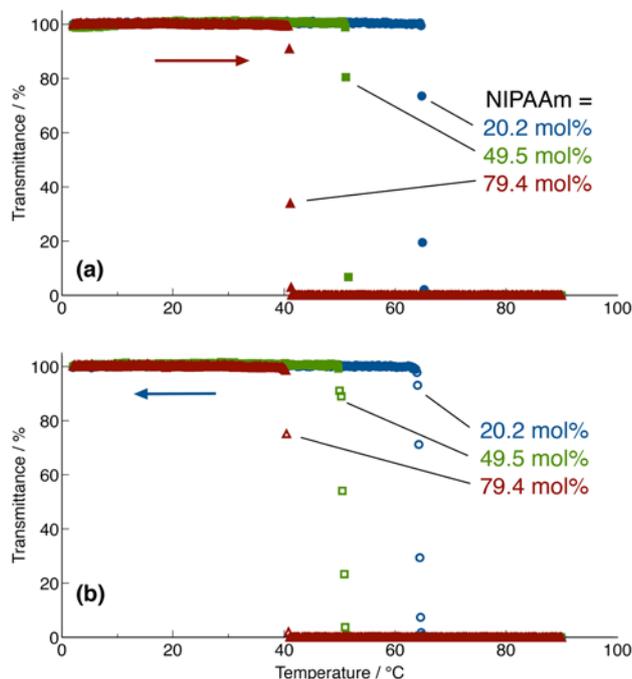


Fig. 4 Effect of chemical composition on temperature dependence of light transmittance of syndiotactic poly(NEAAm-*co*-NIPAAm)s in the (a) heating and (b) cooling processes; 1.0 wt%, heating and cooling rates = 0.5 °C/min.

Fig. 5 shows the relationships of the difference in the phase-transition temperature between heating and cooling processes (ΔT_c) with the \bar{n}_{m-I} values of isotactic copolymers and the average lengths of *r* diad with contiguous NIPAAm units (\bar{n}_{r-I}) of syndiotactic copolymers, respectively. The \bar{n}_{r-I} values were estimated from the diad tacticities and chemical compositions according to a previously-described method [19] because the triad tacticity distribution in poly(NIPAAm) prepared in toluene at low temperatures in the presence of alcohol obeyed Bernoullian statistics [10, 34].

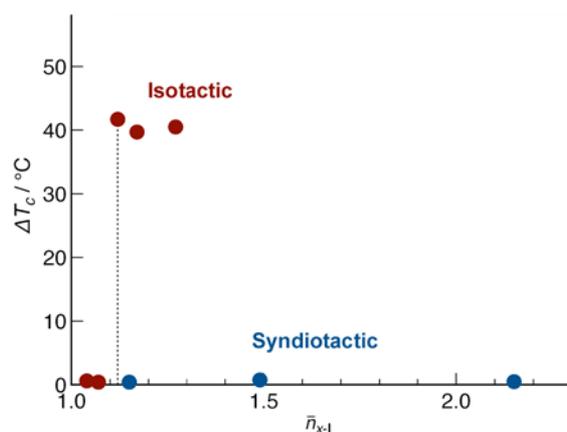


Fig. 5 Relationship between ΔT_c and \bar{n}_{m-I} values in isotactic poly(NEAAm-*co*-NIPAAm)s or \bar{n}_{r-I} values in syndiotactic poly(NEAAm-*co*-NIPAAm)s.

Isotactic copolymers with $\bar{n}_{m-I} \geq 1.12$ exhibited large hystereses (ca. 40 °C), whereas isotactic copolymers with $\bar{n}_{m-I} \leq 1.07$ scarcely exhibited hystereses. A similar tendency was observed for 0.1 wt% aqueous solutions of isotactic poly(NEAAm-*co*-NNPAAm)s, in which clear hystereses (21.9 - 23.9 °C) were observed for the copolymers with average lengths of *m* diad with contiguous NNPAAm units ≥ 1.42 [20]. It is therefore assumed that intramolecular hydrogen-bonding of contiguous NIPAAm units in isotactic stereosequences in the dehydrated state induced the large hysteresis in the isotactic poly(NEAAm-*co*-NIPAAm) system.

3.3. Effect of measurement conditions on the phase-transition behavior of aqueous

solution of isotactic poly(NEAAm-co-NIPAAm)s

The scanning rate significantly influences the T_c observed [4]. Therefore, the effect of the scanning rate was examined in the phase transition behavior of 1.0 wt% aqueous solution of the isotactic poly(NEAAm-co-NIPAAm) with the 13.9 mol% of NIPAAm unit (Fig. 6). The T_c was slightly affected by the scanning rate in both heating and cooling processes. Accordingly, large hystereses were observed regardless of the scanning rate. The phase transition temperatures near the thermodynamic equilibrium (T_{c0}) were determined by extrapolating the scanning rate to zero [29, 35, 36]. ΔT_{c0} remained at 37.7 °C, even near thermodynamic equilibrium conditions. A similar tendency was observed for the 0.1 wt% aqueous solution of syndiotactic poly(NNPAAm) (Fig. S2a), in which the T_c was also scarcely affected by the scanning rate.

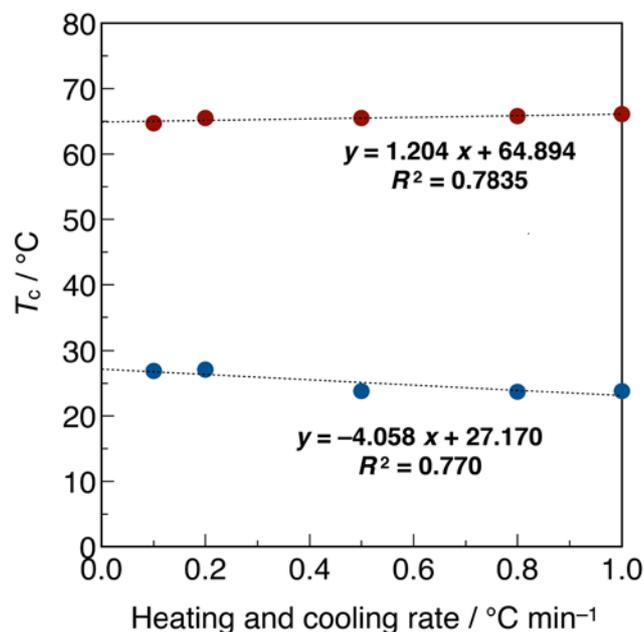


Fig. 6 Effect of scanning rate on the $T_{c, \text{heating}}$ and $T_{c, \text{cooling}}$ in the aqueous solution of isotactic poly(NEAAm-co-NIPAAm) with NIPAAm composition of 13.9 mol%.

The effect of polymer concentration was also examined, because concentration is another important factor for the phase transition behavior (Fig. 7). Large hystereses were observed regardless of the concentration. However, the phase transitions in the cooling process were broadened with decreasing concentration. In particular, stepwise phase transition was observed in the cooling process at 0.1 wt% concentration. Considering that syndiotactic poly(NNPAAm) showed large hystereses regardless of the concentration (Fig. S2b), the mechanism of the induction of the large hysteresis observed in the aqueous solutions of isotactic poly(NEAAm-co-NIPAAm)s is assumed to differ

from that of syndiotactic poly(NNPAAm).

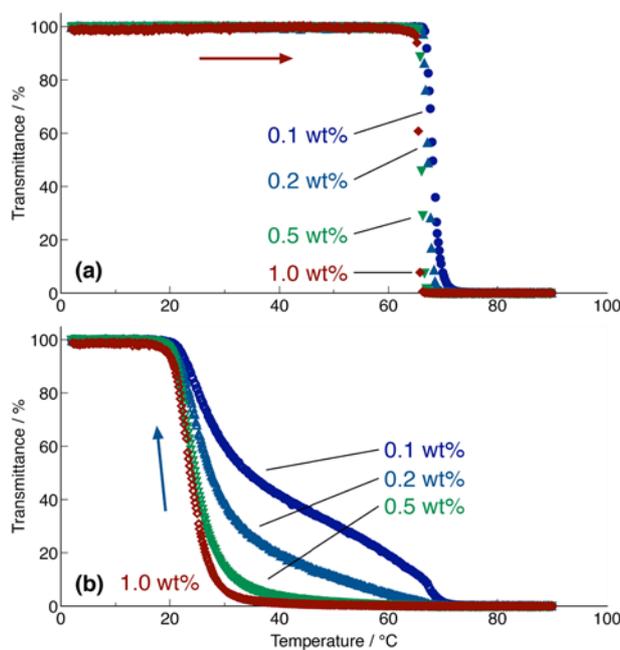


Fig. 7 Effect of concentration on phase transition behavior of the aqueous solution of isotactic poly(NEAAm-co-NIPAAm) with NIPAAm composition of 13.9 mol%; (a) heating and (b) cooling processes, heating and cooling rates = 0.5 °C/min.

3.4. DLS measurement of aqueous solution of poly(NEAAm-co-NIPAAm)s

To investigate the induction of the large hysteresis in more detail, DLS measurements were conducted for the 0.1 wt% aqueous solutions of isotactic (Fig. 8) and syndiotactic (Fig. 9) poly(NEAAm-co-NIPAAm)s, respectively. Isotactic copolymer showed large hysteresis in the hydrodynamic diameter (D_H) distribution, whereas syndiotactic copolymer showed almost the same change tendencies in the D_H distributions in both

heating and cooling processes.

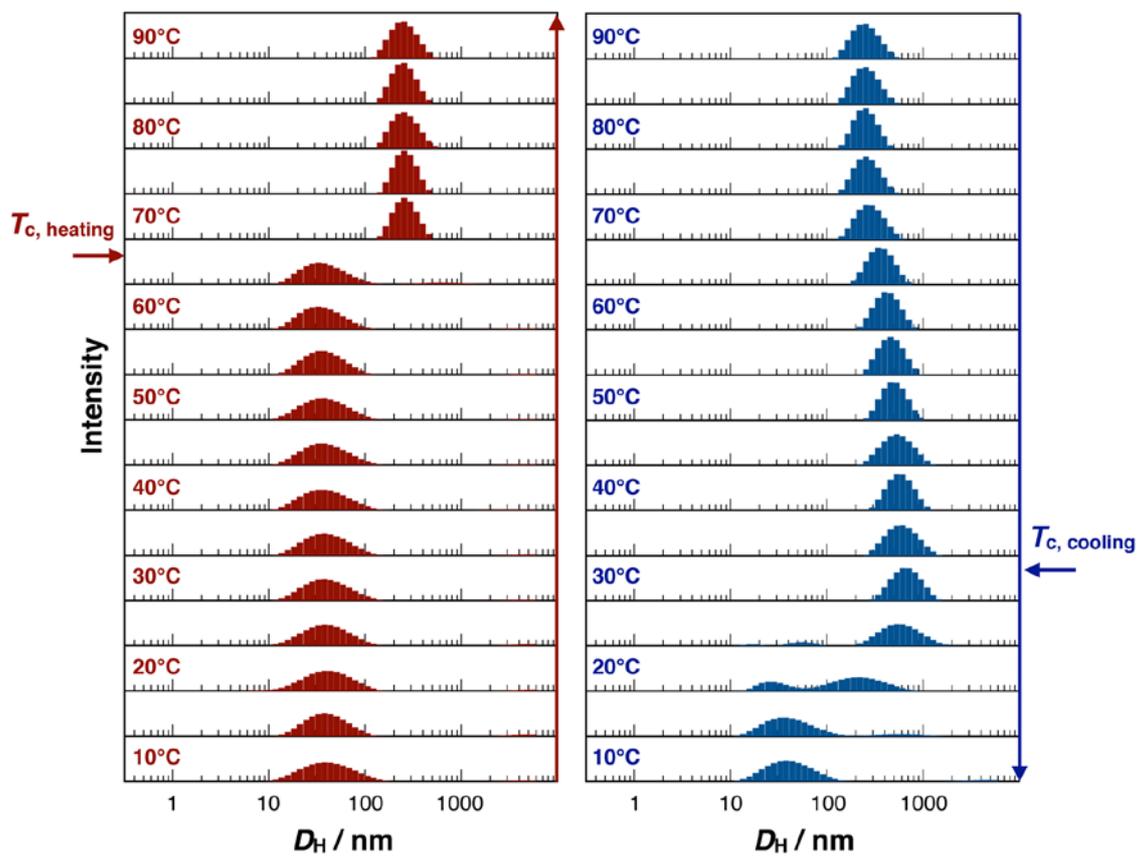


Fig. 8 Temperature dependence of the D_H distribution for the aqueous solution (0.1 wt%) of isotactic poly(NEAAm-co-NIPAAm) with NIPAAm composition of 13.9 mol%.

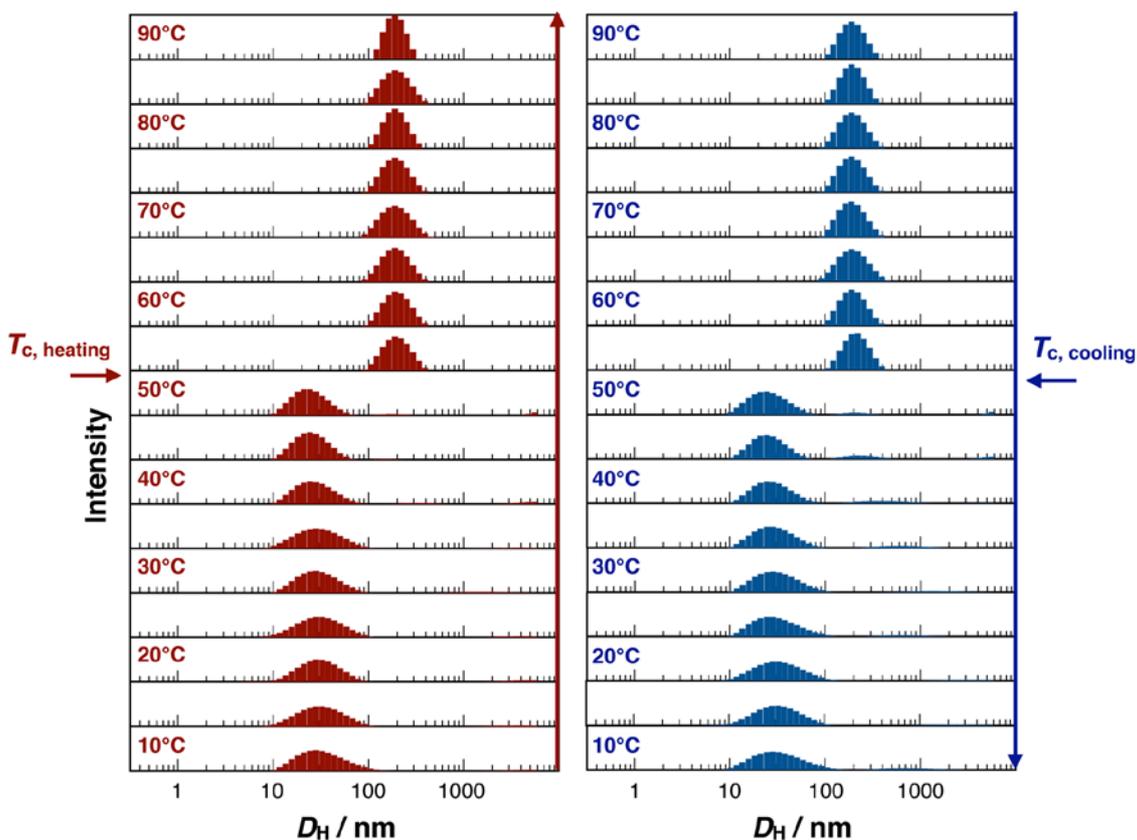
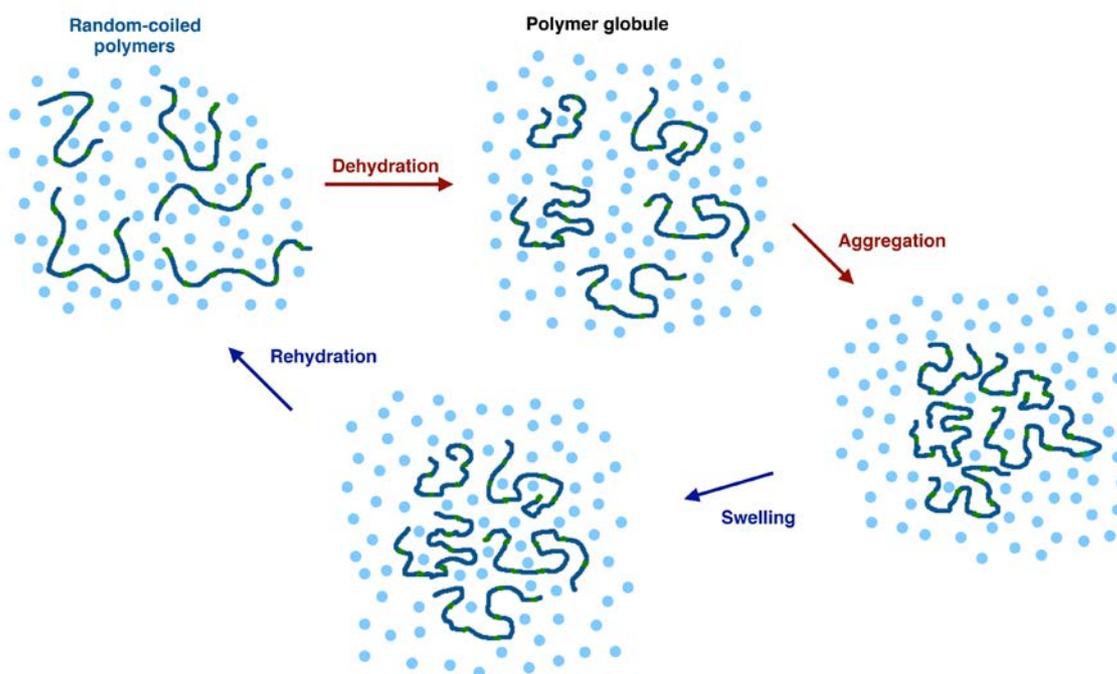


Fig. 9 Temperature dependence of the D_H distribution for the aqueous solution (0.1 wt%) of syndiotactic poly(NEAAm-co-NIPAAm) with NIPAAm composition of 49.5 mol%.

The syndiotactic copolymer showed unimodal distribution at temperatures lower than the $T_{c, \text{heating}}$ (Fig. 9). This is likely assigned to random-coil chains. The average D_H slightly decreased from 37 nm to 26 nm, when temperature increased from 10 °C to 50 °C. The slight decrease in the average D_H would be attributed to partial dehydration, probably in particular of the NIPAAm units, at higher temperatures.

At 55 °C, slightly higher than $T_{c, \text{heating}}$ (Fig. 4), the average D_H dramatically increased to 206 nm with unimodal distribution maintained. This is likely due to the

dehydration of the copolymers followed by aggregation of the dehydrated copolymers (Scheme 3). The average D_H decreased slightly to 196 nm by further increasing the temperature to 90 °C. This is probably because gradual dehydration proceeded even inside the aggregates.

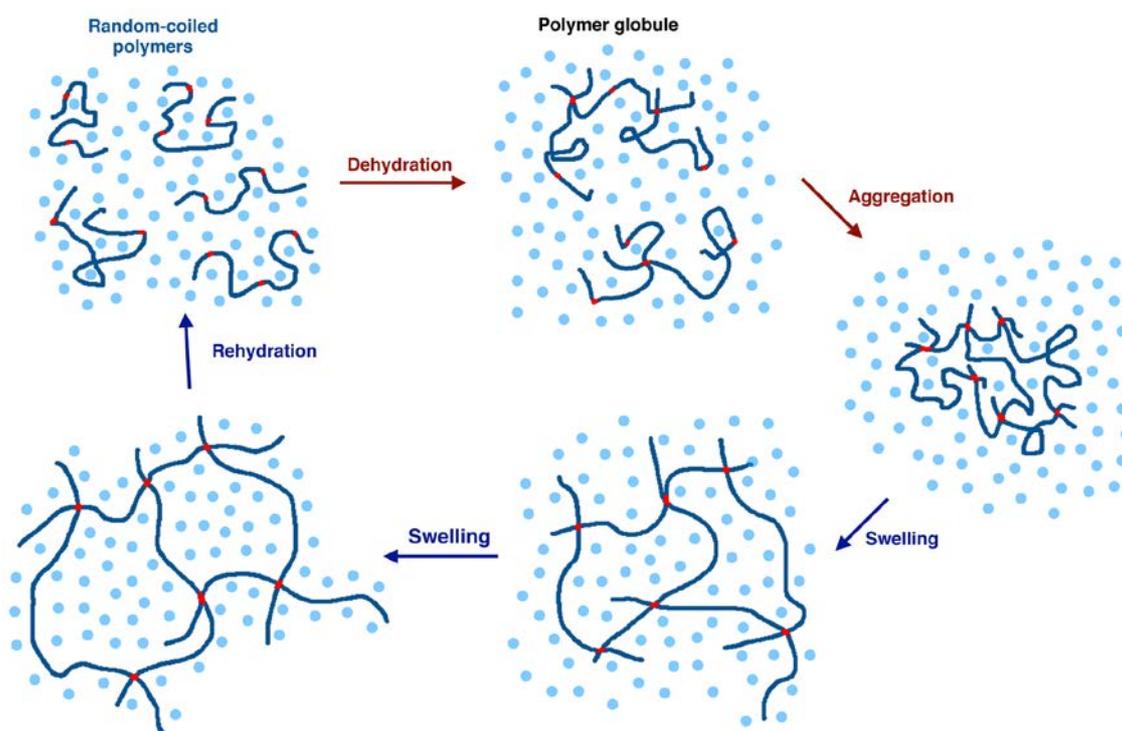


Scheme 3 Possible mechanism for the reversible and sharp phase transition of syndiotactic copolymer containing syndiotactic NIPAAm segments (indicated by green line).

In the cooling process, the average D_H conversely increased to 218 nm by lowering the temperature to 55 °C, and then dramatically decreased to 27 nm at 50 °C. The dissolution of aggregates of dehydrated polymers has been reported to involve two

processes: the swelling of the aggregates as a fast process and the dissociation of the individual polymer chains from the aggregates as a slow process [35, 37]. It is therefore assumed that the dissociation occurs immediately after the swelling process, even though the copolymer contains syndiotactic NIPAAm segments (Scheme 3). Eventually, the average D_H returned to 37 nm at 10 °C.

The isotactic copolymer also showed unimodal distribution at temperatures lower than the $T_{c, \text{heating}}$ (Fig. 8). The average D_H slightly decreased from 47 nm to 40 nm, when temperature increased from 10 °C to 65 °C. The average D_H dramatically increased to 268 nm with unimodal distribution maintained at 70 °C, slightly higher than $T_{c, \text{heating}}$ (Fig. 7), and then decreased slightly to 260 nm by further increasing the temperature to 90 °C. Similar to syndiotactic copolymer, increase in the temperature induced the dehydration, followed by aggregation of the dehydrated copolymers (Scheme 4).



Scheme 4. Possible mechanism for the induction of unusually large hysteresis in which dehydrated polymers are cross-linked by isotactic NIPAAm segments (indicated by red line) with hydrophobic interaction.

In the cooling process, the average D_H conversely increased to 293 nm by lowering the temperature to 70 °C. In contrast to syndiotactic copolymer, the increase in the average D_H was enhanced by further lowering the temperature (515 nm at 50 °C, 699 nm at 30 °C) with the unimodal distribution maintained. The significant increase in the average D_H in the cooling process for the isotactic copolymer suggests the mechanism for the induction of large hysteresis as follows.

The aggregates were swollen with decreasing temperature toward the $T_{c, \text{heating}}$,

which resulted in a slight increase in the average D_H . The segments comprising hydrophilic NEAAm units were rehydrated below the $T_{c, \text{heating}}$. However, the segment comprising NIPAAm units with isotactic stereosequences formed insoluble domain with hydrophobic interaction, because hydrophobicity of isotactic NIPAAm segments were significantly enhanced resulting from favorable intramolecular hydrogen bonding at temperatures higher than $T_{c, \text{heating}}$ (Scheme 4). Accordingly, the swelling behavior continued even below the $T_{c, \text{heating}}$. The average D_H returned to 46 nm by lowering the temperature to 10 °C, likely because rehydration became strong enough to collapse the cross-linking domain formed by isotactic NIPAAm segments with hydrophobic interaction.

In the light transmittance experiments, stepwise phase transition was observed in the cooling process (Fig. 7). The swelling caused the average D_H to reach 699 nm at 30 °C, which suggests that the aggregates gradually changed into coacervates with decreasing temperature. Consequently, the light could transmit through the swollen polymers.

4. Conclusions

The effect of stereoregularity and chemical composition of poly(NEAAm-*co*-NIPAAm)s

on the phase-transition behavior of their aqueous solutions was investigated. The solubility of the copolymers depended on the chemical composition. For example, isotactic copolymers with NIPAAm composition of 37.8 mol% were insoluble in water at a concentration of 1.0 wt%, whereas those of ≤ 28.0 mol% were soluble in water. The aqueous solution of the copolymers showed temperature-induced phase transition. Furthermore, the isotactic copolymers with the NIPAAm components of 13.9 – 28.0 mol% exhibited large hystereses of *ca.* 40 °C. Because the syndiotactic copolymers scarcely showed hystereses, regardless of chemical composition, isotactic stereosequence is essential for the induction of large hystereses in the poly(NEAAm-*co*-NIPAAm) system.

Isotactic copolymers with NIPAAm composition of ≤ 9.1 mol% scarcely showed hystereses. This indicates that monomer sequence is another important factor for the induction of the large hystereses. Stereochemical analysis revealed that isotactic copolymers with $\bar{n}_{m-1} = 1.12 - 1.27$ exhibited large hystereses. This means that an average 2.12 – 2.27 NIPAAm units incorporated contiguously in isotactic stereosequence were responsible for the induction of unusually large hysteresis.

DLS analysis of the aqueous solution revealed that the isotactic copolymers showed normal coil-globule transition in the heating process as well as syndiotactic copolymers. In the cooling process, however, the aggregates of isotactic copolymers were

swollen even by lowering the temperature below the $T_{c, \text{heating}}$. This is likely because the segment comprising contiguous NIPAAm units with isotactic stereosequences formed insoluble domain with hydrophobic interaction, which caused by the hydrogen bonding between the amide groups at the dehydrated state. The insoluble domain behaved like cross-linking domain, leading to the induction of the unusually large hystereses.

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