

The Agglomeration Tendency of Low-molecular-weight Poly(*N*-isopropylacrylamide)

OHTAKA Atsushi¹, IMAI Masato¹, MITSUISHI Takumi¹, NOGI Masaaki¹,

SHIMOMURA Osamu¹, and NOMURA Ryôki^{1,2}

¹Department of Applied Chemistry, and ²NMRC, Osaka Institute of Technology,
Omiya, Asahi, Osaka, 535-8585, Japan

Fax: 81-6-6957-2135, e-mail: otaka@chem.oit.ac.jp

Copolymerization of *N*-isopropylacrylamide (NIPAM) with styrene (2 mol% in feed) was performed using AIBN as an initiator to give a linear copolymer (**1a**) of low-molecular-weight ($M_n = 9.0 \times 10^3$, $M_w/M_n = 1.49$). The content of styrene in **1a** was estimated as 1.8 mol% from ¹H NMR spectra. Cross-linked poly(*N*-isopropylacrylamide) (**2**) was prepared using divinylbenzene (DVB, 2 mol% in feed) as a cross-linker ($M_n = 8.6 \times 10^3$, $M_w/M_n = 1.74$). Lower critical solution temperature (LCST) of **1a** and **2** determined from the transmittance of their 0.35% aqueous solution were 28.9 and 27.0 °C, respectively. When 1.0% aqueous solution of **1a** was heated over LCST, the solution turned cloudy without agglomeration. In contrast, **2** gave agglomerates with heating over LCST. The effect of hydrophobic interaction, cross-linking agent, and cross-linking degree on the agglomeration tendency of **2** was discussed.

Key words: *N*-Isopropylacrylamide, Agglomeration Tendency, Cross-linking, Hydrophobic Interaction

1. INTRODUCTION

Stimuli-responsive polymers and gels, which are sensitive to external stimuli such as pH [1]-[4], temperature [5], ionic concentration [6], etc., have attracted much attention in the field of functional polymer. Especially, temperature sensitive polymer is applied to use as a noviral vector for gene delivery [7] and smart culture surface for controllable cell adhesion and detachment [8].

Poly(*N*-isopropylacrylamide) (PNIPAM) is the most studied water-soluble thermal sensitive polymer [9]. PNIPAM shows a sharp phase transition in water at about 32 °C, which is perhaps derived from a good balance between hydrophilic and hydrophobic interactions in the polymer [10]. The temperature at which such the transition occurs is called lower critical solution temperature (LCST) and this event is accompanied by an endothermic process releasing water from the polymer chains [11]. Much effort has been invested in better understanding the phase transition behavior and the parameters affecting the phase transition temperature. Bergbreiter et al. found that the polydispersity and molecular weight have little effect on LCST as long as the polymer M_w is > 50 kDa [12]. Stöver et al. reported that little effect of the end group on LCST was observed when M_w is above 10 kDa, and that the magnitude of the molecular

weight dependence decreased on using more hydrophobic end groups [13]. Inomata and coworkers investigated the effect of the level of cross-linking on the properties of temperature-sensitive PNIPAM hydrogels in pure water [14], particularly with respect to the issue of a temperature-induced response rate. Chu et al. reported the effect of the cross-linking level on the properties of PNIPAM hydrogels, especially the LCST behavior and the response dynamics [15]. They found that the LCST of PNIPAM hydrogels was virtually independent of the level of cross-linking.

On the other hand, properties of low-molecular-weight PNIPAMs have also been studied by several research groups. Recently, Liu et al. reported the thermoresponsive transition behavior of hydrophobically modified *N*-isopropylacrylamide copolymer ($M_n < 5.0 \times 10^3$) solution [16]. They found that incorporation of poly(vinyl laurate) facilitated the formation of hydrophobic microdomains far below LCST, causing a pronounced aggregation in solutions. Although soluble cross-linked PNIPAMs ($M_n < 1.8 \times 10^4$) synthesized from the conventional radical polymerization using AIBN as an initiator showed an upper critical solution temperature (UCST) in a methanol/water [11:3 (v/v)] mixture, thermal response in pure water did not investigate in the literature [17].

Here we investigate the effect of hydrophobic interaction, cross-linking agent, and cross-linking degree on agglomeration tendency of the low-molecular-weight PNIPAM in water.

2. EXPERIMENTAL

2.1. Materials

N-Isopropylacrylamide (NIPAM) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Styrene and tetrahydrofuran (THF) were obtained from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Divinylbenzene (DVB), *N,N'*-methylenebis(acrylamide) (MBA), and 2,2'-azobis(isobutyronitrile) (AIBN) were obtained from Sigma-Aldrich Co. (Missouri, USA). NIPAM was purified by recrystallization from a toluene/hexane mixture prior to use. Styrene was distilled prior to use.

2.2. Synthesis of poly(*N*-isopropylacrylamide)s

Linear and cross-linked poly(*N*-isopropylacrylamide)s (**1-3**) were synthesized by a conventional radical polymerization using AIBN as an initiator. A typical procedure preparing linear PNIPAM (**1a**: 2 mol% styrene in feed) is as follows. Into a two-necked reaction vessel were added NIPAM (0.55 g, 4.9×10^{-3} mol), AIBN (8.0 mg, 5.0×10^{-5} mol), styrene (10.4 mg, 1.0×10^{-4} mol) and THF (5 mL). After stirring at 60 °C for 24 h under N₂ atmosphere, the solvent was removed *in vacuo* to give a crude product. Reprecipitation was carried out at least three times in a THF-diethyl ether system. The last precipitate was dried under reduced pressure and lyophilized with a freeze dryer to give **1a** (0.41 g, 73% yield) as a white powder. The number-average molecular weight (M_n) and the molecular weight distribution (M_w/M_n) determined by GPC analysis were ca. 9.0×10^3 and 1.49, respectively. The styrene content in **1a** (1.84 mol%) was the average of three measurements using ¹H NMR spectra.

Cross-linked PNIPAM (**2**: 2 mol% DVB in feed) was synthesized using DVB as a cross-linking agent. M_n was 8.6×10^3 , and M_w/M_n was 1.74.

Cross-linked PNIPAM (**3a**: 2 mol% MBA in feed) was synthesized from NIPAM and MBA. M_n was 8.3×10^3 , and M_w/M_n was 1.63.

2.3. General methods

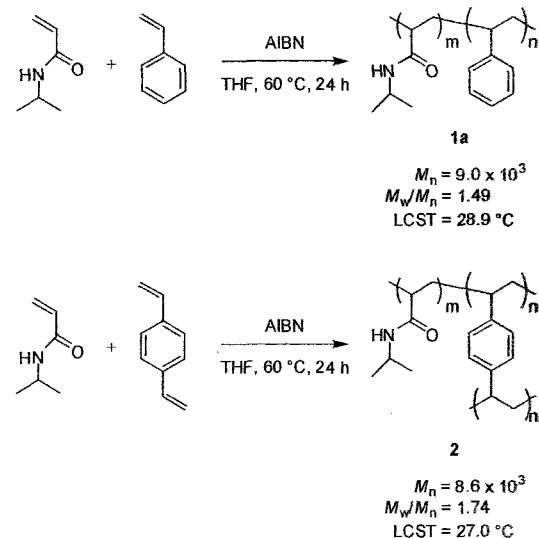
¹H NMR spectra in DMSO-*d*₆ were recorded with a 300 MHz NMR spectrometer (UNITY 300, Varian, Palo Alto, CA) using tetramethylsilane ($\delta = 0$) as an internal standard. Gel permeation chromatographic (GPC) analysis in THF was carried out with a CTO-10A instrument (Shimadzu Co., Kyoto, Japan) (column: SHIMPACK GPC-803). The columns were calibrated with poly(methyl methacrylate) (PMMA) of narrow molecular weight distribution standards. Lyophilization was carried out with a freeze dryer (FDU-830, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Dynamic light scattering

(DLS) measurements have been performed with an LB-500 light scattering photometer (Horiba Ltd. Kyoto, Japan). The data was the average of three runs. LCST was determined from transmittance using UV-vis spectrometer (U-3310, Hitachi High-Technologies Co., Tokyo, Japan) equipped with a thermostatic chamber. The rate of change of temperature was kept as low as possible, and we waited at least 30 min before starting the measurements at the next temperature so that a sufficient equilibrium should be attained.

3. RESULTS AND DISCUSSION

To investigate the agglomeration tendency, linear and cross-linked poly(*N*-isopropylacrylamide)s were synthesized from conventional radical copolymerization. Thus, the copolymerization of *N*-isopropylacrylamide (NIPAM) and styrene was performed at 60 °C in THF for 24 h using AIBN as an initiator to obtain poly(NIPAM-co-styrene) (**1a**, 2 mol% styrene in feed). Cross-linked poly(*N*-isopropylacrylamide) (**2**) was synthesized using divinylbenzene (DVB, 2 mol% in feed) as a cross-linking agent, which will have similar hydrophobicity to styrene (Scheme 1).

Scheme 1. Synthesis of linear and cross-linked poly(*N*-isopropylacrylamide)s (**1** or **2**).



The molecular weights (M_n) of copolymers estimated by gel permeation chromatography (GPC) were low (**1a**: 9.0×10^3 ; **2**: 8.6×10^3) and the molecular weight distributions (M_w/M_n) were slightly broad (**1a**: 1.49; **2**: 1.74). The content of styrene in **1a** which was calculated from ¹H NMR spectra was 1.84 mol%. The lower critical solution temperature (LCST) of **1a** was 28.9 °C determined from the change of the transmittance of 0.35% aqueous solution using UV-vis spectra. On the other hand, the content of DVB in **2** based on ¹H NMR spectra was variable probably because the molecular flexibility at the

cross-linking point was poor. Inomata et al. and Chu et al. pointed out that the LCST of PNIPAM hydrogels was virtually independent of the level of cross-linking [14],[15]. Therefore we estimated the DVB contents in **2** by its LCST (27.0 °C), suggesting that the contents of DVB in **2** was higher than that of styrene in **1a**. The solubility of **2** for water is similar to that of **1a** (10 mg/mL). In addition, both polymers showed a reversible phase change.

When visual observation was performed for 1.0% aqueous solution of **1a** at 40 °C, only turning the solution cloudy was observed. On the contrary, cross-linked copolymer **2** was agglomerated and recovered from the solution by simple filtration on heating the 1.0% aqueous solution at 40 °C (Figure 1).

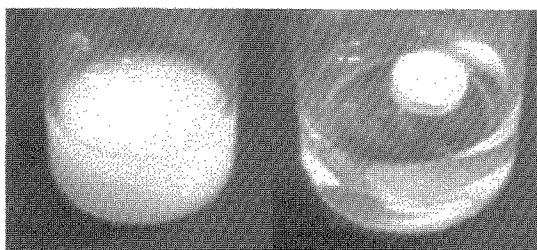


Figure 1. The photographs of visual observation on heating 1.0% aqueous solution of **1a** (left) and **2** (right) at 40 °C.

According to these results, we synthesized linear copolymers with several styrene contents in order to confirm the correlation between hydrophobic interaction and agglomeration. LCST of obtained copolymers were decreased with increasing the styrene contents (**1a**: 28.9 °C; **1b**: 27.4 °C; **1c**: 25.9 °C; **1d**: 25.1 °C [18]). These data are consistent with the previous report [16],[19]. Cross-linked PNIPAMs (**3**) were also prepared using *N,N'*-methylenebis(acrylamide) (MBA) as a cross-linking agent to check the relationship of cross-linking to agglomeration. Unfortunately, the MBA content could not be calculated from ¹H NMR spectra because the signal assignable to MBA was coalesced with that of NIPAM. LCST of **3** (32.0 °C) was independent of the MBA contents in feed ratio which was consistent with the previous papers. Table I listed the characteristics of the obtained copolymers. All of copolymers have similar solubility for water (10 mg/mL).

Table I. Synthesis^a and characteristics of copolymers.

comonomer (mol%) ^b	$M_n^c \times 10^{-3}$	M_w/M_n^c	contents ^d /mol%	LCST ^e /°C	code
styrene	9.0	1.49	1.8	28.9	1a
styrene	7.4	1.47	3.8	27.4	1b
styrene	4.4	2.10	6.5	25.9	1c
styrene	11.1	1.41	9.7	25.1 ^f	1d
MBA (2)	8.3	1.63	—	32.0	3a
MBA (6)	5.3	1.46	—	32.1	3b

^aTotal monomer concentration = 5.0 mol/L in THF, [monomer]/[initiator] = 100, 60 °C, 24 h. ^bfeed composition. ^cDetermined by GPC measurements (calibrated with poly(methyl methacrylate); eluent, THF). ^dDetermined by ¹H NMR spectra (DMSO-*d*₆). ^eDetermined by UV-vis spectra with 0.35% aqueous solution. ^fDetermined by UV-vis spectra with 0.10% aqueous solution.

When 1.0% aqueous solution of **1** was heated at 40 °C, the formation of agglomerate was observed only in the case of **1d** (Figure 2). These results indicate that hydrophobic interaction plays an important role in agglomeration. However, other factors such as cross-linking should influence on the formation of agglomerate since no agglomerate was observed in the case of **1c** which has higher hydrophobicity than **2**. Further, the solution of **3** was turned cloudy without formation of agglomerate. Consequently we tentatively concluded that introduction of cross-linking and cross-linking degree perhaps affected on the agglomeration.

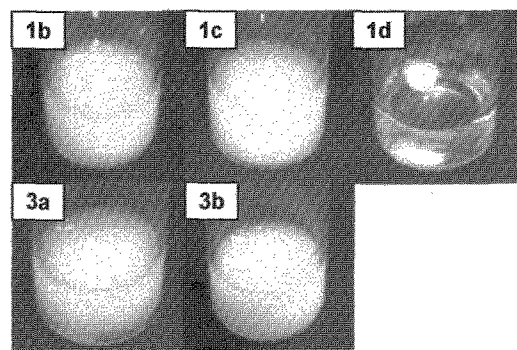


Figure 2. The photographs of visual observation on heating 1.0% aqueous solution of **1b-1d**, **3a**, and **3b** at 40 °C.

To clear the effect of cross-linking on agglomeration, the dynamic light scattering (DLS) measurements was performed for 0.04% aqueous solution at 40 °C. Those results are shown in Table II.

Table II. Hydrodynamic diameter (D_h) for 0.04% aqueous solution of copolymer measured by dynamic light scattering at 40 °C.

code	LCST /°C ^a	$D_h / \mu\text{m}^b$
1a	28.9	0.183 ± 0.004
1b	27.4	0.144 ± 0.004
1c	25.9	0.131 ± 0.001
1d	25.1 ^c	0.148 ± 0.001
2	27.0	0.084 ± 0.003
3a	32.0	0.191 ± 0.006
3b	32.1	0.116 ± 0.003

^aDetermined by UV-vis spectra with 0.35% aqueous solution. ^bAverage of three runs. ^cDetermined by UV-vis spectra with 0.10%

aqueous solution.

Hydrodynamic diameter (D_h) of **2** was $0.084 \pm 0.003 \mu\text{m}$ although the diameter of any other copolymer was larger than $0.1 \mu\text{m}$. The diameters of particles decreased with an increase of their cross-linking degree (**3a**: $0.191 \pm 0.006 \mu\text{m}$; **3b**: $0.116 \pm 0.003 \mu\text{m}$), suggesting the introduction of cross-linking and cross-linking degree would affect on the particle size. It is known that smaller polymer nanoparticles are labile to aggregate. Based on these results, particle size should lead to the difference of visual observation between **1c** and **2**. However, particle size is not a sole reason for agglomeration because no agglomerate was observed in the case of **3b** which formed smaller particle than **1d**. Combining above data, both properties of hydrophobicity and particle size would have influence on agglomeration.

On the other hand, the diameter decreased with increasing the hydrophobicity of copolymer (**1a**: $0.183 \pm 0.004 \mu\text{m}$; **1b**: $0.144 \pm 0.004 \mu\text{m}$; **1c**: $0.131 \pm 0.001 \mu\text{m}$), indicating the polymer chain collapse to compact globular structure. However, the diameter of **1d** ($0.148 \pm 0.001 \mu\text{m}$) was larger than that of **1c**, suggesting the aggregation number would be different [20]. Therefore, we could not discuss about hydrodynamic diameter of copolymers in more detail.

4. SUMMARY

Linear poly(NIPAM-co-styrene) (**1**) and cross-linked PNIPAM (**2** and **3**) were synthesized from radical copolymerization using AIBN as an initiator. The agglomeration was observed in the case of **1d** and **2** on heating 1.0% aqueous solution at 40°C . The hydrodynamic diameter of aggregate of **2** determined by DLS was smaller than that of any other copolymer. It was suggested that both effects of hydrophobicity and cross-linking would affect on agglomeration.

However, we did not discuss about hydrodynamic diameter of copolymers in more detail because aggregation number of copolymer do not measure. Therefore, the determination of aggregation number for all copolymer is now in progress using static light scattering (SLS) measurements. In addition, Engberts et al. reported the aggregation tendency of polysoaps clearly depends on their macromolecular flexibility that varies inversely with the cross-linking agent content [21]. The measurement of molecular flexibility for all copolymers is also now in progress using the differential scanning calorimetry (DSC) measurements. Furthermore, the synthesis of cross-linked poly(NIPAM-co-styrene) using MBA is now in progress in order to confirm the relation between agglomeration and cross-linking.

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