Polymer Vol. 39 No. 16, pp. 3703–3708, 1998
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0032-3861/98/$19.00+0.00

PII: S0032-3861(97)10365-2

Effect of polymer complex formation on the cloud-point of poly(*N*-isopropylacrylamide) (PNIPAAm) in the poly(NIPAAm-*co*-acrylic acid): polyelectrolyte complex between poly(acrylic acid) and poly(L-lysine)

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(Received 8 July 1997; accepted 25 November 1997)

Random copolymers of acrylic acid (AAc) and *N*-isopropylacrylamide (NIPAAm) were synthesized by free-radical polymerization. The copolymers were characterized for their temperature- and pH-responsive behaviour by cloud-point experiments. The influence of polyelectrolyte between poly(allyl amine) (PAA) and poly(L-lysine) (PLL) on the lower critical solution temperature (LCST) of a pH/temperature sensitive polymer was compared in the range of pH 2–12. A polyelectrolyte complex was prepared by mixing poly(NIPAAm-*co*-AAc) and PLL solutions as anionic and cationic polyelectrolytes, respectively. The LCSTs of PNIPAAm/water in the copolymers were strongly affected by the pH, the presence of polyelectrolyte solute and AAc content in the poly(NIPAAm-*co*-AAc). The influence of more hydrophobic PLL as polyelectrolyte on the cloud-point of PNIPAAm/water in the copolymer was stronger than that of PAA. It is thought that as the side chain length increases from C_1 for PAA to C_4 for PLL, the hydrophobicity of polyelectolyte complex increases, indicating that much more dehydration occurs around PNIPAAm. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: pH/temperature sensitive polymer; polyelectrolyte complex; cloud point)

INTRODUCTION

Most of the intelligent polymers studied previously are responsive to only one kind of stimulus[1–6]. But for some applications, independent responsiveness to several factors, such as temperature and pH, may be required[7]. These pH/temperature sensitive polymers which contain both pH and temperature-sensitive components undergo marked solubility changes in water in response to temperature and pH changes[8,9].

Poly(*N*-isopropylacrylamide)(PNIPAAm) is one of the popular temperature sensitive polymers since it exhibits a sharp phase transition at around 32°C[10–13]. Hence this polymer has potential as a sensor in humans, as well as in size-selective separation processes involving materials which can be denatured at high temperatures[14]. pH-sensitive components bearing weakly acidic pendent groups as a anionic polyelectrolyte can be incorporated into the PNIPAAm. When a cationic polyelectrolyte is mixed with an anionic polyelectrolyte solution, a polyelectrolyte complex is formed through electrostatic attraction between two oppositely charged polyelectrolytes. Therefore, it may be expected that the lower critical solution temperature (LCST) of PNIPAAm incorporated with a pH-sensitive component will be affected by a polyelectrolyte complex according to the various pH values. According to the rationale we have proposed above, we can molecularly engineer a pH/temperature sensitive polymer complex with its LCST matching the desired ‘critical region’ by using a polyelectrolyte solute. In a previous study[15], we reported the influence of polyelectrolyte complex formation between poly(acrylic acid) (PAAc) and poly(allylamine) (PAA) on the LCST of PNIPAAm/water in poly(NIPAAm-*co*-AAc). From the results of this study, the LCST of PNIPAAm in poly(NIPAAm-*co*-AAc) solution with PAA increased with pH value and appeared above the pK_a value of PAAc, which was not observed in the absence of PAA. However, it was lower than that of PNIPAAm in the absence of PAA, due to the polyelectrolyte complex formation. Also, it decreased with an increase of AAc content in the copolymer below the pK_a value of PAAc, whereas it increased with AAc content in the copolymer above the pK_a value of PAAc.

The objective of this work is to study the influence of another cationic polyelectrolyte solute, poly(L-lysine) (PLL), on the LCST of PNIPAAm/water in the pH/temperature sensitive polymer, and to study the effect of the more hydrophobic side chain of PLL upon the polyelectrolyte complex formation as compared with PAA. Poly(acrylic acid) (PAAc) as a pH-sensitive component was introduced into the temperature-sensitive component, PNIPAAm, to produce the pH/temperature sensitive polymer, poly(NIPAAm-*co*-AAc).

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EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAAm, Tokyo Kasei) was recrystallized in *n*-hexane. Acrylic acid (AAc, Jensei Chem. Co.) was purified by distillation at 40°C/26 mmHg. *N,N'*-azobisisobutyronitrile (AIBN, Kasida Co.) was purified in methanol. 1,4-Dioxane (Duksan Co.) was purified by distillation. PLL (Aldrich), sodium hydroxide standard solution (Katayama Chem.), hydrochloric acid standard solution (Katayama Chem.) and sodium chloride (Katayama Chem.) were used as recieved.

Synthesis of poly(NIPAAm-co-AAc)

Synthesis of linear poly(NIPAAm-*co*-AAc), containing 0, 10, 20, 30, 40, 50 mol% of AAc, was carried out in 1,4-dioxane(25 wt%) with AIBN as an initiator(0.3 mol% of monomers), as the method previously reported[15]. Dried nitrogen was bubbled through the solution for 20 min prior to polymerization. After polymerization at 70°C for 5 h, the mixtures were dissolved in methanol and precipitated in diethyl ether. The precipitates were filtered and washed with acetone and deionized water to remove the unreacted monomers and homopolymer, respectively. Then, the precipitates were washed again with acetone to substitute the water, and dried in a vacuum desiccator for 2 days.

Preparation of polyelectrolyte complex

poly(NIPAAm-co-AAc) solution (4.2×10^{-2} M), containing 0, 10, 20, 30, 40, 50 mol% of AAc, and 1.1×10^{-1} M of PLL solution was prepared by dissolving polymers in distilled water, respectively. Before mixing two solutions, the pH of each solution was adjusted identically with HCl or NaOH standard solution. To each copolymer solution, PLL solution was added.

Cloud point determination

The absorbance at 450 nm was measured against temperature using a u.v.–vis. spectrophotometer. The temperature of the solutions was raised from 15°C to 70°C in two increments every 10 min. The cloud point was defined as the temperature at the inflection point in the normalized absorbance *versus* temperature curve and the cloud point of solution which does not exhibit inflection point was determined at 10% absorbance in the curve[16].

Titrations

Titrations of the poly(NIPAAm-*co*-AAc) at 0.1 M NaCl solution were performed as follows. Of each linear copolymer, 100 mg was dissolved in 25 ml of 0.1 M NaCl. In order to increase the solubility of the polymers during the titrations, back titrations were performed on ionized polymers. The polymers were fully ionized by adding 26 ml of 0.1 N NaOH. Titrations were performed at 20°C by adding 0.1 N HCl in small quantities and measuring the pH after a stable value has been reached. The temperature was kept constant (±0.3°C) for 30 min before and during each titration, using a waterbath[17].

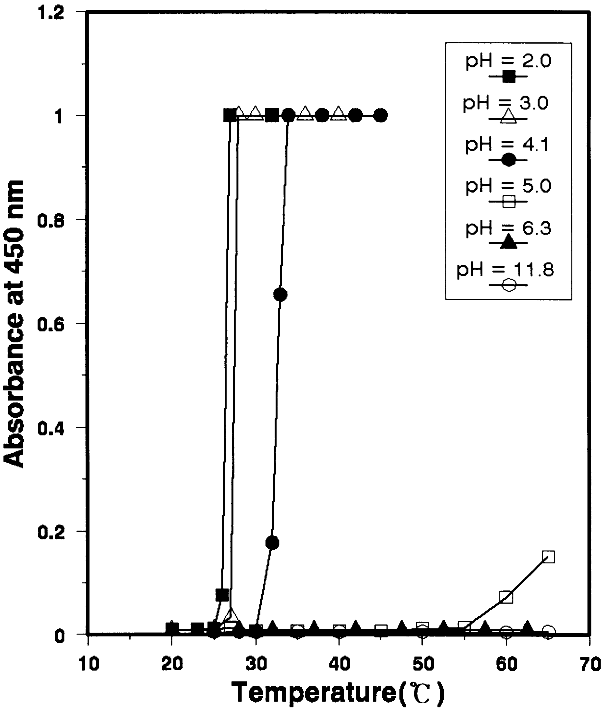

Figure 1 Absorbance at 450 nm of 4.2×10^{-2} M of poly(NIPAAm-*co*-AAc-30) solution against temperature as a function of pH

RESULTS AND DISCUSSION

Poly(NIPAAm-*co*-AAc) was prepared from comonomer feed compositions ranging from 0 to 50 mol%. The copolymer compositions determined from back titration method and elemental analysis are given in *Table 1*.

Figure 1 shows absorbance at 450 nm *versus* temperature for 4.2×10^{-2} M of poly(NIPAAm-*co*-AAc-30) solution according to the various pH values. Distinct cloud points were observed at pH 2.0–4.1 and change of absorbance against temperature became small with an increase of pH. Particularly, a drastic change of absorbance of the solution *versus* temperature was observed around pH 4.3–4.7, which is related to the pK_a value of PAAc. Cloud points of PNIPAAm in poly(NIPAAm-*co*-AAc-30), which are defined as the transition point of absorbance *versus*

Table 1 Monomer ratios in linear poly(NIPAAm-*co*-AAc) compared to monomer feed ratios

Comonomer AAc feed molar composition (mol%)	Copolymer AAc molar composition[a] (mol%)	Copolymer AAc molar composition[b] (mol%)	% Conversion
0	0	0	84.5
10	13.8	13.8	29.6
20	21.8	–	58.5
30	32.9	27.2	89.7
40	35.8	39.8	81.9
50	45.8	–	79.9

[a] obtained by titration
[b] obtained from elemental analysis

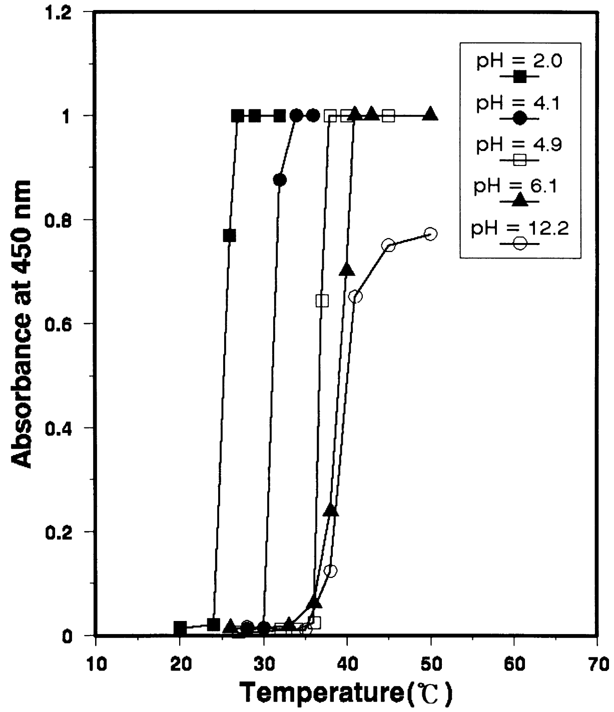

Figure 2 Absorbance at 450 nm of 4.2 × 10^{-2} M of poly(NIPAAm-*co*-AAc-30) solution with 1.1 × 10^{-1} M PLL solution against temperature as a function of pH

temperature, were 26.5, 27.5 and 39.0°C for the pH values 2.0, 3.0 and 4.1, respectively. From these results, it was found that the cloud point of PNIPAAm in the poly(NIPAAm-*co*-AAc-30) solution decreased in the non-ionized state of PAAc whereas its cloud point increased in the partially ionized state of PAAc in comparison with the cloud point of PNIPAAm itself, 31–33°C[10–13]. It is thought that the cloud point of PNIPAAm decreases owing to inter- or intra-hydrogen bonding between the amide group of PNIPAAm and the carboxylic acid group of PAAc below the pK_a value of PAAc. Above the pK_a value of PAAc, the cloud point of PNIPAAm in the poly(NIPAAm-*co*-AAc-30) solution was not observed below 70°C, since the carboxylic acid group of PAAc was completely ionized and resulted in electrostatic repulsion of ionized groups of PAAc.

Figure 2 shows absorbance at 450 nm *versus* temperature for 4.2 × 10^{-2} M of poly(NIPAAm-*co*-AAc-30) solution according to the various pH values in the presence of PLL as a polyelectrolyte solute. Distinct cloud points were observed at all pHs studied. Interestingly, drastic change of absorbance of the solution against temperature was observed even above the pK_a value of PAAc in the presence of PLL. The cloud points of PNIPAAm in poly(NIPAAm-*co*-AAc-30) solution in the presence of PLL were 25.0, 31.0, 37.5 and 39.0°C for the pH value of 2.0, 4.1, 4.9 and 6.1, respectively. From these results, it was found that the cloud point increased with pH value because the degree of ionization of carboxylic acid groups increased with pH value. Also, it was found that the cloud point of PNIPAAm in the solution appeared above the pK_a value of PAAc, which was not observed in the absence of PLL. The cloud point of PNIPAAm in the copolymer solution in the presence of PLL decreased due to the polyelectrolyte complex formation between the carboxylic acid group of PAAc and the amine group of PLL, indicating that dehydration occurred around PNIPAAm. Around a pH value of 7.0, the cloud point of PNIPAAm in the solution was not observed due to the insoluble polyelectrolyte complex formation between them, indicating a compact form of the complex structure. Also, drastic change of absorbance of the solution against temperature was observed at the pH value of 12.2, which is above the pK_b value of PLL. This phenomenon is not a result by

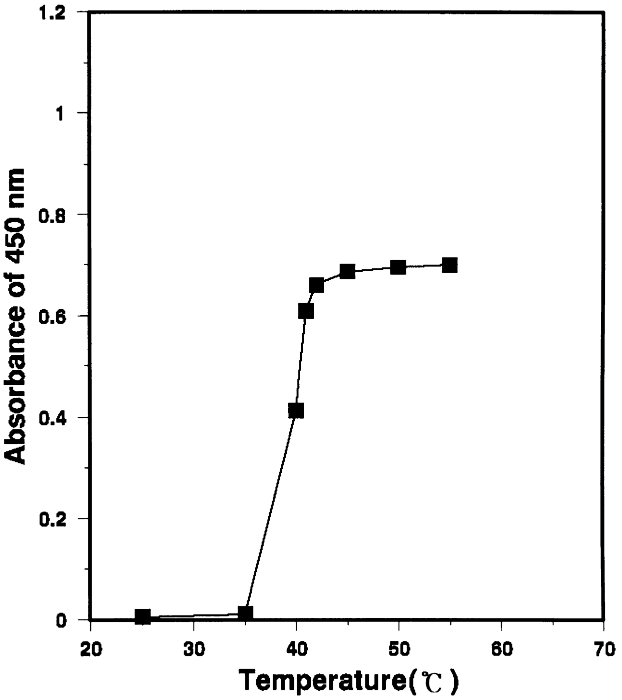

Figure 3 Absorbance at 450 nm of 1.1 × 10^{-1} M of PLL solution against temperature at pH 12

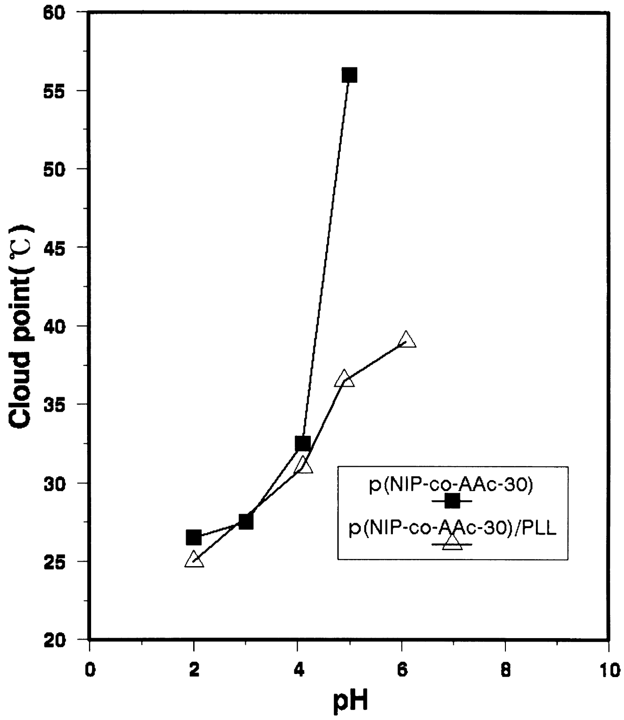

Figure 4 Cloud point of 4.2 × 10^{-2} M of poly(NIPAAm-*co*-AAc-30) solution in the presence of PLL in comparison with 4.2 × 10^{-2} M of poly(NIPAAm-*co*-AAc-30) solution itself against pH

Table 2 Cloud point (°C) of 4.2×10^{-2} M poly(NIPAAm-*co*-AAc) solutions against content of AAc as a function of pH

	AAc content (mol%)				
pH	10	20	30	40	50
2	31.5	29.5.	26.5	23.0	20.5
3	32.0	30.5	27.5	24.5	21.5
4	34.5	35.5	39.0	51.5	[a]
5	44.5	[a]	[a]	[a]	[a]
6	[a]	[a]	[a]	[a]	[a]
12	[a]	[a]	[a]	[a]	[a]

[a] The cloud point was not observed below 70°C

Table 3 Cloud point (°C) of 4.2×10^{-2} M poly(NIPAAm-*co*-AAc) solutions with 1.1×10^{-1} M PAA or PLL aqueous solution against content of AAc as a function of pH

		AAc content (mol%)				
pH	Solute	10	20	30	40	50
2	PAA	30.5	29.0.	24.5	22.5	18.5
	PLL	31.5	28.5	25.0	21.5	18.5
4	PAA	31.0	30.0	29.5	31.0	35.0
	PLL	33.0	31.5	31.0	26.5	24.5
5	PAA	33.0	34.5	37.0	50.0	54.0
	PLL	35.0	37.0	37.5	36.5	34.5
6	PAA	[b]	37.5	46.0	56.5	[a]
	PLL	38.5	38.5	39.0	38.0	[a]
12	PAA	[a]	[a]	[a]	[a]	[a]
	PLL	[a]	[a]	[a]	[a]	[a]

[a] The cloud point was not observed below 70°C

[b] The cloud point was not measured due to the occurrence of precipitate

Table 4 Cloud point (°C) of poly(NIPAAm-*co*-AAc) with PLL against molar ratio of [LL] to [AAc] at pH 5

	[LL]/[AAc]				
AAc content (mol%)	0.375	0.75	1	2	2.5
10	38.0	ppt.	32.5	37	37.5
30	turbid	ppt.	28.5	37	37.5
50	turbid	ppt.	26.5	29	29

ppt., precipitate

polyelectrolyte complex formation but the conformational transition of PLL itself by heating. It was already reported that PLL undergoes a conformational transition from the α-helix to both the random coil and β-form by heating above pH 11, the latter partly giving rise to precipitation[18]. Absorbance at 450 nm of 1.1×10^{-1} M of PLL in aqueous solution against temperature at pH 12 is shown in *Figure 3*.

In *Figure 4*, the cloud points of PNIPAAm of the copolymer in the solution are plotted against pH value in the presence of PLL. At the same pH, the cloud points of PNIPAAm in the aqueous solution with PLL were lower than those of PNIPAAm without PLL. However, not much difference was found below the pK_a value of PAAc. Also, it was found that the cloud points of PNIPAAm in the copolymer solution appeared above the pK_a value of PAAc in the presence of PLL.

Table 2 shows the cloud point of PNIPAAm against AAc mol% in 4.2×10^{-2} M of poly(NIPAAm-*co*-AAc) solution according to the pH values. It was found that the cloud points were affected by pH and AAc content in the copolymer. The cloud points of PNIPAAm in the solution decreased with an increase of AAc content in the copolymer around a pH value of 2.0–3.0, because more hydrogen bonding between the amide group of PNIPAAm and the carboxylic acid group of PAAc occurred with an increase of AAc content in the copolymer. The hydrogen bonding interferes with the access of water molecules to the NIPAAm amide groups[17]. On the other hand, the cloud points of PNIPAAm in the solution increased with AAc content in the copolymer around a pH value of 4.0–5.0, because of increased hydrophilicity with an increase of ionized PAAc.

Table 3 shows the cloud point of PNIPAAm against AAc mol% in 4.2×10^{-2} M of poly(NIPAAm-*co*-AAc) solution according to the various pH values in the presence of PAA and PLL, respectively. Not much difference in the cloud points between the copolymer solutions with PAA and those of PLL was found around a pH value of 2.0. However, the cloud point of PNIPAAm in the copolymer with PAA decreased slightly below 30 mol% of AAc, but increased above 30 mol% of AAc around a pH value of 4.0, and increased with AAc content around a pH value of 5.0–6.0. This phenomenon could be explained as follows: With increasing pH or AAc content, the COO^- groups in the copolymer chain increase. The presence of COO^- groups in the polymer chain increase its rigidity, because of electrostatic interchain repulsions, and makes difficult the efficient polyelectrolyte complex formation between the $-COO^-$ group of the copolymer and the $-NH_3^+$ group of PAA[19]. Hence, the cloud points of PNIPAAm increase with AAc content due to the charged groups not participated in the polymer complex formation. On the other hand, the cloud points of PNIPAAm in the copolymer solutions with PLL decreased with an increase of AAc content around a pH value of 4.0, and did not show an obvious tendency against AAc content around a pH value of 5.0–6.0. This is probably due to the more efficient polyelectrolyte complex formation between ionized PAAc and PLL by the enhanced hydrophobicity of longer side chains in comparison with PAA.

Table 4 shows the cloud points of PNIPAAm in the poly(NIPAAm-*co*-AAc-X)/PLL complex system, $X = 10$, 30, 50 mol%, against molar ratio of [LL] to [AAc]. It was found that the cloud points of PNIPAAm in the three kinds of poly(NIPAAm-*co*-AAc) solutions were not observed at [LL]/[AAc] $= 0.75$, due to the occurrence of insoluble polymer complexes. At [AA]/[AAc] $= 0.35$, the determination of cloud-point by a u.v.–vis. spectrophotometer was impossible because the mixing solution had been turbid, with the exception of poly(NIPAAm-co-AAc-10). On the other hand, the cloud points of PNIPAAm in the three kinds of poly(NIPAAm-*co*-AAc) solutions increased from [LL]/[AAc] $= 1.0$ to [LL]/[AAc] $= 2.0$, but were kept constant above [LL]/[AAc] $= 2.0$. The polyelectrolyte complex formed with equimolarity of each monomer component is neutral, and the solubility of these complex in water is very low because of mutual blocking of oppositely charged ionic groups that are responsible for solubility and enhanced molecular weight, by the formation of networks through the secondary bond. But when nonequimolar compositions are mixed, charges exist on polymer complex and thus these charges can increase solubility. But according to our experimental data neutral polymer complex was formed at nonequimolar ratio, [LL]/[AAc] $= 0.75$, We proposed two explanations in regard to this phenomena. One explanation is that the polymer composition is not equal to feed composition. A second explanation is that two polymer chains have not take ladder-like form during the polymer complex formation.

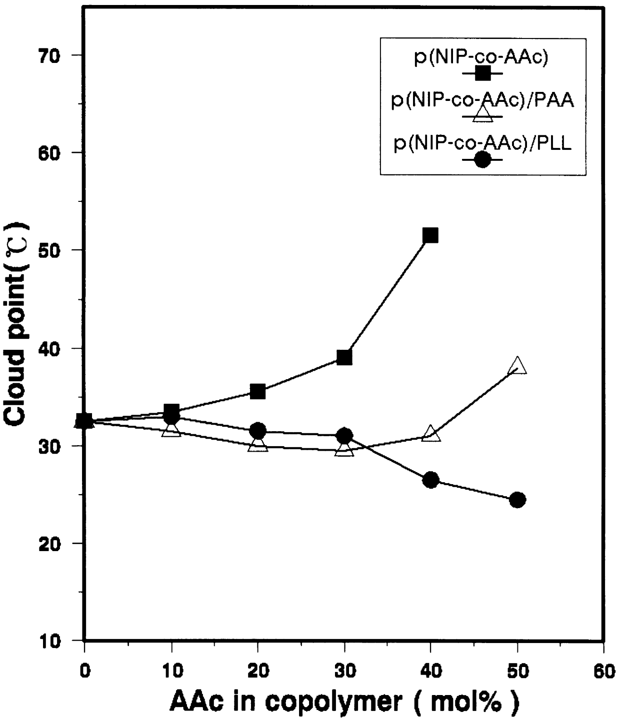

Figure 5 Comparison of cloud point of poly(NIPAAm-*co*-AAc)/PLL with that of poly(NIPAAm-*co*-AAc)/PAA at pH 4

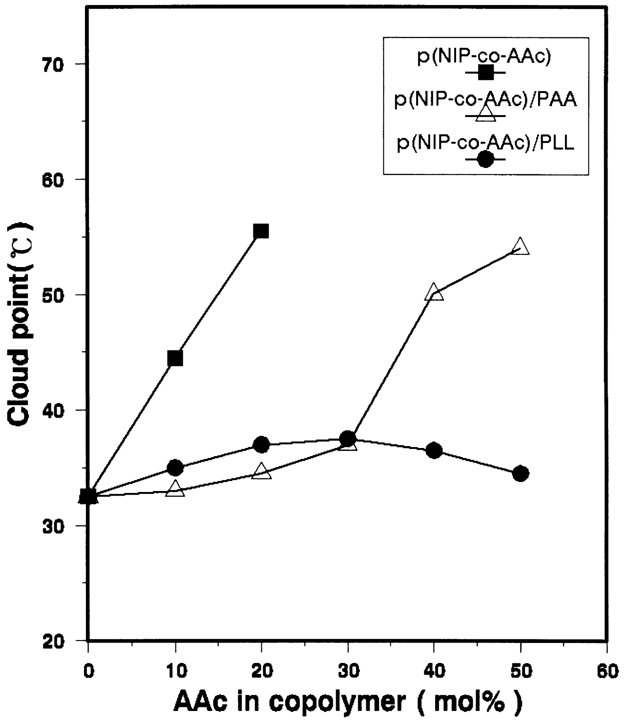

Figure 6 Comparison of cloud point of poly(NIPAAm-*co*-AAc)/PLL with that of poly(NIPAAm-*co*-AAc)/PAA at pH 5

Figures 5–7 show the effect of hydrophobic side chain of PLL on the cloud point of PNIPAAm in the poly(NIPAAm-*co*-AAc) as compared with PAA at pH 4.0, pH 5.0 and pH 6.0, respectively. The influence of PLL on the cloud point of PNIPAAm in the poly(NIPAAm-*co*-AAc) was stronger than that of PAA above 20–30 mol% of AAc content. It is thought that as the side chain length increases from C_1 for PAA to C_4 for PLL, the hydrophobicity of polyelectrolyte complex increases, indicating that more stronger dehydration occurs around PNIPAAm. That is, the $-(CH_2)_4$ group for PLL is more hydrophobic than $-CH_2$ for PAA, contributing to an increased hydrophobicity of copolymer chain and subsequently to the stabilization of the formed interpolymer aggregate by a synergistic hydrophobic effect.

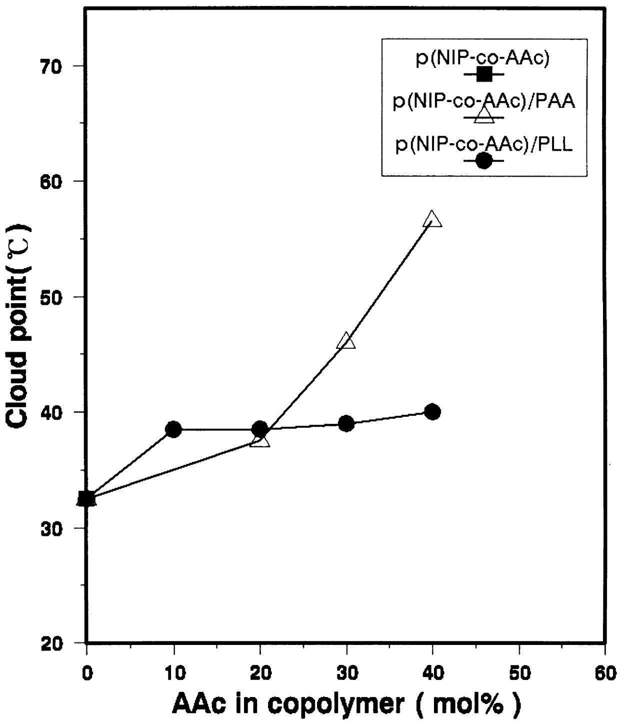

Figure 7 Comparison of cloud point of poly(NIPAAm-*co*-AAc)/PLL with that of poly(NIPAAm-*co*-AAc)/PAA at pH 6

In conclusion, the cloud points of PNIPAAm in poly(NIPAAm-*co*-AAc) were strongly influenced by the pH, AAc content and the type of polyelectrolyte solute. Polyelectrolyte complex in the poly(NIPAAm-*co*-AAc) system with PLL was formed in neutral conditions. The influence of PLL on the cloud point of PNIPAAm in the poly(NIPAAm-*co*-AAc) was stronger than that of PAA above 20–30 mol% of AAc content. These results could be explained by the more hydrophobicity of PLL than PAA in the polyelectrolyte complex formation between PAAc and PLL.

ACKNOWLEDGEMENTS

This work was supported by the Korea Science and Engineering Foundation (95-0300-08-02-3).

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