Characterization of Stimuli-Sensitive Polymers for Biomedical Applications

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Summary: Stimuli-sensitive polymers were synthesized by copolymerizing varying ratios of N-isopropyl acrylamide(NIPAAm) and acrylic acid(AAc). The influence of polyelectrolytes on the lower critical solution temperatures(LCSTs) of these temperature/pH sensitive polymers was investigated in the pH range of 2-12. Polyelectrolyte complexes were prepared by mixing poly(NIPAAm-*co*-AAc) as anionic polyelectrolyte with poly(allyl amine)(PAA) or poly(L-lysine)(PLL) as cationic polyelectrolytes, respectively. Back titration was performed to determine the pK_a values of PAAc in poly(NIPAAm-*co*-AAc) and to study the effect of comonomer ionization on the cloud point temperature. The effect of polyelectrolyte complex formation on the conformation of PLL was studied as a function of temperature by means of circular dichroism(CD). The swelling ratio of poly(NIPAAm-*co*-AAc) hydrogels as a function of pH at various temperature was obtained by measuring the weight of the hydrogels in buffer solutions. The LCSTs of the poly(NIPAAm-*co*-AAc) were strongly affected by pH, polyelectrolyte solutes, AAc content, and charge density. The influence of more hydrophobic PLL as a polyelectrolyte on the cloud point of PNIPAAm/water in the copolymer was stronger than that of poly(allyl amine)(PAA). Indomethacin was loaded into these hydrogels, and controlled release of this molecule from the hydrogel was determined under various temperature and pH conditions using UV/Vis spectrophotometry.

Introduction

Polymers which undergo reversible phase transitions in response to external stimuli such as temperature,[1,2] pH,[3] electric current,[4] ions,[5] and chemical species[6] have been defined as "intelligent materials"[7] having sensor, processor, and effector functions. There are many potential applications of such intelligent polymer system in controlled drug delivery systems(DDS),[8,9] solute separation,[10] and immobilization of enzymes and cells.[11]

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Most intelligent polymers known previously are responsive to only one kind of stimulus,[1-6,12,13] and independent response to several factors such as temperature and pH may be required for some applications.[14] These temperature- and pH-sensitive polymers undergo marked solubility changes in response to temperature and pH changes.[15,16]

A typical temperature-sensitive hydrogel such as crosslinked PNIPAAm has a lower critical solution temperature(LCST) near 32 °C.[17] Below that temperature, the gel is in a swollen, hydrated and hydrophilic state. Above the LCST the gel becomes collapsed, dehydrated and hydrophobic. Furthermore, its phase transition behavior can be controlled by incorporating more hydrophilic or hydrophobic monomers in the gel composition.[18] When a small amount of ionic monomer having pH-sensitivity is incorporated into a PNIPAAm gel, the gel is expected to exhibit a higher LCST and a broader phase transition, in addition to the generation of the pH sensitivity. Poly(NIPAAm-*co*-AAc) hydrogels, in which PAAc is introduced as a pH-sensitive component into the temperature-sensitive component PNIPAAm, has both the pH- and temperature-sensitivity.[14.19] However, for a higher pH than pK_a of PAAc and higher AAc content, the swelling transition disappears.[19] In order to utilize the hydrogels for the drug delivery systems, their temperature- and pH-sensitivity in response to small change in physiological conditions should be maintained.

To retain the temperature-induced transition property over a broad and useful pH range, we have used the method of polyelectrolyte complex formation because PNIPAAm incorporated with pH-sensitive component is an ionic polymer. If the pH-sensitive component bearing weakly acidic pendent group is incorporated into the PNIPAAm, the polymer behaves as an anionic polyelectrolyte. When a cationic polyelectrolyte is mixed with an anionic polyelectrolyte solution, a polyelectrolyte complex is formed through electrostatic attraction between two oppositely charged polyelectrolytes.[20,21] It might be expected that the LCST of poly(NIPAAm-*co*-AAc) will be controlled by polyelectrolyte complexation according to the pH value.

The objectives of this work are to study the influence of polyelectrolyte complex formation on the LCST of PNIPAAm in the poly(NIPAAm-*co*-AAc), and to investigate the influence of polyelectrolyte solute on the swelling property of crosslinked

poly(NIPAAm-*co*-AAc) hydrogel for the application of drug delivery systems.

Experimental

Materials. N-isopropyl acrylamide(NIPAAm, Polysciences Inc.) was recrystallized in n-hexane. Acrylic acid(AAc, Junsei Chem. Co.) was purified by distillation at 40 °C and 26 mm Hg. N,N'-azobisisobutyronitrile(AIBN, Tokyo Kasei Koygo Co.) was purified in methanol. 1,4-Dioxane(Duksan Co.) was purified by distillation. Poly(allyl amine)(PAA, Aldrich), poly(L-lysine)(PLL, Aldrich), ethylene glycol dimethacrylate(EGDMA, Junsei Chem. Co.), sodium hydroxide standard solution(Katayama Chem.), hydrochloric acid standard solution (Katayama Chem.), poly(acrylic acid)(PAAc Aldrich) and indomethacin(Sigma Chem. Co.) were used as received.

Synthesis of poly(NIPAAm-*co*-AAc). Linear poly(NIPAAm-*co*-AAc) and crosslinked poly(NIPAAm-*co*-AAc) hydrogels, containing 0, 10, 20, 30, 40, and 50 mol% of AAc, were prepared by free radical solution polymerization, as described in previous publications.[22-24]

Titrations. Titrations of the poly(NIPAAm-*co*-AAc) were performed as follows. 100 mg of each linear copolymer was dissolved in 25 ml of 0.1M 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 2-6 ml of 0.1N NaOH. Titrations were performed at 20 °C by adding 0.1N HCl in small quantities and measuring the pH after a stable value has been reached. Titrations of the poly(NIPAAm-*co*-AAc), containing 10, 20, and 30 mol% AAc, were performed at temperatures ranging from 4 °C to 45 °C. 100 mg of each linear copolymer was dissolved in 15 ml of 0.2M NaCl. The solutions were titrated with 0.2N HCl after all carboxyl groups were ionized by adding 0.2N NaOH. After each titration 0.2 N NaOH was added to re-ionized all carboxyl groups allowing subsequent titrations at other temperatures. The temperature was kept constant(±0.3 °C) for 30 min before and during each titration using a waterbath.

Preparation of polyelectrolyte complex. 1.7×10^{-1} monomol/ℓ of poly(NIPAAm-*co*-AAc) solution, containing 0, 10, 20, 30, 40, and 50 mol% of AAc, and 4.2×10^{-1}

monomol/ℓ of PAA solution were prepared by dissolving polymers in distilled water, respectively. Before mixing the two solutions, the pH of each solution was adjusted identically with HCl or NaOH standard solution in the range of 2-12. To each copolymer solution, the PAA solution was added with stirring. Each concentration of poly(NIPAAm-*co*-AAc) and PAA was 4.2×10^{-2} monomol/ℓ and 1.1×10^{-1} monomol/ℓ, respectively. The polyelectrolyte complex solution with PLL was prepared by the above method similarly.

Cloud point temperature determination. Turbidity of the solution was determined by measuring the absorbance at 450 nm of each of the solutions prepared above, using a Hewlett-Packard 8452A Diode Array UV/VIS spectrophotometer with HP89090A Peltier Temperature Control Accessory. The temperature of the solution was raised from 15 °C to 70 °C in 2 °C increments every 10 min. Absorbance was normalized against the absorbance of the polymer solution at temperature where precipitation started to take place. The cloud point temperature was defined as the temperature at the inflection point in the absorbance versus temperature curve. The cloud point of solutions not exhibiting an inflection point was determined at 10 % absorbance.[19]

Swelling measurement. For the studies of pH dependent swelling of the hydrogels, the gels in triplicate were incubated in buffer solutions ranging from pH 2 to 11 at an any particular temperature. 0.2M KCl/0.2M HCl buffer was used for pH 2, 0.2M acetic acid/0.2M sodium acetate buffer for pH 4, 5 and 6, and borate buffer for the remaining pH values. The total ionic strength of each buffer was adjusted to 0.2M with a calculated amount of NaCl. The incubation time was approximately 24 hrs. It was confirmed that 24 hrs was enough time to reach the equilibrium swelling. For the studies of temperature dependent swelling of the hydrogels, the gels were swollen in the particular buffer solution at various temperatures in the expected range of the LCST phase transition. Periodically, the gels were withdrawn from the buffer solution and weighed after removal of excessive surface water by lightly blotting with filter paper. After equilibration at one condition, the gels were reequilibrated at the other temperature. The weight swelling ratio was calculated by Ws/Wp, where Ws and Wp were the fully swollen gel and dried gel weights, respectively. The water content(wt%) was also calculated by the following equation, water content(wt%)=[(Ws-Wp)/Ws]×100. To investigate the effect of polyelectrolyte on the LCST, the gels were immersed in 20

°C buffer solutions containing a calculated amount of PAA, and the swelling ratio was determined by weighing gels as described above.

***In vitro* drug release.** Dried cylindrical gel was soaked in the solution of indomethacin(20g/l) in water at 4 °C for 2 days. After washing the gel surface with ethanol, the swollen gel was dried for 2 days at room temperature, and then dried under vacuum for 2 days at 25 °C. Drug loaded gel was placed in 20 ml of phosphate buffer solution(PBS, pH 7.4) release media, and the media were stirred at 37 °C. At 10 min. time intervals, 3 ml aliquots of the aqueous solution were withdrawn from the release media. After the concentration of indomethacin released was monitored using a UV/Vis spectrophotometer at 319 nm, the solution taken as a sample was replaced in the release medium.

Results and Discussion

Effect of AAc content in poly(NIPAAm-*co*-AAc). Poly(NIPAAm-*co*-AAc) was synthesized from comonomer feed compositions ranging from 0 to 50 mol%. Copolymer compositions and pK_a values determined, by back-titration, are given in Table 1.

Table 1. Monomer ratios in P(NIPAAm-*co*-AAc) gels compared to monomer feed ratios

Comonomer AAc feed molar composition(mol%)	Copolymer AAc molar composition(mol%)	pK_a (at 20 °C)
0	0	0
10	14.0	5.14
20	24.2	4.96
30	36.6	4.86
40	45.2	4.79
50	54.0	4.77

Figure 1 exhibits the cloud point temperatures of PNIPAAm against AAc mol % in the poly(NIPAAm-*co*-AAc) copolymer solutions according to the pH values. It was found that the cloud point temperatures of PNIPAAm in the copolymers were affected by pH

and AAc content in the copolymer. The cloud point temperatures of PNIPAAm in the solution decreased with an increase of AAc content in the copolymer around pH value of 2.0-3.0 because more hydrogen bonding between the amide group of PNIPAAm and the carboxylic acid group of PAAc occurs with increasing AAc content in the copolymer. The hydrogen bonding interferes with the access of water molecules to the NIPAAm amide groups.[19] On the other hand, the cloud point temperatures of PNIPAAm in the solution increased with AAc content in the copolymer around pH 4.0 because of increased hydrophilicity resulting from an increase of ionized AAc components.

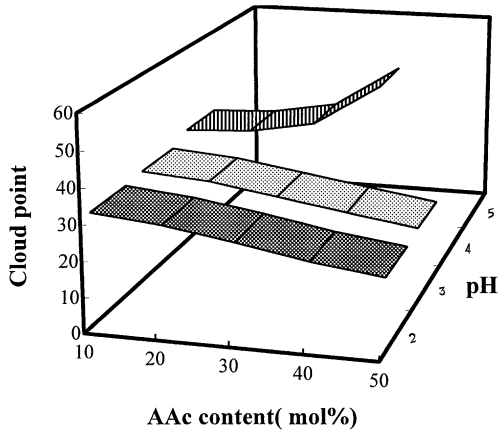

Figure 1. Plot of cloud point of aqueous poly(NIPAAm-*co*-AAc) solution against content of AAc as a function of pH.

In Figure 2, the cloud point temperatures of poly(NIPAAm-*co*-AAc) copolymer solutions PNIPAAm in the presence of PLL at various pH values, are plotted against AAc mol%. The presence or absence of PLL did not have a significant effect on cloud point temperatures for pH values around 2.0, due to hydrogen bonding between PNIPAAm and PAAc. However, at pH 4.0, the cloud point temperature of \poly(NIPAAm-*co*-AAc) with PLL decreased with increasing AAc content in the copolymer, whereas cloud point temperatures of the copolymer solutions without PLL increased with AAc content in the copolymer.

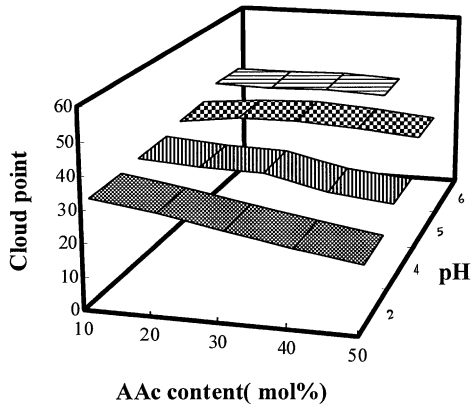

Figure 2. The plot of cloud point of aqueous poly(NIPAAm-*co*-AAc) solutions with PLL against content of AAc, as a function of pH.

When the copolymer of NIPAAm containing partially ionized AAc forms an intermacromolecular complex with oppositely charged PLL, the cloud point temperature of PNIPAAm is affected by the density of complexation.[25] The density of complexing points on poly(NIPAAm-*co*-AAc) increases with AAc content in the copolymer because the complexing points are a carboxylic acid group of AAc in the copolymer and amine group of PLL. An increase of the density of complexing points causes more polyelectrolyte complexes to form between partially ionized PAAc and PLL. This may lead to a decreased cloud point temperature due to the reduced accessibility and mobility of water surrounding the copolymer and dehydration of the copolymer chain by polyelectrolyte complex formation. Therefore, the cloud point temperature of PNIPAAm in the poly(NIPAAm-*co*-AAc) with PLL decreases with increasing AAc content in the copolymer.

On the other hand, the cloud points of the (NIPAAm-*co*-AAc) solutions with PLL did not show an obvious tendency against AAc content around pH 5.0-6.0. With increasing pH or AAc content, the number of $-COO^-$ groups in the copolymer chain increases. The presence of $-COO^-$ groups in the polymer chain increases its rigidity because of electrostatic interchain repulsions and impedes efficient polyelectrolyte complex

formation between $-COO^-$ groups of the copolymer and $-NH_3^+$ groups of PLL.[26] However, the formation of polyelectrolyte complex is not hindered completely because the long side chain of PLL facilitates the approach of $-NH_3^+$ to the $-COO^-$ group. Hence, it is considered that the lack of a clear observed trend in cloud point due to AAc content in the copolymer is due to a balance of hydrophilicity of $-COO^-$ groups not participating in polymer complex formation, and the participation of hydrophobic, alkyl side chains of PLL in polymer complex formation.

Effect of comonomer ionization. In order to examine the specific role of charge on phase separation, the amount of charge on poly(NIPAAm-*co*-AAc) copolymers was determined at their respective cloud point temperatures. By determining the pK_a's of AAc in the copolymers as a function of temperature, the amount of charges on the copolymers as a function of temperature can be calculated from the Henderson-Hasselbalch equation,[27] as shown in Figure 3. A decrease in charges on the polymer chain with increasing temperature was observed due to the decreased acidity of AAc. The amount of charge on poly(NIPAAm-*co*-AAc) chains at their respective cloud point temperature as a function of pH is given in Table 2. It was found that percentage of charged AAc on the copolymers was less than 0.06 % at pH 2. It was also found that the amount of charge increased with increasing pH and AAc content. Increasing the amount of charge leads to a large increase in cloud point temperature, with no phase separation taking place, if more than 2-3 mol% of AAc units are charged.

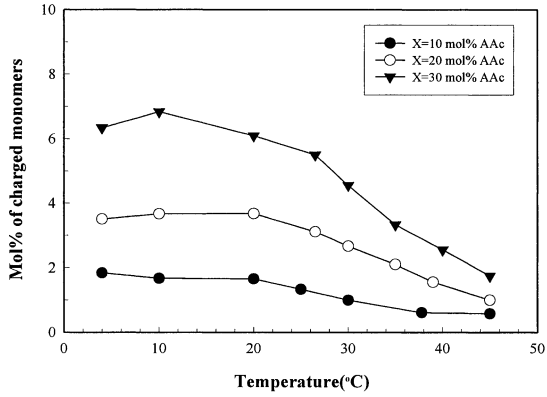

Figure 3. Total amount of charged monomers in poly(NIPAAm-*co*-AAc-X), with X=10, 20, 30 mol%, at pH 4 as a function of temperature.

Table 2. Percentage of charged comonomers in poly(NIPAAm-*co*-AAc) at LCST as a function of pH.

pH	AAc content(mol%)		
	10	20	30
2	0.010%	0.032%	0.065%
3	0.092%	0.298%	0.510%
4	0.768%	2.02%	2.72%
5	3.49%	–	–

Effect of cationic polyelectrolytes and hydrophobicity. Figure 4 shows the effect of polyelectrolytes and the hydrophobic side chain of PLL on the cloud point of PNIPAAm in the poly(NIPAAm-*co*-AAc) as compared with PAA. The cloud point of the copolymer solution in the presence of cationic polyelectrolytes decreased due to polyelectrolyte complex formation between the carboxylic acid group of PAAc and the amine group of PAA or PLL, indicating that dehydration occurred.

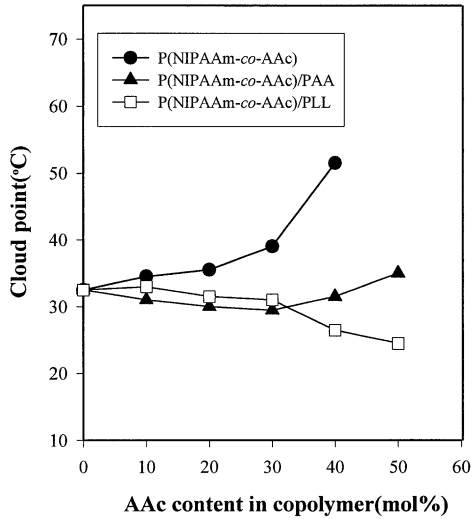

Figure 4. The comparison of cloud point of poly(NIPAAm-*co*-AAc)/PLL with that of poly(NIPAAm-*co*-AAc)/PAA at pH 4.

The influence of PLL on the cloud point of poly(NIPAAm-*co*-AAc) was stronger than that of PAA above 30 mol % of AAc content. It is considered 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 stronger dehydration occurs. 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 synergy with the intrinsic hydrophobicity of NIPAAm.

Effect of polymer complex on conformation of PLL. PLL takes an α-helical conformation at pH above 9.5. Below pH 9.5, PLL exists in an irregular random coil form,[28] in which the flexible backbone undergoes continuous change as a result of thermal motion. This is because at pH below pK_b, all the amine groups of PLL have a positive charge and repel each other so strongly that they overcome the tendency for intrachain hydrogen bonds to form. Ellipticity [θ] at 222 nm is frequently used to estimate the α-helical content, because $[\theta]_{222nm}$ in $deg.cm^2 \cdot dmol^{-1}$ is nearly zero for the random coil and $-4 \cdot 10^4$ for the α-helical conformation, respectively. In a previous paper,[29] we reported that ellipticities at 222 nm in CD spectra of the PLL/PAAc mixture were large negative values at all temperatures, indicating a conformational change of PLL from random-coil to α-helix. It is considered that the positive charges on PLL are blocked by negative charges on PAAc through the polymer-polymer complex, and then the intrachain hydrogen bonds are formed. In the case of PLL/poly(NIPAAm-*co*-AAc-50), the random-coil conformation of PLL did not change, essentially, at all temperatures. It is thought that bulky N-isopropyl groups of PNIPAAm bound in PLL through ionic polymer-polymer interaction inhibit intrachain hydrogen bonding due to steric hinderance.

pH dependent swelling behaviors. Figure 5 shows pH the dependence of swelling ratio for the poly(NIPAAm-*co*-AAc) gels with 10 and 20 mol% of PAAc at 39 °C. These two hydrogels demonstrated a sharp swelling transition according to pH. The equilibrium swelling ratio of poly(NIPAAm-*co*-AAc) gel with 10 mol% AAc greatly increased between pH 5.0 and 6.0, whereas that of poly(NIPAAm-*co*-AAc) gel with 20 mol% of AAc greatly increased between pH 4.0 and 5.0. These swelling transitions are closely related to pK_a values determined by titration curves.

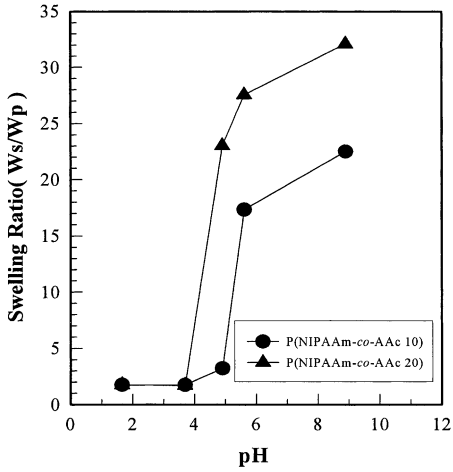

Figure 5. The pH dependent swelling ratio of poly(NIPAAm-*co*-AAc) gels in buffer solution at 39°C.

Figure 6 shows the pH dependence of the swelling ratio of poly(NIPAAm-*co*-AAc) gels with 10 and 20 mol% AAc in the presence of PAA at 39 °C. Comparing Figures 5 and 6, it is seen that the pH sensitivity of these two poly(NIPAAm-*co*-AAc) hydrogels with PAA decreased in comparison with that of the poly(NIPAAm-*co*-AAc) gels in the absence of PAA. Specifically, the swelling pH range widened to 5.6 to 8.9. Also, in the case of poly(NIPAAm-*co*-AAc) gel, there was a large difference between the swelling ratio below pK_a of PAAc (about 4.8)[30] and above swelling ratio above that pK_a, due to the difference in the number of carboxylate side chains resulting from the pH change. In the case of poly(NIPAAm-*co*-AAc) gel with PAA, a polyelectrolyte complex is formed in the pH range from 5.6 to 8.9. This leads to a reduced swelling ratio, since expansion of ionized hydrogel is hindered by the mutual blocking of oppositely charged ionic groups and the accessibility and mobility of water surrounding the hydrogel is decreased. Therefore, the value of Ws/Wp became smaller in spite of the wider pH range.

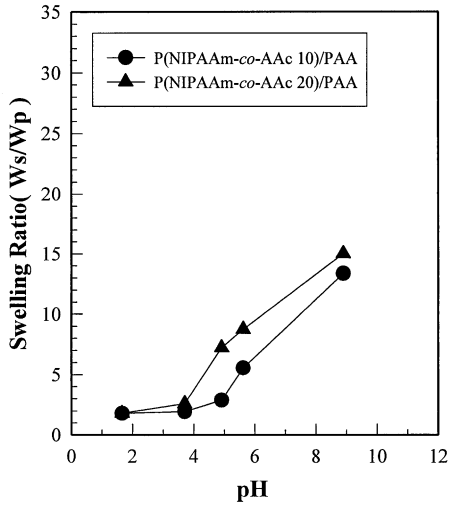

Figure 6. pH dependent swelling ratio of poly(NIPAAm-*co*-AAc) gels with PAA in buffer solution at 39 °C.

Influence of polyelectrolyte on drug release. Figure 7 shows the release profile of indomethacin from poly(NIPAAm-*co*-AAc) hydogel in the absence and presence of PAA, at 37 °C and pH 7.4. In contrast to the rapid release of indomethacin from poly(NIPAAm-*co*-AAc) hydrogel itself, the release of a drug from the hydrogel with PAA was much slower. The swelling ratios increased with pH due to electrostatic repulsion between the carboxylic acid polymer side chain and ions present in the buffer solution. At pH 7.4, the carboxylate side chains are repelled by the anions in the solution, and minimize charge concentration by expanding[31]. Also, the swelling ratios of the hydrogels in the presence of PAA were lower than those of the gels without PAA, since the expansion of ionized hydrogel was hindered by formation of the polyelectrolyte complex, resulting in mutual blocking of oppositely charged ionic groups.

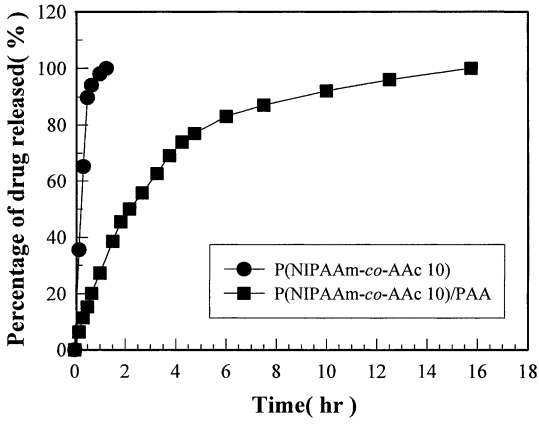

Figure 7. Indomethacin release from poly(NIPAAm-*co*-AAc- 10) and poly(NIPAAm-*co*-AAc 10)/PAA in PBS at 37 °C.

Conclusion

The cloud points of PNIPAAm in aqueous poly(NIPAAm-*co*-AAc) solutions were strongly influenced by pH, AAc content, and the type of added polyelectrolytes such as PAA and PLL. A polyelectrolyte complex in the poly(NIPAAm-*co*-AAc) system with PLL was formed in neutral conditions. The influence of PLL on the LCST of PNIPAAm in the poly(NIPAAm-*co*-AAc) was more stronger than that of PAA above 20 mol% of AAc content due to hydrophobicity of alkyl sidechain of PLL. Although a polymer-polymer complex was formed between poly(NIPAAm-*co*-AAc) and PLL, the conformational change of PLL did not show up clearly due to the steric hinderance of bulky N-isopropyl groups of PNIPAAm.

The LCSTs of the poly(NIPAAm-*co*-AAc) hydrogels increased with pH and disappeared above the pK_a value of AAc, except for the hydrogel containing 10 mol% AAc. The LCSTs of the poly(NIPAAm-*co*-AAc) hydrogels in the presence of PAA were observed even above the pK_a value of AAc. At the same pH, the LCSTs of the poly(NIPAAm-*co*-AAc) hydrogels were lower than those of the same hydrogels in the absence of PAA due to the polyelectrolyte complex formation. Below the pK_a value of AAc, the LCSTs of hydrogels decreased with an increase of AAc content in the

hydrogel. On the other hand, the LCSTs of hydrogels increased with AAc content above pK_a value of PAAc. Furthermore, the swelling ratio of poly(NIPAAm-*co*-AAc) gel was decreased by polyelectrolyte complex formation with PAA. The swelling kinetics of poly(NIPAAm-*co*-AAc) hydrogels are strongly dependent on pH value, temperature, and polyelectrolyte solute. In contrast to the rapid release of indomethacin from poly(NIPAAm-*co*-AAc) hydrogel itself, the release of a drug from the hydrogel with PAA was slowed due to polyelectrolyte complex formation between PAA and the copolymer hydrogels.

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