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pH/temperature-responsive behaviors of semi-IPN and comb-type graft hydrogels composed of alginate and poly(*N*-isopropylacrylamide)

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Abstract

Thermo- and pH-sensitive comb-type graft and semi-IPN hydrogels were prepared by using alginate and poly(*N*-isopropylacrylamide) (PNIPAAm). Comb-type graft hydrogels are composed of crosslinked alginate network and grafted with PNIPAAm. They exhibited fast pH and thermal responses due to free and mobile graft chains. Comb-type graft hydrogels reached an equilibrium swelling and deswelling states within about 10 min. By contrast, semi-interpenetrating polymer network (semi-IPN) hydrogels formed polyelectrolyte complex between carboxyl groups in alginate and amino groups in PNIPAAm-NH_2, resulting in a relatively compact structure and slow swelling and deswelling compared with comb-type graft hydrogel. All the hydrogels exhibited a reasonable sensitivity to temperature, pH and ionic strength of the swelling medium.

Keywords: pH/temperature-responsive; Comb-type graft; Semi-interpenetrating polymer network

1. Introduction

Hydrogels are three-dimensional and hydrophilic polymer networks capable of imbibing large amounts of water or biological fluids. There are numerous applications of these hydrogels, in particular in the medical and pharmaceutical fields. Hydrogels resemble natural living tissue due to their high water contents and soft touch. Hydrogels may also show a swelling behavior depending on the external environments. These stimuli-sensitive hydrogels can exhibit dramatic changes in their swelling behavior of the network structure, permeability or mechanical strength in response to changes in the pH, ionic strength, temperature and electromagnetic radiation. The most commonly studied hydrogels having environmental sensitivity are either pH- or temperature-sensitive [1,2].

In the case of thermosensitive hydrogels, poly(*N*-isopropylacryamide) (PNIPAAm) is well-known to the best example due to its lower critical solution temperature (LCST) behavior at around 32°C in aqueous solution. PNIPAAm chains hydrate to form expanded structures in water when the solution temperature is below its LCST but becomes compact structure by dehydration when heated up above the LCST [3,4].

For pH-sensitive hydrogels, either acid or basic pendent groups were contained in the network. We choose alginate having carboxylic acid groups as ionic hydrogels. Alginate has several unique properties: (i) a relatively inert aqueous environment within the matrix, (ii) a mild room temperature encapsulation process free of organic solvents, (iii) a high gel porosity which allows for high diffusion rates of macromolecules, (iv) dissolution and biodegradation of the system under normal physiological conditions — that enables it to be used as a matrix for the entrapment and delivery of proteins, drugs and cells [5–7].

Many researchers have investigated on the fast response hydrogels according to the surrounding environment. Hoffman et al. synthesized fast temperature responsive, macroporous PNIPAAm gels [8]. Okano et al. prepared a thermosensitive PNIPAAm hydrogels having PNIPAAm chains grafted on the backbone PNIPAAm network [9,10]. They also reported comb-type graft hydrogels composed of poly(ethylene oxide) (PEO) graft chains in PNIPAAm crosslinked network [11]. These results showed rapid gel swelling–deswelling kinetics. A fast response is necessary for applications such as artificial muscles and/or rapidly acting actuators.

In this study, we prepared alginate/PNIPAAm-NH_2 comb-type graft hydrogels which were able to respond rapidly to both temperature and pH changes and compare

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with properties of semi-IPN hydrogels containing the same components.

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAAm) (Aldrich Chem. Milwaukee, WI) was purified by recrystallization from *n* -hexane/toluene (Duksan Pure Chemical, Korea). Sodium alginate (mannuronate/gluronate ratio of alginate (M/G) = 1.56) and 2-aminoethanethiol hydrochloride (AESH) were purchased from Aldrich Chem. (Milwaukee, WI). The molecular weight distribution of alginate was determined by gel permeation chromatography (GPC, Waters Model 510 HPLC pump, Milford, USA) with a Millennium software program in water. The number-average (M_n) and weight-average (M_w) molecular weights were 339,000 and 1,073,000, respectively. 1-Ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma Chem. (St. Louis, MO). *N*,*N*′-Azobisisobutyronitrile (AIBN) (Aldrich Chem.) was recrystallized from methanol (Duksan Pure Chemical, Korea). *N*,*N*-Dimethylformamide (DMF) (Duksan Pure Chemical, Korea) was purified by distillation. Calcium chloride ($CaCl_2$), tetrahydrofuran (THF) and ethyl ether (Duksan Pure Chemical. Korea) were used as received. Water was first treated with a reverse osmosis system (Sambo Glove, Ansan, Korea) and further purified with a Milli-Q Plus system (Waters, Millipore, MA, USA).

2.2. Synthesis of semi-telechelic PNIPAAm

Amino semi-telechelic PNIPAAm was synthesized by radical polymerization using AESH as a chain transfer agent and AIBN as an initiator. NIPAAm (9.7×10^{-2} mol), AESH (4.85×10^{-3} mol), AIBN (5.5×10^{-5} mol) were dissolved in DMF (110 ml). Dried nitrogen was bubbled into the solution for 20 min prior to polymerization. Polymerization was carried out at 75°C for 8 h under vacuum. After the reaction, the reactant was precipitated into an excess of ethyl ether and dried in vacuo at room temperature. The dried polymer was purified by precipitation in hot water and dissolved in water. Polymer product was obtained by freeze-drying.

2.3. Preparation of alginate/PNIPAAm-NH_2 semi-IPN hydrogels

The semi-IPN hydrogels composed of PNIPAAm-NH_2 and alginate were prepared by the method reported in our study [12]. The aqueous solution of alginate (5 wt%) and PNIPAAm-NH_2 (20 wt%) were mixed in various compositions (80/20, 50/50, 20/80 by weight). Mixed solution was poured into a petri-dish and dried to constant weight at room temperature in a vacuum oven.

To prepare semi-IPN hydrogels, the dry film was cut into the size of 1.0×1.0 cm^2 and immersed in 10 ml of $CaCl_2$ aqueous solution (0.5 wt%). After shaking for 10 min at room temperature, semi-IPN was washed in water and dried to constant weight at room temperature in a vacuum oven.

2.4. Preparation of alginate/PNIPAAm-NH_2 comb-type graft hydrogels

To form amide bonds between carboxyl group in alginate and amino group in PNIPAAm-NH_2, alginate, PNIPAAm-NH_2, EDC and NHS with various compositions were dissolved in water at room temperature. Each solution had a molar ratio of alginate:EDC:NHS of 2:2:1 on the basis of carboxyl group in alginate. The solution mixture was continuously stirred at room temperature overnight. After dialysis for two days against water, the reactant was precipitated into THF-hexane (4:1) and dried in vacuo. The product was dissolved in water to prepare 5 wt% (w/w) solution. This solution was poured into a petri-dish and dried to constant weight at room temperature in a vaccum oven. Crosslinking with calcium ions was done by the same procedure as that for the above prepared semi-IPN hydrogels. The composition and designation of each sample are listed in Table 1. Elemental analysis (Carbo Erba model EA 1110) of N was used to determine the actual reaction composition. As shown in Table 1, element nitrogen (wt%) in the gel increased as the amount of PNIPAAm-NH_2 in the hydrogel increased. Note that alginate does not contain N.

2.5. TGA measurements

Thermogravimetric analysis (TGA) was done using a Perkin–Elmer System 7 (Connecticut, USA) to study the

Table 1
Designation and composition of alginate/PNIPAAm-NH_2 hydrogels

Hydrogel type	Sample code	Molar ratio		Weight ratio (wt%)		N (wt%)
		[COOH]	[NH_2]	Alginate	PNIPAAm-NH_2	
Comb-type	GAN28	69.23	30.77	20	80	8.7
Graft	GAN55	90.14	9.86	50	50	5.8
Hydrogel	GAN82	97.33	2.67	80	20	4.3
Semi-IPN	IPN28	69.23	30.77	20	80	7.8
	IPN55	90.14	9.86	50	50	4.6
Hydrogel	IPN82	97.33	2.67	80	20	2.1

thermal stability of the polymer. Decomposition profiles of TGA were recorded with a heating rate of 10°C/min in nitrogen between 25°C and 500°C.

2.6. Determination of swelling property

Swelling studies were conducted on alginate/PNIPAAm-NH_2 semi-IPNs and comb-type graft hydrogels as functions of temperature, pH and ionic strength of swelling medium. The swelling ratio $[(W_s - W_d)/W_d]$ was defined as the weight of absorbed water per weight of dried gel, where W_s and W_d were the weight of the hydrogels at the swelling state and dry state. To measure the swelling ratio, preweighed dry samples were immersed in water. After the excessive surface water was removed with filter paper, the weight of swollen samples was measured. For swelling and deswelling kinetics studies, swollen gels in water at 25°C were weighed at each given time. After confirming no further changes in swelling ratios over time, the gels were transferred into water at 40°C and weighed at various time intervals.

3. Results and discussions

3.1. Preparation of semi-IPN and comb-type graft hydrogels

To confirm the polymerization of semi-telechelic PNIPAAm-NH_2, FT-IR spectroscopy (Nicolet Model Magna IR 550) and ^{1}H-NMR spectroscopy (Bruker AMX-500) measurements were carried out. Characteristic peaks of PNIPAAm-NH_2 are located at 1655 and 1542 cm^{-1} of amide I and II, and 3436 and 3300 cm^{-1} of primary amine, while those of NIPAAm monomer at 1617 cm^{-1} (C=C) and 1409 cm^{-1} (CH_2=) disappeared. In ^{1}H-NMR spectroscopy measurement, the spectrum of PNIPAAm-NH_2 exhibited peaks at 1 ppm (–CH_3) and 3.85 ppm (–NH–CH), while peaks at 2.7 and 2.8 ppm were due to chain transfer agent (AESH). Also, two broad signals at 1.4 and 1.8 ppm due to methylene proton and methyne proton were observed while the peaks of vinyl proton at 5.5–6 ppm were not detected. From these results, the synthesis of semi-telechelic PNIPAAm-NH_2 could be confirmed.

The molecular weight distribution of PNIPAAm-NH_2 was determined by GPC apparatus (Waters Model 510 HPLC pump, Milford, MA, USA) in tetrahydrofuran (THF). The number-average (M_n) and weight-average (M_w) molecular weights were 11,100 and 15,600, respectively.

In the preparation of semi-IPN composed of alginate and PNIPAAm-NH_2, each solution of alginate and PNIPAAm-NH_2 was mixed well. Then, alginate was crosslinked with divalent calcium ions and resultantly formed an alginate network. It is expected that amino groups of PNIPAAm-NH_2 form polyelectrolyte complex with carboxylic acid groups of the alginate network. On the other hand, comb-type graft hydrogels composed of alginate and PNIPAAm-NH_2 were prepared by amide bonds between carboxyl groups in alginate and amino groups in PNIPAAm-NH_2. To form amide bonds, EDC and NHS were used. Then, unreacted carboxylic acid groups in alginate were crosslinked with $CaCl_2$. Structures for comb-type graft: (a) and semi-IPN hydrogels (b) are schematically illustrated in Fig. 1. As shown in semi-IPN hydrogel, although one site is available in PNIPAAm-NH_2 for ion complex with alginate, PNIPAAm-NH_2 would be stabilized, because PNIPAAm-NH_2 was fixed between crosslinked alginate chains.

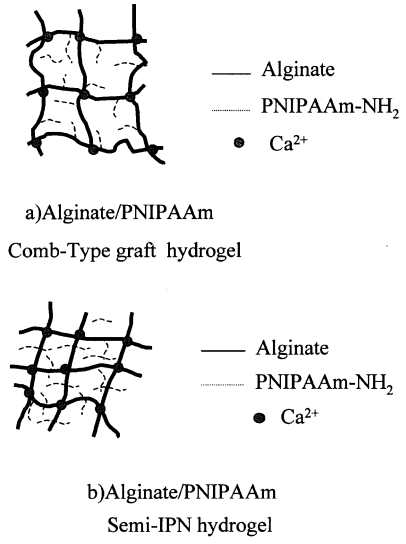

Fig. 1. Schematic illustration of structures for: (a) alginate/PNIPAAm-NH_2 comb-type graft hydrogel and (b) semi-IPN hydrogel.

3.2. Thermal analysis

Thermal stabilities of alginate, NIPAAm, alginate/PNIPAAm-NH_2 semi-IPN and comb-type graft hydrogel were measured using TGA analysis. As shown in Fig. 2, thermal stability of semi-IPN and comb-type graft hydrogels decreases in comparison with alginate. This result may

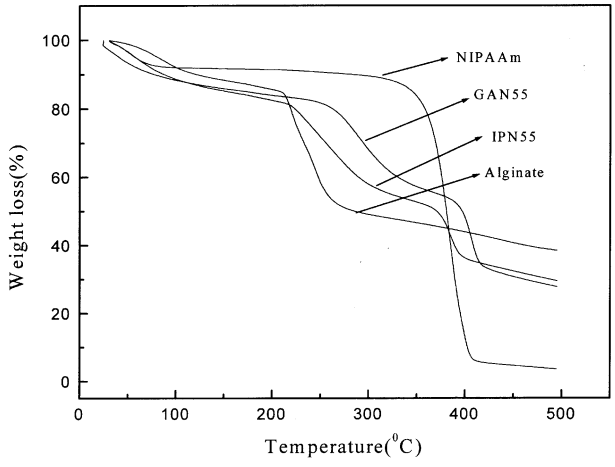

Fig. 2. TGA of NIPAAm, GAN55, IPN55 and alginate.

be due to the introduction of NIPAAm which shows an initial thermal decomposition temperature of 135°C. Also, GAN55 shows a faster thermal decomposition than IPN55 which has more compact structure due to the reaction between carboxyl groups in alginate and amino groups in PNIPAAm-NH_2.

3.3. Swelling–deswelling kinetics of hydrogels

Fig. 3 shows swelling kinetics of semi-IPN and comb-type graft hydrogels composed of alginate and PNIPAAm-NH_2. Note that the unit of *x*-axis in Fig. 3(a) is in minute, while that in Fig. 3(b) is in hour. In the alginate/PNIPAAm-NH_2 comb-type graft hydrogels, their swelling ratio reached an equilibrium swelling state within about 10 min. By contrast, semi-IPN hydrogels reached an equilibrium swelling state within 12 h. Rapid swelling kinetics of GAN series was because of the fast and strong hydration of PNIPAAm-NH_2 graft chain. GAN82 with 20 wt% PNIPAAm-NH_2 graft chain slowly reached an equilibrium swelling state. It was reported that grafted chains maintain high mobility as opposed to polymer networks crosslinked on each chain because they were free-end polymer. Okano et al. reported that rapid and strong graft chain hydration did not occur because these chains surrounded by the hydrophobic collapsed backbone network sterically hindered hydration [9–11]. The difference in swelling kinetics may result from the component of the backbone network. That is, we obtained the fast swelling kinetics because alginate does not have hydrophobic groups such as isopropyl group in PNIPAAm. The swelling ratio of GAN series depends on the degree of crosslinking. The degree of crosslinking of hydrogels increases with the ratio of alginate to PNIPAAm-NH_2 increases. As a result, GAN28 has the highest swelling ratio while GAN82 exhibits the lowest swelling ratio depending on the amount of alginate for crosslinking.

On the other hand, in the semi-IPN hydrogels, IPN55 shows lower swelling ratio than IPN82 having 80 wt% alginate. This is due to the reaction between carboxyl groups in alginate and amino groups in PNIPAAm-NH_2. The polyelectrolyte complex captured the hydrophilic group and produced the tight and ionic bonded structures. Namely, the swelling ratio of IPN series was influenced by the degree of complexation and crosslinking. Therefore, the lowest swelling ratio of IPN55 should be attributed to the compact complex structures.

Fig. 4 shows the deswelling kinetics of hydrogels pre-equilibrated at 25°C by elevating the temperature to 40°C.

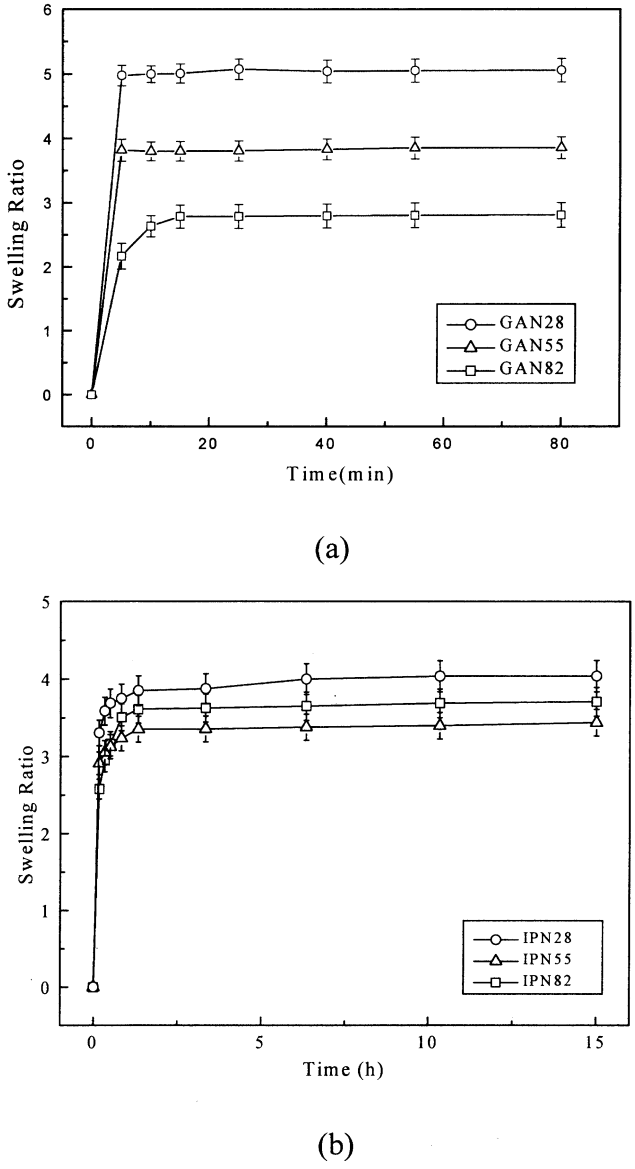

Fig. 3. Swelling kinetics of alginte/PNIPAAm-NH_2 comb-type graft hydrogels (GAN28, GAN55, GAN82) (a) and semi-IPN hydrogels (IPN28, IPN55, IPN82) (b) in water (pH = 5.4) at 25°C.

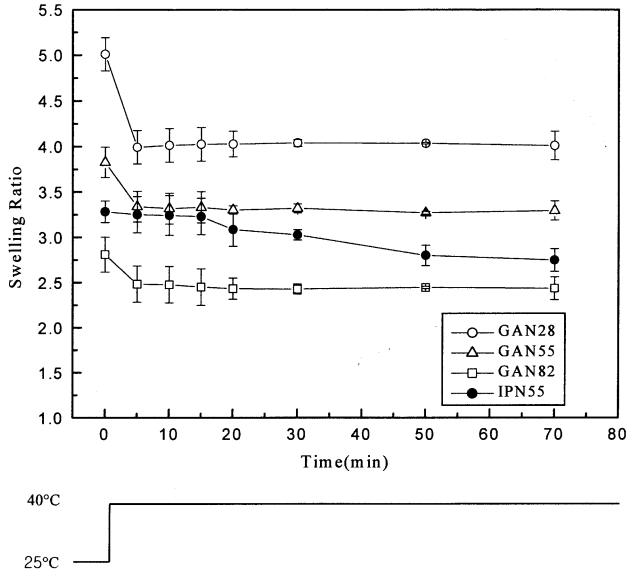

Fig. 4. Deswelling kinetics of alginte/PNIPAAm-NH_2 comb-type graft hydrogels (GAN28, GAN55, GAN82) and semi-IPN hydrogel (IPN55) in water at 40°C from the equilibrium swelling state at 25°C.

GAN series exhibit a rapid deswelling and reach the equilibrium deswelling state within about 5 min. However, IPN55 takes several hours to reach its equilibrium swelling state. These results are due to the difference of gel structure. Generally, PNIPAAm gel shrunk slowly because of formed skin layer during the deswelling process [13]. However, comb-type graft hydrogels with temperature-sensitive PNIPAAm-NH_2 chains show a rapid deswelling due to the mobility of grafted chains. At temperature above 32°C, grafted chains are dehydrated, then hydrophobic aggregation force forms between dehydrated grafted chains. These strong aggregation forces contribute to an increase in void volume within a gel, resulting in a rapid release of water. From the results of swelling and deswelling kinetics, we could expect that freely mobile chains in comb-type graft hydrogels affect a fast rate of swelling and deswelling. In the swelling/deswelling kinetics experiment, weight loss of the gel was not observed, because calcium alginate was not dissolved in water at zero ionic strength but at higher ionic strength or pH 7.

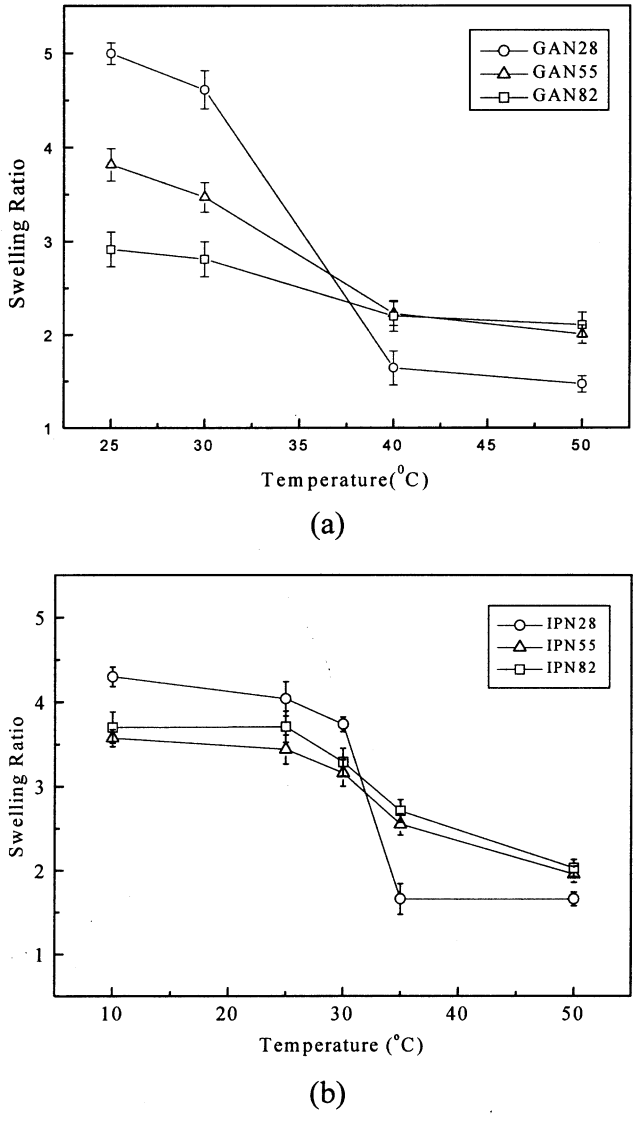

Fig. 5. Swelling ratio of: (a) alginte/PNIPAAm-NH_2 comb-type graft hydrogels (GAN28, GAN55, GAN82) and (b) semi-IPN hydrogels (IPN28, IPN55, IPN82) as a function of temperature in water.

3.4. Stimuli-responsive swelling behaviors

Temperature dependence of equilibrium swelling of: (a) alginate/PNIPAAm-NH_2 comb-type graft hydrogels and (b) semi-IPN hydrogels is shown in Fig. 5. All the hydrogels had significant changes in swelling ratio over the temperature range between 30 and 40°C. Among the hydrogels, GAN28 and IPN28 with 80 wt% PNIPAAm-NH_2 in GAN and IPN series, respectively, showed the most drastic volume phase transition among the hydrogels. This is due to the temperature-responsive properties of PNIPAAm at around LCST (32°C).

The equilibrium swelling ratio is shown in Fig. 6 as a function of the ionic strength of the external solution. Unlike chemically crosslinked hydrogels, hydrogels crosslinked with calcium ions exhibit the increase of the number of charged groups as the ionic strength of the medium increases, resulting from the ion-exchange between Ca^{2+} ions and Na^+ ions. The large number of the charged groups decreased the crosslink density and increased the hydrophilicity of the network. The swelling ratios of IPN55 and GAN55 hydrogels increase up to about 0.05N NaCl concentration, but reduce at high NaCl concentration due to the decrease of the crosslink density and destruction of the alginate network. It could be seen that high swelling ratio of IPN55 was caused by the large number of charged groups (COO^-, NH^{3+}) within IPN55 hydrogel network than those within GAN55 (COO^-).

pH-sensitive characteristics of hydrogels were studied by swelling test under pH range between 2 and 5 (NaCl concentration = 0.01N). As shown in Fig. 7, the swelling

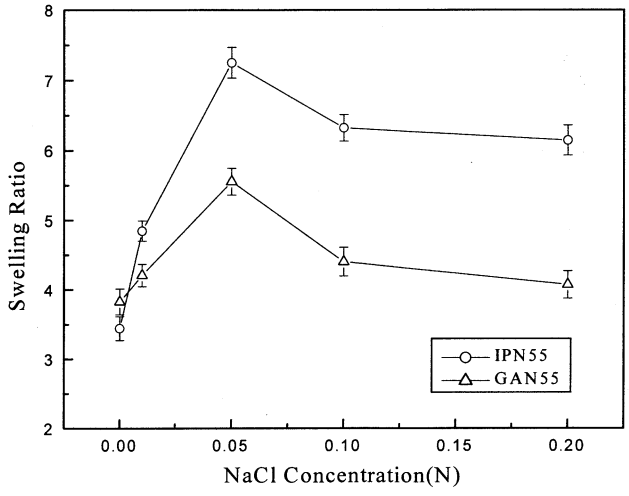

Fig. 6. Effect of ionic strength on the swelling behaviors of alginte/PNIPAAm-NH_2 comb-type graft hydrogel (GAN55) and semi-IPN hydrogel (IPN55) at 25°C in water.

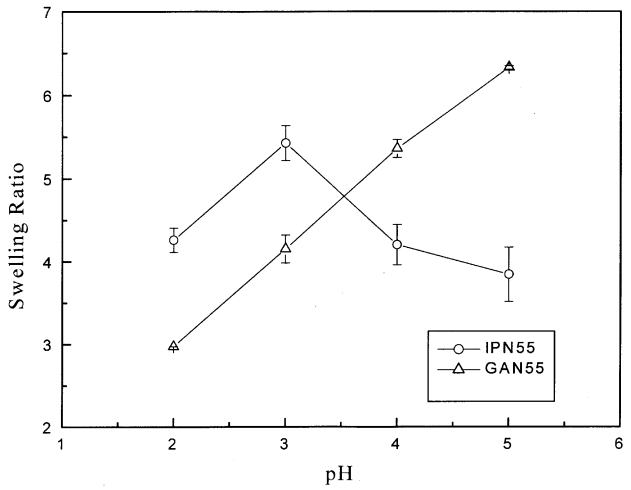

Fig. 7. pH-dependent swelling behaviors of alginte/PNIPAAm-NH_2 comb-type graft hydrogel (GAN55) and semi-IPN hydrogel (IPN55) at 25°C (NaCl concentration = 0.01N).

ratio of GAN55 continuously increased with increasing pH values, while the swelling ratio of IPN55 decreased at pH ranging between 3 and 5. GAN55 has only carboxylic acid groups of alginate in gel networks. It is well known that below pK_a values, carboxylic acid groups are in the form of COOH. As pH of the solution increases, COOH becomes ionized (COO^-), and the resulting electrostatic repulsion causes the hydrogels to swell. However, in the case of IPN55 hydrogels, carboxylic acid in alginate and ammonium ions in PNIPAAm-NH_2 coexist. Above the pK_a of alginate (about 3.2 and 4 for guluronic and mannuronic acids, respectively.), polyelectrolyte complexes between COO^- in alginate and NH^{3+} in PNIPAAm-NH_2 are formed, resulting in the decrease of the swelling ratio of the hydrogel. As mentioned before, this means that IPN55 forms the compact complex structure.

Stepwise swelling behaviors were observed in water with temperature alternating between 25 and 40°C, as shown in Fig. 8. Swelling ratio of: (a) GAN series with temperature changes was measured in every 5 min, and that of (b) IPN series was calculated in every 30 min, as temperature was switched in every 2 h. Their swelling processes are proved to be repeatable with temperature changes. Comb-type graft hydrogels respond to temperature change more rapidly than semi-IPN. These results are in agreement with those obtained by fast swelling/deswelling behaviors of comb-type graft hydrogels composed of alginate and PNIPAAm-NH_2.

Fig. 9 shows a stepwise swelling behavior of comb-type graft hydrogels at 25°C with alternating pH between 2 and 7 (NaCl concentration = 0.01N). Swelling ratio was measured in every 5 min, because pH was switched in every 5 min. pH-sensitive behaviors of comb-type hydrogels occur more slowly and are less dramatic than temperature-sensitive response. This is because comb-type graft hydrogels are composed of temperature-sensitive grafted PNIPAAm-NH_2 chains and pH-sensitive crosslinked alginate backbone.

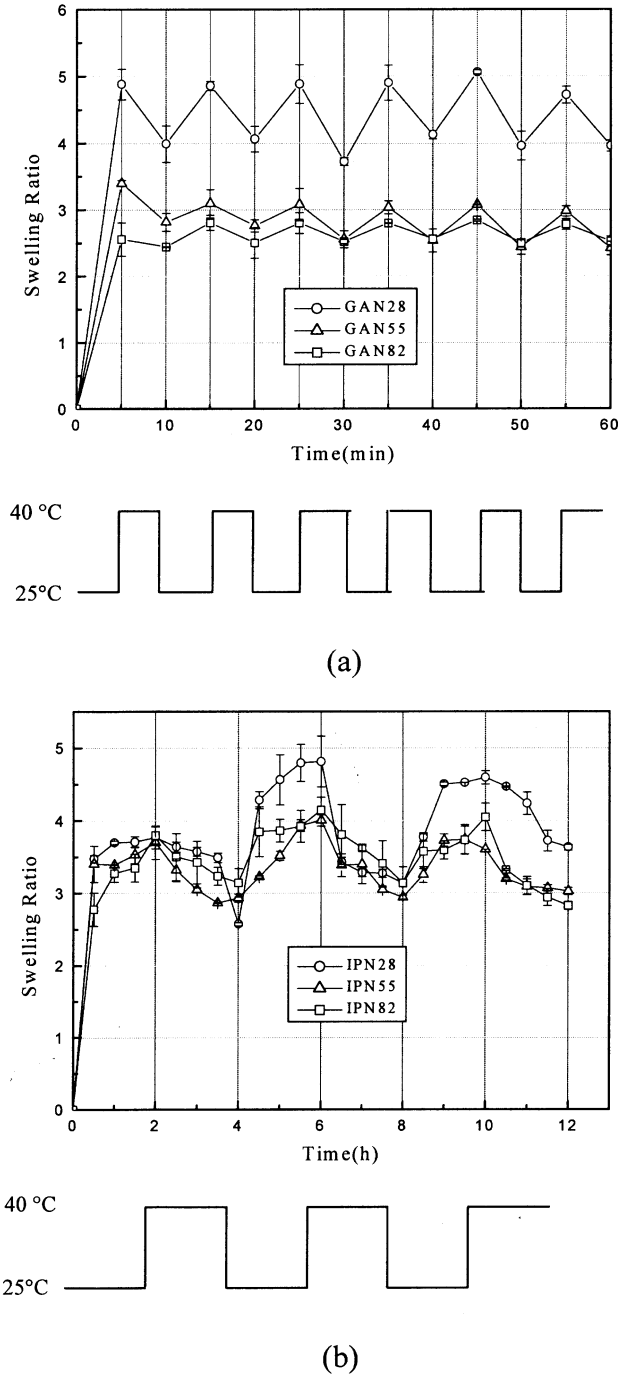

Fig. 8. Pulsatile temperature-dependent swelling behaviors of: (a) alginte/PNIPAAm-NH_2 comb-type graft hydrogels (GAN28, GAN55, GAN82) and (b) semi-IPN hydrogels (IPN28, IPN55, IPN82) in water.

Therefore, PNIPAAm-NH_2 chains maintain the chain mobility, but alginate networks are anchored at several points by crosslinking on each chain restricting the mobility. We could not see the rapid pH-sensitive behaviors of comb-type hydrogels because the fast response is due to the freely mobile grafted chains. After 20 min, pH-dependent pulsatile swelling behaviors were observed. GAN55 with

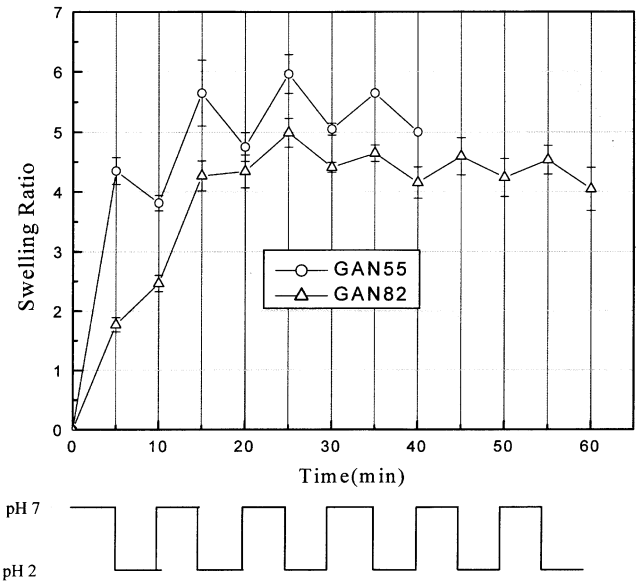

Fig. 9. Pulsatile pH-dependent swelling behaviors of alginate/PNIPAAm-NH_2 comb-type graft hydrogels (GAN55, GAN82) at 25°C.

low crosslink density dissolved after 40 min, so that we were unable to measure the swelling ratio after that. Dissolution of the hydrogel is due to the well-known fact that the calcium alginate (crosslinked sodium algiante) disintegrated into sodium alginate in EDTA (ethylenediaminetetracetic acid) or in alkaline solution because of the exchange reaction between Ca^{2+} and Na^{+} [14]. But, the dissociation of carboxyl groups in calcium alginate is suppressed at lower pH, because suppressed dissociation of carboxyl groups is thermodynamically favorable under this condition [6]. Therefore, dissolution of calcium alginate is reduced at pH 2 with neutral carboxyl groups.

4. Conculsions

We prepared the comb-type and semi-IPN hydrogels composed of alginate and amine-terminated PNIPAAm with various compositions by crosslinking with calcium ions. Comb-type graft polymer was synthesized by the formation of amide bond between carboxyl groups in alginate and amino groups in PNIPAAm-NH_2 using EDC as a coupling agent. We also prepared semi-IPNs which formed polyelectrolyte complex due to the reaction between carboxyl and amino groups within the network. Comb-type graft hydrogels rapidly reached the equilibrium swelling and deswelling states and exhibited the fast response to the temperature changes due to freely mobile PNIPAAm-NH_2 chains. Both comb-type graft and semi-IPN hydrogels showed the change in swelling ratio at around 32°C, because of PNIPAAm exhibiting the LCST behaviors. By increasing the amounts of PNIPAAm-NH_2, swelling behavior of hydrogels displayed a sharp volume phase transition. In the pH-sensitive swelling behaviors, the swelling ratio of comb-type graft hydrogels increased continuously with increasing pH values. However, the swelling ratio of semi-IPN decreased at pH ranging between 3 and 5 because of the compact structure resulting from formation of polyelectrolyte complex. Also, the swelling ratio of all the hydrogels was affected by the ionic strength in solution. Alginate/PNIPAAm-NH_2 comb-type and semi-IPN hydrogels could be useful as stimuli-responsive drug delivery system or biomimetic actuators in biomedical fields.

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