Thermo- and pH-Responsive Behaviors of Graft Copolymer and Blend Based on Chitosan and *N*-Isopropylacrylamide

SO YEON KIM,[1] SUNG MIN CHO,[1] YOUNG MOO LEE,[1] SEON JEONG KIM[2]

[1] Department of Industrial Chemistry, College of Engineering, Hanyang University, Seoul 133-791, Korea

[2] Korea Orthopedics and Rehabilitation Engineering Center, 47-3, Koosan-dong, Pupyung-ku, Inchon, 430-120, Korea

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ABSTRACT: Thermo- and pH-sensitive polymers were prepared by graft polymerization or blending of chitosan and poly(*N*-isopropylacrylamide) (PNIPAAm). The graft copolymer and blend were characterized by Fourier transform-infrared, thermogravimetric analysis, X-ray diffraction measurements, and solubility test. The maximum grafting (%) of chitosan-*g*-(*N*-isopropylacrylamide) (NIPAAm) was obtained at the 0.5 *M* NIPAAm monomer concentration, 2×10^{-3} *M* of ceric ammonium nitrate initiator and 2 h of reaction time at 25°C. The percentage of grafting (%) and the efficiency of grafting (%) gradually increased with the concentration of NIPAAm up to 0.5 *M*, and then decreased at above 0.5 *M* NIPAAm concentration due to the increase in the homopolymerization of NIPAAm. Both crosslinked chitosan-*g*-NIPAAm and chitosan/PNIPAAm blend reached an equilibrium state within 30 min. The equilibrium water content of all IPN samples dropped sharply at pH $>$ 6 and temperature $>$ 30°C. In the buffer solutions of various pH and temperature, the chitosan/PNIPAAm blend IPN has a somewhat higher swelling than that of the chitosan-*g*-NIPAAm IPN. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 1381–1391, 2000

Key words: chitosan; *N*-isopropylacrylamide; graft copolymer; blend; interpenetrating polymer network

INTRODUCTION

Considerable attention has been drawn to various responsive polymer gels, depending on external signals, because of their application for stimuli-sensitive drug delivery as well as their technological and scientific importance.[1,2] The stimuli-sensitive systems using polymer can change their volume and shape reversibly according to various external physicochemical factors.[1,2] Chemical signals, such as pH, metabolites, and ionic factors, will alter the molecular interactions between polymer chains or between polymer chain and solutes present in a system. The physical stimuli, such as temperature or electrical potential, may provide various energy sources for molecular motions and altering molecular interactions. These interactions will change properties of polymer materials such as swelling, solubility, configuration or conformational change, redox states, and crystalline/amorphous transition.[3–8] pH- and thermo-sensitive polymers especially have been extensively studied because these two factors are important environments inside the human body.[9–14]

For pH-sensitive hydrogels, their swelling is influenced by various factors, such as charge and pKa of the ionizable monomer; the crosslinking density and hydrophilicity of the polymer; and pH, ionic strength, and composition of the surrounding solution.

Correspondence to: Y. M. Lee.

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The water swelling of most hydrogels is influenced by temperature in a different degree in terms of sensitivity and dependency: an increase (positive thermosensitivity) or a decrease (negative thermosensitivity) of swelling with increasing temperature. In a positive temperature-sensitive system, hydrogels with upper critical solution temperature (UCST) shrink by cooling below the UCST, while the hydrogels with lower critical solution temperature (LCST) contract by heating above the LCST in a negative temperature-sensitive system. Many hydrophilic/hydrophobic (HPL/HPB)-balanced water-soluble polymers exhibit LCSTs, and HPL/HPB balance is from either the monomeric structure of homopolymers or the polymer composition of copolymers.

Chitosan is a poly[β(1→4)-2-amido-2-deoxy-*D*-glucopyranose], and can be obtained by the *N*-deacetylation of chitin, poly[β(1→4)-2-acetamido-2-deoxy-*D*-glucopyranose], being second only to cellulose in the amount produced annually by biosynthesis. Chitosan has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions. Chitosan and its derivatives have become useful polysaccharides in the biomedical area because of their numerous and interesting biological properties such as biocompatibility, biodegradability, and nontoxic properties. Chitosan in particular exhibits pH-responsive behavior as a weak polybase due to the large quantities of amino groups on its chain.[15–20]

Poly(*N*-isopropylacrylamide) (PNIPAAm) is well known to exhibit a lower critical solution temperature (LCST) at around 32°C in aqueous solution; that is, it dissolves in water below the LCST and precipitates from solution above the LCST. Poly(NIPAAm) hydrogels with crosslinked structure are characterized by a temperature-responsive nature in which they swell in water below and shrink above the LCST.[11–14]

Recently, our previous studies reported on pH- and temperature-sensitive IPN hydrogels, their responsive properties, and drug-release behaviors of IPN hydrogels under electric stimulus.[21–24]

In the present study, we prepared graft copolymers, a blend and their interpenetrating polymer network (IPN) hydrogels, based on chitosan having pH sensitivity and PNIPAAm exhibiting LCST behaviors, to develop dual sensitive system against both pH and temperature. This study deals with the synthesis, characterization, and the pH/temperature dependence of swelling behavior for the IPN hydrogels composed either of chitosan-*g*-NIPAAm copolymer or chitosan/PNIPAAm blend.

EXPERIMENTAL

Materials

N-Isopropylacrylamide (NIPAAm) (Tokyo Kasei, Japan) was recrystallized from mixture of hexane (Junsei Chemical Co. Ltd, Tokyo, Japan) and toluene (Junsei Chemical Co. Ltd.) (65:35 v/v). Chitosan (degree of deacetylation was 0.76 and viscosity-average molecular weight was 5×10^5) was purchased from Tokyo Kasei Organic Chemicals. Ceric ammonium nitrate (Junsei Chemical Co., Ltd) and azobis-isobutyronitrile (AIBN) (Aldrich Chem. Co., Milwaukee, WI) were used without further purification. Acetic acid, formic acid, and dioxane were supplied from Duksan Phamaceutical Co. Ltd. (Tokyo, Japan), Janssen Chimica (Belgium), and Sigma Chem. Co. (St. Louis, MO), respectively. Anhydrous ethyl ether was purchased form J. T. Baker Inc. (Phillipsburg, Canada). Water was first treated with a reverse osmosis system (Sambo Glove Co., Ansan, Korea) and further purified with a Milli-Q Plus system (Waters, Millipore, MA). All other chemicals were reagent grades and were used as purchased without further purification.

Deacetylation of Chitosan

To increase the amine content of chitosan, chitosan with 76% of deacetylation degree was further deacetylated according to the modified Mima's method.[19] Chitosan was treated for 2 h in 47% NaOH solution in a reactor at 110°C under nitrogen atmosphere. The deacetylated chitosan obtained by the alkali treatment was washed in water to neutrality. The alkali treatment and washing processes were repeated three times to obtain chitosan products, which showed a deacetylation degree of over 90%. The product was washed with water, ethanol, and diethylether, in turn, and then dried in a vacuum oven at 60°C.

The degree of deacetylation of chitosan was determined by the acid-base titration method. Chitosan was dissolved in 0.5 mol/L of 0.3 *N* HCl and the solution was titrated potentiometrically with a standard solution of 0.1 *N* NaOH. This gives a titration curve having two inflection points, the difference between the two along the

abscissa corresponding to the amount of acid required to protonate the amine groups. The degree of deacetylation was calculated from the amount of NaOH consumed between two inflection points by the following equation.[20]

$$\text{Degree of deacetylation (D.A.)} = 16.1(y - x)f/w \quad (1)$$

where f = molarity of the NaOH solution, y, x = two inflection points, and w = weight of the sample.

Preparation of Chitosan-graft-NIPAAm Copolymer

Graft polymerization of NIPAAm onto chitosan was carried out using ceric ammonium nitrate (CAN) as an initiator under a nitrogen atmosphere. Chitosan flake treated by further deacetylation was dissolved in 10 wt % aqueous acetic acid solution. While bubbling nitrogen gas, NIPAAm monomer and 2×10^{-3} M of CAN were added into the chitosan solution and the reaction mixture was stirred at 25°C for 2 h. The optimum condition was determined by varying the concentration of NIPAAm monomer to prepare chitosan having high content of the NIPAAm moiety. After graft polymerization, the products were precipitated in excess acetone and separated by filtration. To remove the NIPAAm homopolymer formed during the reaction, the grafted chitosan was followed by a Soxhlet extraction with methanol for 48 h. The resulting product was then dried under vacuum at 40°C until a constant weight was attained.

Preparation of Chitosan/Poly(NIPAAm) Blend

The linear poly(*N*-isopropylacrylamide) (PNIPAAm) was obtained by free radical polymerization of NIPAAm monomer using AIBN as a free radical initiator. NIPAAm (6.0 g) was dissolved in 100 mL of 1,4-dioxane, followed by the addition of AIBN (0.72 mmol). Dried nitrogen was bubbled through the solution for 15 min prior to polymerization. After polymerization at 60°C for 12 h, the resulting product was dissolved in acetone, from which the polymer was precipitated by adding an equal volume of water. PNIPAAm, like polyacrylamide, is insoluble in acetone–water mixtures but differs from the latter by being readily soluble in pure acetone. Dissolution in acetone and precipitation with water were repeated to purify the polymer, after which the polymer was dried *in vacuo* at room temperature.

Blend samples were prepared by dissolving PNIPAAm in an aqueous 10 wt % acetic acid solution of chitosan with further deacetylation, and then poured into a glass Petri dish. The blending ratio of two components was adjusted according to the composition of chitosan-*g*-NIPAAm copolymer with highest grafting percentage (%) for comparison. The solvent was allowed to evaporate at room temperature overnight, then it was kept at 40°C under a vacuum for 24 h. After the above procedure, film of the chitosan/PNIPAAm blend was obtained.

Preparation of Interpenetrating Polymer Network (IPN) Hydrogels of Chitosan-*g*-NIPAAm Copolymer and Chitosan/PNIPAAm Blend

To investigate the swelling kinetics and compare the thermo- and pH-responsive sensitivities of chitosan-*g*-NIPAAm copolymer and chitosan/PNIPAAm blends, we prepared IPNs composed of these polymers. Chitosan-*g*-NIPAAm IPN was prepared by dissolving the material in formic acid and adding 10^{-3} mol of glutaraldehyde as a crosslinking agent under agitation. Subsequently, it was spread on a glass Petri dish and maintained at room temperature in a dust-free environment for 24 h, followed by drying under vacuum for 2 days. The IPN obtained was washed with distilled water throughout and dried according to the above drying process.

For the preparation of IPN composed of a chitosan/PNIPAAm blend, PNIPAAm was dissolved in aqueous 10 wt % acetic acid solution of chitosan, and glutaraldehyde as a crosslinking agent was added. The blended mixture was treated by the same procedure as that for the above crosslinked chitosan-*g*-NIPAAm IPN.

Measurements

The changes in the chemical structure of the chitosan-*g*-NIPAAm copolymer and chitosan/PNIPAAm blend were investigated by Fourier transform infrared (FTIR) (Nicolet Model Magma IR 550). Wide-angle X-ray diffraction (XRD) patterns were recorded using nickel-filtered CuKα radiation produced by a Rigaku Denki Model RAD-C diffractometer. Their thermal properties were measured by thermogravimetric analysis (TGA) (Perkin-Elmer TGA-7). Decomposition profiles of TGA were recorded with a heating rate of 10°/min in nitrogen between 50 and 500°C.

The molecular weight distribution of PNIPAAm homopolymers synthesized by radical

polymerization was characterized by elution time relative to polystyrene monodisperse standards from gel permeation chromatograph (GPC) apparatus (Waters Model 510 HPLC pump, Milford, MA) with the Millennium software program. Three ultrastyragel tetrahydrofuran (THF) columns (each 30 cm × 7.8 mm i.d. HR-0.5, HR-4, HR-5, all waters) and a Waters R410 differential refractometric detector were used. The mobile phase was THF with a flow rate of 1 mL/min. The injection volume was usually 100 of stock solutions (0.1–0.5 w/v %). The calibration curve was prepared before measurements by using standard polystyrene (molecular weight: 1.28×10^3, 2.96×10^3, 1.13×10^4, 2.85×10^4, and 6.50×10^4, respectively, Shodex standard SM-105, Showa Denko, Tokyo, Japan). In addition, structural analysis of PNIPAAm homopolymer was performed by a 500 MHz ^{1}H-NMR (Bruker AMX-500) using $CDCl_3$ solution.

Solubility tests for chitosan, PNIPAAm, chitosan-*g*-NIPAAm copolymer and the chitosan/PNIPAAm blend were carried out in various solvents such as water, methanol, dioxane, acetic acid, and formic acid. Samples (0.01 g) were dispersed in 10 mL of each solvent, then the mixtures were continuously shaken at room temperature for 7 days.

Determination of Grafting Parameters for Chitosan-*g*-NIPAAm Copolymer

For chitosan-*g*-NIPAAm copolymer, grafting parameters such as the percentage of grafting (%), efficiency of grafting (%). and percentage of homopolymer (%) were calculated as follows.

Percentage of grafting (%)

$$= \frac{W_2 - W_1}{W_1} \times 100 \quad (2)$$

Efficiency of grafting (%)

$$= \frac{W_2 - W_1}{W_3} \times 100 \quad (3)$$

Percentage of homopolymer (%)

$$= \frac{W_4 - W_2}{W_3} \times 100 \quad (4)$$

where W_1, W_2, W_3, and W_4 denote the weight of initial chitosan, grafted chitosan after methanol extraction, NIPAAm, and grafted chitosan before methanol extraction, respectively.

Measurement of Equilibrium Water Content (EWC)

The IPN of chitosan-*g*-NIPAAm and chitosan/PNIPAAm blend were immersed in buffer solutions ranging from pH 4 to 9 at various temperatures, and maintained for 24 h until equilibrium swelling was reached. The swollen samples were then removed from the solution, excess water on the IPN was wiped off by filter paper, and then the IPN was weighed. Their equilibrium water content (EWC) was calculated from the following equation:

$$\text{EWC}\ (\%) = [(W_s - W_d)/W_s] \times 100 \quad (5)$$

where W_s and W_d are the weights of the IPN sample at the equilibrium swelling state and dry state, respectively.

RESULTS AND DISCUSSION

Preparation of Chitosan-*g*-NIPAAm Copolymer and Chitosan/ PNIPAAm Blend

Chitosan exists in different forms as a random type or a block type of copolymer of *N*-acetyl-*D*-glucosamine units. We performed further deacetylation of chitosan to enhance the grafting of PNIPAAm onto chitosan by increasing its amine content. Chitosan, with a 76% deacetylation degree, was further deacetylated by treating for 2 h in 47% NaOH solution in a reactor at 110°C under nitrogen atmosphere, and this procedure was repeated three times. The degree of deacetylation (D.A.), for chitosan sample treated by further deacetylation, was determined by acid-base titration. The D.A. value calculated using eq. (1) was about 97%.

To confirm the changes in chemical structure of the chitosan-*g*-NIPAAm copolymer and chitosan/PNIPAAm blend, FTIR spectroscopy measurement was carried out. Figure 1 illustrates the FTIR spectra for chitosan (a), chitosan-*g*-NIPAAm copolymer (b), chitosan/PNIPAAm blend (c), NIPAAm (d) and PNIPAAm (e). The FTIR spectrum of chitosan with a 76% deacetylation degree indicated that peaks appeared at 3450 cm^{-1}, 1653 cm^{-1}, and 1558 cm^{-1} could be assigned to a hydroxyl group, carbonyl stretching vibration (amide I), and N—H bending vibration

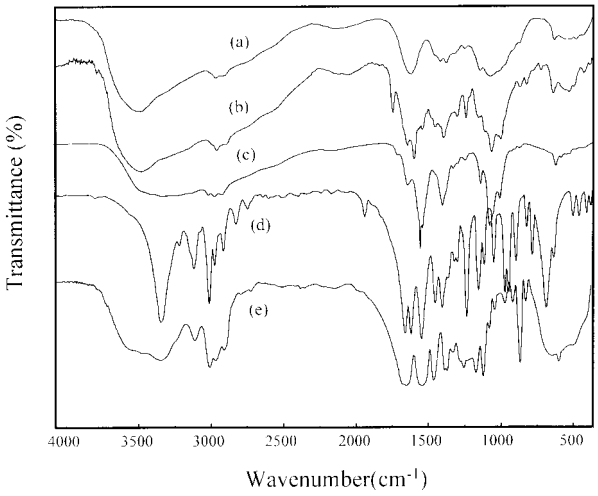

Figure 1 Fourier transform infrared spectra for (a) chitosan, (b) chitosan-*g*-NIPAAm copolymer, (c) chitosan/PNIAAm blend, (d) NIPAAm monomer, and (e) PNIPAAm hopolymer.

(amide II) of a primary amino group, respectively (not shown here). For the chitosan sample treated by further deacetylation [Fig. 1(a)], however, the characteristic peak of the amide II band at 1558 cm^{-1} shifted to 1622 cm^{-1}, while the absorption bands at 1653 cm^{-1} and 1322 cm^{-1}, which were a characteristic peak of amide I and III bands of chitin, decreased. This may be due to the fact that the degree of deacetylation of chitosan is significantly higher after further deacetylation reaction. Figure 1(e), obtained from linear NIPAAm homopolymer, shows a significant peak at 1654 cm^{-1}, 1542 cm^{-1}, and 1379 cm^{-1} , which can be attributed to the characteristic peaks of amide I, amide II, and methyl group in —$CH(CH_3)_2$, respectively. Also, the characteristic peaks at 1631 cm^{-1} (C=C), 1413 cm^{-1} (CH_2=), and C—H vinyl out-of-plane bending vibrations, observed in the spectrum of the monomer [Fig. 1 (d)], disappeared.

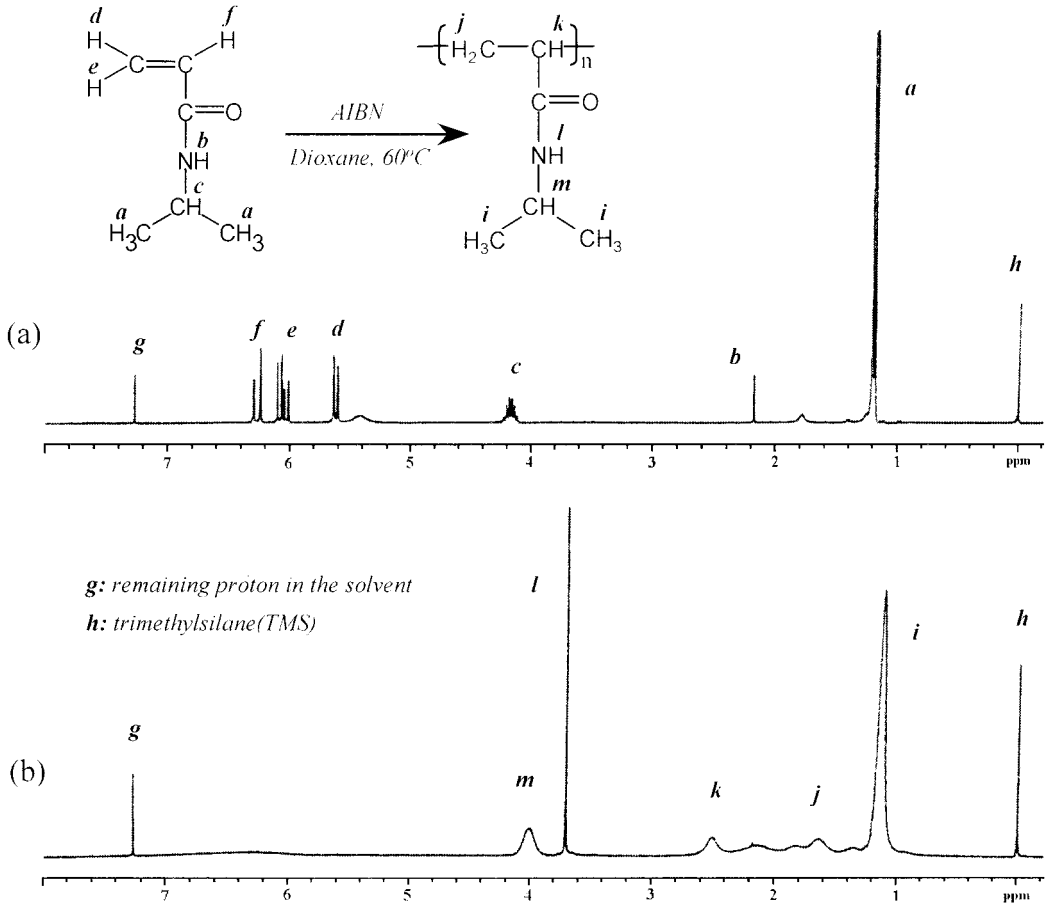

Figure 2 ^{1}H-Nuclear magnetic resonance spectra of (a) NIPAAm monomer, and (b) PNIPAAm homopolymer.

Furthermore, ^{1}H-NMR spectroscopy measurement was carried out to identify the polymerization of NIPAAm monomer. As shown in Figure 2, the spectrum of NIPAAm monomer exhibited peaks at 5.63–6.29 ppm due to CH_2=CH—, at 4.18 ppm due to —NH—CH, and at 1.21 ppm due to —CH_3. On the other hand, PNIPAAm homopolymer exhibited two broad signals for the methylene proton and one for methyne proton in the vicinity of the strong methyl signals at 1.21 ppm. This broadening of ^{1}H signals can be attributed to a distribution of PNIPAAm chain conformations, which are somewhat fixed due to the lack of mobility. Also, the peaks of the vinyl proton were not detected in the spectrum of PNIPAAm. From these results, the synthesis of PNIPAAm homopolymer could be confirmed.

The molecular weight distribution of PNIPAAm synthesized was determined by GPC measurement. From these results, the weight-average molecular weight (M_w) and polydispersity (M_w/M_n) of PNIPAAm were 21,600 and 1.88, respectively.

In the case of the chitosan-*g*-NIPAAm copolymer, an increase of the characteristic peaks of amide I and II compared with chitosan itself was observed, and the peak due to the methyl group in —$CH(CH_3)_2$ at 1379 cm^{-1} significantly increased. This result clearly indicated that NIPAAm was introduced into the chitosan. In Figure 1(c), it can be seen that the spectrum of a physical mixture of both components differs appreciably from that of the grafted chitosan. This further confirms that PNIPAAm homopolymer chains are covalently bonded to the chitosan backbone.

The crosslinking of chitosan via glutaraldehyde has been investigated by many researchers. The crosslinking mechanism probably involved the formation of imine bonds between amino groups on chitosan and aldehyde groups on glutaraldehyde. In the FTIR spectra, however, there was no obvious difference between uncrosslinked samples and crosslinked samples.

Graft Parameters for Chitosan-*g*-NIPAAm Copolymer

The grafting parameters of the chitosan-*g*-NIPAAm copolymer, such as percentage of grafting (%), efficiency of grafting (%), and percentage of homopolymer (%), were calculated by eqs. (2)–(4). These results are summarized in Figure 3, which plots the percentage of each parameter as a function of the concentration of NIPAAm monomer.

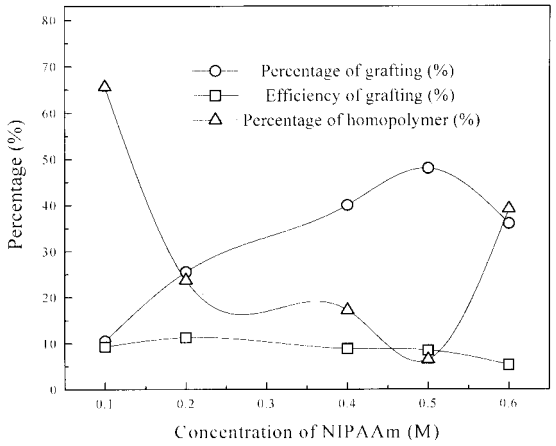

Figure 3 Graft parameters for chitosan-*g*-NIPAAm copolymer.

As can be seen in Figure 3, the concentration of the NIPAAm monomer significantly affects the grafting reaction onto chitosan backbone. The percentage of grafting (%) and the efficiency of grafting (%) gradually increase with the concentration of NIPAAm up to 0.5 *M*. However, at higher monomer concentration, the percentage of grafting begins to decrease. This can be attributed to the substantial amount of polymer grafted onto the substrate backbone, which inhibits the diffusion of CAN and monomer into the chitosan for further grafting. As a result, this effect stimulates the formation of homopolymer, which increases with an increase in monomer concentration. From the increase in the percentage of homopolymer (%), as shown in Figure 3, it can be confirmed that the rate of production of NIPAAm homopolymer is faster than that of graft polymerization when excess monomer is present.

Several research articles have been published reporting on the graft copolymerization of various acrylic monomers onto polymer backbone containing hydroxyl groups, using Ce(IV) ion as an initiator.[25–27] The grafting was assumed to proceed via a redox mechanism in three steps: (1) the solvation of water to chitosan, (2) the formation of the complex between solvated chitosan and Ce(IV), and (3) grafting initiation by radicals from the complex. Considering the effect of concentration of CAN as an initiator, high initiator concentration, compared with that of NIPAAm monomer (NIPAAm concentration: 0.1 and 0.2 *M*), resulted in a relatively high percentage of homopolymerization and low percentage of grafting. If the con-

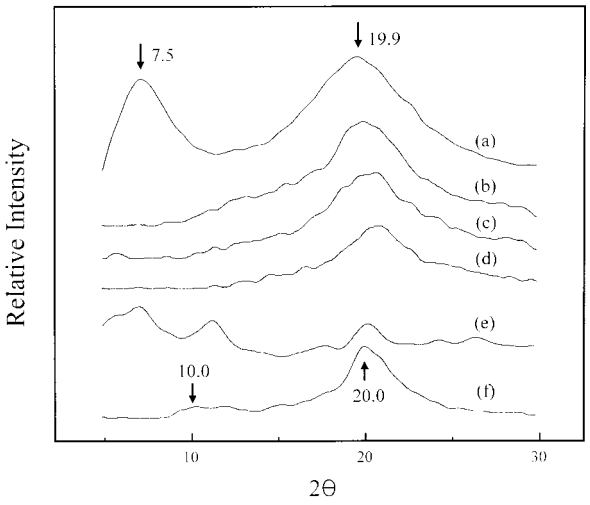

Figure 4 Wide-angle X-ray diffraction patterns of (a) PNIPAAm, (b) chitosan-*g*-NIPAAm) the percentage of grafting = 10.5%), (c) chitosan-*g*-NIPAAm (the percentage of grafting = 25.5%), (d) chitosan-*g*-NIPAAm (the percentage of grafting = 48%, (e) chitosan/PNIPAAm blend, and (f) chitosan.

centration of initiator CAN was above the critical concentration, initial points of graft polymerization were reduced, or the polymerization was terminated by Ce(IV) reacting with primary radicals or propagational radicals of chitosan. Therefore, the lower percentage of the grafting (%) at high CAN concentration, compared with that of NIPAAm monomer, was due to the increase in the homopolymerization of NIPAAm and the oxidation of chitosan by the presence of excessive CAN. Finally, the maximum percentage of grafting (%) of 48.0% was obtained at 0.5 *M* of NIPAAm concentration, 2×10^{-3} *M* of initiator (CAN) and 2 h of reaction time at 25°C. This graft copolymer with the maximum percentage of grafting (%) will be used later in all experiments. In the preparation of the chitosan/PNIPAAm blend sample, the blending ratio of the two components was also adjusted according to the composition of the chitosan-*g*-NIPAAm copolymer with 48% maximum percentage of grafting for comparison.

Crystallinity of Chitosan-*g*-NIPAAm Copolymer and Chitosan/ PNIPAAm Blend

The crystal structure and crystallinity of chitosan, PNIPAAm, chitosan-*g*-NIPAAm copolymer and the chitosan/PNIPAAm blend were investigated by the wide-angle X-ray diffraction (WAXD) measurement. In the WAXD pattern in Figure 4, chitosan gives somewhat broader patterns at around $2\theta = 10.0°$ and 20.0°, indicating lower crystallinity. These peaks were assigned to be a mixture of (001) and (100), of (101) and (002), respectively. It has been reported that the crystalline structure of chitin derivatives depends on the degree of deacetylation. Crystalline structure of shrimp chitosan is retained up to 70% of deacetylation degree. On further deacetylation, crystalline regions of chitosan are destroyed and become amorphous.[20] PNIPAAm homopolymer [(a) in Fig. 4) shows two diffraction peaks at $2\theta = 7.5°$ and 19.0°. As shown in Figure 4(b)–(d), the diffraction intensity of chitosan-*g*-NIPAAm was somewhat reduced compared with those of chitosan and PNIPAAm, and peak positions were similar to that of chitosan itself (f). Their diffraction intensities decreased with increasing content of NIPAAm in chitosan-*g*-NIPAAm. It could be confirmed that the crystalline content of chitosan was gradually decreased by grafting bulky NIPAAm chains into chitosan. In the case of the chitosan/PNIPAAm blend (e), it showed different diffraction patterns from those of chitosan-*g*-NIPAAm, which exhibited split characteristic peaks of chitosan and NIPAAm. This indicates that the chitosan/PNIPAAm blend had different crystalline structure from chitosan-*g*-NIPAAm copolymer.

Thermal Stability

To examine the thermal properties of chitosan, PNIPAAm, chitosan-*g*-NIPAAm copolymer and the chitosan/PNIPAAm blend, thermogravimetric

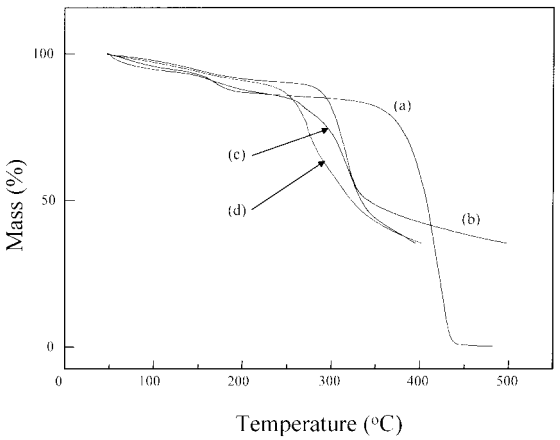

Figure 5 Thermalgravimetric analysis of (a) PNIPAAm, (b) chitosan-*g*-NIPAAm, (c) chitosan/PNIPAAm blend, and (d) chitosan.

Table I Solubility of Chitosan, PNIPAAm, Chitosan-*g*-NIPAAm, and Chitosan/PNIPAAm Blend

Sample[a]	Water	Methanol	Dioxane	Acetic Acid	Formic Acid
Chitosan	I.S.[b]	I.S.	I.S.	S.	S.
PNIPAAm	S.[b]	S.	S.	S.	S.
Chitosan-*g*-NIPAAm	I.S.	I.S.	I.S.	I.S.	S.
Chitosan/PNIPAAm blend	I.S.	I.S.	I.S.	I.S.	S.

[a] Sample (0.01 g) was dispersed in 10 mL of each solvent, then the mixtures were continuously shaken at room temperature for 7 days.
[b] I.S.: insoluble, S.: soluble.

analysis (TGA) was carried out. Figure 5 shows the weight loss curves recorded with a heating rate of 10°C/min in nitrogen between 50 and 500°C. The initial thermal decompositions of chitosan took place at 286°C, whereas the chitosan-*g*-NIPAAm copolymer and chitosan/PNIPAAm blend exhibited their initial thermal decomposition at 232 and 128°C, respectively. The results obtained from TGA curves indicate a decrease of thermal stability by copolymerization and blending. This may be due to the introduction of PNIPAAm groups, which show a lower initial thermal decomposition temperature of 135°C. These results are in agreement with those obtained by X-ray diffraction.

Solubility of Chitosan-*g*-NIPAAm Copolymer and Chitosan/ PNIPAAm Blend

The solubilities of chitosan, PNIPAAm, chitosan-*g*-NIPAAm copolymer, and the chitosan/PNIPAAm blend were investigated in several solvents, and the results are given in Table I. Because of its pendant hydroxyl groups, chitosan forms strong hydrogen bonding. Therefore, chitosan is insoluble in water and in common solvents. Due to the effect of the amine group, it is also insoluble in alkaline solution and dissolves in acidic solutions such as formic acid or acetic acid. As shown in Table I, however, the solubility of chitosan was significantly reduced after grafting and blending with NIPAAm. This is probably due to the formation of inter- and intramolecular hydrogen bonding between PNIPAAm and chitosan.

Equilibrium Water Content (EWC) of IPN for Chitosan-*g*-NIPAAm Copolymer and Chitosan/NIPAAm Blend

Swelling kinetics of IPN hydrogels prepared from chitosan-*g*-NIPAAm and the chitosan/PNIPAAm blend were investigated as functions of both temperature (25 and 35°C) and pH (pH 4 and pH 7). As shown in Figure 6, the IPN hydrogels of chitosan-*g*-NIPAAm and chitosan/PNIPAAm blend exhibited relatively high equilibrium water content (EWC), and their swelling ratios reached the equilibrium state within about 30 min. Moreover, their EWC was significantly influenced by the external stimuli such as temperature and pH. All the IPN samples exhibited higher swelling ratios at pH 4 than that at pH 7. The chitosan/PNIPAAm blend IPN especially showed the highest EWC value in swelling medium of pH 4 at 25°C. It was considered that high pH sensitivity was induced mainly by chitosan, which is a weak base with an intrinsic pKa of about 6.5;[20] namely, the IPN hydrogels swelled at low pH due to the ionic repulsion of the protonated amine groups in chitosan, and collapsed at high pH because of the influence of unprotonated amine groups.

At 35°C, which is above the LCST of NIPAAm [Fig. 6 (b)], the EWC of both chitosan-*g*-NIPAAm IPN and the chitosan/PNIPAAm blend IPN dramatically decreased compared with that at 25°C. For example, at pH 4, the EWC of chitosan/PNIPAAm blend IPN was 89.9% at 25°C and 62.3% at 35°C.

It is well known that PNIPAAm in water undergoes a coil-to-globule transition at about 32°C with elevating temperature. PNIPAAm contains hydrophilic amide and hydrophobic isopropyl groups in its side chain, rendering this polymer soluble in water and in low polarity solvents such as tetrahydrofuran. Therefore, it could be expected that the thermal sensitivity of PNIPAAm was mainly due to the dissociation of ordered water molecules surrounding hydrophobic *N*-isopropyl groups. As a result, in the case of IPN hydrogels composed of chitosan-*g*-NIPAAm or the chitosan/PNIPAAm blend, they also underwent a volume phase transition in water at the transi-

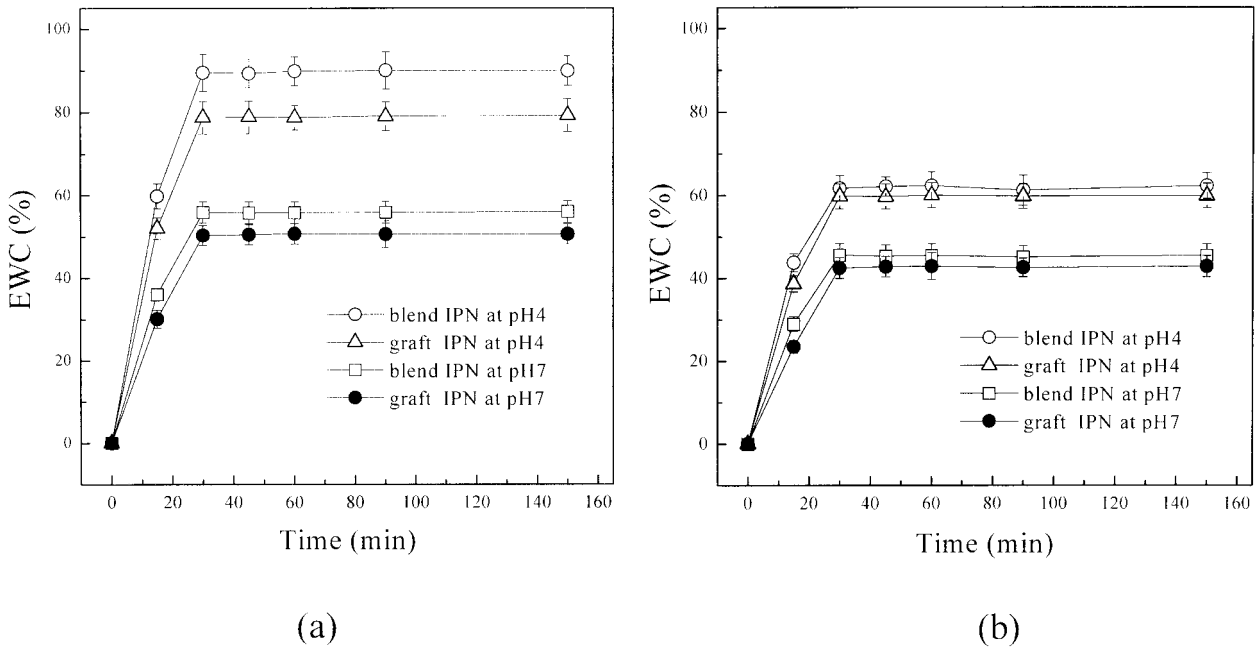

Figure 6 Swelling kinetics of chitosan-*g*-NIPAAm IPN and chitosan/PNIPAAm blend IPN at different pH and temperature conditions; (a) swelling in medium with pH 4 and 7 at 24°C, and (b) swelling in medium with pH 4 and 7 at 34°C.

tion temperature from a swollen state to a shrunken state with increasing temperature from 25 to 35°C. In all cases, the EWC of the chitosan/PNIPAAm blend IPN was higher than that of the chitosan-*g*-NIPAAm copolymer IPN. The reason might be due to the decrease of the number of amine groups in the chitosan backbone by grafting of the NIPAAm, i.e., the hydrophilic nature of

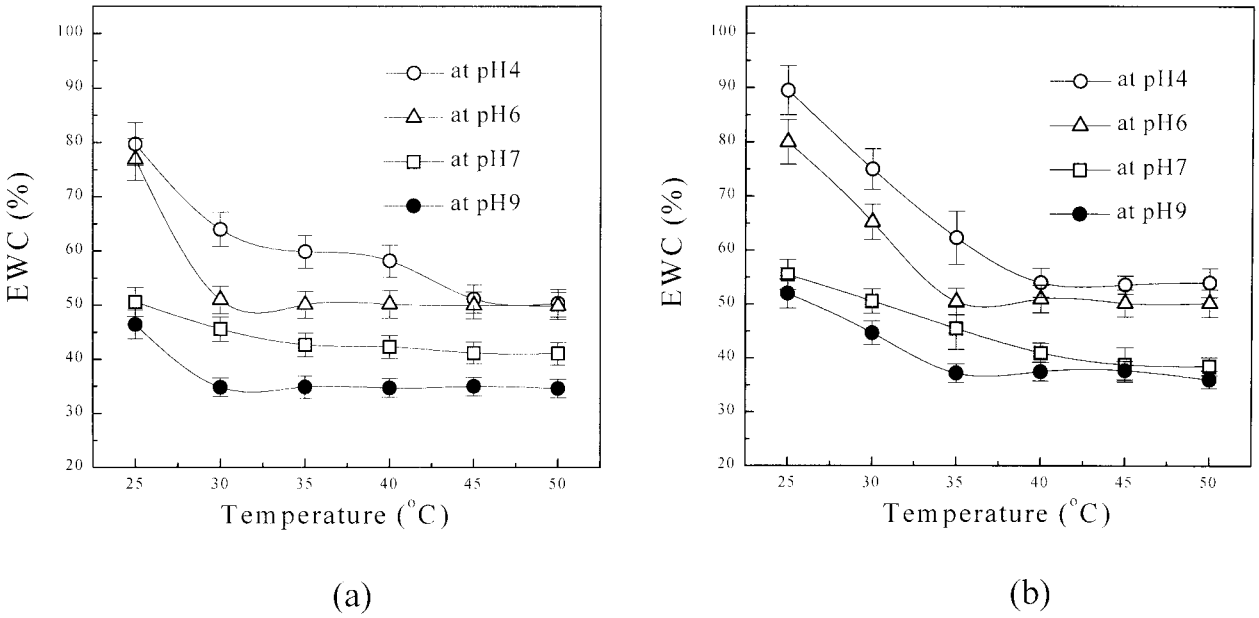

Figure 7 Equilibrium water content of chitosan-*g*-NIPAAm IPN and chitosan/PNIPAAm blend IPN at differnt temperatures as a function of pH; (a) chitosan-*g*-NIPAAm IPN, and (b) chitosan/PNIPAAm blend IPN.

chitosan is due to the presence of free amino groups on the C-2 carbon. During the grafting process, substantial number of free amino groups are blocked by the growing chains of PNIPAAm.

The changes of EWC for IPN hydrogels of the graft copolymer and blend at different pH values are shown in Figure 7 as a function of temperature. We observed that the EWC of the chitosan-*g*-NIPAAm and chitosan/PNIPAAm IPN hydrogels was strongly dependent on both pH and temperature, which was similar to the results of swelling kinetics. As shown in Figure 7, the phase transition of IPN hydrogels can be clearly seen in all IPN hydrogels as the temperature increased. Also, the decrease of EWC induced by increasing temperature measured at pH 4 and 6 was more drastic than that at pH 7 and 9. Furthermore, chitosan-*g*-NIPAAm copolymer IPN hydrogels showed a decrease of EWC as the temperature increased, while the chitosan/PNIPAAm blend IPN sample exhibited a sharp volume phase transition. This could be induced by the lower EWC value of chitosan-*g*-NIPAAm IPN hydrogel than that of the chitosan/PNIPAAm blend IPN, clearly due to the decrease of the number free amine groups in the chitosan backbone after grafting.

CONCLUSIONS

We prepared graft copolymers, blends, and their IPN hydrogels based on the chitosan and NIPAAm to develop dual pH- and temperature-responsive systems. The chitosan-*g*-NIPAAm copolymer was synthesized by graft polymerization of NIPAAm onto chitosan using ceric ammonium nitrate (CAN) as an initiator. We also prepared chitosan/PNIPAAm blends composed of chitosan and linear PNIPAAm homopolymer synthesized by radical polymerization, which had the same composition ratios with the graft copolymer. Thermo- and pH-sensitive IPN hydrogels were prepared by crosslinking the chitosan-*g*-NIPAAm copolymer and chitosan/PNIPAAm blend using glutaraldehyde. The changes in chemical structure of the chitosan-*g*-NIPAAm copolymers and chitosan/PNIPAAm blend were confirmed by FTIR, ^{1}H-NMR, GPC, WAXD, and TGA measurements. The solubility of chitosan was significantly reduced after grafting and blending with NIPAAm. IPN hydrogels of chitosan-*g*-NIPAAm and chitosan/PNIPAAm blend exhibited relatively high equilibrium water content (EWC), and their swelling ratios reached the equilibrium state within about 30 min. All IPN samples exhibited higher swelling ratios at pH 4 than at pH 7. At 35°C, which is above the LCST of NIPAAm, the EWC of both the chitosan-*g*-NIPAAm IPN and chitosan/ PNIPAAm blend IPN dramatically decreased compared with those at 25°C. In addition, their pH-dependent swelling behaviors were more significantly at 25 than at 35°C. From the plot of equilibrium water content vs. temperature as a function of pH, the phase transition of IPN hydrogels could be clearly seen as the temperature increased in all IPN hydrogels. Also, the decrease of EWC induced by increasing temperature at pH 4 and 6 was more intensive than that at pH 7 and 9. Furthermore, chitosan-*g*-NIPAAm copolymer IPN hydrogels showed a decrease of EWC as the temperature increased, while chitosan/PNIPAAm blend samples exhibited a sharp volume phase transition. Both chitosan-*g*-NIPAAm and the chitosan/PNIPAAm blend IPN hydrogels exhibited swelling/deswelling changes in response to external stimuli such as pH and temperature, and could be useful as novel modulation systems in biomedical fields.

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REFERENCES

1. Mikos, A. G.; Murphy, R. M.; Bernstein, H.; Peppas, N. A. Materials Research Society Symposium Proceedings: Vol. 331; Materials Research Society: U.S.A., 1994.
2. Park, K. Controlled Drug Delivery; Challenges and Strategies; ACS Professional Reference Book: U.S.A., 1997.
3. Iwata, H.; Matsuda, T. J Membr Sci 1988, 38, 185.
4. Okahata, Y.; Nouguchi, H.; Seki, T. Macromolecules 1987, 20, 15.
5. Hoffman, A. S.; Dong, L. C.; J Controlled Release, 1991, 15, 141.
6. Gudeman, L. F.; Peppas, N. A. J Membr Sci 1995, 107, 239.
7. Afrassiabi, A.; Dong, L. C. J Controlled Release 1986, 4, 213.
8. Nozawa, I.; Suzuki, Y.; Sato, S.; Sugibayashi, K.; Morimoto, Y. J Biomed Mater Res 1991, 25, 243.
9. Nishi, S.; Kotaka, T. Polym J 1989, 21, 393.
10. Yao, K. D.; Peng, T.; M. Goosen, F. A.; Min, J. M.; He, Y. Y. J Appl Polym Sci 1993, 48, 343.

11. Tanaka, Y.; Kagami, Y.; Matsuda, A.; Osada, Y. Macromolecules 1995, 28, 2574.
12. Tanaka, T. Phys Rev Lett 1980, 45, 1636.
13. Yoshida, R.; Sakai, K.; Okano, T.; Sakurai, Y. J Biomater Sci Polym Ed 1992, 3, 243.
14. Aoki, T.; Kawashima, M.; Katono, H.; Sanui, K.; Ogata, N.; Okano, T.; Sakurai, Y. Macromolecules 1994, 27, 947.
15. Cerrai, P.; Guerra, G. D.; Tricoli, M. Macromol Chem Phys 1996, 197, 3367.
16. Denuziere, A.; Ferrier, D.; Domard, A. Carbohydr Polym 1996, 29, 317.
17. Park, W. H. Macromol Chem Phys 1996, 197, 2175.
18. Lee, K. Y.; Park, W. H.; Ha, W. S. J Appl Polym Sci 1997, 63, 425.
19. Mima, S.; Miya, M.; Iwato, R.; Yoshikawa, S. J Appl Polym Sci 1983, 28, 1909.
20. Roberts, G. A. F. Chitin Chemistry; The Macmillan Press Ltd.: London, UK, 1992.
21. Shin, H. S.; Kim, S. Y.; Lee, Y. M. J Appl Polym Sci 1997, 65, 685.
22. Shin, H. S.; Kim, S. Y.; Lee, Y. M.; Lee, K. H.; Kim, S. J.; Rogers, C. E. J Appl Polym Sci 1998, 69, 479.
23. Kim, S. Y.; Shin, H. S.; Lee, Y. M. J Appl Polym Sci 1999, 73, 1675.
24. Kim, S. Y.; Lee, Y. M. J Appl Polym Sci 1999, 74, 1752.
25. Gurruchaga, M.; Goni, I.; Vazquez, M. B.; Valero, M.; Guzman, G. M. J Polym Sci: Part C Polym Lett 1989, 27, 149.
26. Goni, I.; Gurruchaga, M.; Valero, M.; Guzman, M. J Polym Sci Polym Chem Ed, 1983, 21, 2573.
27. Nonaka, T.; Hashimoto, K.; Kurihara, S. J Appl Polym Sci 1997, 66, 209.