Thermosensitive Properties of Semi-IPN Gel Composed of Amphiphilic Gel and Thermosensitive Polymer in Buffer Solution

Atsushi Takahashi,[1,2] Shuji Sakohara[1]

[1]*Department of Chemical Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan*
[2]*Yokohama Corporate Research Laboratories, Mitsubishi Rayon Co. Ltd., 10-1 Daikoku-cho, Tsurumi-ku, Yokohama 230-0053, Japan*

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ABSTRACT: In this study, a semi-interpenetrating polymer network (semi-IPN) gel comprising a crosslinked amphiphilic *N,N*-dimethylacrylamide-*co*-*N*-isopropylacrylamide gel and interpenetrating thermosensitive poly(*N*-isopropylacrylamide) (poly(NIPAM)) was prepared. Their thermosensitive behaviors in a buffer solution comprising sodium chloride, sodium citrate, and sodium dodecyl sulfate, which are generally used in biochips, were investigated. At low temperatures, the gel in the buffer solution was absolutely transparent. However, when the gel was heated, it became milky white or opaque without changes in the gel size. It is well known that the network or crosslink distribution of the transparent gel is homogeneous macroscopically, whereas that of the opaque gel is inhomogeneous; in other words, the network of the opaque gel consists of coarse and dense parts. The structural changes in the gel network were confirmed by the temperature dependence of the permeability of the buffer solution through the semi-IPN gel membrane. The permeability increased stepwise when the gel became opaque due to heating. The structural changes in the semi-IPN gel network depended on the compositions of the buffer solution and gel, i.e., the copolymerization ratio of NIPAM in the gel and the content of the poly(NIPAM) molecules in the gel. These results suggest that the thermosensitive behaviors of the semi-IPN gel strongly depend on the interactions between the copolymerized NIPAM in the gel network and the poly(NIPAM) molecules. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1530–1537, 2011

Key words: semi-IPN gel; poly(*N*-isopropylacrylamide); *N,N*-dimethylacrylamide gel; thermosensitive property; permeability

INTRODUCTION

Recently, the application of polymeric hydrogels to biochips such as DNA chips and protein chips has attracted special attention in the field of biotechnology.[1–5] In hydrogel-type biochips, capture probes are immobilized on a gel network, and the target probes diffuse through the gel network and react with the capture probes. The hydrogel-type biochip has the following advantages. The immobilization capacity of hydrogel-type biochips is larger than that of conventional biochips, in which the capture probes are immobilized two-dimensionally on a flat plate. Furthermore, in hydrogel-type biochips, the gel network, which functions as a matrix that holds water, prevents the chips from drying.

Hydrogel-type biochips have certain important requirements as follows:

a. High diffusivity or permeability of capture probes such as DNA through the gel network in the reaction process. The reaction is performed at relatively high temperatures, i.e., 50–70°C.
b. High transparency of the gel at room temperature. The reaction is usually detected by means of a fluorescence technique, and the detection is performed at room temperature. The target probes are previously labeled with a fluorescent material. The hybridization is detected by the fluorescence emitted from the target probes, which react with the capture probes in the gel. Therefore, the gel should be transparent at room temperature.

Existing hydrogel-type biochips satisfy requirement (b). However, the diffusivity or permeability of the target probes or buffer solution through the gel network is low, and the analysis time required is long. To alleviate this problem, we have proposed a semi-interpenetrating polymer network (semi-IPN) gel comprising crosslinked *N,N*-dimethylacrylamide-

Correspondence to: A. Takahashi (takahashi_at@mrc.co.jp).

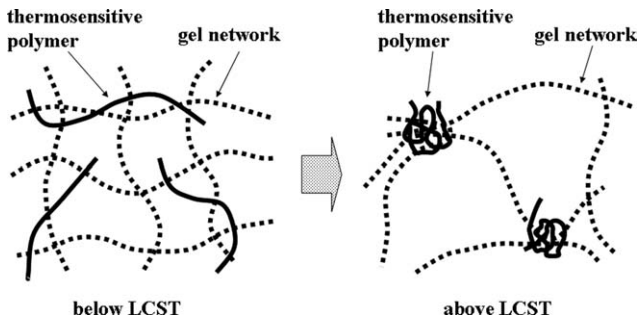

Figure 1 Concept of structural changes in semi-IPN gel networks.

co-*N*-isopropylacrylamide (DMAA-*co*-NIPAM) gel and interpenetrating thermosensitive poly(*N*-isopropylacrylamide) (poly(NIPAM)). Thermosensitive polymers are soluble in water below their lower critical solution temperature (LCST) but they are insoluble above the LCST because they become hydrophobic. Poly(NIPAM) is a representative nonionic thermosensitive polymer with an LCST of $\sim$ 32°C. The DMAA gel is an amphiphilic gel, and its swelling degree is almost independent of the salt concentration in solutions such as buffer solutions. Furthermore, the copolymerization of NIPAM with DMAA enhances the interaction between the NIPAM components in the gel network and the interpenetrated poly(NIPAM) molecules. Hence, it can be expected to prevent poly(NIPAM) molecules from flowing out of the gel.

These semi-IPN gels might be transparent because the polymer density in the gel is homogeneous macroscopically. However, when the gel is heated, the poly(NIPAM) molecules get entangled in the gel network and shrinks a part of the gel network, as shown in Figure 1. As a result, the gel network collapses partially, and coarse and dense networks are formed. Although such an inhomogeneous gel might be opaque,[6] the formation of the coarse network promotes the diffusion of materials.

There have been several reports on semi-IPN gels comprising a hydrophilic gel and an interpenetrating thermosensitive polymer; the responsiveness, mechanical strength, and partition of hydrophobic materials in these gels have been improved by controlling the temperature.[7–10] The thermosensitive properties of these semi-IPN gels have been examined only in water. However, biochips such as DNA chips are used in buffer solutions comprising salts and surfactants having relatively high concentrations. Typically, buffer solutions of sodium chloride, sodium citrate, and sodium dodecyl sulfate (SDS) are used. The composition of the buffer solution is usually expressed in terms of SDS and a mixture of sodium chloride and sodium citrate (SSC). The solution represented as 1 $\times$ SSC consists of a mixture of 0.15-mol/L sodium chloride and 0.015-mol/L sodium citrate. Although many studies on the effects of salts and surfactants on the LCST of poly(NIPAM) have been conducted,[11–14] there have been very few studies on the thermosensitive properties of the semi-IPN gel in buffer solutions. In this study, the effects of the preparation conditions of the semi-IPN gel, such as the copolymerization ratio of NIPAM and the content of poly(NIPAM), on the thermosensitive behaviors of the semi-IPN gel in a buffer solution have been examined experimentally. The thermosensitive behaviors have been examined by measuring the changes in the transmittance through the semi-IPN gel; the changes in the permeability of the buffer solution through the semi-IPN gel with temperature have also been examined.

EXPERIMENTAL

Synthesis of poly(NIPAM)

NIPAM was kindly supplied by Kohjin Co., Ltd. (Japan) and was purified by recrystallization from hexane before use. Poly(NIPAM) was prepared by radical polymerization in the same manner as that described in our previous paper.[15] *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS) were used as an accelerator and an initiator, respectively. The prepared poly(NIPAM) was purified by carrying out dialysis using a membrane (Cellu Sep T3, Nominal MWCO: 12000-14000, Membrane Filtration Products, Inc.) for one week. The average molecular weight of poly(NIPAM) was estimated from its intrinsic viscosity [η] as previously reported.[15] The intrinsic viscosity measurement was performed by using a solution obtained by dissolving poly(NIPAM) in tetrahydrofuran at 27°C, and the following equation proposed by Fujishige was used to calculate the number–average molecular weight $\overline{M_n}$[16]:

$$[\eta] = 9.59 \times 10^{-6} \overline{M_n}^{0.65} [\mathrm{m^3/kg}] \quad (1)$$

$\overline{M_n}$ of poly(NIPAM) prepared in this study was $\sim 7.42 \times 10^6$.

Synthesis of semi-IPN gels

The semi-IPN gel was prepared by copolymerizing DMAA as the primary monomer, NIPAM as the comonomer, and *N*,*N*′-methylenebisacrylamide (MBAA) as the crosslinker in an aqueous solution containing the poly(NIPAM) molecules. The preparation was carried out at 20°C by free-radical polymerization using TEMED and APS. The synthesis conditions for the semi-IPN gels are listed in Table I. The gels were washed with deionized water and

TABLE I
Synthesis Conditions of Semi-IPN Gels (mol/m^3)

		NP0	NP10	NP20	NP30
Monomer:	*N*,*N*-dimethylacrylamide (DMAA)	400	360	320	280
Co-monomer:	*N*-isopropylacrylamide (NIPAM)	0	40	80	120
	Copolymerization ratio of NIPAM	0 mol %	10 mol %	20 mol %	30 mol %
Linker:	*N*,*N*′-methylenebisacrylamide (MBAA)		4		
Accelerator:	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED)		10		
Initiator:	ammonium peroxodisulfate (APS)		0.5		

Solvent: aqueous poly(NIPAM) solution: 0.1, 0.3, 0.5 wt %; Temperature: 20°C; Reaction time: 6 h.

kept in water or in buffer solutions of the desired composition.

To measure the transmittance through the semi-IPN gels, plate-type gels with thicknesses of 3 mm were prepared. The synthesis of these gels was carried out between two glass plates separated by a 3-mm-thick spacer. The gel was cut into pieces having dimensions of 10 × 20 mm. Cylindrical gels were also synthesized to measure the swelling ratio. The synthesis was carried out in a glass tube with an inner diameter of 6 mm. The gels were cut into pieces having lengths equal to the diameter, washed with deionized water, and then dried at room temperature. During the drying process, the gels were placed on a Teflon sheet that was spread on a Petri dish. Because the gels break if they are dried quickly, the dish was covered with a thin plastic film having small holes to decrease the drying speed.

Measurement of transmittance through buffer solutions containing poly(NIPAM) molecules and through semi-IPN gels immersed in buffer solutions

To investigate the transition behaviors of poly(NIPAM) in the aqueous solutions of SSC and SDS (these solutions together constitute the buffer solution), the changes in the transmittance with temperature were measured. At temperatures lower than the LCST of poly(NIPAM), the aqueous solutions are absolutely transparent and the transmittance is almost 100% because poly(NIPAM) is hydrophilic and soluble in the solutions. However, when the solutions are heated above the LCST, the solutions become milky white and the transmittance decreases because poly(NIPAM) becomes hydrophobic and is insoluble in the solutions. Therefore, the LCST can be estimated by monitoring the changes in the transmittance through the solutions with temperature. The transmittance was measured at 600 nm using a spectrophotometer equipped with a temperature-control system (V-530, Japan Spectroscopy Co., Ltd.). The concentration of poly(NIPAM) molecules in the solutions was adjusted to 0.5 wt %, and the measurements were performed for solutions with various concentrations of SSC and SDS.

The temperature dependence of the transmittance through the semi-IPN gels immersed in the buffer solution was also measured to examine the thermosensitive behaviors of the gels in the buffer solution. The measurement was performed at 600 nm using the aforementioned spectrophotometer. In the measurement, a quartz cell with a path length of 10 mm was used, and the gel was placed perpendicular to the light path and gently pressed on the inner wall of the quartz cell filled with the buffer solution.

Measurement of swelling properties of semi-IPN gels

The effect of temperature on the swelling degree of the semi-IPN gels in the buffer solution was examined by using the cylindrical gels. The dried gels were immersed in the buffer solution at 25°C until the swelling of the gels reached equilibrium, and then, the swelling diameter of the gel was measured with a cathetometer. Next, the temperature was increased to the desired temperature, and the swelling diameter at equilibrium was measured again. This procedure was repeated until the temperature reached 70°C.

Measurement of temperature dependence of permeability of buffer solution through semi-IPN gel membrane

A semi-IPN gel membrane was synthesized in the hole of a gel holder comprising three glass plates with a total thickness of 3.6 mm, as shown in Figure 2(a). The diameter of the hole in the center plate was 30 mm, and a cut was made in a section of the center plate to introduce the reactant solution. The diameter of the hole in the glass plates on either side of the center plate was 20 mm. The walls of the glass plates with holes were treated with chlorodimethylvinylsilane to introduce double bonds on the wall surface.[17] The gel was synthesized by radical polymerization. The preparation procedure was as follows. The hole was covered by two slide glasses,

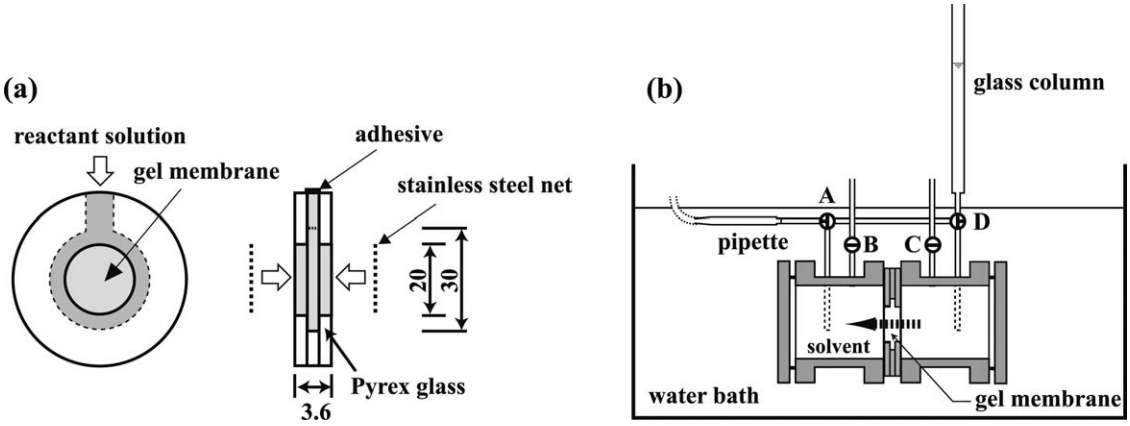

Figure 2 (a) Gel holder and (b) apparatus for permeability measurements.

and the reactant solution, which was previously degassed, was poured into the hole through a cut in the center plate. The reactant solution was composed of two aqueous solutions. One was a monomer solution containing DMAA, NIPAM, MBAA, TEMED, and poly(NIPAM), and the other was an APS solution. After the polymerization of the gel was complete, the slide glasses covering the holes were removed, and the gel membrane surface was reinforced by a stainless steel net, which was attached onto the glass plate using quick-drying glue, as shown in Figure 2(a). Subsequently, the membrane was washed by immersing it in distilled water to remove unreacted monomers. A DMAA-*co*-NIPAM gel membrane without poly(NIPAM) (NP30) was also prepared for comparison. The gel containing poly(NIPAM) was prepared with the following composition: DMAA/NIPAM/MBAA: 280/120/4 mol/m^3 (NP30). The poly(NIPAM) concentration was 0.5 wt %.

The measurement of the permeability of the buffer solution through the gel membrane was performed using the apparatus shown in Figure 2(b). The apparatus consisted of two permeation cells made of stainless steel, a 500-mm glass column to generate hydrostatic pressure, and a calibrated glass pipette to measure the flow rate. The gel holder shown in Figure 2(a) was fixed between the two permeation cells. The apparatus was immersed in a water bath. Subsequently, the buffer solution was poured into both the cells, and the apparatus was left overnight at 30°C without applying pressure to allow the swelling of the gel to reach the equilibrium state in the buffer solution. When the measurement was started, valves B and C were closed and valves A and D were opened; this resulted in a hydrostatic pressure being applied to the gel membrane. The position of the meniscus in the pipette was measured as a function of time. Subsequently, the temperature was raised to 40°C; the apparatus was again left overnight without applying pressure; and the measurement was performed. These procedures were repeated until the temperature reached 60°C.

The permeability K was estimated by using the following equation:

$$\nu = \frac{V}{A} = K \cdot \frac{\Delta P}{d \cdot \mu} \tag{2}$$

Here, ν is the velocity of the buffer solution; V, the volumetric flow rate of the buffer solution; A, the cross-sectional area of the gel membrane; ΔP, the pressure applied on the gel membrane; d, the thickness of the gel membrane; and μ, the viscosity of the buffer solution.

RESULTS AND DISCUSSION

Transition behaviors of poly(NIPAM) in buffer solutions

Figure 3 shows the temperature dependence of the transmittance through the poly(NIPAM) solutions containing sodium chloride [Fig. 3(a)], sodium citrate [Fig. 3(b)], and SDS [Fig. 3(c)] with various concentrations. The concentration of poly(NIPAM) in the solutions was adjusted to 0.5 wt %. In water, the transmittance decreased abruptly at around 32°C, whereas in the sodium chloride and sodium citrate solutions, the transmittance decreased at lower temperatures when the concentration of these salts was increased. This implies that the LCST of poly(NIPAM) decreases as the concentration of these salts increases. Furthermore, the LCST of poly(NIPAM) in the sodium citrate solution is lower than that in the sodium chloride solution even though the salt concentration in both the solutions is the same. These phenomena can be explained by the fact that the poly(NIPAM) molecules become more hydrophobic in salt solutions because the salt molecules destroy the hydration layer around the poly

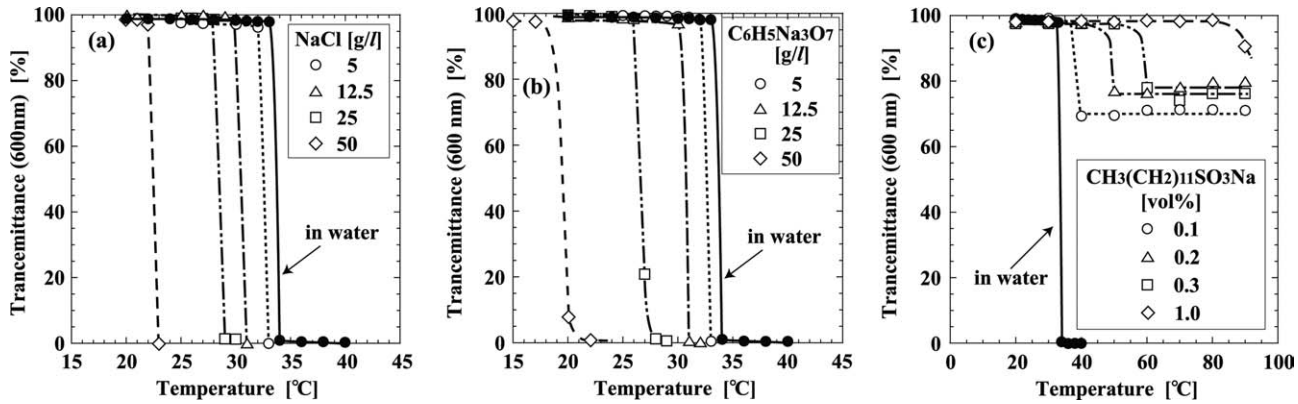

Figure 3 Transition behaviors of poly(NIPAM) in (a) sodium chloride, (b) sodium citrate, and (c) sodium dodecyl sulfate. The concentration of poly(NIPAM) is 0.5 wt %.

(NIPAM) molecules and the electric charge of sodium citrate is twice that of sodium chloride.[14] On the other hand, in the SDS solution, the LCST increased with the SDS concentration. Furthermore, the magnitude of the decrease in the transmittance reduced as the SDS concentration increased. These phenomena can be attributed to the interaction between the hydrophobic group of SDS and the isopropyl group of poly(NIPAM).[11,12]

Figure 4 shows the temperature dependence of the transmittance through the buffer solution containing poly(NIPAM). The concentration of poly(NIPAM) in the solutions was adjusted to 0.5 wt %. The concentration of SDS in the buffer solution was fixed at 0.2 wt %, and the concentration of SSC was changed from 0.25 × SSC to 2 × SSC. The decrease in the transmittance in the buffer solutions containing 0.25 ×, 0.5 ×, and 1 × SSC was observed to start from ~ 40°C, which was higher than that in water (~ 32°C). This implies that the LCSTs of poly(NIPAM) in these SSC solutions were higher than that in water. However, in the solution containing 2 × SSC, the decrease in the transmittance was observed to start from ~ 30°C; in other words, the LCST was lower. Furthermore, the sharpness of the transition also decreased. These phenomena can be attributed to the fact that the effects of changes in the concentration of SSC and SDS on the LCST of poly(NIPAM) are not similar.

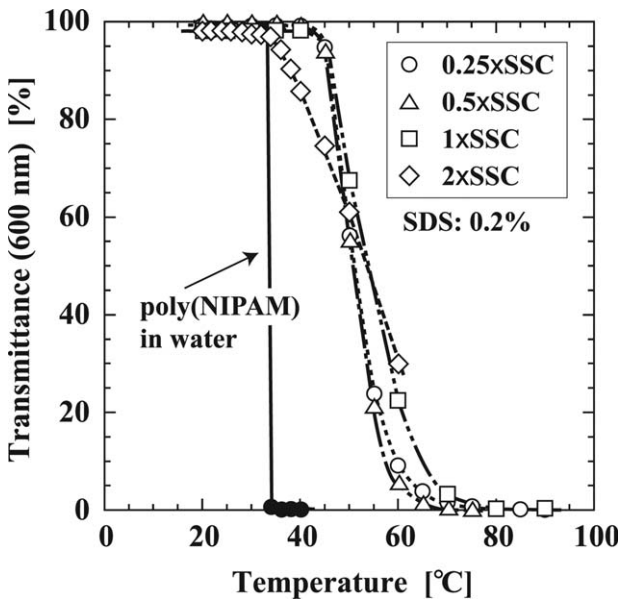

Figure 4 Transition behaviors of poly(NIAPM) in buffer solution consisting of SSC and SDS. The SDS concentration was fixed at 0.2 wt %, whereas that of SSC was varied from 0.25 × SSC to 2 × SSC. The concentration of poly(NIPAM) is 0.5 wt %.

Swelling properties of semi-IPN gel

Figure 5 shows the temperature dependence of the swelling diameter of the cylindrical semi-IPN gels in the buffer solution containing 2 × SSC and 0.2% SDS. For the preparation of the gels, NIPAM was copolymerized with various copolymerization ratios, which are denoted by NP0, NP10, NP20, and NP30, and the poly(NIPAM) concentration in preparation was fixed at 0.5 wt %. The diameter of the gel prepared without copolymerizing NIPAM, i.e., the semi-IPN gel composed of DMAA gel (NP0) decreased slightly as the temperature increased, but the changes were very small. The decrease in the gel diameter prepared by copolymerizing NIPAM became larger than that of the DMAA gel. However, in the experimental range of the copolymerization ratio of NIPAM, the decrease in the diameter was sufficiently small, and it did not affect the diffusivity or permeability of the target probes.

The effects of the content of poly(NIPAM) were also examined but the data are not presented in this article. The copolymerization ratio of NIPAM was fixed at 30 mol %, and the content of poly(NIPAM) was varied from 0 to 0.5 wt %. The temperature dependence of the diameter of these gels was also sufficiently small.

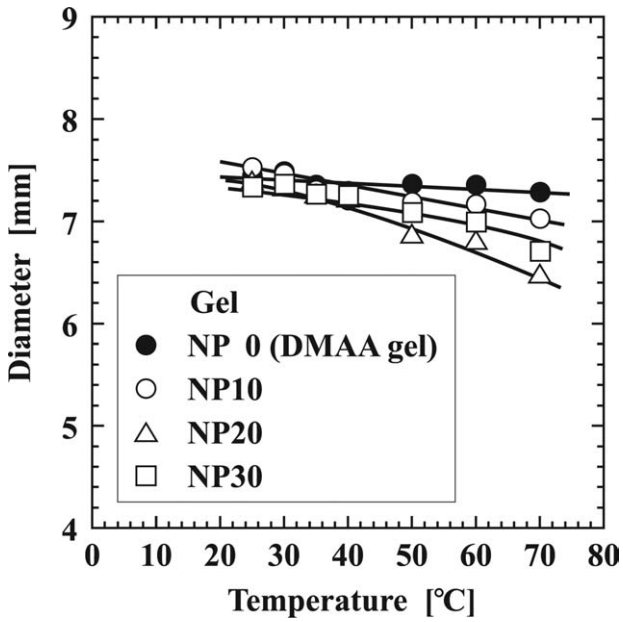

Figure 5 Effect of the copolymerization ratio of NIPAM on the temperature dependence of the swelling diameter of semi-IPN gel. The poly(NIPAM) concentration in preparation is 0.5 wt %.

Thermosensitive properties of semi-IPN gel in buffer solutions

The semi-IPN gel in the buffer solution was transparent at lower temperatures and when the solution was heated, the gel became milky white or opaque. An example is shown in Figure 6. The NP30 gel prepared in the 0.5 wt % poly(NIPAM) solution was used. The measurement of the thermosensitive properties of the gel was performed in a buffer solution containing 2 × SSC and 0.2% SDS. At 30°C, the gel was absolutely transparent, whereas at 50°C, the gel was milky white; however, the changes in the gel size were sufficiently small. It is well known that the network distribution of the transparent gel is homogeneous macroscopically, whereas that of the opaque gel consists of coarse and dense networks, i.e., an inhomogeneous structure. This result implies that the network structure of the semi-IPN gel can change without sufficient changes in the gel size by heating. The formation of the inhomogeneous network structure can be attributed to the fact that the poly(NIPAM) molecules get entangled in the gel network to shrink a part of the gel network, as shown in Figure 1.

The changes in the gel state from transparent to opaque were investigated systematically by measuring the transmittance through the gels. Figure 7 shows the temperature dependence of the transmittance through the semi-IPN gels immersed in the buffer solution containing 2 × SSC and 0.2% SDS. For the preparation of the semi-IPN gel, NIPAM was copolymerized with various copolymerization ratios (NP0, NP10, NP20, and NP30), and the poly (NIPAM) concentration was also varied.

Figure 7(a) shows the effect of the copolymerization ratio of NIPAM on the temperature dependence of the transmittance. The poly(NIPAM) concentration for the gel preparation was fixed at 0.5 wt %. In the case of the NP0 gel prepared without copolymerizing NIPAM, the decrease in the transmittance was observed to start from ~ 65°C, and as the copolymerization ratio of NIPAM increased, the decrease in the transmittance started from lower temperatures. On the other hand, the transition of poly(NIPAM) in the buffer solution was observed to start from ~ 35°C. The temperature from which the transmittance through the gel was observed to decrease did not correspond to the transition temperature of poly(NIPAM).

Figure 7(b) shows the effect of the poly(NIPAM) concentration on the temperature dependence of the transmittance. The copolymerization ratio of NIPAM was fixed at 30 mol % (NP30). The NP0 gel was transparent in the experimental range of the temperature. The decrease in the transmittance was observed at lower temperatures as the poly(NIPAM) concentration increased.

These results suggest that the structural changes in the gel network depend on the interaction between the poly(NIAPM) molecules and gel network. Incorporating the NIPAM component into the gel network strengthens these interactions.

Temperature dependence of permeability of buffer solution through semi-IPN gel

Figure 8 shows the temperature dependence of the permeability of the buffer solution containing 2 × SSC and 0.2% SDS through the semi-IPN gel membrane. The NP30 gel prepared in the solution containing 0.5 wt % poly(NIPAM) was used. The results for the NP30 gel membrane without poly(NIPAM) are also shown for comparison. In addition, the temperature dependence of the transmittance through

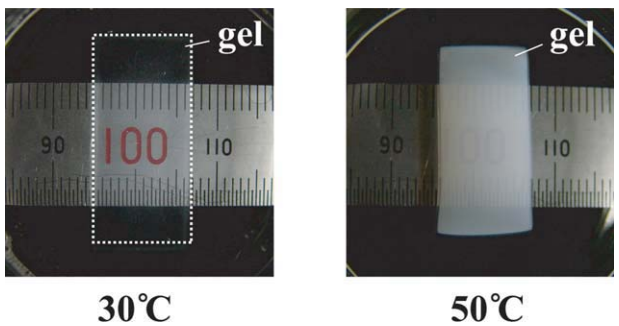

Figure 6 Photographs of NP30 semi-IPN gel at 30 and 50°C. The poly(NIPAM) concentration in preparation is 0.5 wt %. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

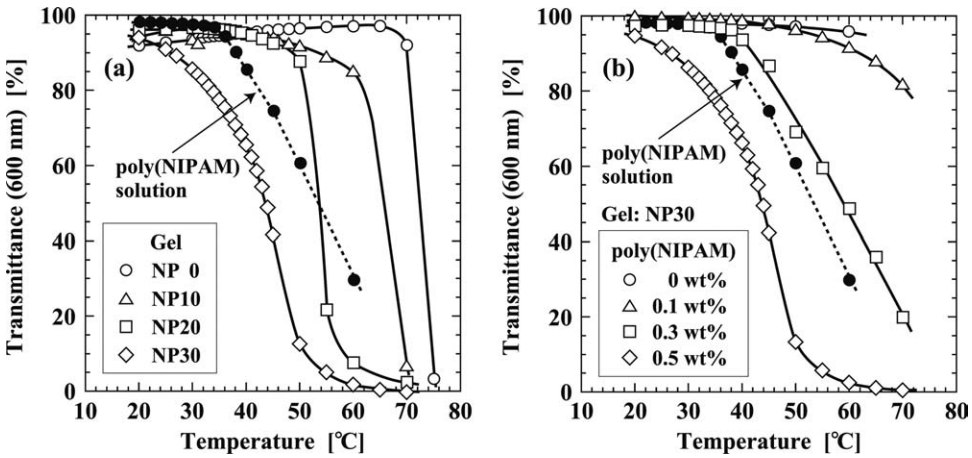

Figure 7 Temperature dependence of the transmittance through semi-IPN gels. The gel prepared with various copolymerization ratios of NIPAM (a) in the solution containing 0.5 wt % poly(NIPAM) and (b) with various concentrations of poly(NIPAM).

the semi-IPN gel is also shown. The permeability through the NP30 gel membrane without poly(NIPAM) decreased as the temperature increased. This result can be attributed to the fact that the volume of the NP30 gel without poly(NIPAM) slightly decreased as the temperature increased; in other words, the effective pore size of the gel network slightly decreased. On the other hand, the permeability through the semi-IPN gel increased stepwise between 40 and 50°C; at 50°C, the gel became opaque. At 25°C, the permeability through the NP30 semi-IPN gel was smaller than that of the NP30 gel without poly(NIPAM) gel. This difference can be attributed to the fact that the total polymer concentration of the NP30 semi-IPN gel was higher than that of the NP30 gel without poly(NIPAM) because of the addition of poly(NIPAM) to the DMAA-*co*-NIPAM gel. From these results, it can be inferred that the changes in the network structure of the semi-IPN gel, i.e., the formation of the inhomogeneous structure comprising coarse and dense networks, as shown in Figure 1, enables the buffer solution to permeate easily through the semi-IPN gel.

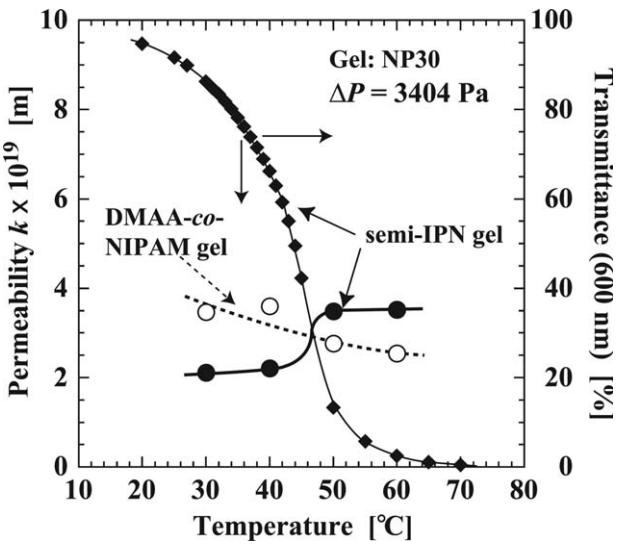

Figure 8 Temperature dependence of permeability of buffer solution through NP30 gel without poly(NIPAM) and NP30 semi-IPN gel.

CONCLUSIONS

The transition behaviors of a semi-IPN gel composed of a DMAA-*co*-NIPAM gel and interpenetrating poly(NIPAM) molecules in a buffer solution containing SSC and SDS were examined. At low temperatures, the semi-IPN gel was absolutely transparent, but when the gel was heated, it became milky white or opaque without sufficient changes in the gel size. This result implies that because of heating, the structure of the gel network changes to an inhomogeneous structure, i.e., a coarse and dense network structure. This structural change was confirmed by the changes in the permeability of the buffer solution through the semi-IPN gel membrane after heating. The thermosensitive behaviors of the semi-IPN gel strongly depend on the interactions between the NIPAM component copolymerized in the network and the poly(NIPAM) molecules. The semi-IPN gel proposed in this study can be used as the base gel for hydrogel-type biochips such as DNA chips.

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