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Effect of synthesis temperature on characteristics of PNIPAM/alginate IPN hydrogel beads

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ABSTRACT: Poly(*N*-isopropylacrylamide)/alginate interpenetrating polymer network hydrogel beads (IPN beads) were prepared via modified inverse emulsion polymerization, and the effect of the synthesis temperature on the characteristics of the IPN beads was investigated. Varying the synthesis temperature from 10 to 40°C led to slight differences in the FTIR and elemental analysis results, whereas significant differences in optical color, thermoresponsive behavior, and release properties of vitamin B_{12} were observed. The IPN beads prepared at low temperature were transparent and exhibited a large and drastic thermoresponsive volume change. On the other hand, the IPN beads prepared at high temperature were opaque and exhibited a small and gradual thermoresponsive volume change. In addition, the diffusion coefficient in the IPN beads prepared at 10°C decreased with increasing solution temperature, whereas the diffusion coefficient in IPN beads prepared at 40°C increased with increasing solution temperature. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41814.

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INTRODUCTION

Poly(*N*-isopropylacrylamide) (PNIPAM) hydrogel is a well-known, thermoresponsive hydrogel that exhibits a sharp volume phase transition at around 33°C in aqueous solution.[1–3] Below the volume phase transition temperature (VPTT), the hydrogel swells, whereas above the VPTT, the hydrogel shrinks because it dehydrates to a collapsed state due to the breakdown of the delicate hydrophilic/hydrophobic balance in the network structure. Because of their unique properties, the potential of thermoresponsive hydrogels for use in various applications, including drug delivery, enzyme immobilization, biosensors, soft actuator, and oil recovery, has been investigated by many researchers.[4–9] In general, PNIPAM hydrogels prepared via free radical polymerization of an *N*-isopropylacrylamide (NIPAM) monomer and a crosslinker. The monomer concentration, and crosslinker type and concentration are the important parameters that determine the properties of PNIPAM hydrogels, and thus they have been extensively investigated.[10–13] The synthesis temperature is also a significant parameter; when PNIPAM hydrogels are prepared at low temperature, a homogeneous network is obtained, whereas at high temperature, a heterogeneous network is obtained.[13–15]

On the other hand, a chemically independent interpenetrating polymer network (IPN) comprising a PNIPAM network and another polymer network, especially natural polymer such as alginate, chitosan, and cellulose, has also been proposed by many researchers.[16–20] By combining the natural polymer, the biocompatibility and the mechanical strength were enhanced. In these types of IPN hydrogels, the choice of the combined polymer in addition to PNIPAM, the concentrations of the polymer, the crosslinker as well as PNIPAM are the main attention, because these parameters were important to determine the properties of the IPN hydrogels. However, to the best of our knowledge, there have been no reports describing the effects of the synthesis temperature on the characteristics of IPN hydrogels to date, despite the fact that the synthesis temperature is known to be an important factor in the preparation of PNIPAM hydrogels.

Regarding the size of the hydrogel, microspheres, and millimeter-sized gel beads are suitable for column operation, such as packed bed, moving bed, and fluidized bed operation that are used in reaction and separation processes because of the easiness in handling and negligible pressure drop involved. In addition, monodisperse spheres are advantageous for the design and execution of the separation, reaction, adsorption, and diffusion processes, e.g., the solution of the Fickian diffusion equation within a sphere.[21]

Therefore, in this study, the monodisperse millimeter size PNIPAM/alginate IPN hydrogel beads were prepared via modified

inverse emulsion polymerization, and the effect of the synthesis temperature on the characteristics of IPN beads was investigated. The obtained beads were characterized using Fourier transform infrared (FTIR) spectroscopy, elemental analysis, and stereomicroscopy. The thermoresponsive properties of the hydrogels were then evaluated by measuring the wet weight of the IPN beads at several temperatures. In addition, the release properties for vitamin B_{12} (VB_{12}) as a model drug were evaluated below and above the VPTT.

EXPERIMENTAL

Materials

NIPAM was kindly supplied by Kohjin. *N,N′*-methylenebisacrylamide (MBAA), sodium alginate (SA), calcium chloride ($CaCl_2$), *n*-octane, and SPAN 20 were purchased from Kanto Chemical. Ammonium persulfate (APS), *N,N,N′,N′*-tetramethylethylenediamine (TEMED), and vitamin B_{12} (VB_{12}) were purchased from Wako Pure Chemical Industries. Tris-HCl buffer (pH 8.6) was used as the solvent in all the experiments.

Preparation of IPN Beads

The IPN beads were prepared via modified inverse emulsion in which calcium alginate hydrogel was used as water phase. First, calcium alginate beads were synthesized via the dripping method. An SA solution (1.0–3.0 wt %) was pumped from a nozzle (OD = 1.26 mm, ID = 0.90 mm) using a syringe pump (30 mL/h) into a $CaCl_2$ solution (1.1 wt %). The obtained calcium alginate beads were then left to stand in the $CaCl_2$ solution for more than 24 h. The thermoresponsive IPN beads were then synthesized by the modified inverse emulsion polymerization technique. The calcium alginate beads (approximately 2 mL) were immersed in 10 mL of a buffer solution containing NIPAM monomer (4.8–14.4 wt %), MBAA (crosslinker, 0.2–0.6 wt %), APS (initiator, 0.1 wt %), and $CaCl_2$ (1.1 wt %). The solution was degassed for 1 h using N_2 at 0°C. The calcium alginate beads were then collected, the excess surface water was removed by wiping, and the beads were redispersed in *n*-octane (40 mL) containing SPAN 20 (emulsifier, 0.25 vol %). Polymerization was initiated by adding TEMED (10 μL) and continued for 24 h at 10–40°C. After polymerization, the beads were separated from the *n*-octane and washed several times with water. Finally, the beads were respectively shrunken and swollen three times at 50 and 10°C for 2 h each in a $CaCl_2$ solution to harden the calcium alginate and then stored at 10°C until used. We performed shrinking/swelling pretreatment of the IPN beads before carrying out characterizations, because the size of the beads irreversibly decreases by repeating the shrinking/swelling process during first 2–3 cycles probably due to hardening of calcium alginate. After 2–3 cycles, thermoresponsive properties were stabilized, then, following experiments were conducted.

Characterization

The chemical structure of the IPN beads was confirmed by FTIR spectroscopy (IRPrestige-21, Shimadzu) using the KBr tablet method.

Elemental analyses were performed to determine the PNIPAM content in the IPN beads using a CHN elemental analyzer (EA1110, CE Instruments). The PNIPAM/alginate weight ratio was calculated based on the obtained N/C ratio by assuming that NIPAM and MBAA were polymerized at the input ratio. It should be noted that alginate does not contain N.

Furthermore, the IPN beads were observed under a stereomicroscope (WILD M3Z, Leica), and the outer diameters were determined using image analysis software (Azokun, Asahi Kasei Engineering).

Measurement of Thermoresponsive Properties

The thermoresponsive properties of the IPN beads were measured over the temperature range from 10 to 50°C. The IPN beads were maintained in a 1.1 wt % $CaCl_2$ solution at each temperature for 10 h before measurement. The beads were then collected, excess surface water was removed by wiping, and their wet weight was measured. The thermoresponsive properties of the IPN beads were evaluated based on the relative wet weight (W_T/W_{10}), where W_T and W_{10} are the wet weights of the IPN beads at temperature T (°C) and 10°C, respectively.

Release of Vitamin B_{12}

Release experiments with vitamin B_{12} (VB_{12}) were performed at 25°C (below VPTT) and 40°C (above VPTT), respectively. To load the VB_{12} into the IPN beads, the beads were dialyzed against a 50 mg/L VB_{12} solution for 2 days with replacing the solution every 12 h. It should be noted here that the solution temperature during VB_{12} loading was set at the same temperature as release experiment. That means, when the release experiment was performed at 25°C, the loading was carried out at 25°C. For the release experiment at 40°C, the loading was done at 40°C. Once collected, the beads were gently wiped to remove excess surface water and then immersed in a 1.1 wt % $CaCl_2$ solution, which was shaken at 120 rpm in a horizontal laboratory shaker (Personal-11, TAITEC). The VB_{12} concentration in the surrounding solution was measured with time using a spectrophotometer (V-530, JASCO) at 361 nm. As mentioned above, since the solution was kept at the same temperature of 25 or 40°C during the load and release experiments, there was no volume change of the IPN beads between the load and release experiments. The diffusion coefficient with VB_{12} in the IPN hydrogel was expressed as D_g and calculated using the unsteady–state transport model derived for molecular diffusion in a homogenous sphere, as shown in the following equation[22]:

$$\frac{C_t}{C_e} = 1 - \sum_{n=1}^{\infty} \frac{6\alpha(1+\alpha)}{9+9\alpha+\alpha^2 q_n^2} \exp\left(\frac{D_g q_n^2 t}{r^2}\right) \quad (1)$$

where C_t and C_e are the VB_{12} concentrations of the surrounding solution at time t and equilibrium, respectively, r is the radius of IPN beads, α is the effective volume ratio as described in eq. (2) below, and q_n is the positive, nonzero root of eq. (3) below:

$$\alpha = \frac{3V}{4\pi r^3 K} \quad (2)$$

and

$$\tan(q_n) = \frac{3q_n}{3+\alpha q_n^2} \quad (3)$$

where V is the volume of the surrounding solution and K is the equilibrium partition coefficient, which is the ratio of the

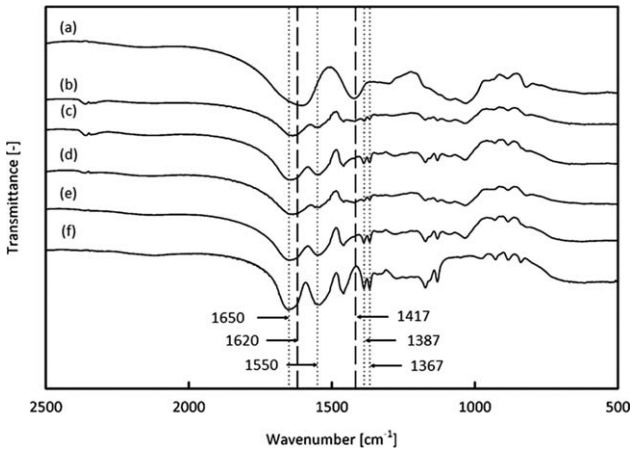

Figure 1. FTIR spectra of (a) calcium alginate and IPN beads prepared with initial NIPAM monomer concentrations and synthesis temperatures of (b) 4.8 wt % and 10°C, (c) 14.4 wt % and 10°C, (d) 4.8 wt % and 40°C, (e) 14.4 wt % and 40°C, and (f) PNIPAM (dashed lines: alginate peaks, dotted lines: PNIPAM peaks).

equilibrium VB_{12} concentration in the IPN beads to that in the surrounding solution.

RESULTS AND DISCUSSION

FTIR Analysis

Figure 1 presents FTIR spectra of the calcium alginate beads, IPN beads, and PNIPAM. The spectrum of the calcium alginate beads contained characteristic peaks at 1620 and 1417 cm^{-1}, which correspond to the asymmetric and symmetric stretching peaks of the carboxylate salt groups, respectively.[23] The spectrum of PNIPAM showed significant peaks at 1650, 1550, and 1387 and 1367 cm^{-1}, which are attributed to the characteristic peaks for the amide I, amide II, and methyl groups, respectively.[24] In the IPN beads, the characteristic peaks for both PNIPAM and calcium alginate were observed. Furthermore, the intensities of the characteristic peaks for PNIPAM in the IPN beads increased with increasing initial NIPAM concentration, whereas those of the characteristic peaks for calcium alginate became weaker. In contrast, however, no difference was observed in the FTIR spectra of the IPN beads prepared at different synthesis temperatures.

Elemental Analysis

Figure 2 shows the PNIPAM/alginate weight ratio in the IPN beads, which was calculated using the elemental analysis results. As can be observed in the figure, the PNIPAM/alginate weight ratio increased linearly with increasing initial NIPAM monomer concentration. This result indicated that the NIPAM yield was nearly the same regardless of the NIPAM monomer concentration. In addition, the PNIPAM/alginate weight ratio was barely affected by the synthesis temperature. Therefore, IPN beads with nearly the same PNIPAM/alginate weight ratio can be prepared at different synthesis temperatures.

Stereomicroscope Observation

The IPN beads in the $CaCl_2$ solution at 10°C were observed under a stereomicroscope, and the resultant photos are shown in Figure 3. The monodisperse IPN beads were obtained in the all experimental conditions and the average size were 2.2–2.7 mm. As can be observed in the figure, the color of the IPN beads was significantly different at different synthesis temperatures. The IPN beads prepared at 10 and 20°C were transparent, whereas those prepared at 30 and 40°C were opaque. The differences in the optical properties of PNIPAM hydrogels have been investigated by some researchers.[15,25] They synthesized chemically crosslinked PNIPAM hydrogel at several temperatures. According to these studies, opaque PNIPAM hydrogels were obtained at temperatures above around 24°C because of the phase separation in the reaction solution. When PNIPAM hydrogel was prepared at low temperature (<24°C), the propagation of polymers proceeded in a thermodynamically stable solution, thereby, homogeneous PNIPAM network was obtained. On the other hand, when PNIPAM hydrogel was prepared at high temperature (≥24°C), the phase separation during polymerization process was caused by the change of thermodynamic interaction between the polymer segment and water, thereby, heterogeneous network was obtained. In our case, therefore, when the IPN beads were synthesized at 30 and 40°C, the PNIPAM structure was also likely more spatially inhomogeneous, which is probably caused by the phase separation during polymerization.

The photos of the IPN beads in the $CaCl_2$ solution at 50°C were shown in Figure 4. Compared with low temperature surrounding solution (Figure 3), the optical color of the IPN beads prepared at 10 and 20°C were changed from transparent to opaque due to phase transition of PNIPAM in the gel beads. In addition, IPN beads were drastically shrunk isotropically and kept spherical shape. On the other hand, optical color of the IPN beads prepared at 30 and 40°C were almost same (opaque), and the IPN beads shrunk slightly.

Thermoresponsive Properties

In this section thermoresponsive properties of IPN beads synthesized at different conditions (synthesis temperature, NIPAM monomer and SA concentration) were examined. The relative wet weight of the IPN beads as a function of temperature is plotted in Figures 5–7. Thermoresponsive shrinkage was

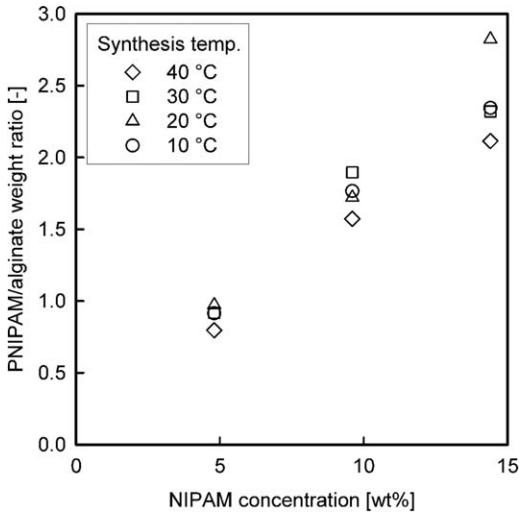

Figure 2. PNIPAM/alginate weight ratio of the IPN beads.

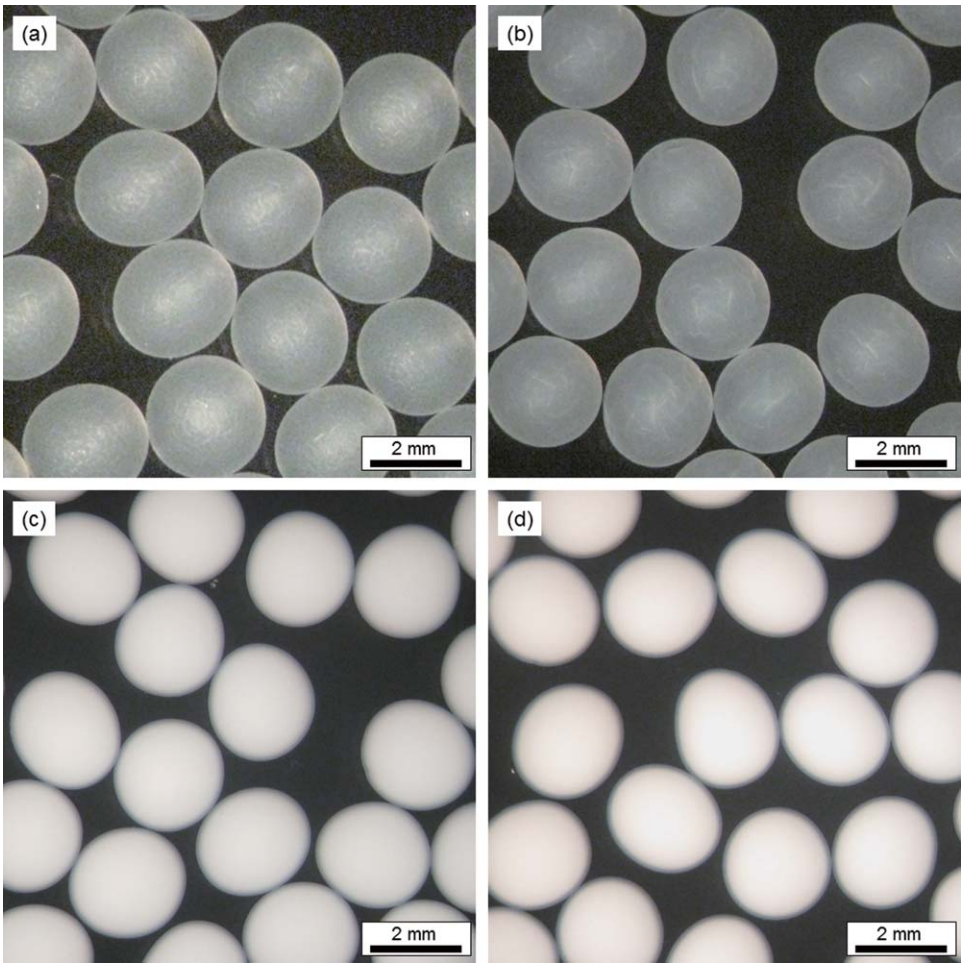

Figure 3. Stereoscopic photos taken at 10°C of IPN beads prepared at (a) 10°C, (b) 20°C, (c) 30°C, and (d) 40°C (NIPAM concentration: 9.6 wt %, alginate concentration: 2.0 wt %). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed for all the IPN beads; however, the shrinkage behaviors were different depending on the experimental parameters. The effect of synthesis temperature was shown in Figure 5. A large and drastic shrinkage occurred for the IPN beads synthesized at 10 and 20°C, whereas only a small and gradual shrinkage occurred for the IPN beads synthesized at 30 and 40°C. Sayil *et al.* and Takata *et al.* reported the thermoresponsive volume changes for pure PNIPAM hydrogels prepared at 2–29°C and 5–35°C, respectively.[26,27] They reported that the thermoresponsive behavior was only slightly different as a function of synthesis temperature, and still drastic shrinkage of PNIPAM hydrogels were occurred at temperature close to VPTT. Therefore, the gradual shrinkage of the IPN beads synthesized at 30 and 40°C in the current study is a special feature of this IPN hydrogel comprised of both PNIPAM and calcium alginate. Since distinctive difference was observed for the IPN beads prepared below and above VPTT, two representative temperatures of 20 and 30°C were selected and following experiments were performed at the two temperatures.

The effect of initial NIPAM monomer concentration was shown in Figure 6. In the IPN beads prepared at 20°C [Figure 6(a)], thermoresponsive shrinkage was significantly affected by the NIPAM concentration. In the IPN beads prepared with 14.4 wt % NIPAM concentration, large, and drastic thermoresponsive shrinkage was observed. However, the shrinkage ratio became small with the decrease of NIPAM concentration, and merely gradual shrinkage was observed in the IPN beads prepared with 4.8 wt % NIPAM concentration. This was probably due to insufficient amount of PNIPAM in the IPN beads to cause shrinkage. Similar trends of the effect of NIPAM monomer concentration on the thermoresponsive properties were also observed in the PNIPAM/alginate hydrogel prepared at RT and reported by de Moura *et al.*[16] On the other hand, IPN beads prepared at 30°C [Figure 6(b)], thermoresponsive volume change was rarely changed with the difference of NIPAM concentration and showed a small and gradual shrinkage.

Then, the effect of initial alginate concentration was shown in Figure 7. In the IPN beads prepared at 20°C [Figure 7(a)], a characteristic shrinkage near the VPTT was observed in all alginate concentration, but the shrinkage ratio was decreased with the increase of alginate concentration. These shrinking trends were also observed in the PNIPAM/alginate hydrogel films prepared in refrigerator.[17] For this reason, it was considered that the rigid structure of alginate was enhanced with the increase of

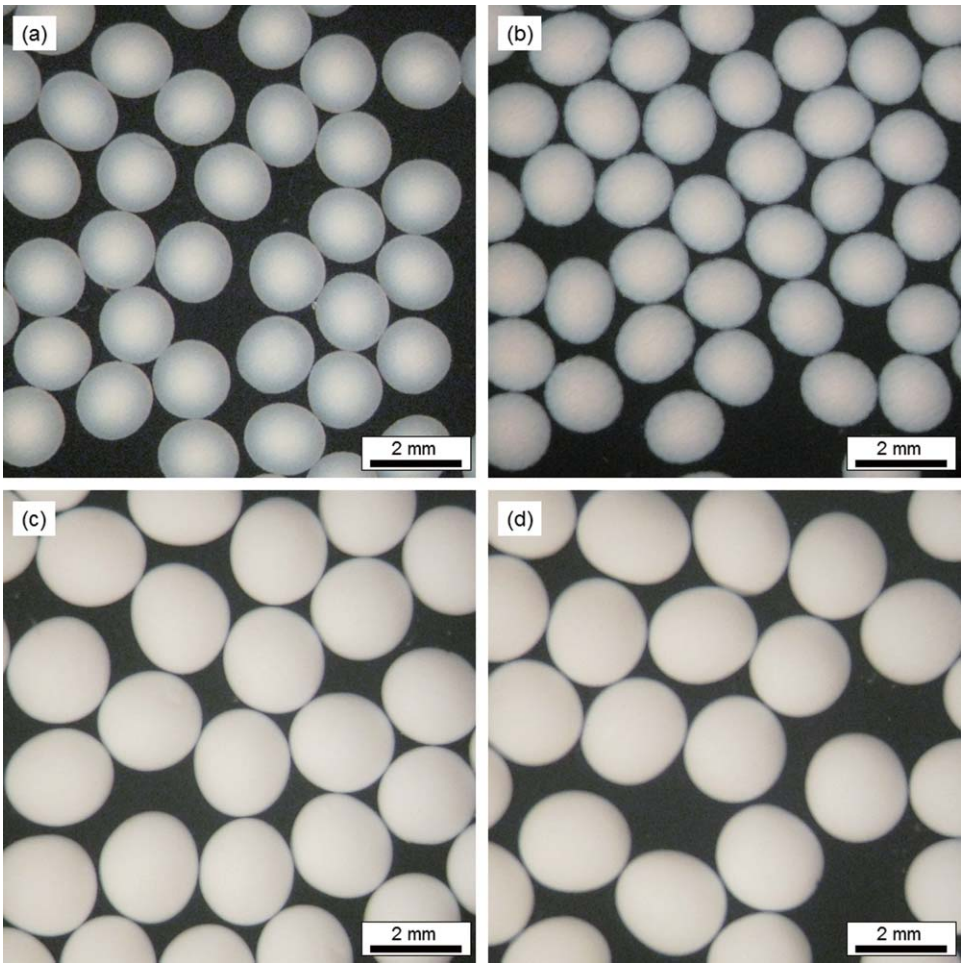

Figure 4. Stereoscopic photos taken at 50°C of IPN beads prepared at (a) 10°C, (b) 20°C, (c) 30°C, and (d) 40°C (NIPAM concentration: 9.6 wt %, alginate concentration: 2.0 wt %). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

alginate concentration and PNIPAM shrinkage was suppressed. On the contrary, in the IPN beads prepared at 30°C [Figure 6(b)], thermoresponsive shrinkage was also small and gradual

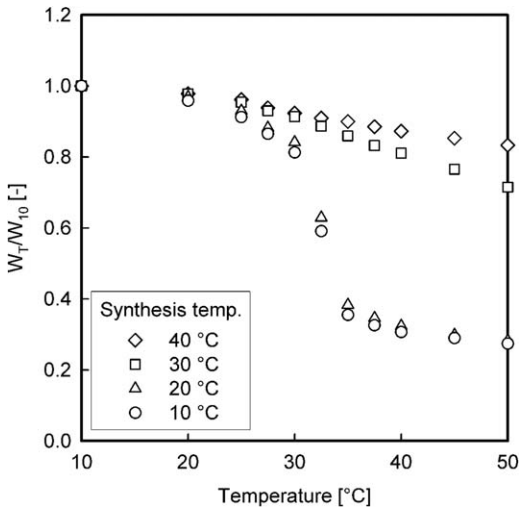

Figure 5. Effect of synthesis temperature on thermoresponsive volume change of IPN beads (NIPAM concentration: 9.6 wt %, alginate concentration: 2.0 wt %).

except for 1.0 wt % alginate concentration. In the IPN beads prepared with 1.0 wt % alginate, a characteristic shrinkage near the VPTT was slightly observed, and this was probably due to low density alginate network to suppress PNIPAM aggregation.

To summarize the effect of experimental parameters, the relationship between the PNIPAM/alginate weight ratio and the shrinkage ratio from 10 to 50°C (i.e., $1 - W_{50}/W_{10}$) was shown in Figure 8. It can be observed that the shrinkage ratio increased with the PNIPAM/alginate weight ratio. Here again, it can be seen that the IPN beads synthesized at 10 and 20°C showed a larger shrinkage ratio. For the IPN beads prepared at these temperatures, the shrinkage ratio increased with the PNIPAM/alginate weight ratio until the weight ratio became 1.7, and after which point the shrinkage ratio was nearly constant. In the IPN beads synthesized at 30 and 40°C, the shrinkage ratio increased with the increase of PNIPAM/alginate weight ratio, but the increase ratio was very small except for the experimental condition of the low alginate concentration (PNIPAM/alginate weight ratio was 2.54). These differences in the thermoresponsive behavior of the IPN beads may be due to the inhomogeneous distribution of PNIPAM in the IPN hydrogels which were prepared at 30 and 40°C as discussed in previous section. Hirokawa *et al.* reported that homogeneous PNIPAM hydrogels were

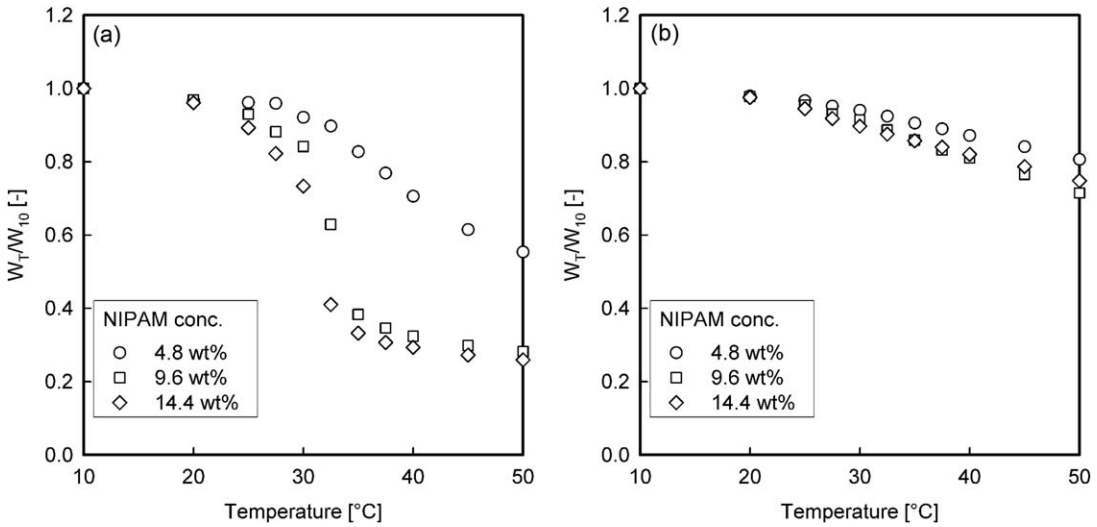

Figure 6. Effect of initial NIPAM monomer concentration on thermoresponsive volume change of IPN beads prepared at synthesis temperature of (a) 20°C and (b) 30°C (alginate concentration: 2.0 wt %).

obtained at low synthesis temperatures (<24°C), whereas heterogeneous PNIPAM hydrogels in which tight and loose PNIPAM polymer networks coexisted were formed at high temperatures (≥24°C).[15] Therefore, in the IPN beads synthesized at 10 and 20°C, it is considered that the PNIPAM polymer network is homogeneously distributed and entangled with the calcium alginate network. As a result, the shrinkage force of the PNIPAM is equally transmitted to the entire calcium alginate network, and the IPN beads can shrink to a large extent. On the other hand, in the IPN beads synthesized at 30 and 40°C, it is considered that the PNIPAM polymer network is heterogeneously distributed in the calcium alginate network with less entanglement with the networks; thus, the shrinkage force of the PNIPAM is not equally transmitted to the entire calcium alginate network. In the regions where PNIPAM is localized with a tight network, the IPN shrinks locally as the PNIPAM shrinks, but this local shrinks may little affect to the entire networks. In the regions where the PNIPAM network is not localized in the IPN hydrogel, the PNIPAM shrinkage force affect to the entire networks; however, the force is too weak to shrink whole IPN networks due to less existence of PNIPAM in the region. As a result, the overall shrinkage ratio for the IPN beads synthesized at 30 and 40°C is much smaller.

Release of Vitamin B_{12}

The temporal release of VB_{12} at the surrounding solution temperature of 25 and 40°C is shown in Figure 9. VB_{12} was diffused from IPN beads to the surrounding solution, and the VB_{12} concentration in the surrounding solution reached equilibrium. In the IPN beads prepared at 10°C [Figure 9(a)], the release trends at 40 and 25°C were not very different. On the other hand, in the IPN beads prepared at 40°C [Figure 9(b)], the release of VB_{12} at 40°C was clearly faster than that at 25°C. From these data, the fitting curves were calculated using eq. (1)

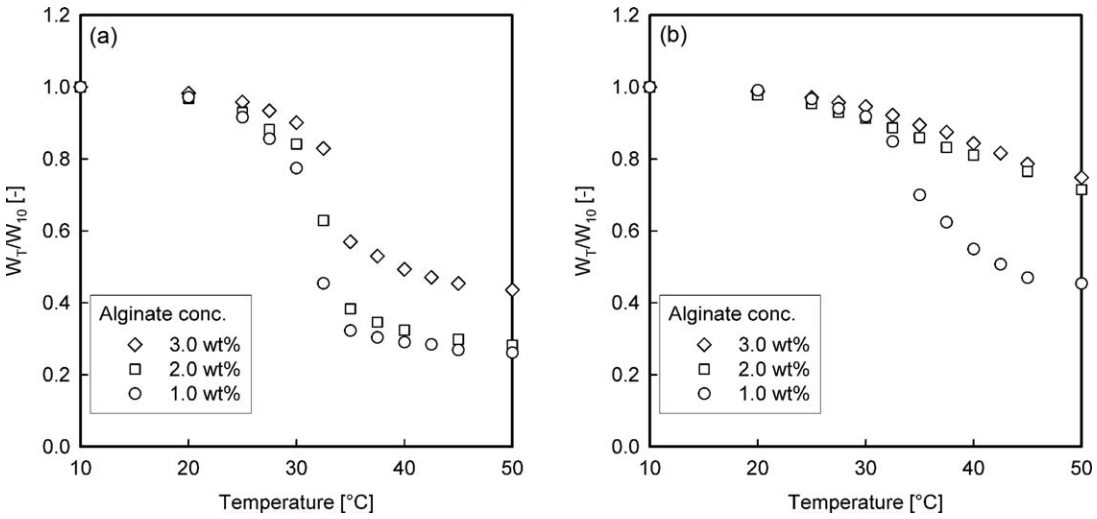

Figure 7. Effect of initial alginate concentration on thermoresponsive volume change of IPN beads prepared at synthesis temperature of (a) 20°C and (b) 30°C (NIPAM concentration: 2.0 wt %).

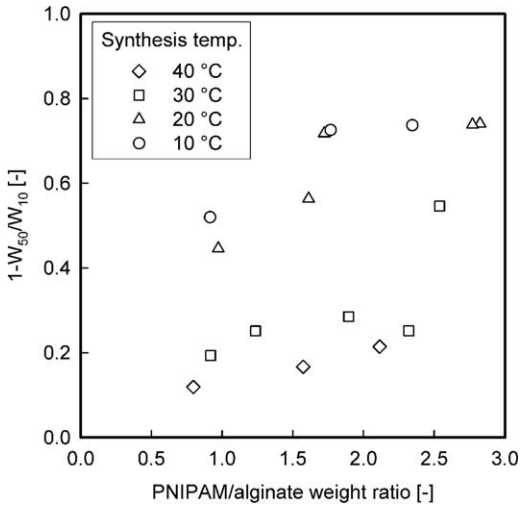

Figure 8. Relationship between the PNIPAM/alginate weight ratio and shrinkage ratio of the IPN beads at 50°C.

and also shown in Figure 9. These curves showed good agreement with the experimental data. The calculated diffusion coefficients for VB_{12} in the IPN beads, D_g, were listed in Table I. The diffusion coefficient for VB_{12} in the pure water, D_w, was estimated using Stokes-Einstein equation as VB_{12} radius of 0.87 nm[28] and also listed. In both IPN beads, the value of D_g were smaller than D_w, which means that diffusion of VB_{12} in the IPN beads was restricted by the polymer networks and the restrictions were depended on the synthesis temperature because of homogeneous/heterogeneous distribution of PNIPAM as discussed in the previous session. In the IPN beads prepared at 10°C, the polymer networks of IPN beads were uniform and equally shrunk with the increase of solution temperature. Therefore, D_g became smaller with the increase of solution temperature. On the other hands, in the IPN beads prepared at 40°C, D_g became larger with the increase of solution temperature in spite that the whole IPN beads volume was slightly shrunk. Lorén *et al.* reported that diffusion coefficient in the

Table I. Calculated Diffusion Coefficients for VB_{12} in the IPN Beads

Synthesis temperature (°C)	Solution temperature (°C)	D_g or D_w ($\times 10^{-10}$ m^2/s)
10	25	0.81 ± 0.04
	40	0.55 ± 0.09
40	25	1.12 ± 0.04
	40	2.59 ± 0.15
Water	25	2.82
	40	4.04

heterogeneous κ-carrageenan gel increased with the increase of KCl concentration even if gel concentrations were same. They explained that the low KCl κ-carrageenan gel has finer strands with small voids as compared to the high KCl gel that has more aggregated strands.[29] In our study, since polymer network of the IPN beads was formed at 40°C, the polymer networks would be also heterogeneous and became more aggregated with the increase of solution temperature. This is probably the reason for the large value of D_g in the release experiment at 40°C.

CONCLUSIONS

The monodisperse PNIPAM/alginate IPN hydrogel beads were prepared via modified inverse emulsion polymerization, and the effect of the synthesis temperature on their characteristics were investigated. Negligible differences in the FTIR and elemental analysis results were observed for the IPN beads prepared at different synthesis temperatures, whereas significant differences in the optical color, thermoresponsive behavior, and VB_{12} release properties of the hydrogels were observed due to the synthesis temperature difference. The IPN beads prepared at 10 and 20°C were transparent, whereas those prepared at 30 and 40°C were opaque, indicating the presence of spatial inhomogeneities in the PNIPAM structure. Furthermore, a large and drastic shrinkage occurred for the IPN beads synthesized at 10 and 20°C. On the other hand, only a small and gradual shrinkage occurred for the

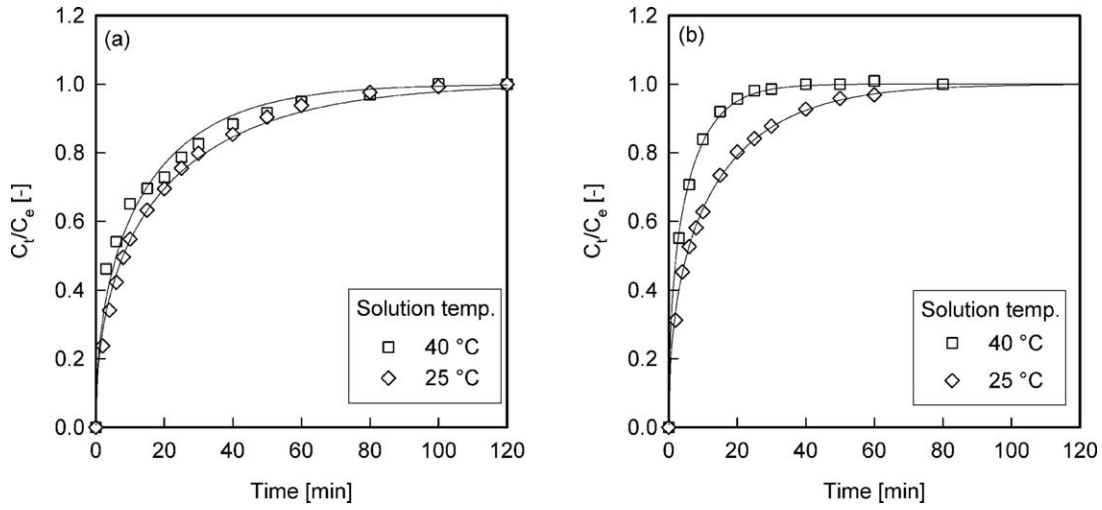

Figure 9. Temporal release of VB_{12} at 25 and 40°C from IPN beads prepared at (a) 10°C and (b) 40°C (NIPAM concentration: 9.6 wt %, alginate concentration: 2.0 wt %).

IPN beads synthesized at 30 and 40°C based on the analysis of wet weight measurements over a wide temperature range. Notably, the influence of the synthesis temperature on the shrinkage behavior was greater than the effect of the PNIPAM/alginate ratio. Furthermore, based on the results of VB_{12} release experiments, it was determined that the diffusion coefficient in the IPN beads prepared at 10°C decreased with increasing solution temperature, whereas the diffusion coefficient in the IPN beads prepared at 40°C increased with increasing solution temperature.

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