Temperature-Dependent Permeability of Polyelectrolyte Complex Capsule Membranes Having *N*-Isopropylacrylamide Domains

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ABSTRACT: As a microcapsule with temperature sensitivity, poly(methacrylic acid)–polyethylenimine complex capsules containing *N*-isopropylacrylamide units were designed. Two kinds of copolymers of methacrylic acid and *N*-isopropylacrylamide were synthesized by free-radical copolymerization. Partly crosslinked poly(methacrylic acid)–polyethylenimine complex capsules containing the methacrylic acid–*N*-isopropylacrylamide copolymers were prepared at 40 or 25°C. The permeation of phenylethylene glycol through the capsule membranes was investigated. Permeability of the capsules prepared at 25°C increased monotonously with increasing temperature from 10 to 50°C. Permeability of the capsules prepared at 40°C also increased with increasing temperature up to 25°C but decreased above 30°C. Also, the degree of swelling of the membranes prepared at 40°C decreased above 30°C. Differential scanning calorimetry measurement showed that *N*-isopropylacrylamide units underwent more efficient transition in the capsule membranes prepared at 40°C than in the membranes prepared at 25°C. The capsule membranes prepared at 40°C might have domains in which *N*-isopropylacrylamide units are concentrated, whereas these units should distribute uniformly in the capsule membranes made at 25°C. Such a difference in distribution of *N*-isopropylacrylamide units might result in the different permeation property of the capsule membranes. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 2703–2710, 2000

Key words: polyelectrolyte complex; microcapsule; temperature sensitivity; poly(*N*-isopropylacrylamide); lower critical solution temperature

INTRODUCTION

A variety of stimuli-responsive systems have been developed for application to drug-delivery systems.[1–6] Among them, temperature-responsive systems, such as temperature-responsive polymeric gels,[7–9] microcapsules,[10,11] microspheres,[12] and liposomes,[13–15] have been extensively studied.

Because microcapsules have large spaces inside them, they can entrap large amounts of drugs. The release rate of the drugs from the microcapsules is generally controlled by the diffusion rate of the drugs across the thin microcapsule membranes. Therefore, if the structure of the microcapsule membrane can be changed by applying stimuli, a quick release response from the microcapsule is expected to be achieved. For these

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reasons, microcapsules are suitable for stimuli-responsive drug-delivery systems.

We have shown that the polyelectrolyte complex is useful for the preparation of stimuli-responsive microcapsules.[16] When weak polyacids and/or weak polybases are used as the membrane components, the capsule membranes reveal a pH-responsive release property.[17–19] Also, functional molecules can be incorporated into the capsule membranes by using polyelectrolytes to which these molecules are combined. In a previous study, we prepared polyelectrolyte complex capsules containing triphenylmethane leucohydroxide, which dissociates into an ion pair under UV light irradiation, and demonstrated their photo-responsive release property.[20]

It is well known that poly(*N*-isopropylacrylamide) [poly(NIPAM)] exhibits a lower critical solution temperature (LCST) at about 32°C.[21] The polymer is highly hydrophilic and soluble in water below the LCST. However, the polymer becomes hydrophobic and insoluble in water above that temperature. Also, this hydrophilic–hydrophobic alteration occurs drastically, responding to a small temperature change. If poly(NIPAM) chains are incorporated into polyelectrolyte complex capsule membranes, the membranes are expected to exhibit a temperature-sensitive release property.

To obtain functional microcapsules with a temperature-sensitive release property, we designed polyelectrolyte complex capsules containing NIPAM units. In this study, two kinds of copolymers of NIPAM and methacrylic acid (MA) were synthesized. Poly(methacrylic acid) (PMA)–polyethylenimine (PEI) complex capsules containing NIPAM units were prepared by using these NIPAM–MA copolymers as the membrane components. The temperature-dependent release property of these capsules was investigated.

EXPERIMENTAL

Chemicals

MA and azobisisobutyronitrile (AIBN) were purchased from Kishida Chemical Co., Ltd. (Osaka, Japan). PEI (branched type having primary, secondary, and tertiary nitrogens in the ratio of 1:2:1; molecular weight 40,000–50,000), NIPAM, and phenylethylene glycol were supplied by Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide–HCl salt (EDC) was obtained from Nacalai Tesque Co. Ltd. (Kyoto, Japan). MA was purified by vacuum-distillation before use. Phenylethylene glycol and NIPAM were purified by recrystallization from ligroin and benzene/*n*-hexane, respectively. Other chemicals were used without further purification unless otherwise stated.

Poly(NIPAM-*co*-MA)

Poly(NIPAM-*co*-MA)s were prepared by free-radical copolymerization of NIPAM and MA using AIBN as the initiator. NIPAM (74 or 133 mmol), MA (8.2 or 7.0 mmol), and AIBN (0.16 or 0.35 mmol) were dissolved in freshly distilled tetrahydrofuran (15 mL) in test tubes. The solutions were degassed by bubbling with N_2 for 15 min. The test tubes were sealed, heated to 65°C for 12 h, and then cooled to room temperature. The copolymers were dissolved in methanol and precipitated by adding the copolymer solutions into diethyl ether. The copolymers were dialyzed for 5 days against distilled water and then isolated by lyophilization. The homopolymer of MA was also prepared in the same manner.

Capsule Preparation

Polyelectrolyte complex capsules were prepared according to the method described previously.[17–19] An aqueous PMA solution containing poly(NIPAM-*co*-MA) (PMA 4.9 wt %, copolymer 2.1 wt %, pH 6) was added dropwise from a pipette into an aqueous PEI solution (0.5 wt %, pH 6) at 25 or 40°C. The solution was incubated with gentle agitation for 3 h to form the polyelectrolyte complex membrane at the droplet surface. The resultant capsules were washed three times with distilled water. Then, the capsule membranes were crosslinked by incubation in an aqueous 50 mM phosphate-buffered solution containing EDC (11.4 g/L) at pH 4.4 for 1 h at 40°C or 2 h at 25°C with gentle stirring. Finally, the crosslinked capsules were kept in distilled water for 2 days and washed three times with water to remove EDC remaining in the capsules. The purified capsules were immersed in an aqueous 40 mM phenylethylene glycol solution for more than 1 day. PMA–PEI complex capsules were prepared in the same manner except that an aqueous 7.0 wt % PMA solution was used as the polyanion solution.

Estimation of Capsule Diameter

The capsule diameter was estimated as previously reported.[17] The diameter d of the capsule

was determined by calculation according to Eq. (1):

$$d = [6(M_c - M_m)/\pi\rho]^{1/3} \quad (1)$$

where M_c, M_m, and ρ represent the weight of the whole capsule, the weight of the capsule membrane in the dry state, and the density of the solution inside the capsule, respectively.

Permeation Measurement

The permeation measurement was performed as previously reported.[17] A phenylethylene glycol-loaded capsule was put into 30 mL of a 5 mM acetate-buffered solution (pH 4.5) at 25°C. The permeation of phenylethylene glycol through the capsule membrane was detected by following the absorbance of the outer aqueous phase at 210 nm using a spectrophotometer (Jasco Ubest-30).

The permeability constant P (cm s^{-1}) was determined using Eq. (2) obtained from Fick's first law of diffusion:

$$ln[(C^f - C^t)/(C^f - C^i)] = -(V + V_c)APt/VV_c \quad (2)$$

where t represents the time since the start of the experiment. C^i, C^t, and C^f are the initial, intermediary, and final concentrations of the permeant in volume V of the surrounding phase, respectively. V_c and A are the volume and the surface area of the capsule, respectively. Generally, more than 90% of the amount of phenylethylene glycol calculated from the volume of the capsule and the concentration of the loading solution was finally released from the capsules.

Swelling of Capsule Membranes

Dry capsule membranes (ca. 20 mg) were immersed in a 5 mM acetate-buffered solution (pH 4.5) for 24 h at a given temperature. The swollen membranes were taken out of the solution and excess water on their surfaces was removed by wiping with a filter paper. Then, the samples were weighed. The swelling ratio was defined by Eq. (3):

$$\text{Swelling ratio} = (W - W_0)/W_0 \quad (3)$$

where W and W_0 represent the weight of the swollen membranes and the weight of the dry membranes, respectively.

Other Methods

The molecular weight of the copolymers was estimated by gel permeation chromatography on a Shodex SB-804 HQ column using 5 mM of a phosphate-buffered solution containing 0.3M Na_2SO_4 (pH 7.0). Polyethylene glycol was used as the standard for the determination of molecular weight. Differential scanning calorimetry (DSC) was performed with a Seiko Electronics DSC 120 microcalorimeter. The samples were analyzed at a heating rate of 0.5°C/min.

RESULTS AND DISCUSSION

Characterization of Polyelectrolytes

Two kinds of poly(NIPAM-*co*-MA)s were prepared by free-radical copolymerization of NIPAM and MA. The compositions of the copolymers were evaluated by elemental analysis to be in the NIPAM/MA ratio of 82/18 and 89/11. These copolymers were named CP-82 and CP-89, respectively. The weight-average molecular weights of CP-82 and CP-89 were estimated by gel permeation chromatography to be 49,000 and 41,000, respectively. PMA was also prepared in this study. Its weight-average molecular weight was 360,000.

Figure 1 shows microcalorimetric endotherms for an aqueous solution of CP-89 at various pH. The copolymer did not exhibit an endotherm clearly at pH 7.0. However, a broad peak was seen around 42°C at pH 6.0, indicating the occurrence of the conformational transition of the copolymer. This temperature is higher than the LCST of poly(NIPAM). Because MA units enhanced hydration of the copolymer chain, the LCST of the copolymer was elevated from the LCST of poly(NIPAM).[22,23] However, as the pH of the solution was lowered, the transition temperature decreased. At pH 3.0, the transition occurred at about 31°C, which is the same as the LCST of poly(NIPAM). Because more carboxylate groups on the copolymer chains became protonated with decreasing pH, a decrease in the charge density of the copolymer lowered its LCST. Also, the Δ H for the transition increased with decreasing pH, indicating that the copolymer undergoes the transition more efficiently at lower pH.

Release Property of Capsules

PMA–PEI complex capsules containing the NIPAM–MA copolymers, CP-82 and CP-89, were

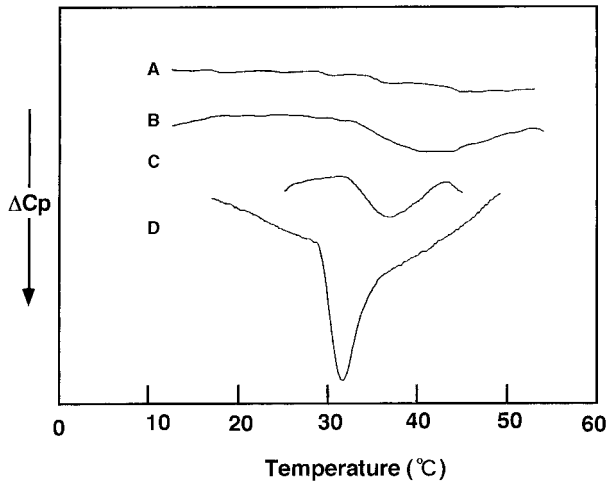

Figure 1 Microcalorimetric endotherms for CP-89 dissolved in 5 mM acetate-buffered solution at various pH: (A) pH 7.0; (B) pH 6.0; (C) pH 5.5; (D) pH 3.0. ΔH's for curves were about (B) 253, (C) 473, and (D) 886 cal/mol NIPAM. ΔH for curve (A) could not be estimated.

prepared at 25 or 40°C. Because negative charges on the copolymer chains are neutralized by complex formation with the positively charged polymer chains, NIPAM units in the copolymer are considered to undergo the transition during the capsule preparation above the LCST of poly(NIPAM). Thus, we expected that the capsule preparation temperature affects the structure and permeation property of the capsule membranes. The diameter of the capsules was about 5.6 mm. The capsules containing CP-89 prepared at 25 and 40°C were estimated to have membranes with MA/ethylenimine (EI) (mol/mol) ratios of 38.2/61.8 and 41.3/58.7, respectively, by elemental analysis on the assumption that the PMA/CP-89 ratio in the capsule membranes is the same as that of the polyanion solution used for the capsule preparation. The capsule prepared at 40°C had a slightly higher PEI content than that prepared at 25°C.

Permeation of phenylethylene glycol through the capsule membranes was investigated. Because the copolymers undergo the phase transition efficiently in the acidic pH region (Fig. 1) and the polyelectrolyte complex membrane is stable above pH 4.5,[18] the permeation measurement was performed at pH 4.5. Figure 2(A) shows the release profiles of phenylethylene glycol from the capsule containing CP-82 (CP-82 capsule) prepared at 40°C. The release was enhanced with temperature from 10 to 25°C, but decreased with temperature above 35°C.

The plots of $\ln[(C^f - C^t)/(C^f - C^i)]$ against time t for the data given in Figure 2(A) are shown in Figure 2(B). These plots fit into straight lines. From the slopes of the lines, the permeability constants were evaluated using Eq. (2).

Figure 3 represents the Arrhenius plots of phenylethylene glycol permeation through the CP-82 capsule membrane. For comparison, data for the PMA–PEI complex capsule which does not contain NIPAM units were also shown in the same figure. As is seen in Figure 3, the permeability of

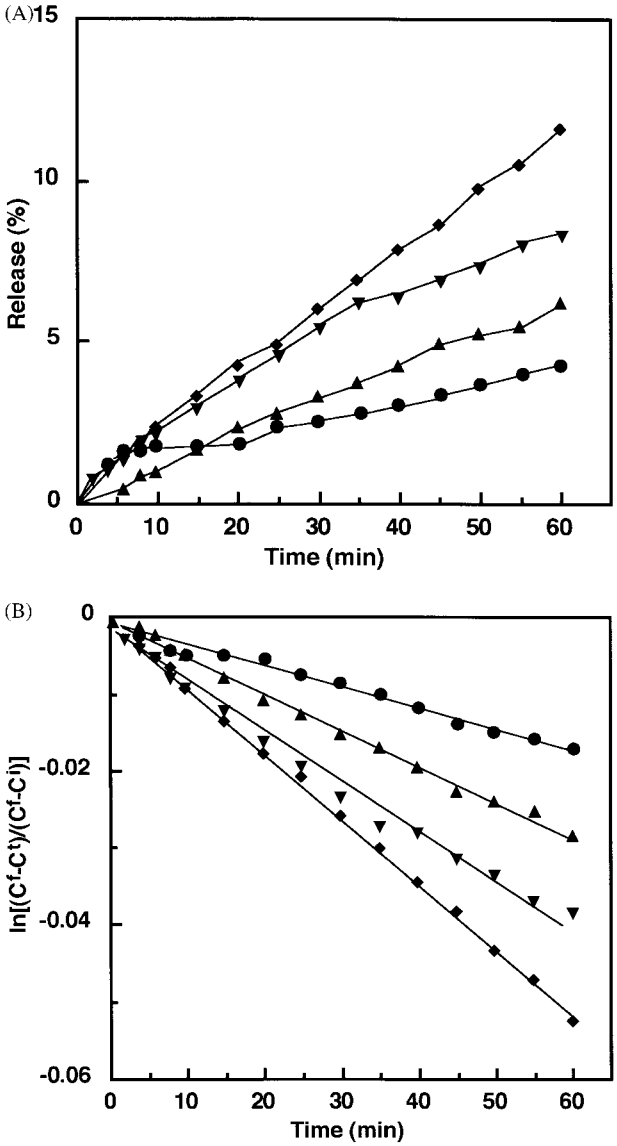

Figure 2 Temperature-dependent release of phenylethylene glycol from CP-82 capsules at various temperatures: (●) 10°C; (▲) 20°C; (◆) 25°C; (▼) 45°C. (A) release profiles; (B) plot of $\ln[(C^f - C^t)/(C^f - C^i)]$ against time t for the data in (A).

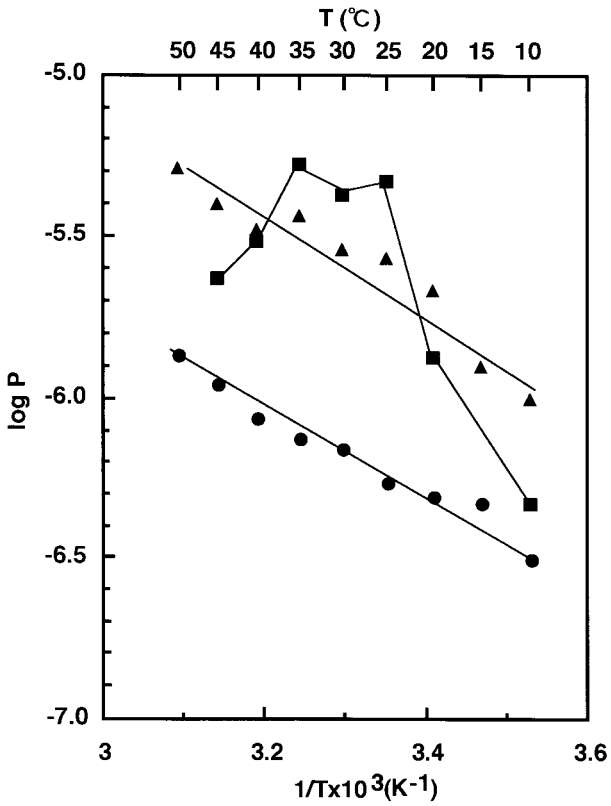

Figure 3 Arrhenius plot for the permeation of phenylethylene glycol through various polyelectrolyte complex capsules: (▲) CP-82 capsule prepared at 25°C; (■) CP-82 capsule prepared at 40°C; (●) PMA–PEI capsule.

the PMA–PEI capsules increased monotonously with increasing temperature. Similarly, the permeability of the CP-82 capsules prepared at 25°C increased with increasing temperature from 10 to 50°C and did not exhibit any remarkable change in the temperature dependence of the permeability. For the CP-82 capsules prepared at 40°C, the permeability increased with increasing temperature up to 25°C. However, the temperature dependence of the permeability changed around 30°C, and above this temperature, the permeability decreased with the temperature. Because this temperature is approximately the same as the LCST of poly(NIPAM), dehydration of the NIPAM units in the capsule membrane might induce the decrease in permeability. As already mentioned, NIPAM units in the copolymer chains form aggregates in the solution at 40°C and, hence, the NIPAM units should be incorporated as aggregates in the capsule membrane when the capsule was prepared at this temperature. However, when the capsule was prepared at 25°C, the NIPAM units distribute uniformly in the capsule membrane because these units are hydrated and do not form aggregate in the solution at this temperature. This difference of the NIPAM unit distribution might provide the capsule membranes with a different permeation property, as discussed below.

As seen in Figure 3, the permeability of the CP-82 capsule membrane prepared at 40°C decreased with increasing temperature from 35 to 45°C, although this temperature region is higher than is the LCST of poly(NIPAM). While most of MA units of the copolymer might form ion pairs with the EI units, unpaired MA units should still remain in the copolymer. Because these units increase the transition temperature of the copolymer chain, the transition would occur in a broad temperature region near and above the LCST of poly(NIPAM). Thus, the permeability continued to change above the LCST.

In comparison between the PMA–PEI capsule and the CP-82 capsule prepared at 25°C, the latter exhibited higher permeability than that of the former. Because the NIPAM units in the copolymer chains cannot participate in complex formation, the CP-82 capsule membrane might contain many defects in the polyelectrolyte complex structure. Such defects should increase the water content of the membrane and, hence, the capsule membrane showed the higher permeability.

The capsules prepared at 40°C exhibited a permeability change above 30°C because of the transition of the NIPAM units. However, the capsules were stored at room temperature (ca. 20°C), which is lower than is the LCST of poly(NIPAM), until the permeation measurement. Therefore, distribution of the NIPAM units may change during that period. Thus, we examined the influence of the storage temperature.

Figure 4 shows the Arrhenius plots for phenylethylene glycol permeation through the CP-89 capsules stored at 40°C or at room temperature. While both capsules exhibited a change in the temperature dependence of the permeability between 25 and 30°C, the capsule stored at 40°C revealed a more drastic change. This result indicates that the structure of the capsule membranes was altered during the storage below the LCST. Since the NIPAM units in the capsule membrane become hydrophilic at room temperature, the NIPAM domain in the membrane should swell and expand during the storage. It is likely that such a change of the NIPAM domains enhanced recombination of the polyelectrolyte complex and induced gradual alteration of the capsule membrane structure. The fact that the capsule

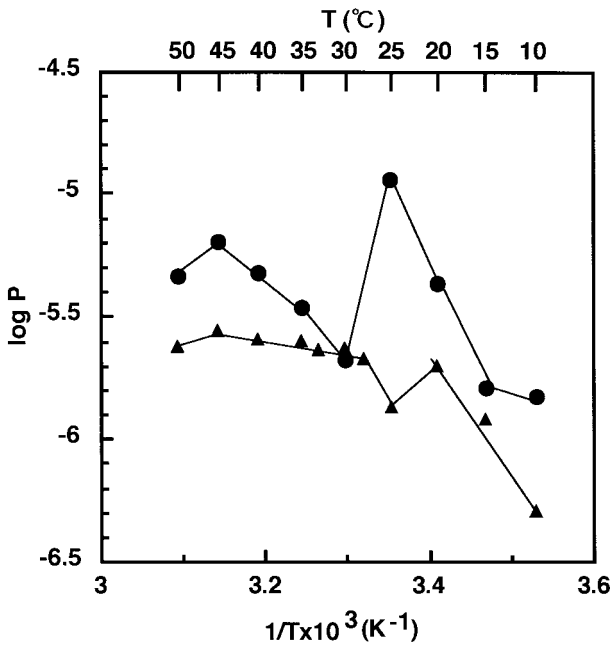

Figure 4 Arrhenius plot for the permeation of phenylethylene glycol through CP-89 capsules: (●) prepared and stored at 40°C; (▲) prepared at 40°C and stored at room temperature for 7 days.

membrane stored at room temperature exhibited lower permeability than that stored at 40°C suggests the occurrence of the recombination of the polyelectrolyte complex.

In comparison between the CP-82 and CP-89 capsules (Figs. 3 and 4), it seems that their temperature dependence of the permeability is different above the LCST. Because CP-82 has a larger number of MA units than has CP-89, more unpaired MA units should remain in the chains of CP-82 in the capsule membrane than those of CP-89 chains. Because these units increase the transition temperature of the copolymer chains, the CP-82 capsule membrane exhibited the permeability change in a broader temperature region than did the CP-89 capsule membrane.

Influence of Capsule Preparation Temperature on Its Membrane Structure

Temperature sensitivity of the capsule membranes was affected by their preparation and storage temperatures. To determine the influence of these temperature conditions on the capsule membrane structure, the transition of NIPAM units in the capsule membranes was investigated using DSC. Figure 5 depicts microcalorimetric endotherms for the CP-89 capsule membranes prepared and stored differently. As is seen in Figure 5, the temperature region and ΔH of the transition varied remarkably, depending on these conditions. The capsule membrane prepared at 25°C and stored at room temperature showed a broad and small endotherm around 36°C. However, the membrane prepared at 40°C and stored at room temperature exhibited a larger endotherm around 33.5°C, indicating a more efficient transition of NIPAM units in the latter membrane. Moreover, for the capsule membrane prepared and stored at 40°C, the peak corresponding to the NIPAM transition was much larger than that of the other capsule membranes. As shown above, the permeability change above the transition temperature became more significant in the order of capsules prepared at 25°C < the capsule prepared at 40°C and stored at room temperature < the capsule prepared and stored at 40°C. Therefore, this result indicates that the efficiency of the NIPAM unit transition correlates with the permeability change of the capsule membrane above the transition temperature.

The NIPAM unit transition in the capsule prepared at 25°C occurred at a higher temperature than that of the capsule prepared at 40°C. Because poly(NIPAM) having hydrophilic units exhibits a higher transition temperature than does poly(NIPAM),[24] the difference in the transition temperature suggests that unpaired MA units remain in the chain of CP-89 in the capsule prepared at 25°C. It is likely that the polyelectrolyte

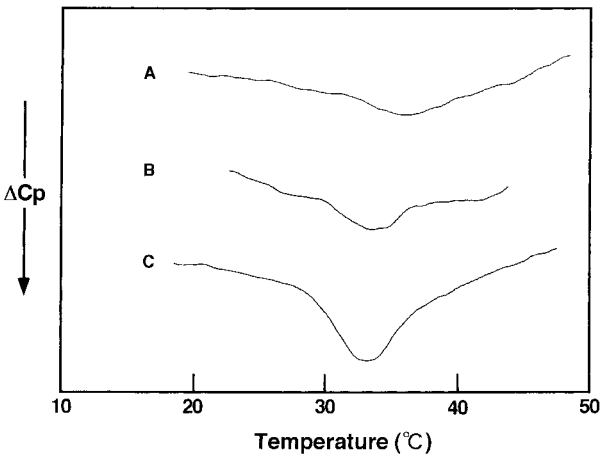

Figure 5 Microcalorimetric endotherms for various CP-89 capsule membranes in 5 mM acetate-buffered solution at pH 4.5: (A) prepared at 25°C; (B) prepared at 40°C and stored at room temperature for 7 days; (C) prepared and stored at 40°C. ΔH for curves were about (A) 98, (B) 195, and (C) 455cal/mol NIPAM.

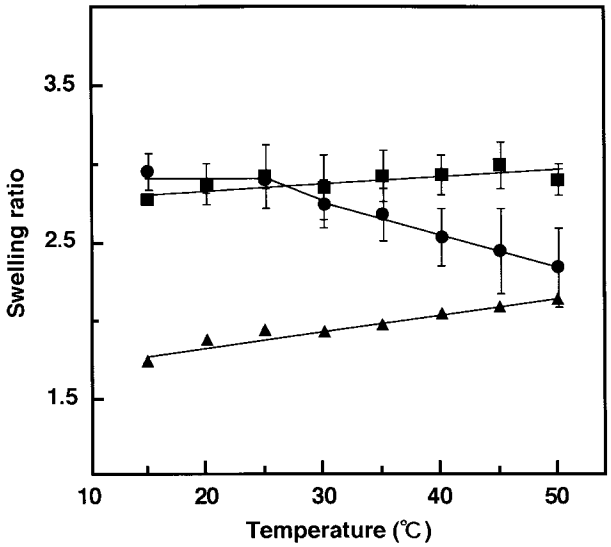

Figure 6 Swelling ratio of various capsule membranes as a function of temperature: (■) CP-89 capsule prepared at 25°C; (●) CP-89 capsule prepared at 40°C; (▲) PMA-PEI capsule.

complex formation is suppressed by an irregular alignment of MA units in the copolymer chain. However, at 40°C, the dehydrated NIPAM units gather and may produce a better alignment of MA units.

Permeation of phenylethylene glycol through the capsule membranes is known to be influenced by the water content of the membranes.[17] Thus, swelling of the capsule membranes was investigated (Fig. 6). The swelling ratio of the PMA–PEI capsule membrane increased slightly with increasing temperature. The CP-89 capsule membrane prepared at 25°C also showed a similar tendency. Although this membrane contains NIPAM units, the swelling ratio did not change around 30°C, suggesting that NIPAM units hardly undergo the transition in this membrane. The swelling ratio of the CP-89 capsule membrane was significantly higher than that of the PMA capsule membrane. As mentioned above, when the NIPAM units in the copolymer chain were hydrated, efficient complex formation could not occur during the capsule preparation at 25°C. Therefore, more charged groups should remain in the CP-89 capsule membrane than in the PMA capsule membrane. These groups as well as the NIPAM units might increase the water content of the membrane.

The CP-89 capsule membranes prepared at 40°C exhibited a swelling ratio almost the same as that of the CP-89 capsule membrane made at 25°C, below 25°C. However, its swelling ratio decreased above 30°C, due to the NIPAM unit transition. This result is consistent with the larger transition endotherm shown in Figure 5 and indicates that the NIPAM domains in the CP-89 capsule membrane prepared at 40°C could undergo the transition and caused a decrease in water content of the membrane.

Temperature-dependent Permeability of Capsule Membrane Having NIPAM Domains

A schematic illustration of the capsule membranes prepared at 25 and 40°C are shown in Figure 7. As shown in Figures 5 and 6, neither a remarkable endotherm nor a change of swelling occurred near the LCST of poly(NIPAM) for the capsule membrane prepared at 25°C. Because the NIPAM units in the copolymer chain are hydrated at 25°C, the NIPAM units might be incorporated uniformly in the capsule membrane during the capsule formation at 25°C [Fig. 7(A)]. For the occurrence of the transition, hydrophobic interactions and hydrogen-bond formation between NIPAM units are necessary. However, a large fraction of the NIPAM units might exist separately in the membrane and, hence, these units could not interact with each other efficiently. Thus, the transition of NIPAM units did not occur in this membrane and this might be the reason why the capsule membranes prepared at 25°C did not exhibit a change in the permeability around the LCST of poly(NIPAM).

On the other hand, when the capsule was prepared at 40°C, the NIPAM units form aggregates during the capsule formation and, hence, domains in which the NIPAM units were concentrated should exist in the capsule membrane [Fig. 7(B)]. Because in these domains NIPAM units are able to interact with neighboring NIPAM units, they can undergo the transition above the LCST of poly(NIPAM) (Fig. 5). This transition causes a decrease in the water content of the membrane (Fig. 6), resulting in suppression of permeation above that temperature.

CONCLUSIONS

We prepared polyelectrolyte complex capsules containing NIPAM units at 25 and 40°C. The capsules prepared at 25°C did not show a permeability change around the LCST of poly(NIPAM), whereas the capsule prepared at 40°C exhibited a

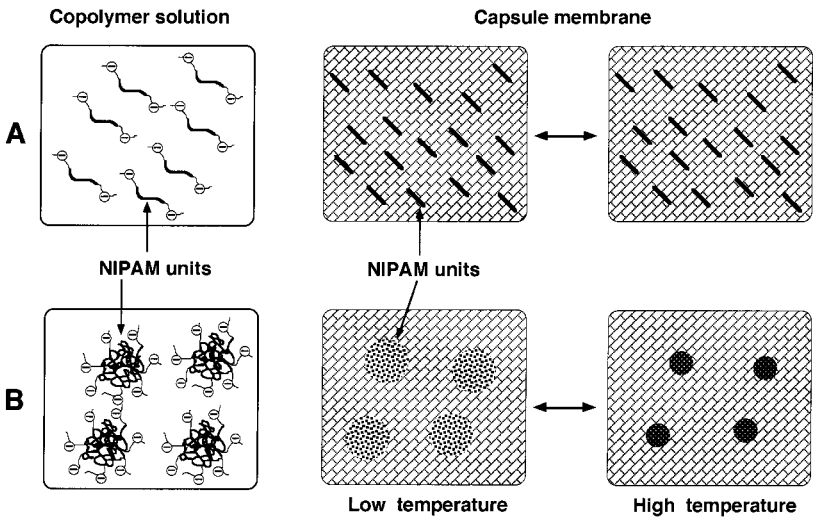

Figure 7 Schematic illustration of the MA–NIPAM copolymer solutions and the capsule membranes containing the copolymer: (A) (left) copolymer solution at 25°C and (right) capsule membrane prepared at 25°C; (B) (left) copolymer solution at 40°C and (right) capsule membrane prepared at 40°C. Hydration and dehydration of domains of the NIPAM units occur, depending on temperature.

significant permeability change above the LCST. Also, the swelling ratio of the capsule membrane prepared at 40°C decreased remarkably above that temperature, in contrast to the capsule membrane prepared at 25°C, which did not show a change of the swelling ratio. The NIPAM units should exist as aggregates in the capsule membrane prepared at 40°C, but distribute uniformly in the membrane prepared at 25°C. Such a difference in the distribution of the NIPAM units might result in the different permeation properties of these capsule membranes.

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