

# Permeability Control of Poly(methacrylic acid)–Poly(ethylenimine) Complex Capsule Membrane Responding to External pH

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## SYNOPSIS

Previously, permeability of partly crosslinked poly(acrylic acid)–poly(ethylenimine) complex capsule membranes has been shown to be controlled responding to external pH [K. Kono, F. Tabata, and T. Takagishi, *J. Membr. Sci.*, **76**, 233 (1993)]. In order to improve pH-dependent permeation behavior of the polyelectrolyte complex capsule membranes, poly(methacrylic acid), which has hydrophobic side groups, was used as a polyanion component instead of poly(acrylic acid). The polyelectrolyte complex capsules composed of poly(methacrylic acid)–poly(ethylenimine) with a diameter of 5.2 mm were prepared. Permeation of phenylethylene glycol, which was used as a permeant, through the capsule membrane was quite limited at pH between 4 and 8. The permeation of the permeant across the capsule membrane in these pH regions was much less than that of poly(acrylic acid)–poly(ethylenimine) complex capsule membrane. However, the permeability constant was drastically increased in the order of 1 or 2 above pH 8 and below pH 4, depending on temperature. The Arrhenius plot for the permeation of phenylethylene glycol through the capsule membrane showed that the activation energy for the permeation is almost constant in the pH region between 4.5 and 8, but greatly decreases below pH 4 and above pH 8.8, suggesting occurrence of a significant change of the capsule membrane structure near these pH regions due to dissociation of polyelectrolyte complex. When environmental pH of the capsule was changed, release rate of phenylethylene glycol from the capsule was altered quickly and reversibly under acidic condition. © 1995 John Wiley & Sons, Inc.

## INTRODUCTION

In recent years, in order to optimize delivery of drugs, various systems that can control drug release in response to environmental stimuli have been developed.<sup>1</sup> A number of responsive systems have been reported by using polymers.<sup>2</sup> Microcapsules are suitable for such systems because they entrap a variety of chemicals in a large inner space and release them at a suitable rate. Since the release rate from microcapsules is controlled by diffusion rate of chemicals in their thin membranes, if structure of the microcapsule membranes is changed by applying various stimuli, quick response of release to the

stimuli might be expected compared with other systems such as polymer gels.

Several capsules and microcapsules in response to various stimuli, such as pH,<sup>3–5</sup> temperature,<sup>6,7</sup> light,<sup>8</sup> and so on, have been developed. These stimuli-responsive properties of the microcapsules have been achieved by modification of semipermeable capsule membranes with lipid membranes and/or polyelectrolytes. As another technique to prepare stimuli-responsive microcapsules, use of polyelectrolyte complex has been attempted.<sup>9,10</sup>

In a previous study,<sup>9</sup> a partly crosslinked poly(acrylic acid)–poly(ethylenimine) complex capsule membrane was shown to reveal a pH-responsive permeation behavior. Release rate of a neutral permeant, phenylethylene glycol, from the capsule was low under neutral and weak acidic conditions but increased under acidic and alkaline conditions.

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However, the permeation through the capsule membrane under neutral and weak acidic conditions was still remarkable and, therefore, the capsule could not regulate release of the permeant in the complete on-off fashion. Since the capsule membrane is considered to be rather hydrophilic due to a great ability of poly(acrylic acid) to bind water even when the polyelectrolyte complex is fully formed under neutral and weak acidic conditions,<sup>11</sup> hydrophilic molecules can easily permeate through macromolecular networks of polyelectrolyte complex.

In the present work, in order to improve pH-dependent control of permeability of the polyelectrolyte complex capsule membranes, polyelectrolyte complex capsules composed of poly(ethylenimine) and poly(methacrylic acid), which has hydrophobic side groups, have been prepared. The capsules are expected to form a dense polyelectrolyte complex membrane due to the hydrophobic interaction between the side chains<sup>12</sup> as well as ionic bonds between polyelectrolytes. Effects of pH on the permeation across the capsule membrane were investigated.

## EXPERIMENTAL

### Chemicals

Poly(sodium acrylate) (molecular weight 44,000) was purchased from Kishida Chemical Co., Ltd. (Japan). Poly(methacrylic acid) (molecular weight 35,000) was obtained from Polysciences, Inc. (U.S.A.). Poly(ethylenimine) (branched type having primary, secondary, and tertiary nitrogens in the ratio of 1 : 2 : 1; molecular weight 40,000–50,000) and phenylethylene glycol were supplied from Tokyo Kasei Kogyo Co., Ltd. (Japan). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Nacalai Tesque Co. Ltd. (Japan). Phenylethylene glycol was purified by recrystallization from ligroin. Other chemicals were used without further purification.

### Capsule Preparation

Partly crosslinked poly(methacrylic acid)–poly(ethylenimine) complex capsules and poly(acrylic acid)–poly(ethylenimine) capsules were prepared as follows. An aqueous solution of poly(methacrylic acid) (7.0 wt %, pH 5.0) was added dropwise from a pipette to 0.5 wt % aqueous poly(ethylenimine) solution (pH 7.0). The mixture was incubated for 2 h with gentle agitation to form

the polyelectrolyte complex membrane at the droplet surface. The resultant polyelectrolyte complex capsules were washed several times with distilled water. The capsule membranes were then crosslinked by incubating in an aqueous 50mM phosphate-buffered solution containing EDC (11.4 g/L) at pH 4.4 for 7 h with shaking. Finally, the crosslinked capsules were kept in distilled water and washed repeatedly in order to remove EDC remaining in the capsules. The purified capsules were put in an aqueous 40mM phenylethylene glycol solution at pH 7.0 for several days. Poly(acrylic acid)–poly(ethylenimine) complex capsules were prepared via the procedure previously reported<sup>9</sup> using poly(acrylic acid) solution (1.5 wt %, pH 7.0) instead of poly(methacrylic acid) solution.

### Determination of Residual Poly(Methacrylic Acid) inside Capsule

A 20- $\mu$ L portion of inner aqueous solution of the capsule was analyzed by gel permeation chromatography through an Asahipak GS-510 column (Asahi Chemical Industry Co. Ltd.) using 5mM phosphate solution of pH 7.0 as an effluent. Poly(methacrylic acid) was detected with a UV detector (Gilson, model 115) at 210 nm.

### Estimation of Capsule Diameter

Capsule diameter was estimated as previously reported.<sup>9</sup> 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  $\rho$  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 Measurements

Permeability measurements were performed as previously reported.<sup>9</sup> A permeant-loaded capsule was preincubated in a permeant solution adjusted at the pH of the measurement for more than 10 h. The capsule was taken out of the solution and then put into 20 mL of aqueous HCl solution (pH 2.5–3.0), 5mM acetate-buffered solution (pH 3.5–6.0), 5mM phosphate-buffered solution (pH 7.0), or 5mM Tris-HCl-buffered solution (pH 8.0–9.0) as the outer aqueous phase at varying temperature with agitation by a stirrer. Permeation of phenylethylene glycol

through the capsule membrane was detected by following the absorbance of the outer aqueous phase at 210 nm.

The permeability constant  $P$  ( $\text{cm s}^{-1}$ ) was determined by 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 in volume  $V$  of the surrounding phase, respectively.  $V_c$  and  $A$  are the volume and the surface area of one capsule, respectively.

### Potentiometric Titrations

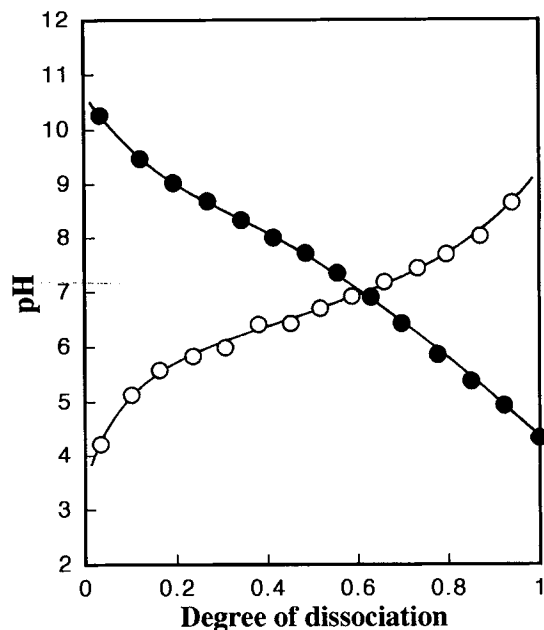
Potentiometric titrations were carried out using a pH meter (Horiba M-8). Poly(methacrylic acid) (100 mL) solution (10mM) containing 10mM NaCl and 100 mL of poly(ethylenimine) solution (10mM) containing 10mM NaCl were neutralized by adding 0.1N NaOH or 0.1N HCl solution at room temperature.

## RESULTS AND DISCUSSION

The acid-base titration of poly(methacrylic acid) and poly(ethylenimine) used in this study was performed in order to reveal the effect of pH on the charge density of the polyelectrolytes. The titration curves are shown in Figure 1. These curves indicate that poly(methacrylic acid) changes charge density in the pH region between 9 and 4 and poly(ethylenimine) changes between 10.5 and 4.

The diameter of the capsule prepared here was determined to be  $5.2 \pm 0.3$  mm and the thickness of the capsule membrane lyophilized was estimated to be  $38 \pm 6$   $\mu\text{m}$ . The composition of the capsule membrane was estimated to have a ratio of 46.2 mol % of poly(methacrylic acid) to 53.8 mol % of poly(ethylenimine) by elemental analysis. Residual poly(methacrylic acid) in the capsule was evaluated by the high-performance liquid chromatography analysis, which showed that the concentration of poly(methacrylic acid) in the inner aqueous phase was 0.49 wt %.

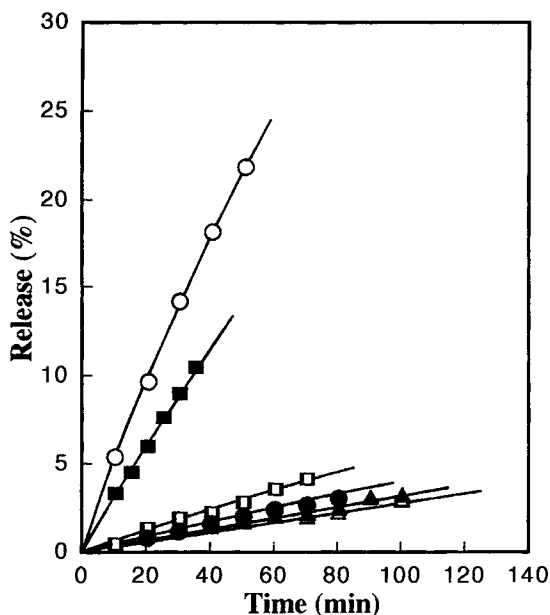
The release behavior of the poly(methacrylic acid)-poly(ethylenimine) complex capsule was investigated. Figure 2 shows typical examples of the release profiles of phenylethylene glycol loaded inside the capsule at various pHs. The plots of  $\ln[(C^f$



**Figure 1** The pH dependence of degree of dissociation of (○) poly(methacrylic acid) and (●) poly(ethylenimine) in aqueous 10 mM NaCl solution.

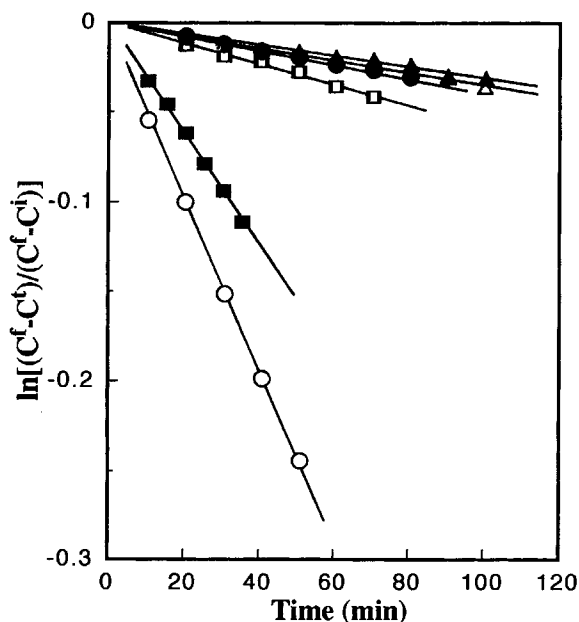
$- C^t)/(C^f - C^i)]$  against time  $t$  for the data given in Figure 2 are illustrated in Figure 3. From the slopes of the lines in Figure 3 the permeability constants were calculated using Eq. (2).

The pH dependence of permeability constant of phenylethylene glycol through the capsule membrane at various temperatures is shown in Figure 4. The permeability was low at pH between 5 and 7 at any temperature examined, but drastically increased below pH 4.0 and above pH 8.0. The permeability constant at pH 3.5 was about 35 times that at pH 4.0 and also about 64 times that at pH 5.1, where the permeability was the lowest, at 10°C. The permeation of the permeant across the capsule membrane was further increased with decreasing pH below 3.5 or with raising pH above 8.8. However, the permeability constant at these pHs could not be determined because the capsule became nonspherical at these pHs due to swelling of the polyelectrolyte capsule membrane.<sup>9,13</sup> Since the capsule membrane was highly swollen at higher pHs, the capsule became deformable but was not destroyed because of crosslinking of the capsule membrane. At lower pHs where the permeability of the capsule membrane was remarkably increased as well as at higher pHs, mechanical strength of the capsule membrane was higher than that at higher pHs, possibly due to formation of hydrogen bondings between carboxyl groups on poly(methacrylic acid).<sup>14</sup> It has been al-

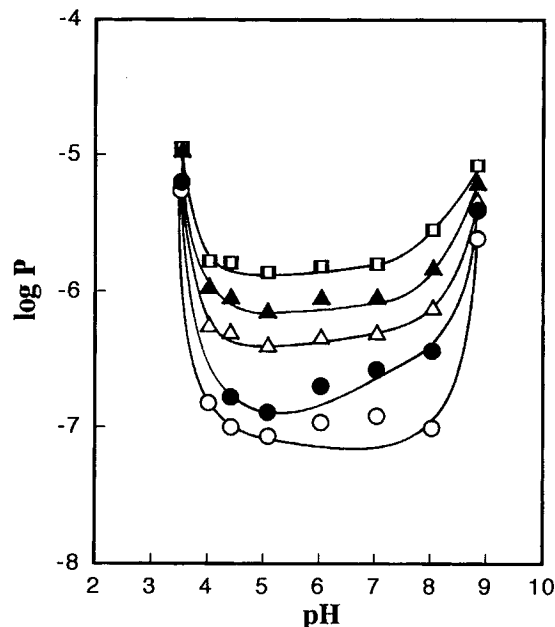


**Figure 2** Release profiles of phenylethylene glycol-loaded poly(methacrylic acid)-poly(ethylenimine) complex capsule at various pHs: (○) pH 3.5, (●) pH 4.0, (△) pH 4.4, (▲) pH 5.1, (□) pH 8.0, (■) pH 8.8.

ready shown that permeability of polyelectrolyte complex capsule membrane changes pH dependently due to the alteration of the capsule membrane structure.<sup>9</sup> The permeability was low under the conditions where both polyelectrolytes possess sufficient charges to form polyelectrolyte complex, but under



**Figure 3** Plot of  $\ln[(C^f - C^t)/(C^f - C^i)]$  against time for the data in Figure 2: (○) pH 3.5, (●) pH 4.0, (△) pH 4.4, (▲) pH 5.1, (□) pH 8.0, (■) pH 8.8.

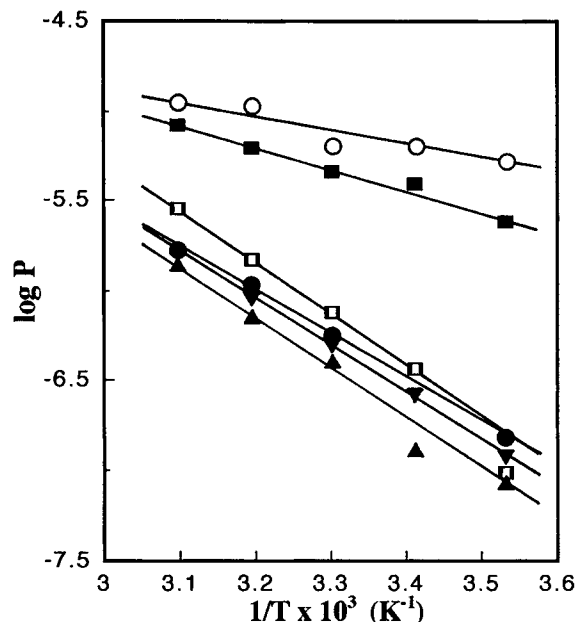


**Figure 4** The pH dependence of permeability constant of phenylethylene glycol through poly(methacrylic acid)-poly(ethylenimine) complex capsule membrane at various temperatures: (○) 10°C, (●) 20°C, (△) 30°C, (▲) 40°C, (□) 50°C.

the conditions where the polyelectrolyte complex dissociates, swelling of the capsule membrane occurred, resulting in a remarkable increase in permeability.

It is also apparent in Figure 4 that permeability of the capsule membrane depends on temperature as well as pH. Although a slight increase in the permeability was seen at pHs 3.5 and 8.8 with increasing temperature, the increase in permeability was more significant under neutral and weak acidic condition where the capsule membrane consists of complete polyelectrolyte complex. This temperature-dependent increase in permeability may suggest a partial dissociation of the polyelectrolyte complex and the resultant increase in hydration of poly(methacrylic acid)-poly(ethylenimine) complex membrane, because formation,<sup>12</sup> mobility,<sup>15</sup> and mechanical strength<sup>14</sup> of polyelectrolyte complex is known to be temperature-dependent. Alternatively, it may be due to the difference in the activation energy for diffusion of the permeant across the polyelectrolyte complex capsule membrane at various pHs.

In order to obtain information about the temperature-dependent permeation property of the capsule membrane, the Arrhenius plots of phenylethylene glycol permeation through the capsule membrane at various pHs were made and are given in Figure 5. These plots fit fairly well on the straight



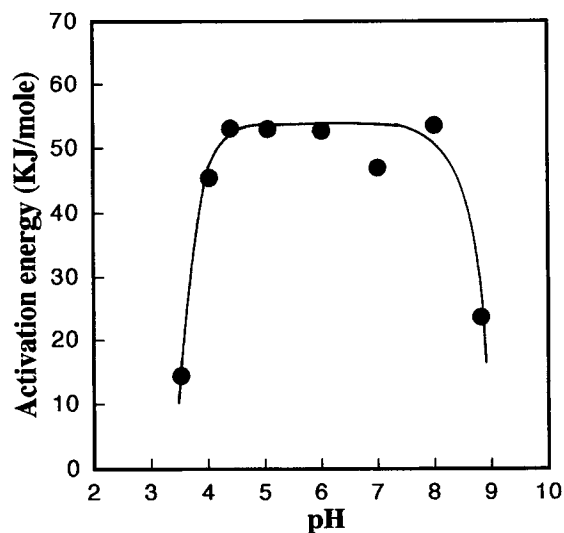
**Figure 5** Arrhenius plot for the permeation of phenylethylene glycol through poly(methacrylic acid)-poly(ethylenimine) complex capsule membrane: (○) pH 3.5, (●) pH 4.0, (▲) pH 5.1, (▼) pH 7.0, (□) pH 8.0, (■) pH 8.8.

line at each pH measured. This indicates that at a particular pH the activation energy for the permeation is constant and hence that structure of the capsule membrane does not change with temperature.

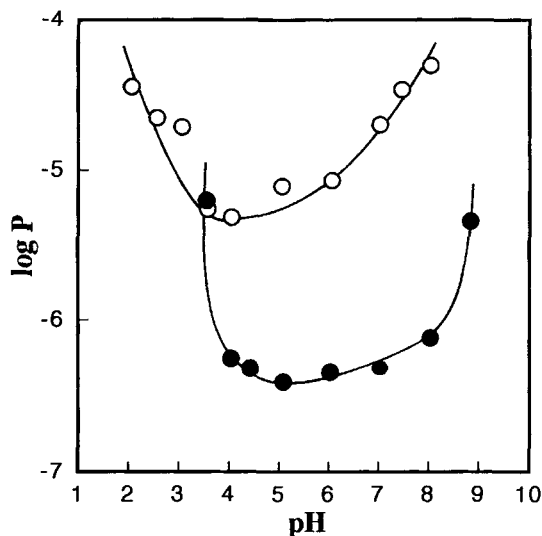
By contrast, the activation energy for the permeation was pH-dependent. Figure 6 represents pH dependence of the activation energy for the permeation of phenylethylene glycol across the capsule membrane. While the activation energy between pH 4.5 and pH 8.0 was approximately constant, a drastic decrease was seen above pH 8 and below pH 4. The former activation energy value was ca. 3-4-fold larger than the latter one. Therefore, it is considered that in neutral and weak acidic regions the capsule membrane consists of dense networks of polyelectrolyte complex and hence phenylethylene glycol molecules are difficult to permeate through the membrane. However, significant alteration of structure of the capsule membrane occurs in the acidic and alkaline regions, where macromolecular networks of the capsule membrane become swollen due to dissociation of the polyelectrolyte complex,<sup>9</sup> and therefore the molecules can permeate smoothly across the capsule membrane.

Permeation property of phenylethylene glycol through a capsule membrane made of poly(acrylic acid)-poly(ethylenimine) complex was also inves-

tigated to clarify difference between poly(acrylic acid) and poly(methacrylic acid) as a polyanion component. The pH dependence of permeability of poly(acrylic acid)-poly(ethylenimine) complex capsule as well as that of poly(methacrylic acid)-poly(ethylenimine) complex capsule is shown in Figure 7. The diameter and thickness of poly(acrylic acid)-poly(ethylenimine) complex capsule used here were  $4.9 \pm 0.5$  mm and  $24.9 \pm 2.2$   $\mu$ m, respectively. Although the capsules used here were prepared under similar conditions, it was impossible to make capsules by using the same concentration and pH of polyanion solutions because viscosity of the polyanion solutions, which is a very important factor for the formation of interface between a droplet of the polyanion solution and the outer polycation solution in the capsule preparation, was quite different between poly(acrylic acid) and poly(methacrylic acid). Therefore, exact comparison between these capsules is impossible, but it is apparent in Figure 7 that permeability of phenylethylene glycol across the capsule membrane containing poly(methacrylic acid) was roughly 1/10 of that containing poly(acrylic acid) at the same pH. Moreover, the capsule containing poly(methacrylic acid) showed more sharp change in permeability under acidic or alkaline pH. The composition of the poly(acrylic acid)-poly(ethylenimine) capsule membrane was shown to have a ratio of 43 mol % of poly(acrylic acid) to 57 mol % of poly(ethylenimine). Thus, both capsule membranes are considered to contain similar amounts of crosslinkage formed by ionic bonds in



**Figure 6** The pH dependence of the activation energy for the permeation of phenylethylene glycol through poly(methacrylic acid)-poly(ethylenimine) complex capsule membrane.

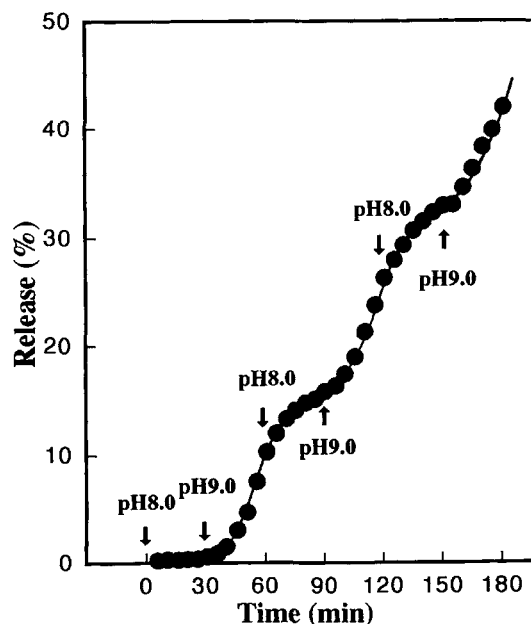


**Figure 7** The pH dependence of permeability constant of phenylethylene glycol through capsule membrane consisting of (○) poly(acrylic acid)-poly(ethylenimine) complex and (●) poly(methacrylic acid)-poly(ethylenimine) complex.

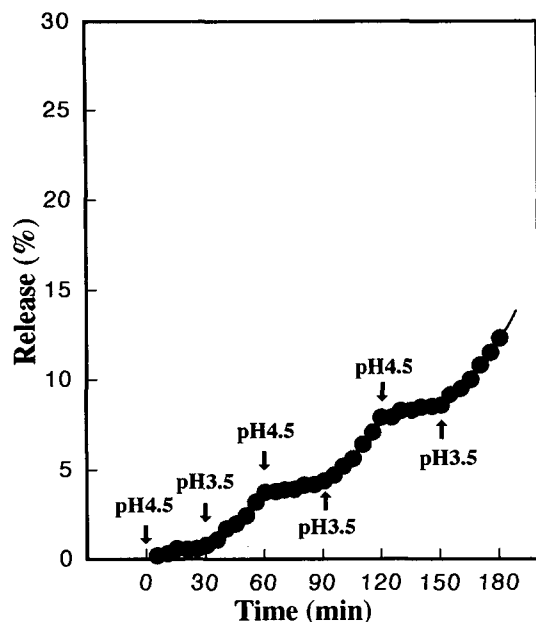
the polyelectrolyte complex networks. Thickness of the membrane of poly(methacrylic acid)-poly(ethylenimine) complex capsule is about 1.5 times that of poly(acrylic acid)-poly(ethylenimine) capsule. Because permeability constant is reversely proportional to the membrane thickness when the whole capsule membrane has a constant diffusion coefficient value, the great difference in permeability between the capsule containing poly(methacrylic acid) and that containing poly(acrylic acid) cannot be explained completely by thickness of these capsule membranes, although the difference in thickness might partly contribute to the difference in permeability. Also if permeability of the capsule membranes is controlled by a limited portion consisting of the capsule membranes such as the surface layer of the capsule membranes, thickness of the capsule membranes might hardly affect the permeability of the capsule membranes. Therefore, it is likely that this permeation characteristic of poly(methacrylic acid)-poly(ethylenimine) complex capsule might be attributable mainly to the hydrophobic nature of the polyanion, which should promote close packing of polyelectrolyte complex and formation of dense networks of the polyelectrolyte complex. In fact, it was shown that hydrophobic interactions are the main cause for interpolyelectrolyte complex association.<sup>12,16</sup>

Release control of poly(methacrylic acid)-poly(ethylenimine) complex capsule responding to pH was examined. Figures 8 and 9 show the profiles

of phenylethylene glycol permeation through the capsule membrane when the capsule containing phenylethylene glycol in the inner aqueous phase was alternatively soaked in outer solutions of various pHs. Since the volume of the outer phase is much larger than the inner volume of the capsule, there is no change in pH by adding the capsule. As is seen in Figure 8, release rate of phenylethylene glycol from the capsule was quite low at pH 8.0. However, when pH of the outer phase was changed from 8.0 to 9.0, permeation of the solute increased immediately. After several minutes the pH of the outer phase was returned to 8.0 and then a decrease in the permeation rate was observed after a several minute time lag. However, the permeation rate did not revert completely to the slow rate observed as before. Since capsule membrane was extremely swollen when the polyelectrolyte complex was dissociated at alkaline pH, it might be difficult to return its original state although ambient pH was reverted to pH 8.0. By contrast, reversible change of the permeation was observed when pH value of the outer phase of the capsule was changed alternatively to 3.5 or 4.5 as shown in Figure 9. While a very low rate of permeation was seen at pH 4.5, an immediate increase in permeation was induced by changing environmental pH to 3.5. When pH of the solution was returned to 4.5, the permeation reverted to the same



**Figure 8** The pH sensitive release of phenylethylene glycol from poly(methacrylic acid)-poly(ethylenimine) complex capsule at 20°C under alkaline condition. The capsule was alternatively dipped in aqueous solution of pH 8.0 or 9.0. The arrows show the change of the media.



**Figure 9** The pH sensitive release of phenylethylene glycol from poly(methacrylic acid)-poly(ethylenimine) complex capsule at 20°C under acidic condition. The capsule was alternatively dipped in aqueous solution of pH 4.5 or 3.5. The arrows show the change of the media.

low rate. In acidic region even when the polyelectrolyte complex was broken, significant alteration of arrangement of polymer networks might not occur in the capsule membrane because of hydrogen bondings between carboxyl groups on poly(methacrylic acid). In fact, the observation that mechanical strength of the capsule was much higher under acidic condition than alkaline one suggests the formation of hydrogen bondings under acidic condition.<sup>14</sup> Moreover, since polycations promote dissociation of poly(methacrylic acid) to form the polyelectrolyte complex,<sup>17,18</sup> effective complex formation could occur at pH 4.5, where few carboxyl groups on poly(methacrylic acid) dissociate into ions. As a result, when pH of the outer phase was returned from 3.5 to 4.5, structure of the polyelectrolyte complex membrane was reproduced and hence permeability of the capsule membrane returned to the same low level as before.

In conclusion, the partly crosslinked poly(methacrylic acid)-poly(ethylenimine) capsule prepared in this study was found to be a functional capsule with pH-sensitive release property. Release of phenylethylene glycol from the capsule was controlled in the on-off-like fashion responding to ambient pH under acidic region. Moreover, the capsule might be sensitive to various stimuli by introducing functional molecules and enzymes, which transduce

various stimuli to pH change,<sup>19-23</sup> into the capsule membrane. Therefore, the capsule is expected to be useful as a stimuli-responsive drug delivery system.

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