Permeability Characteristic of Polyelectrolyte Complex Capsule Membranes: Effect of Preparation Condition on Permeability

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SYNOPSIS

Because polyelectrolyte complex formation is known to depend on charge densities of polyelectrolytes, influence of pH condition in the preparation of the polyelectrolyte complex capsules on their permeability characteristic for phenylethylene glycol was examined. Partly crosslinked polyelectrolyte complex capsules were prepared by addition of an aqueous poly(acrylic acid) solution into an aqueous poly(ethylenimine) or L-histidine-attached poly(ethylenimine) (ethylenimine unit/L-histidine residue: 5/1, mol/mol) solution and subsequent crosslinking under various pH conditions. Although poly(acrylic acid) and poly(ethylenimine) change their charge density depending on pH in an aqueous solution, the resultant capsules prepared at pHs 7, 6, and 5 show similar permeation properties under neutral and acidic conditions. By contrast, when L-histidine-attached poly(ethylenimine) was used instead of the unmodified polymer as the capsule membrane component, the resultant capsules prepared at pHs 8, 7, and 6 revealed remarkable difference in the permeability under neutral and acidic conditions. Because the ionization of an imidazolyl group, whose pKa is 6, on the L-histidine-attached poly(ethylenimine) is influenced significantly by pH near its pKa, such situation might affect density of networks formed by the ionic bonds in the polyelectrolyte complex membrane and resulted in the permeability difference. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

In recent years, as a new type of microcapsules, stimuli-responsive microcapsules have been proposed. These microcapsules can control release of their contents responding to external stimuli, such as pH,[1-6] light,[7,8] and temperature.[9,10] These microcapsules are considered to have potential usefulness as a drug delivery system because they can entrap a variety of chemicals in a large inner space and release them at a suitable time and rate for a desired period. So far, several stimuli-responsive microcapsules and capsules have been prepared by modification of semipermeable capsule membranes.[1,2,7-10] Microcapsules made of stimuli-sensitive membranes were also reported.[3-6]

We have demonstrated that polyelectrolyte complex is useful for preparation of stimuli-responsive microcapsules.[5,6,8] The polyelectrolyte complex is formed by the electrostatic interaction of oppositely charged polyelectrolytes. When weak polyacids and/or weak polybases are used as components of polyelectrolyte complex capsules, permeability of the capsule membranes reveals pH response because the structure of the polyelectrolyte complex membranes changes drastically near pKa of ionizable groups on the polyelectrolytes.[5,6] Moreover, incorporation of functional molecules into the capsule membranes is possible by using the polyelectrolytes to which these molecules are attached.[8]

In the previous study[5,6] it has been shown that permeability of partly crosslinked poly(acrylic acid)–or poly(methacrylic acid)–poly(ethylenimine) complex capsule membranes for phenylethylene glycol, which is a nonionic molecule, varied,

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depending on the environmental pH. The permeability increase of the capsule membranes was induced by swelling of the membranes due to dissociation of the polyelectrolyte complexes. The protonation of carboxyl groups that do not participate in the polyelectrolyte complex formation in the membranes also affects their permeability. Because polyelectrolyte complex formation is known to be influenced by charge densities or degree of dissociation of the polyelectrolytes,[11-13] when weak polyacids and/or polybases are used as components of polyelectrolyte complex capsules, permeability of the capsule membranes is expected to be affected by pH condition where the capsules are prepared.

In the present study, polyelectrolyte complex capsule membranes consisting of poly(acrylic acid) and poly(ethylenimine) or poly(ethylenimine) having L-histidine residues have been prepared under various pH conditions. Effect of the pH condition of the capsule preparation on the permeability characteristic of the capsule membranes was investigated.

EXPERIMENTAL

Chemicals

Poly(sodium acrylate) (molecular weight was estimated to be 44,000 using gel permeation chromatography) and L-histidine were purchased from Kishida Chemical Co., Ltd. (Japan). 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 by Tokyo Kasei Kogyo Co., Ltd. (Japan). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide–HCl salt (EDC) was obtained from Nacalai Tesque Co. Ltd. (Japan). Phenylethylene glycol was purified by recrystallization from ligroin. Other chemicals were used without further purification.

Preparation of Poly(ethylenimine) Having L-Histidine Residues

L-Histidine (0.023 mol) and poly(ethylenimine) (0.046 unit mol) were dissolved in water. The solution was adjusted to pH 4.5. EDC (0.023 mol) was added to the solution at 0°C and stirred for 24 h at pH 4.5. The resultant polymer was dialyzed for 7 days against water and then was lyophilized. The ratio of L-histidine residue to ethylenimine unit in the polymer was determined to be 1/5 (mol/mol) using ^{1}H NMR (JEOL JNM-GX-270).

Potentiometric Titrations

Potentiometric titrations were carried out using a pH meter (Horiba M-8). Poly(acrylic acid) solution (10 mM) containing 10 mM NaCl, poly(ethylenimine) solution (10 mM) containing 10 mM NaCl, and L-histidine-attached poly(ethylenimine) solution (10 mM) containing 10 mM NaCl, each volume being 100 mL, were neutralized by adding 0.1 N NaOH or 0.1 N HCl solution at room temperature.

Capsule Preparation

Polyelectrolyte complex capsules were prepared according to the method described in the previous article.[5] An aqueous poly(sodium acrylate) solution (1.5 wt %) of a given pH (pH 8, 7, or 6) was added drop-wise from a pipette into an aqueous solution of a given pH (pH 8,7,6, or 5) containing poly(ethylenimine) with or without L-histidine residues (0.5 wt %) at 30°C. The solution was incubated with gentle agitation for 2 h to form the polyelectrolyte complex membrane at the droplet surface. The resultant capsules were washed with distilled water, followed by contact with 0.15 wt % aqueous poly(sodium acrylate) solution and 0.05 wt % of aqueous histidine-attached or unmodified poly(ethylenimine) solution for 2 h each. After washing with distilled water, the capsule membranes were crosslinked by incubation in an aqueous 50 mM phosphate-buffered solution containing EDC (11.4 g/L) at pH 4.5 overnight with gentle stirring. Finally, the crosslinked capsules were kept in distilled water and washed 3 times in order to remove EDC remaining in the capsules. The purified capsules were put in an aqueous 38.5 mM phenylethylene glycol solution at pH 2.5 for more than 3 days.

Estimation of Capsule Diameter

Capsule diameter was estimated as previously reported.[5] Diameter d of the capsule was determined by calculation according to eq. (1):

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

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.

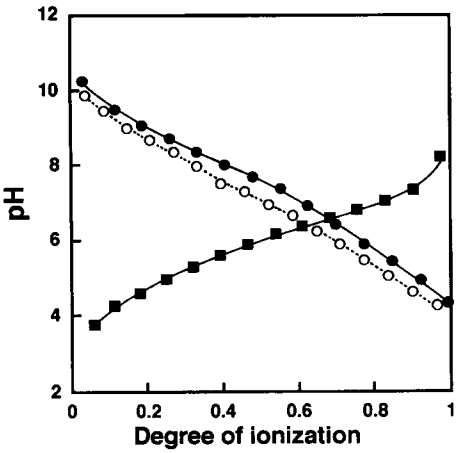

Figure 1 The pH dependence of degree of ionization of (■) poly(acrylic acid), (●) poly(ethylenimine), and (○) L-histidine-attached poly(ethylenimine) in aqueous 10 m*M* NaCl solution.

Permeation Measurements

Permeation measurements were performed as previously reported.[5] A permeant-loaded capsule was preincubated in the permeant solution adjusted at the pH value of the measurement for more than 10 h at 30°C. The capsule was taken out of the solution and then was put into 30 mL of aqueous HCl solution (pH 1.5–2.5), 5 m*M* acetate-buffered solution (pH 3.0–6.0), or 5 m*M* Tris-HCl-buffered solution (pH 7.0–9.0), as the outer aqueous phase at 30°C. 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 (Hitachi U-3200).

The permeability constant P (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.

RESULTS AND DISCUSSION

Because charge density of weak polyelectrolytes changes depending on pH, permeation characteristic of polyelectrolyte complex is expected to be controlled by pH condition in the capsule preparation. The purpose of this study is to clarify the influence of pH condition in the preparation of polyelectrolyte complex capsules on their membrane permeability characteristic. Poly(acrylic acid) and poly(ethylenimine) were used in this study because polyelectrolyte complex capsule membrane consisting of these polyelectrolytes was already characterized in the previous study.[5] Poly(ethylenimine) having L-histidine residues was synthesized and also used as a polycation with ionizable groups that have pKa in weak acidic region, as mentioned below.

In order to know an effect of pH on charge density of the polyelectrolytes, the acid-base titration was performed. The result is shown in Figure 1. Poly(acrylic acid) changes its charge density in the pH region between 3.5 and 8. Although both poly(ethylenimine) and the L-histidine-attached derivative vary their charge density in the pH region between 4 and 10, the derivative reveals lower charge density at a particular pH than unmodified poly(ethylenimine) does.

Permeability Characteristic of Poly(acrylic acid)–Poly(ethylenimine) Complex Capsule Membrane

The influence of pH condition of the capsule preparation on the permeability of poly(acrylic acid)–poly(ethylenimine) complex capsule membrane was investigated. Three kinds of partly crosslinked poly(acrylic acid)–poly(ethylenimine) complex capsules were prepared at various pHs. The preparation conditions and mean radii of these capsules are listed in Table I.

The formation of polyelectrolyte complex capsule was quite pH dependent. The stable capsule membranes were formed in the pH region between 7.0 and 6.0. However, it was difficult to obtain the stable capsule membranes in the pH regions above 8.0 and below 5.0. Because above pH 8.0 the degree of ion-

Table I Preparation Conditions and Radii of Partly Crosslinked Poly(acrylic acid)–Poly(ethylenimine) Complex Capsules

	pH of Polyelectrolyte Solution		
Capsule	Poly-(acrylic acid)	Poly-(ethylenimine)	Radius (mm)
CP-7	7.0	7.0	2.45 ± 0.25
CP-6	6.0	6.0	2.12 ± 0.15
CP-5	6.0	5.0	2.96 ± 0.24

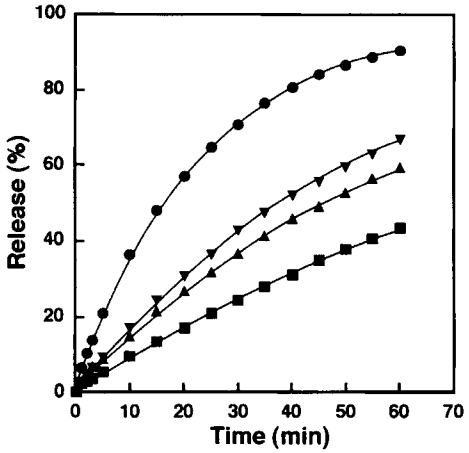

Figure 2 Release profiles of phenylethylene glycol-loaded CP-6 at various pHs: (●) pH 2.5, (▲) pH 3.5, (■) pH 5.0, (▼) pH 6.0.

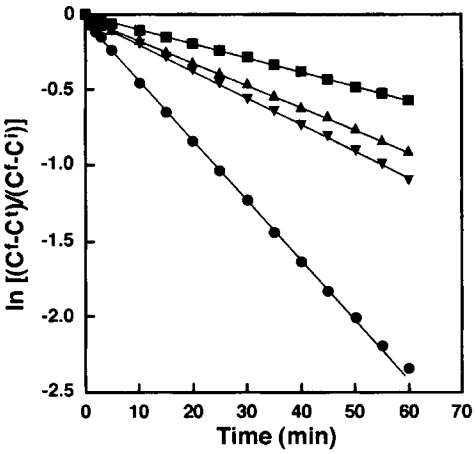

Figure 3 Plot of $\ln[(C^f - C^t)/(C^f - C^i)]$ against time t for the data in Figure 2: (●) pH 2.5, (▲) pH 3.5, (■) pH 5.0, (▼) pH 6.0.

ization of poly(ethylenimine) is less than 0.4, charge density of the polycation would not be sufficient to form stable polyelectrolyte complex membranes under the condition. Alternatively, a considerable hydration of poly(acrylic acid) under the alkaline condition may make the membrane highly swollen and weak.[11] By contrast, the polyelectrolyte complex capsules could not be obtained below pH 5 because viscosity of the poly(acrylic acid) solution decreased below pH 5. Viscosity of the solution is important for formation of the interface between the polyanion and polycation solutions. In this pH region, a droplet of the aqueous poly(acrylic acid) solution added into the aqueous poly(ethylenimine) solution was diffused without formation of the interface. Therefore, in order to obtain the polyelectrolyte complex capsules at pH 5, poly(acrylic acid) solution of pH 6 and the polycation solution of pH 5 were used. In this procedure, final pH of the mixed solution became 5 and, hence, the resultant capsule membrane may be considered to consist of the polyelectrolyte complex formed at pH 5.

Figure 2 shows typical examples of release profiles of phenylethylene glycol from CP-6 at various pHs. The permeation of the permeant through the capsule membrane changes remarkably, depending on pH. For the quantitative evaluation of the permeability characteristic of the capsule membrane, permeability constants of the membrane at various pHs were determined using eq. (2). The plots of $\ln[(C^f - C^t)/(C^f - C^i)]$ against time t for the data given in Figure 2 are illustrated in Figure 3. These plots fit well into straight lines, indicating that the permeation obeys Fick's first low of diffusion. The permeability constants of CP-6 membrane were obtained from the slopes of the lines in Figure 3. A similar pH dependence was observed in the permeation of CP-7 and CP-5. The permeability constants of these capsules were also estimated in the same manner. Figure 4 represents pH dependence of the permeability constant of CP-5, CP-6, and CP-7. These capsule membranes reveal virtually the same permeability, in spite of difference in the preparation condition. The permeability of these capsule membranes shows minimum in the pH region between 3.5 and 5, and increases below pH 3 or above pH 7. This pH dependence of the permeability is attributable to the change in degree of swelling of the capsule membranes, depending on pH.[5]

The compositions of these capsule membranes were determined by elemental analysis and the

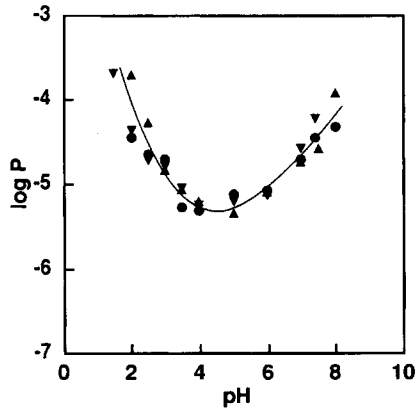

Figure 4 pH Dependence of the permeability constant of phenylethylene glycol through partly crosslinked poly(acrylic acid)–poly(ethylenimine) complex capsule membranes prepared at varying pHs: (●) CP-7, (▼) CP-6, (▲) CP-5.

results are listed in Table II. Unit mol ratio of poly(acrylic acid) to poly(ethylenimine) in the capsule membrane increases as pH of the capsule preparation decreases, because charge density of poly(acrylic acid) decreases and that of poly (ethylenimine) increases with decreasing pH. However, the pH condition of the capsule preparation does not influence the membrane composition remarkably and the ratio is much closer to unity than expected by considering the degree of ionization of these polyelectrolytes from the standpoint of interchain neutralization of ionic charges on the polyelectrolytes. In general, ionization of a given group on polyelectrolytes is suppressed by neighboring sites already charged. However, when charged groups on the polyelectrolyte bind to oppositely charged groups of the counter polyelectrolyte upon the formation of the polyelectrolyte complex, ionization of the polyelectrolyte is enhanced.[11] Thus, the ionization of poly(acrylic acid) and poly (ethylenimine) should be promoted during the capsule formation and, as a result, the membrane composition and, hence, the permeability are not significantly affected by the pH condition of the capsule preparation.

Permeability Characteristic of Poly(acrylic acid)–L-Histidine-Attached Poly(ethylenimine) Complex Capsule Membrane

As mentioned above, although charge densities of poly(ethylenimine) and poly(acrylic acid) in aqueous solutions altered depending on pH, permeability characteristic of the capsule membranes was hardly affected by the pH condition of the capsule preparation, because pKa values of the ionizable groups on these polyelectrolytes are out of the pH region where the capsules were prepared. Therefore, when a polyelectrolyte complex capsule is prepared using a polyelectrolyte having ionizable groups that have a pKa within the pH region, permeation characteristic of the capsule membrane is expected to be affected by the pH condition of the capsule preparation. For this purpose, we selected L-histidine, which has an imidazolyl group, whose pKa is 6, and L-histidine-attached poly(ethylenimine) was used as a membrane component. The ionization of imidazolyl groups hardly occurs above pH 7, even when a large portion of ionizable sites on the polyelectrolyte form ion bonds. However, imidazolyl groups becomes charged and participates in polyelectrolyte formation at and below pH 6. These situations might give a significant difference in permeability characteristic of the capsule membranes.

Table II Composition of Partly Crosslinked Poly(acrylic acid)–Poly(ethylenimine) Complex Capsule Membranes

	Unit mol %	
Capsule	Poly(acrylic acid)	Poly(ethylenimine)
CP-7	46	54
CP-6	55	45
CP-5	56	44

Although L-histidine was reacted with poly(ethylenimine) with a varying ratio of L-histidine to the ethylenimine unit from 0.5 to 1.0, all of the polymers obtained revealed approximately a same composition; the ratio of L-histidine residue to ethylenimine unit was 1/5 (mol/mol). Poly(ethylenimine) used in this study is highly branched and has primary, secondary, and tertiary amino groups in the ratio of 1 : 2 : 1. The ratio of L-histidine residue to ethylenimine unit in the modified poly(ethylenimine) was close to the content of primary amino group of poly(ethylenimine), possibly because the reaction of secondary amino groups is suppressed by the steric hindrance and, therefore, primary amino groups might exclusively react with L-histidine.

Using the L-histidine-attached poly(ethylenimine) derivative instead of the unmodified polymer, three kinds of partly crosslinked polyelectrolyte complex capsules, namely CP-8(His), CP-7(His), and CP-6(His) were prepared at pHs 8, 7, and 6, respectively. The preparation conditions and mean radii of these capsules are listed in Table III. Although poly(acrylic acid)–poly(ethylenimine) capsule was not obtained at pH 8, the polyelectrolyte complex capsule could be prepared using L-histidine-attached poly(ethylenimine) at the same pH. The ability of imidazolyl groups and amide groups on the L-histidine–attached polymer to form hydrogen bondings may contribute to the membrane stability.

Figure 5 shows pH dependence of the permeability constant of phenylethylene glycol through the capsule membranes. In contrast with the polyelectrolyte complex capsules without L-histidine residues shown in Figure 4, the permeability characteristic of these polyelectrolyte complex capsules is clearly affected by the pH condition of the capsule preparation. Generally, the permeability of these capsules is low in the pH region between 4 and 7.5, and increases below pH 4 or above pH 8. However, these capsules reveal a remarkable difference in

the permeability constant values especially under a weak acidic condition. The permeability of these capsules decreases in the order of CP-8(His), CP-7(His), and CP-6(His). Because charge density of poly(ethylenimine) is insufficient to form a stable polyelectrolyte complex membrane at pH 8 as mentioned above, it is likely that there exist many ionizable sites unpaired in CP-8(His) membrane and, therefore, the density of networks formed by the ionic bonds in the membrane should be relatively low. Thus, CP-8(His) membrane revealed higher permeability, compared with CP-7(His) and CP-6(His) membranes. On the other hand, CP-7(His) is considered to have more dense networks in the membrane. However, because imidazolyl groups of L-histidine residues are hardly protonated at pH 7 where the capsule was prepared, these groups should be incorporated in the membrane in the uncharged state and, hence, do not participate in polyelectrolyte complex formation. By contrast, in the case of CP-6(His), imidazolyl groups becoming protonated under the preparation condition should participate in formation of the polyelectrolyte complex, resulting in formation of the membrane with much more dense networks. These situation might give the difference in the permeability of these capsule membranes.

Moreover, among these capsules a distinct tendency for the pH dependence of the permeability can be seen in the weak acidic region. While the permeability of CP-6(His) membrane decreases with decreasing pH in the pH region between 7.4 and 3.5, the permeabilities of CP-7(His) and CP-8(His) membranes show a tendency to increase slightly with decreasing pH from 7.4 to 4. As mentioned above, protonation of carboxylate groups that do not participate in the formation of polyelectrolyte complex occurs in the weak acidic region upon decreasing pH, resulting in decrease in swelling and, hence, decrease in permeability of the membrane. However, because the capsule membranes of CP-7(His) and CP-8(His) contain imidazolyl groups that do not form ionic bonds, these groups become protonated in the pH region as pH decreases. The generation of the positive charges in the capsule membranes should enhance swelling of the membranes, and as a result, the permeability of these membranes increased slightly.

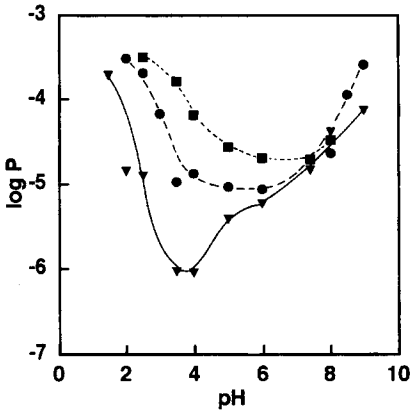

Figure 5 pH Dependence of the permeability constant of phenylethylene glycol through partly crosslinked poly(acrylic acid)–L-histidine-attached poly(ethylenimine) complex capsule membranes prepared at varying pHs: (■) CP-8(His), (●) CP-7(His), (▼) CP-6(His).

pH Responsive Permeability Change of Polyelectrolyte Complex Capsule Membrane Having L-Histidine Residues

As is seen in Figure 5, the permeability of CP-6(His) membrane varies to a great extent depending on the external pH, especially under an acidic condition. The permeability changes by a factor of 200 between pHs 1.5 and 3.5. Then, pH-induced regulation of release from CP-6(His) was examined. Figure 6 shows permeation profile of phenylethylene glycol from CP-6(His) when pH of the outer phase is changed between pH 3.5 and 1.5. When the capsule was put in the medium of pH 3.5, release rate of phenylethylene glycol was very low. However, pH

Table III Preparation Conditions and Radii of Partly Crosslinked Poly(acrylic acid)–L-Histidine-Attached Poly(ethylenimine) Complex Capsules

	pH of Polyelectrolyte Solution		
Capsule	Poly(acrylic acid)	L-Histidine-Attached Poly(ethylenimine)	Radius (mm)
CP-8 (His)	8.0	8.0	2.22 ± 0.15
CP-7 (His)	7.0	7.0	2.55 ± 0.33
CP-6 (His)	6.0	6.0	2.47 ± 0.20

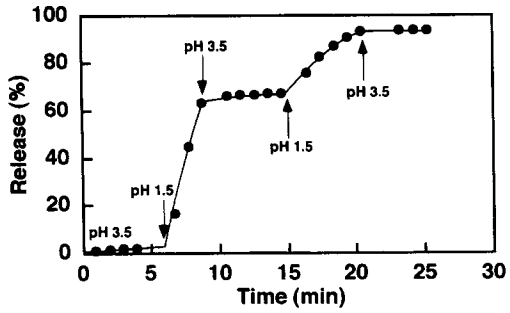

Figure 6 pH Induced control of release from phenylethylene glycol-loaded CP-6(His). The capsule was alternatively dipped in aqueous solution of pH 3.5 or 1.5.

of the outer phase was changed to 1.5 and then, extensive release of phenylethylene glycol was induced immediately. When pH of the outer phase was returned to 3.5, the release reverted to the same low rate as before. This pH regulation of release from the capsule could be achieved reversibly.

In conclusion, it was shown that the permeability characteristic of partly crosslinked poly(acrylic acid)–poly(ethylenimine) complex capsules was hardly affected by the pH condition of the capsule preparation; however, when L-histidine-attached poly(ethylenimine) was used as a membrane component instead of the unmodified polymer, a significant influence of the preparation condition on the permeability characteristic was observed. The different influence of the pH condition of the capsule preparation on their permeability can be interpreted by pKas of ionizable groups on the polyelectrolytes.

While the polyelectrolyte complex capsules so far prepared reveals pH responsive permeability, the pH region where permeability of the capsules changes is limited in the acidic or alkaline one.[5,6] However, it is important to prepare capsules with pH response in weak acidic region for their application to the drug delivery system. For example, when enzymes such as glucose oxidase that transduces glucose concentration to the pH change by converting glucose into gluconic acid are used for glucose sensitization of the capsule membranes,[14,15] the capsule membranes are required to reveal pH response in the weak acidic region. The findings obtained in this study should provide important information for the design of functional capsules that shows pH response in a desired pH region.

REFERENCES

1. Y. Okahata, H. Noguchi, and T. Seki, *Macromolecules*, **20**, 15 (1987).
2. E. Kokufuta, N. Shimizu, and I. Nakamura, *Biotech. Bioeng.*, **32**, 289 (1988).
3. K. Kono, S. Kimura, and Y. Imanishi, *J. Membr. Sci.*, **58**, 1 (1991).
4. E. Miyauchi, Y. Togawa, K. Makino, H. Ohshima, and T. Kondo, *J. Microencap.*, **9**, 329 (1992).
5. K. Kono, F. Tabata, and T. Takagishi, *J. Membr. Sci.*, **76**, 233 (1993).
6. N. S. Yoon, K. Kono, and T. Takagishi, *J. Appl. Polym. Sci.*, **55**, 351 (1995).
7. Y. Okahata, H.-J. Lim, and S. Hachiya, *J. Chem. Soc., Perkin Trans.*, **II**, 989 (1984).
8. K. Kono, Y. Nishihara, and T. Takagishi, *J. Appl. Polym. Sci.*, **56**, 707 (1995).
9. Y. Okahata, H.-J. Lim, G. Nakamura, and S. Hachiya, *J. Am. Chem. Soc.*, **105**, 4855 (1983).
10. Y. Okahata, H. Noguchi, and T. Seki, *Macromolecules*, **19**, 493 (1986).
11. K. Abe, H. Ohno, and E. Tsuchida, *Makromol. Chem.*, **178**, 2285 (1977).
12. J. Koetz, K.-J. Linow, B. Philipp, L. P. Hu, and O. Vogl, *Polymer*, **27**, 1574 (1986).
13. S. K. Chatterjee, D. Yadav, S. Ghosh, and A. M. Khan, *J. Polym. Sci., Part A: Polym. Chem. Ed.*, **27**, 3855 (1989).
14. G. Albin, T. A. Horbett, and B. D. Ratner, *J. Controlled Rel.*, **2**, 153 (1985).
15. Y. Ito, M. Casolaro, K. Kono, and Y. Imanishi, *J. Controlled Rel.*, **10**, 195 (1989).

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