# Effect of Hydrophobic Units on the pH-Responsive Release Property of Polyelectrolyte Complex Capsules

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**ABSTRACT**: Copolymers of acrylic acid and styrene with styrene unit contents of 2.7, 5.7, and 9.5% were synthesized by free radical copolymerization. Poly(ethylenimine)s with benzylated unit contents of 2.4, 6.0, 10.6, and 16.7% were obtained by the reaction of poly(ethylenimine) with benzyl bromide. Polyelectrolyte complex capsules consisting of these polymers were prepared. Influence of the hydrophobic units on pH-responsive release property of the capsules was studied using phenylethylene glycol as a permeant. When the copolymer with styrene unit content of 5.7% or the poly(ethylenimine) with the benzylated unit content of 2.4-10.6% was used as the membrane components, the permeability of the capsule membrane became minimum and was 10-20 fold lower than that of the poly(acrylic acid)-poly(ethylenimine) complex capsule membrane in the pH region between 3 and 7. In contrast, the hydrophobic units did not lower the permeability of the capsule membranes significantly below pH 3 and above pH 7. Thus, the capsule membranes containing hydrophobic units exhibited remarkable permeability changes in the narrow pH regions of 2–3 and 7–9. Also, the capsule containing the benzylated PEI in the membrane changed the release rate of the contents very quickly, in response to the ambient pH alteration. Therefore, polyelectrolyte complex capsules, which are highly sensitive to pH change, were obtained by using the polyelectrolytes with the hydrophobic units as membrane components. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 72: 1763-1773, 1999

**Key words:** polyelectrolyte complex; microcapsule; pH-responsive release; poly-(acrylic acid); poly(ethylenimine); hydrophobicity

# **INTRODUCTION**

In recent years, responsive controlled release systems have become of interest because these systems are considered to optimize the delivery of drugs.<sup>1,2</sup> So far, a variety of responsive drug delivery systems have been developed, such as stimuli-responsive or stimuli-sensitive membranes, gels, liposomes, microspheres, and microcapsules.<sup>1-4</sup> Because the release rate from microcapsules is generally controlled by the diffusion rate of the permeants across the thin microcapsule membranes, a quicker change in the release rate responding to the stimuli may be expected, compared to gels or microspheres. Also, microcapsules have large inner spaces and can entrap a large quantity of drugs. Therefore, microcapsules

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are useful as the stimuli-responsive drug delivery systems.

Various stimuli-responsive microcapsules have been developed.<sup>5,6</sup> These microcapsules release their contents in response to pH,<sup>7-9</sup> temperature,<sup>10,11</sup> and so on. We have demonstrated that polyelectrolyte complex formation is useful for preparation of stimuli-responsive microcapsules.<sup>12–15</sup> The polyelectrolyte complex is formed by the electrostatic interaction between oppositely charged polyelectrolytes. When weak polyacids and/or weak polybases are used as components of polyelectrolyte complex microcapsules, permeability of the microcapsule membrane changes, depending on the environmental pH, because formation or dissociation of the polyelectrolyte complex occurs, depending on pH.<sup>12-14</sup> Also, polyelectrolyte complex microcapsules can be prepared by mild reactions, such as complex formation at the interface between polyanion and polycation solutions<sup>16</sup> or complexation on the surface of gelled polyelectrolyte droplets.<sup>17</sup> Therefore, they are favorable for encapsulation of bioactive molecules. Microencapsulation of drugs,<sup>18,19</sup> proteins,<sup>20,21</sup> liposomes,<sup>22</sup> and living cells<sup>23,24</sup> using a polyelectrolyte complex has been reported.

In previous studies,<sup>12,13</sup> we have shown that permeability of partly crosslinked poly(acrvlic acid), or poly(methacrylic acid), poly(ethylenimine) complex capsule membranes changes, depending on the ambient pH; permeability of these membranes is low under neutral and weakly acidic conditions but increases significantly under acidic and alkaline conditions. However, the poly-(methacrylic acid)-poly(ethylenimine) complex capsule membrane exhibited lower permeability than the poly(acrylic acid)-poly(ethylenimine) complex capsule membrane and, hence, the former capsule controlled release of the contents in the on-off-like fashion, responding to pH change.<sup>13</sup> Because hydrophobicity of polyelectrolytes affects polyelectrolyte complex formation,<sup>25</sup> it is considered that the pH-responsive release property of polyelectrolyte complex capsules will be improved by controlling hydrophobicity of the polyelectrolyte components. Thus, in this study, we synthesized copolymers of acrylic acid and styrene and poly(ethylenimine)s with benzylated units. Polyelectrolyte complex capsules were prepared using these polyelectrolytes as the membrane components. The influence of the hydrophobic units on the pH-responsive release property of the capsules was investigated.

# **EXPERIMENTAL**

# Chemicals

Poly(acrylic acid) (PAA) (viscosity-average molecular weight, 115,000), acrylic acid, styrene, benzyl bromide, and azobis(isobutyronitrile) (AIBN) were purchased from Kishida Chemical Co., Ltd., Japan. Poly(ethylenimine) (PEI) (the 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. Acrylic acid and styrene were purified by vacuum distillation. Phenylethylene glycol was purified by recrystallization from ligroin. Other chemicals were used without further purification.

# Poly(acrylic acid-co-styrene)

Poly(acrylic acid-co-styrene) [PAA(St)] was prepared by free radical copolymerization of acrylic acid and styrene using AIBN as the initiator. Acrylic acid and styrene were mixed in various ratios in test tubes. AIBN  $(2 \times 10^{-4} \text{ mol})$  was added in the mixture, and the mixture was degassed by bubbling with N<sub>2</sub> for 15 min. The test tubes were sealed, heated to 60°C for 6 h, and then cooled to room temperature. The copolymers were dissolved in an aqueous 1*M* NaOH solution. The copolymer solutions were neutralized using 1*M* HCl and dialyzed for 3 days against distilled water. The copolymers were isolated by lyophilization of these solutions.

# **Benzylated Poly(ethylenimine)**

Benzylated PEIs [PEI(Bzl)s] were synthesized according to the method reported by Nango and Klotz.<sup>26</sup> PEI was isolated from an aqueous 30 wt % PEI solution by lyophilization and dissolved in ethanol (100 g L). Various amounts of benzyl bromide were added to the PEI solutions and stirred at 50°C for 24 h. Then, the solutions were dialyzed against an ethanol-water mixture (1 : 4, v/v) for 1 day and then against distilled water for 3 days. The polymers were recovered by lyophilization of the solutions.

# **Potentiometric Titrations**

Potentiometric titration was carried out using a pH meter (Horiba M-8). By adding excess NaOH,

PAA or PAA(St) was dissolved in aqueous 10 mM NaCl solution (10 mM, 100 mL). Then, to the polymer solution, an aqueous 0.1N HCl solution was added. Protonation of the polymer was detected by monitoring electric conductivity and pH of the solution. Titration of PEI and PEI(Bzl) was performed in the same manner.

## **Capsule Preparation**

Polyelectrolyte complex capsules were prepared according to the method described previously.<sup>12-14</sup> An aqueous PAA (1.5 wt %, pH 6) or PAA(St) (7.0 wt %, pH 6) solution was added dropwise from a pipette into an aqueous PEI (0.5 wt %, pH 5) or PEI(Bzl) solution (0.5 wt %, pH 5) at 25°C. The solution was incubated with gentle agitation for 2 h (PAA-PEI capsules) or 5 h (other capsules) to form the polyelectrolyte complex membrane at the droplet surface. The resultant capsules were washed three times with distilled water. After washing, the capsule membranes were crosslinked by the incubation in an aqueous 50 mM phosphatebuffered solution containing EDC (11.4 g  $L^{-1}$ ) at pH 4.4 for 12–15 h with gentle stirring. Finally, the crosslinked capsules were kept in distilled water for 2 days and washed 3 times with water in order to remove EDC remaining in the capsules. The purified capsules were immersed in an aqueous 20 mM phenylethylene glycol solution for more than 3 days. The capsules were visually confirmed to have an inner aqueous space. Generally, the concentration of polyanions in the inner aqueous phase was less than 0.35 wt %, which is less than 5% of the concentration of the original solution.

## **Estimation of Capsule Diameter**

Capsule diameter was estimated as previously reported.<sup>12</sup> Diameter d of the capsule was determined by calculation according to eq. (1), as follows:

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

Permeation measurements were performed as previously reported.<sup>12</sup> A phenylethylene glycol-

loaded capsule was preincubated in the permeant solution adjusted at the pH value of the measurement for more than 10 h at 25 °C. The capsule was taken out of the solution and then was put into 30 mL of aqueous HCl solution (pH 2–3), 1 mM acetate-buffered solution (pH 4–6), or 1 mM Tris-HCl-buffered solution (pH 7–9) at 25 °C. Permeation of phenylethylene glycol through the capsule membrane was detected by following the absorbance of the outer aqueous phase at 215 nm using a spectrophotometer (Jasco V-520-SR).

The permeability constant P (cm s<sup>-1</sup>) was determined by using eq. (2) obtained from Fick's first law of diffusion, as follows:

$$\ln[(C^{f} - C^{t})/(C^{f} - C^{i})] = -(V + V_{c})APt/VV_{c} \quad (2)$$

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; and  $V_c$  and A are the volume and the surface area of a capsule, respectively. Permeation measurements were carried out at least duplicate for individual conditions and P values are the average of these points. The errors in the permeability experiments among different capsules of the same kind was less than  $\pm 15\%$ . 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 capsules.

## Water Content Measurement

Dry capsule membranes (ca. 80 mg) were immersed in aqueous solutions used for the permeation measurements for 12 h at  $25^{\circ}$ C. The swollen membranes were taken out of the solution, and excess water on their surface was removed by wiping with a filter paper. Then the samples were weighed. The water content was defined by eq. (3), as follows:

water content (%) = 
$$(W - W_0)/W \times 100$$
 (3)

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

### **Other Methods**

Nuclear magnetic resonance (NMR) spectra were taken with a JEOL JNM-GX 270 MHz instrument. The molecular weight of the copolymers

Copolymer	Feed			Copolymer	
	Acrylic Acid (mol)	Styrene $(10^{-2} \text{ mol})$	Styrene (mol %)	Styrene Unit (mol %)	Weight-Average Molecular Weight <sup>a</sup>
PAA (St-2.7) PAA (St-5.7) PAA (St-9.5)	$0.274 \\ 0.325 \\ 0.245$	$0.691 \\ 1.74 \\ 2.08$	$2.5 \\ 5.1 \\ 7.8$	2.7 5.7 9.5	448,000 466,000 62,000

Table I Synthesis and Characterization of Poly(acrylic acid-co-styrene)s

<sup>a</sup> Estimated using polyethylene glycol as the standard.

was estimated by gel permeation chromatography analysis on a Shodex SB-804 HQ column using a 50 mM sodium acetate-buffered solution containing 0.1M Na<sub>2</sub>SO<sub>4</sub> and 20 vol % acetonitrile (pH 7.0) at 40°C.

# **RESULTS AND DISCUSSION**

## **Characterization of Polyelectrolytes**

As polyanions having hydrophobic units, three kinds of PAA(St)s with different compositions were synthesized by feeding acrylic acid and styrene in varying ratios in the copolymerization (Table I). Composition and weight-average molecular weight of these copolymers were evaluated from <sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra and by gel permeation chromatography (GPC), respectively, and are shown in Table I. The styrene unit content of the copolymers increased with an increasing amount of styrene monomer supplied in the reaction. The copolymers had styrene-acrylic acid unit ratios close to those in feed. Also, we prepared four kinds of PEI(Bzl)s as polycations with hydrophobic groups by the reaction of PEI with benzyl bromide. The contents of benzylated unit in the resultant polymers were estimated from <sup>1</sup>H-NMR spectra and

are listed in Table II. For PEI(Bzl-6.0), PEI(Bzl-10.6), and PEI(Bzl-16.7), the benzylated unit contents in the polymers were slightly higher than those fed in their synthesis, probably because PEI samples used for the synthesis were not completely dry.

The acid-base titration was performed for these polyelectrolytes to know the pH-dependence of ionization of these polyelectrolytes (Fig. 1). As is seen in Figure 1(A), PAA(St) and PAA varied the charge density depending on pH in the pH region between 3 and 10. However, dissociation of carboxyl groups on PAA(St) was slightly suppressed in the acidic region, compared with the case of PAA. Because a large fraction of carboxyl groups are protonated in the acidic region, PAA(St) should take a compact conformation due to the existence of hydrophobic units and form hydrophobic domains, which promote protonation of carboxylate anions. Similarly, slight suppression of ionization of PEI(Bzl) was seen in the alkaline region [Fig. 1(B)], compared to the case of PEI, possibly due to the same reason.

# Permeability of PAA(St)-PEI Complex Capsule Membranes

Three kinds of polyelectrolyte complex capsules were prepared using PAA(St) and PEI. The PAA–

Table II Sy	nthesis o	f Benzylated	Poly(ethy	lenimine)
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		Polymer		
Polymer	Poly(ethyleneimine) (Unit Mol)	Benzyl Bromide (mol)	Benzyl Bromide (mol %)	Benzylated Unit (mol %)
PEI (Bzl-2.4)	0.63	0.034	2.6	2.4
PEI (Bzl-6.0)	1.20	0.032	5.1	6.0
PEI (Bzl-10.6)	1.42	0.154	9.8	10.6
PEI (Bzl-16.7)	1.32	0.213	13.9	16.7



**Figure 1** The pH-dependence of ionization of polyelectrolytes used in this study: (A) (■) PAA; (●) PAA(St-9.5). (B) (■) PEI; (●) PEI(Bzl-16.7).

PEI complex capsule was also prepared. Viscosity of a polyanion solution was very important for the capsule formation. Aqueous PAA(St) solutions were much less viscous than aqueous PAA solution. Thus, we used PAA(St) solutions of 7.0 wt % for the preparation of PAA(St)-PEI complex capsules and 1.5 wt % PAA solution for the preparation of a PAA-PEI complex capsule. Diameters of these capsules are listed in Table III. The diameters of PAA(St)-PEI capsules were larger than that of the PAA-PEI capsule because the concentration of the PAA(St) droplet was high and expansion of the droplet occurred during the capsule preparation. Compositions of the capsule membranes estimated by elemental analysis are also shown in Table III. The unit ratio of acrylic acid to ethylenimine in these capsule membranes was close to 1:1, irrespective of difference in the styrene unit content.

Permeation of phenylethylene glycol through the capsule membranes was investigated (Fig. 2). Figure 2(A) shows release profiles of the permeant from PAA(St-9.5)–PEI capsule at various pHs. The capsule hardly released the contents at pHs 7 and 4, but the release was enhanced at pH 8.5 and, more significantly, at pH 2.0. We have already shown that the polyelectrolyte complex between PAA and PEI is stable under neutral and weakly acidic conditions, but dissociation of the complex occurs at acidic and alkaline pHs.<sup>12</sup>

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). From the slopes of the lines in Figure 2(B), the permeability constants were evaluated using eq. (2).

Figure 3 represents the pH-dependence of permeability constant of phenylethylene glycol through polyelectrolyte complex capsules consist-

		Composition		
Capsule	Diameter (mm)	Acrylic Acid (mol %)	Ethylenimine (mol %)	
PAA-PEI	$2.1\pm0.2$	55	45	
PAA(St-2.7)-PEI	$5.8\pm0.1$	48	52	
PAA(St-5.7)-PEI	$6.2\pm0.4$	49	51	
PAA(St-9.5)-PEI	$6.0\pm0.3$	53	47	

Table III Characterization of PAA(St)-PEI Complex Capsules



**Figure 2** pH-dependent release of phenylethylene glycol from the PAA(St-9.5)–PEI complex capsule at various pHs: ( $\blacksquare$ ) 2.0, ( $\bullet$ ) 4.0, ( $\blacktriangle$ ) 7.0, and ( $\bullet$ ) 8.5 pH. (A) Release profiles. (B) Plot of  $\ln[(C^f - C^t)/(C^f - C^i)]$  against time for the data in (A).

ing of PEI and PAA(St) with varying styrene unit contents. All capsules revealed a lower permeability in neutral and weakly acidic regions than in acidic and alkaline conditions. For the PAA–PEI complex capsule membrane, the permeability was minimum around pH 5 and increased with decreasing pH or increasing pH from that pH. The PAA(St-2.7)-PEI complex capsule membrane exhibited a similar pH-dependence of the permeability, and it became minimum at pH 4. However, the permeability in the neutral and weakly acidic regions was much lower than the capsule membrane without the styrene unit. The permeability constant of the PAA(St-2.7)-PEI capsule membrane around pH 4-5 was about six times lower than that of the PAA-PEI capsule. A further decrease in permeability was seen for the PAA(St-5.7)-PEI capsule membrane, of which the permeability constant in the weakly acidic pH region was about 20-fold lower than the PAA-PEI capsule. Therefore, it is considered that inclusion of styrene units decreases permeability of the polyelectrolyte complex capsule membranes. The polyelectrolyte complex containing PAA(St) becomes more hydrophobic with increasing styrene content. Because hydrophobic interactions between styrene units should make the capsule membrane more dense, the permeability decreases with styrene content. However, the PAA(St-9.5)-PEI capsule exhibited a higher permeability than PAA(St-5.7)-PEI capsule mem-



**Figure 3** The pH-dependence of permeability constant of phenylethylene glycol through polyelectrolyte complex capsule membranes consisting of PEI and various PAA(St): ( $\blacksquare$ ) PAA; ( $\bigoplus$ ) PAA(St-2.7); ( $\blacktriangle$ ) PAA(St-5.7); ( $\bigstar$ ) PAA(St-9.5).

		Composition		
Capsule	Diameter	Acrylic Acid	Ethylenimine	
	(mm)	(mol %)	(mol %)	
PAA-PEI(Bzl-2.4)	$egin{array}{llllllllllllllllllllllllllllllllllll$	52	48	
PAA-PEI(Bzl-6.0)		53	47	
PAA-PEI(Bzl-10.1)		53	47	
PAA-PEI(Bzl-16.7)		53	47	

Table IV Characterization of PAA-PEI(Bzl) Complex Capsules

brane. It is suggested that the PAA(St) having too many styrene units could not form polyelectrolyte complex efficiently with PEI because of its irregularity of negatively charged units in the copolymer chain.

The permeability of PAA-PEI capsule membrane changed to some extent in the weakly acidic and neutral regions depending on pH, although the polyelectrolyte complex was stable. Because the result of elemental analysis indicates that the number of carboxyl group in the membrane was slightly greater than that of amino group (Table III), protonation or deprotonation of carboxyl groups, which do not form the ion pair, might cause a slight change in the membrane structure. An alternative explanation may be that partial dissociation of polyelectrolyte complex occurred depending on pH. In contrast, permeabilities of the PAA(St-5.7)–PEI and the PAA(St-9.5)–PEI capsule membranes were kept low and hardly changed in weakly acidic and neutral pH regions. This fact suggests that hydrophobic interactions between styrene units stabilized polyelectrolyte complex membranes and make them more dense.

As mentioned above, permeability of all of the capsule membranes increased remarkably under acidic and alkaline conditions because dissociation of the polyelectrolyte complex took place. However, as is seen in Figure 3, the change in permeability in these pH regions became more significant for the PAA(St)-PEI capsule membranes than for the PAA-PEI capsule membrane. The polyelectrolyte complex was stabilized by hydrophobic interactions between styrene units and, hence, partial dissociation of the polyelectrolyte complex, depending on pH, was suppressed in the pH region between 3 and 7. However, when the ambient pH was decreased below 3 or increased above 8, dissociation of the polyelectrolyte complex took place suddenly, and, as a result, the drastic permeability change was induced in

the very narrow pH regions. The permeability in these pH regions was suppressed to some extent by the existence of styrene units. However, the influence of these units was not so significant as it was in the weakly acidic and neutral pH regions. Thus, the capsule membranes containing styrene units revealed a large magnitude of permeability change in the acidic and the alkaline regions.

## Permeability of PAA–PEI(Bzl) Complex Capsule Membranes

We also prepared polyelectrolyte complex capsules using PEI(Bzl)s as polycations with hydrophobic units because every monomer unit can be charged in these polymers, unlike PAA(St). These polymers are expected to form a polyelectrolyte complex so efficiently with polyanions as unmodified PEI does. Four kinds of PAA-PEI(Bzl) complex capsules were prepared using PEI(Bzl-2.4), PEI(Bzl-6.0), PEI(Bzl-10.1), and PEI(Bzl-16.7). Diameter and composition of these capsules are summarized in Table IV. These capsules had similar diameters, of approximately 4.5 mm, and the diameter was not affected by the content of benzylated unit of the polycation. However, these diameters were larger than the PAA-PEI capsule (ca. 2.1 mm diameter). Because the viscosity of PEI(Bzl) solutions was higher than the unmodified PEI, complexation of PEI(Bzl) with PAA proceeded more slowly than was the case of PEI. Therefore, the size of the droplet of PAA solution increased during the capsule formation due to the osmotic pressure of PAA solution. The unit ratio of acrylic acid to ethylenimine in the PAA-PEI(Bzl) capsule membranes was almost same to that of PAA-PEI complex capsule and hardly affected by the hydrophobic units of the polycation.

Figure 4 represents the pH-dependence of the permeability of PAA–PEI(Bzl) capsule membranes. The capsule membranes containing the



**Figure 4** The pH-dependence of the permeability constant of phenylethylene glycol through polyelectrolyte complex capsule membranes consisting of PAA and various PEI(Bzl): ( $\blacksquare$ ) unmodified PEI; ( $\bigcirc$ ) PEI(Bzl-2.4); ( $\blacktriangle$ ) PEI(Bzl-6.0); ( $\diamondsuit$ ) PEI(Bzl-10.6); ( $\blacktriangledown$ ) PEI(Bzl-16.7).

benzylated units revealed much lower permeability than the PAA-PEI complex capsule membrane. As shown in the above section, the use of PAA(St) with too many styrene units increased the permeability of the membrane in the weakly acidic and neutral pH regions (Fig. 3). In the case of PAA-PEI(Bzl) capsules, however, such a significant increase in the permeability was not observed, even when PEI(Bzl-16.7) was used as the membrane component, indicating that efficient polyelectrolyte complex formation occurred between PAA and PEI(Bzl) with the high benzylated unit content.

## Water Content of Capsule Membranes

In the previous study,<sup>12</sup> we showed that permeability of the polyelectrolyte complex capsule membrane is controlled by its water content. Thus, the effect of hydrophobic units on the water content of the capsule membrane was investigated. Figure 5 depicts the pH-dependence of water content of PAA(St)–PEI complex capsule membranes. We expected that the water content will decrease with increasing hydrophobic unit content because the hydrophobic units would

make the membrane dense by their hydrophobic interactions. In fact, the permeability of PAA(St)-PEI capsule membranes was much lower than that of the PAA–PEI capsule membrane (Fig. 3). However, the water content of the PAA(St)–PEI capsule membrane was higher than that of PAA-PEI capsules. Also, the PAA(St)-PEI complex capsule membranes exhibited similar water content values under weakly acidic conditions, where a significant difference in the permeability was observed among these capsule membranes. This discrepancy may be explained by means of difference in parts of the membrane, which mainly influence the water content and the permeability. In the process of capsule formation, the capsule membranes were formed by complexation of the polyelectrolytes at the interface of their solutions. The polyelectrolyte chains were necessary to diffuse across the growing polyelectrolyte complex membrane. However, diffusion of the chains might be depressed with increasing membrane thickness. Therefore, the resultant membranes are considered to have asymmetrical structures, as follows: a PAA-rich inner surface and a PEIrich outer surface. Also, diffusion of the polyelectrolytes should be suppressed more strongly when the growing membrane is less permeable. Thus,



Figure 5 The pH-dependence of water content of polyelectrolyte complex capsule membranes consisting of PEI and various PAA(St): (■) PAA; (●) PAA(St-2.7); (▲) PAA(St-5.7); (◆) PAA(St-9.5).



**Figure 6** The pH-responsive release of phenylethylene glycol from various polyelectrolyte complex capsules: (A) PAA–PEI; (B) PAA(St-5.7)–PEI; (C) PAA–PEI(Bzl-6.0).

the capsule membranes having hydrophobic groups, which revealed a lower permeability, might be more asymmetrical. The polyelectrolyte chains located around the surface of the membrane could not form polyelectrolyte complex so efficiently as those in the center of the capsule membrane. The water content of the membrane might be reflected mainly by the surface region because many charged groups, which were highly hydrated, remained in that region, in contrast to the permeability, which is considered to be controlled by the dense part of the membrane, probably the center of the membrane.

### pH-Responsive Release from Capsules

We examined the influence of the hydrophobic units on responsiveness of the capsule. The capsule was alternately soaked in solutions of different pHs and the release of phenylethylene glycol from the capsule was monitored. Figure 6 shows the pH-responsive release of the contents from the PAA-PEI, the PAA(St)-PEI, and the PAA-PEI(Bzl) complex capsules. As is seen in Figure 6(A), the release from the PAA–PEI complex capsule was enhanced immediately after changing the pH of the outer phase from 4 to 2. However, when the pH was returned to 4, a few minutes' time lag was observed before the release rate decreased, indicating that several minutes are needed for reformation of the polyelectrolyte complex membrane.

The release profile of PAA(St-5.7)-PEI complex capsule is shown in Figure 6(B). The capsule started to release immediately after the alteration of pH from 4.0 to 2.0. However, the release did not stop by returning the pH to 4.0. When the capsule was immersed in the solution of pH 2.0, protonation of carboxylate groups and simultaneous dissociation of the complex occurred, resulting in the release. However, when the pH was returned to 4.0, this pH condition was not high enough to induce hydration and ionization of the polyanion, which had taken a compact conformation due to the hydrophobic interaction between styrene units at pH 2.0. Thus, reformation of the polyelectrolyte complex membrane did not take place, and the capsule did not stop the release of the contents.

In contrast, the PAA–PEI(Bzl-6.0) capsule could regulate initiation and cessation of the release, responding to the pH alteration [Fig. 6(C)]. Because of the protonation of carboxylate groups on PAA chains, dissociation of the polyelectrolyte complex occurred. However, because the PAA chain does not have hydrophobic groups, it might not undergo such a significant conformational change as PAA(St-5.7) did at pH 2.0. Therefore, PAA could react with the polycation and reform the polyelectrolyte complex membrane immediately after returning pH to 3.0. Considering the result of titration measurement (Fig. 1), it is supposed that the PAA chain does not possess negative charges at this pH condition. However, existence of PEI may promote ionization of PAA by forming the polyelectrolyte complex.<sup>27,28</sup> Also, it is likely that hydrophobic groups on the PEI(Bzl) chain enhanced ionization of carboxyl groups by stabilizing the polyelectrolyte complex.<sup>25</sup> Thus, the capsule achieved self-regulation of the release in the on-off-like fashion, responding quickly to the environmental pH alteration.

In conclusion, it was found that the permeability of the PAA–PEI complex capsule membranes decreased greatly in the weakly acidic and neutral pH regions by the incorporation of hydrophobic units in the polyelectrolytes. In contrast, the hydrophobic units affected permeability of the capsule membranes slightly in the acidic and the alkaline regions. As a result, the capsule membranes containing hydrophobic groups revealed the drastic permeability change in the narrow pH regions. It was also shown that responsiveness of the capsule to environmental pH can be improved by the use of polyelectrolyte having hydrophobic units.

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