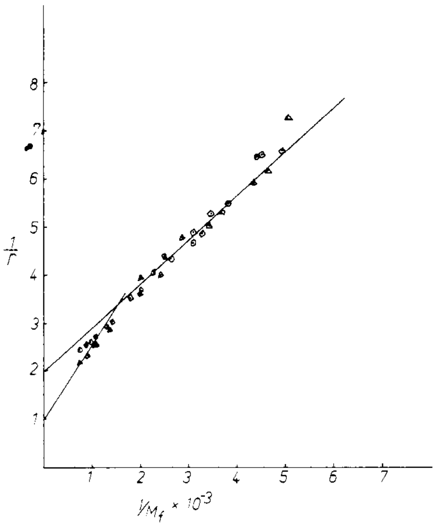

Figure 7. Determination of stability constants and coordination numbers for Zn-PMA complexes (2.5×10^{-3} M polymer concentration, $I = 0.10$). α: (○) 0.45; (△) 0.60; (□) 0.75.

tions are in perfect agreement with the results obtained from conductance titrations.

Leading from the above suggestions further, we have attempted to find support for the above suggestions from dialysis studies. In this respect, we apply the general multiple equilibrium theory to the following equilibria:

$$\text{-COO}^- + \text{Zn}^{2+} \overset{K_1}{\rightleftharpoons} \text{-COOZn]}^+ \quad (2)$$

(at high $[\text{Zn}^{2+}]_f$)

$$\text{-COOZn]}^+ + \text{-COO}^- \overset{K_2}{\rightleftharpoons} \text{-COOZnOOC-} \quad (3)$$

(at low and medium $[\text{Zn}^{2+}]_f$)

It can be shown that

$$\frac{1}{r} = \frac{n}{k}\frac{1}{[\text{Zn}^{2+}]_f} + n \quad (8)$$

where n is the number of carboxylate groups combining with the zinc ion, r is the ratio of carboxylate concentration in the solution to the concentration of the combining zinc ions, and k is the apparent equilibrium constant. n should have more than one value if more than one type of complex is formed in the system. From eq 8 the value of k can be obtained once the value of n is obtained from the intercept on the ordinate. A plot of $1/r$ against $1/[\text{Zn}^{2+}]_f$ is shown in Figure 7. $1/r$ rises linearly with $1/[\text{Zn}^{2+}]_f$, but with two distinct segments. This reveals the existence of two complexes having different binding constants. The intercept gave values of 2 and 1, respectively, corresponding to an initial formation of a 2:1 complex at low and medium $[\text{Zn}^{2+}]_f$ and then some 1:1 complex at high $[\text{Zn}^{2+}]_f$. But, as can be seen from Figure 7, the 2:1 complex predominates. Values of $K_1 = 625$ and $K_2 = 2.183 \times 10^3$ have been obtained, confirming that the 2:1 complex is the more stable complex species present.

In addition, a plot of the maximum amount of $[\text{-COO}^-]_b$ against the maximum amount of $[\text{Zn}^{2+}]_b$ at varying degrees of ionization gave a slope of 2, showing clearly that the only stable complex formed is a 2:1 complex and that such a complex would lead to dimerization of the molecules in solution.[6]

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Hydrophobic Interaction between Alkyl Side Chains of Polyions and Alkyl Quaternary Ammonium Ions

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ABSTRACT: The degree of hydrophobic interaction between the alkyl side chain of polyion and alkyl quaternary ammonium ion was investigated by conductivity, potentiometric titration, and viscosity measurements. Alternating maleic acid-ethylene, -methyl vinyl ether, -ethyl vinyl ether, -butyl vinyl ether, and -isobutyl vinyl ether copolymers were used as specific polyions and $(CH_3)_4N^+$, $(C_2H_5)_4N^+$, $(C_3H_7)_4N^+$, and $(C_4H_9)_4N^+$ were employed as hydrophobic counterions. According to conductivity measurements, the larger the alkyl side chain, the more effective the hydrophobic interaction at full neutralization.

Introduction

The hydrophobic interaction remains one of the most important unsolved problems in understanding biological functions and the molecular structure of biological systems.

For example, for the ordering of lipid molecules in the biomembrane and the regulation of protein association and dissociation, it is well-known that hydrophobic interactions play an important role.[1,2] In the study of these subjects polyelectrolytes having both hydrophobic side chains and ionizable groups are believed to be good models of biopolymers. In this work, alternating copolymers of maleic acid and various alkyl vinyl ethers were used as polye-

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lectrolytes satisfying these conditions. As hydrophobic counterions, various tetraalkylammonium ions, which have been used in the study of hydrophobic interactions with a chain polymer[3] and ion-transport phenomena in biomembranes[4] were employed.

Generally, in the study of hydrophobic interaction from the standpoint of thermodynamics, the method of microcalorimetry has been often used.[2,5] In this work, as a rather convenient experimental method to measure both hydrophobic interaction and electrostatic interaction simultaneously, conductivity measurements have been used, and by measuring a feature of counterion binding, the strength of the hydrophobic interaction was analyzed as a function of the length of the alkyl chain.

Experimental Section

A. Materials. Alternating copolymers of ethyl vinyl ether and maleic anhydride [p(EVE-co-MA)], isobutyl vinyl ether and maleic anhydride [p(iBVE-co-MA)], and *n*-butyl vinyl ether and maleic anhydride [p(nBVE-co-MA)] were synthesized by free radical polymerization in the presence of equimolar alkyl vinyl ether and maleic anhydride in benzene solution, initiated with 0.1 g (per 100 mL) of azobis(isobutyronitrile). The detailed method of polymerization has been described elsewhere.[6]

Alternating copolymer of ethylene and maleic anhydride [p(Et-co-MA)] and methyl vinyl ether and maleic anhydride [p(MVE-co-MA)] were purchased from Scientific Polymer Products Inc. The bromides of methyl, ethyl, propyl, and butyl quaternary ammonium ions, and the hydroxides of these, were Merck Special Grade. Contamination of these salts and hydroxides by other ions was checked by comparing the electrical conductivity value of the simple salt and hydroxide solution with the literature value.

The sodium hydroxide employed for standarization and potentiometric titration for the various systems investigated was prepared by diluting a saturated solution of NaOH with redistilled water. About 1.0 g of these copolymers was completely hydrolyzed in 50 mL of 1 M NaOH solution at 5 °C in the dark. In aqueous solutions the anhydride units of these copolymers hydrolyze to form dicarboxylic acids. The sodium salts of these copolymers were dialyzed against deionized redistilled water at 5 °C to remove excess hydroxide and low molecular weight fragments.

Sodium salts of the copolymers were then converted completely to the acid form by exchanging H^+ counterions for Na^+ ions with Dowex-50 resin. Removal of the main impurities and low molecular weight fragments was performed by diafiltration in a 65-mL stirred ultrafiltration cell (52 Amicon Corp.), using a Diaflow membrane (10 μm, molecular weight cutoff of 10 000) under nitrogen gas at 5 °C. This procedure was repeated several times by adding redistilled water. This ultrafiltration method was also very convenient for concentrating the polyelectrolyte solution. Standardization of the acid form of these polyelectrolytes was performed after ultrafiltration by titration against standard $Ba(OH)_2$ in the presence of $BaCl_2$.

The neutralization of the acid form of the polyelectrolyte by various quaternary ammonium hydroxides was also performed by titration against standardized quaternary ammonium hydroxide.

B. Measurements. Conductivity measurements were performed by the method described in the previous paper,[7] and all measurements were carried out 8-kHz at 25.0 ± 0.01 °C. Viscosity measurements and potentiometric titrations were carried out by a method described elsewhere.[6,7]

Results and Discussion

A. Potentiometric Titration of the Polymer Solution. In Figure 1, values of pH + log $[(1 - \alpha)/\alpha]$ for p(iBVE-co-MA) and p(nBVE-co-MA) at a constant polyion concentration (0.0035 mol/L per mole of monomer unit) are plotted against the degree of dissociation, α, neutralized with Bu_4NOH in the presence of 0.01 mol/L Bu_4NBr.

Abnormalities are observed in this titration curve, which is different from that of poly(acrylic acid). First, there is an abnormal rise of pH + log $[(1 - \alpha)/\alpha]$ in the low α region ($\alpha < 0.1$), which is also observed for poly(methacrylic acid)[8] and maleic acid copolymers having large alkyl side chains in a simple salt.[9,10] Second, the plots of pH + log $[(1 - \alpha)/\alpha]$ vs. α show a downward concavity in the broad α region (α = 0.2–0.5). These results indicate that these copolymers undergo a conformational transition from a compact globular form to an extended coil conformation in the α range $0.2 < \alpha < 0.5$. Finally, in the very low α region ($\alpha > 0.15$), these copolymers have a kind of supercompact conformation and in the high α region ($\alpha > 0.5$) an expanded rodlike coil conformation, which has been found for the other copolymers of maleic acid and various alkyl vinyl ethers, independent of the length of the alkyl chains.[5,9]

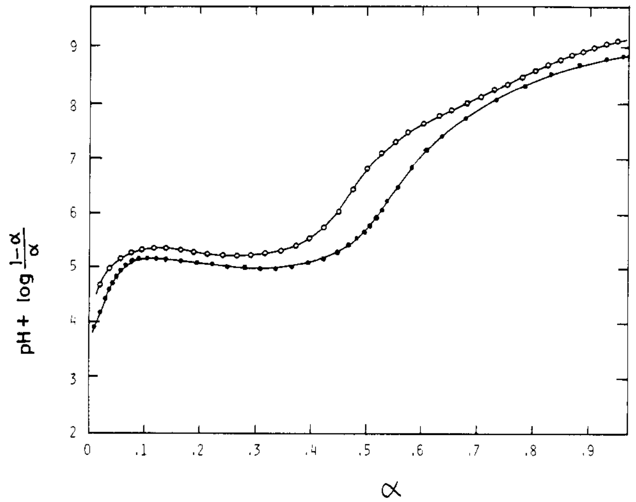

Figure 1. pH + log [(1 $(CH_3)_4NBr$ α)/α] vs. degree of dissociation, α, neutralized with Bu_4NOH for p(iBVE-co-MA) and p(nBVE-co-MA) at a constant polyion concentration of 0.0035 mol/L per monomole in the presence of 0.01 mol/L Bu_4NBr at 25 °C: (○) p(nBVE-co-MA); (●) p(iBVE-co-MA).

B. Viscometric Study of the Polymer Solution. In Figure 2, values of the reduced viscosity, η_{sp}/C_p, for p(iBVE-co-MA) neutralized with Bu_4NOH and Et_4NOH are shown as a function of the degree of neutralization β in the presence of Bu_4NBr and Et_4NBr, respectively. In the case of Bu_4N^+ counterion, this polyion shows a precipitation phase in the low β region ($\beta < 0.3$); on the other hand, with the Et_4N^+ counterion, precipitation is never observed over the whole β region. This result demonstrates that the Bu_4N^+ ion interacts with the alkyl side chain of the polyion more hydrophobically than the Et_4N^+ ion in the low β region, where the charged groups are barely exposed to the outside, so that the solubility of this polyion with Bu_4N^+ counterions is very small. On the other hand, in the high β region, these quaternary ammonium ions are bound to the polyion only by the electrostatic interaction in spite of the high charge density, being different from the case for simple monovalent counterions such as Na^+. The higher values of η_{sp}/C_p for the Bu_4N^+ counterion compared to those for Et_4N^+ counterion may be due to the fact that the shielding effect against the electric field around the polyion by the Et_4N^+ counterion is slightly greater than that by Bu_4N^+ counterion because the Et_4N^+ ion is smaller.

C. Conductometric Study of the Polymer Solution. In Figure 3, the specific conductivities of the tetramethyl quaternary ammonium ion salt solutions of various polyelectrolytes are shown as a function of increasing $(CH_3)_4NBr$ concentration. Obviously, the additivity law of specific conductivity for the p(Et-co-MA) and $(CH_3)_4NBr$ system holds, while for the other polyelectrolytes it does not. Similar results were obtained for the other

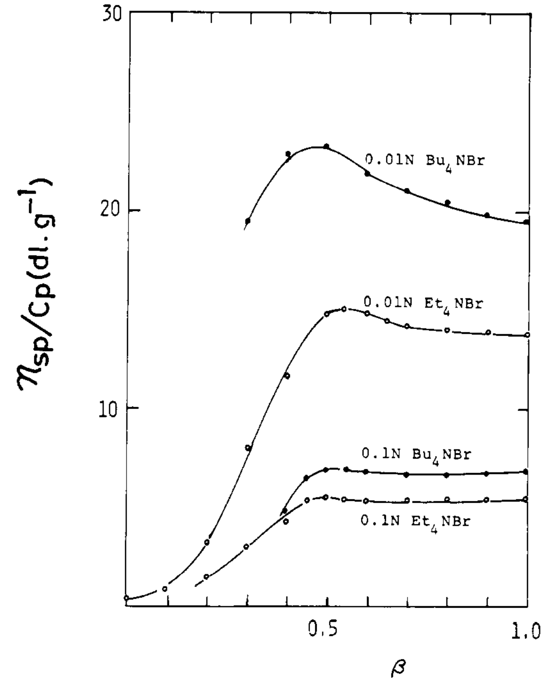

Figure 2. Reduced viscosity, η_{sp}/C_p, vs. degree of neutralization β neutralized with Bu_4NOH and Et_4NOH at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C in the presence of Bu_4NBr and Et_4NBr as indicated: (●) Bu_4N^+ counterion; (○) Et_4N^+ counterion.

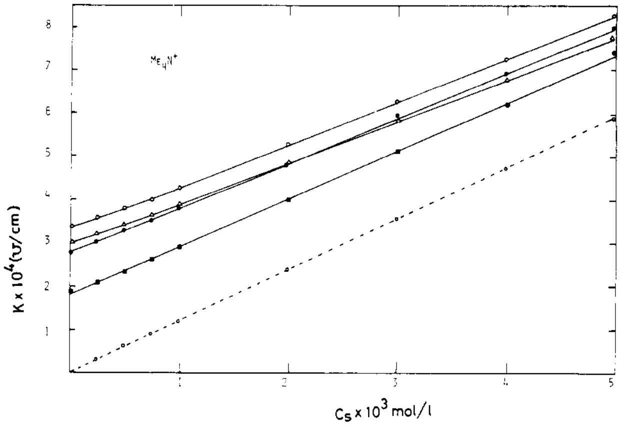

Figure 3. Specific conductivities vs. added $(CH_3)_4NBr$ concentration for p(Et-co-MA), p(MVE-co-MA), p(iBVE-co-MA), and p(nBVE-co-MA) at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C: (■) p(Et-co-MA); (●) p(MVE-co-MA); (△) p(iBVE-co-MA); (○) p(nBVE-co-MA).

tetraalkylammonium ions, $(C_2H_5)_4N^+$, $(C_3H_7)_4N^+$, and $(C_4H_9)_4N^+$.

In Figure 4, the specific conductivities of the various quaternary ammonium ion salt solutions of p(Et-co-MA) and those of simple quaternary ammonium bromide salts are shown as a function of the added quaternary ammonium bromide. These results show that a specific interaction between these quaternary ammonium ions and p(Et-co-MA), which has no alkyl side group, does not occur. As is well-known, the specific interactions observed for the other polyelectrolytes are thought to be due to hydrophobic forces between alkyl side chains on the polyion and the alkyl groups of the quaternary ammonium ions.[11]

Generally, the specific conductivity K_1 (in S/cm) of a polyelectrolyte solution in the presence of added low molecular weight salt (salt concentration, C_s, in mol/L) at a degree of neutralization β may be expressed by the limiting equivalent conductivities of the positive and negative ions of the added salt, $\lambda_+°$ [$=10^{-3}\Lambda_+°$ in (S·L)/(mol·cm)] and $\lambda_-°$ [$=10^{-3}\Lambda_-°$ in (S·L)/(mol·cm)], and that of the originally bound counterions, $\lambda_c°$ [$=10^{-3}\Lambda_c°$ in (S·L)/(mol·cm)], as follows:[11]

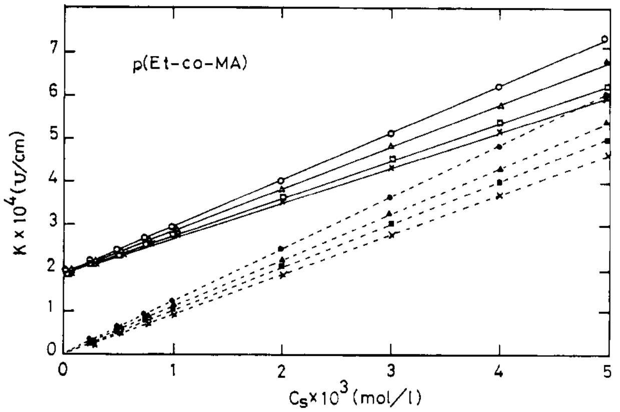

Figure 4. Specific conductivities (S/cm) of various quaternary ammonium ion salts of p(Et-co-MA) vs. added $(CH_3)_4NBr$, $(C_2H_5)_4NBr$, $(C_3H_7)_4NBr$, and $(C_4H_9)_4NBr$ concentration (mol/L) at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C: (○) $(CH_3)_4N^+$ salt of p(Et-co-MA); (△) $(C_2H_5)_4N^+$ salt of p(Et-co-MA); (□) $(C_3H_7)_4N^+$ salt of p(Et-co-MA); (-×-) $(C_4H_9)_4N^+$ salt of p(Et-co-MA); (●) $(CH_3)_4NBr$; (▲) $(C_2H_5)_4NBr$; (■) $(C_3H_7)_4NBr$; (--×--) $(C_4H_9)_4NBr$.

$$K_1 = [z(\beta C_p - C_b)^2/C_p]\lambda_{se} + (\beta C_p - C_{bg})\lambda_c° + \gamma_+(C_s - C_{bs})\lambda_+° + \gamma_- C_s\lambda_-° + a_H\lambda_H° + a_{OH}\lambda_{OH}° \quad (1)$$

where λ_{se} is the equivalent conductivity of a polymer segment with z ionizable groups, C_{bg} (in mol/L) is the concentration of the bound counterions in the absence of salt, C_{bs} (in mol/L) is the increase of the bound counterions resulting from added salt, γ_+ and γ_- are the activity coefficients of positive and negative low molecular weight ions, and $\lambda_H°$ ($=10^{-3}\Lambda_H°$) and $\lambda_{OH}°$ ($=10^{-3}\Lambda_{OH}°$) are limiting equivalent conductivities [in (S·L)/(mol·cm)] of the H^+ ion and OH^- ion whose activities are a_H and a_{OH}, respectively. The concentration of total bound low molecular ions to the polyion, C_b (in mol/L), can be given by

$$C_b = C_{bg} + C_{bs} \quad (2)$$

On the other hand, when the additivity law is perfectly obeyed, the specific conductivity of a polyelectrolyte solution with added low molecular salt can be expressed as follows:

$$K_2 = fC_p(\lambda_p + \lambda_+°) + \gamma_+ C_s\lambda_+° + \gamma_- C_s\lambda_-° + a_H\lambda_H° + a_{OH}\lambda_{OH}° \quad (3)$$

Here f is the fraction of free counterion which is independent of the added salt concentration. The subtraction of K_1 from K_2 gives

$$K_2 - K_1 = \{(f - 1)C_p + C_b\}(\lambda_p + \lambda_+°) \quad (4)$$

The equivalent conductivity of the polyion, λ_p, is believed to be determined by the molecular structure of the polyion and to be almost independent of the species of counterion. Actually, according to the results of the potentiometric titration, it can be concluded that the conformation of the polyion is almost independent of the counterion species at $\alpha = 1.0$, as indicated in Figure 1. Accordingly, by the analysis of $K_2 - K_1$ behavior as a function of C_s, the feature of the specific counterion binding (hydrophobic interaction

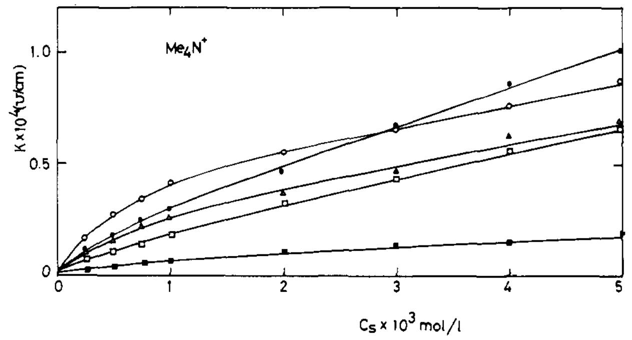

Figure 5. Difference of specific conductivity, $K_2 - K_1$, vs. added $(CH_3)_4NBr$ concentration at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C: (■) p(Et-co-MA); (□) p(MVE-co-MA); (△) p(EVE-co-MA); (○) p(iBVE-co-MA); (●) p(nBVE-co-MA).

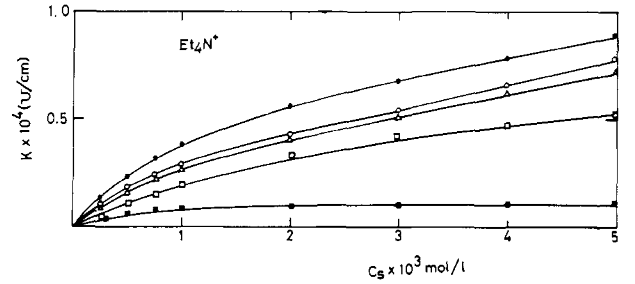

Figure 6. Difference of specific conductivity, $K_2 - K_1$, vs. added Et_4NBr concentration at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C: (■) p(Et-co-MA); (□) p(MVE-co-MA); (△) p(EVE-co-MA); (○) p(iBVE-co-MA); (●) p(nBVE-co-MA).

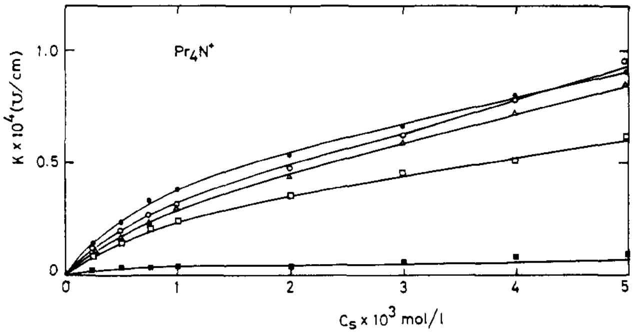

Figure 7. Difference of specific conductivity, $K_2 - K_1$, vs. added Pr_4NBr concentration at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C: (■) p(Et-co-MA); (□) p(MVE-co-MA); (△) p(EVE-co-MA); (○) p(iBVE-co-MA); (●) p(nBVE-co-MA).

in this case) can be obtained.

In Figures 5 (Me_4N^+), 6 (Et_4N^+), 7 (Pr_4N^+), and 8 (Bu_4N^+), the values of the left side of eq 4 are shown as a function of the added salt concentration. As seen in these figures, the values of $K_2 - K_1$ for p(Et-co-MA) are negligibly small in comparison with those of the other copolymers. These results show that no hydrophobic interaction occurs between quaternary ammonium ions and p(Et-co-MA), which has no alkyl side chain, as observed by conductivity measurements.

Discussion

At full neutralization, charged groups of polyions are expected to form layers with a special water structure around them. The formation of this hydration layer is cooperatively accelerated due to overlapping of the special water structure around neighboring charged sites, and this hydration layer is expected to form a uniform cylindrical

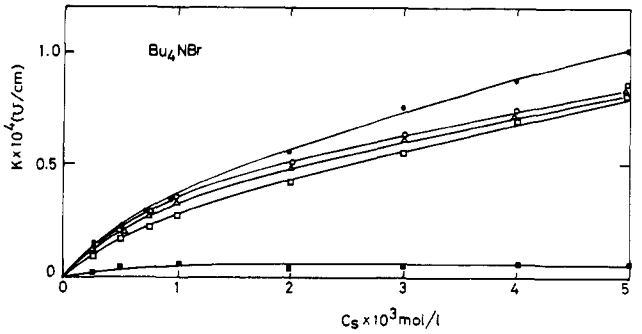

Figure 8. Difference of specific conductivity, $K_2 - K_1$, vs. added Bu_4NBr concentration at a constant polyion concentration of 0.0018 mol/L per monomole at 25 °C: (■) p(Et-co-MA); (□) p(MVE-co-MA); (△) p(EVE-co-MA); (○) p(iBVE-co-MA); (●) p(nBVE-co-MA).

region around this polyion. A simple monovalent counterion such as Na^+ or H^+ is supposed to enter the hydration layer and destroy the special water structure. On the other hand, a large hydrophobic counterion such as Bu_4N^+ approaches the polyion due to electrostatic interaction but never destroys the special water structure. It is therefore observed that quaternary ammonium ions are never bound to the polyion, which has a uniform cylindrical hydration layer, as observed in the case of poly(acrylic acid).[12]

Special water structures, as observed in the case of the above polyion, are never formed around a tetraalkylammonium ions, which is instead surrounded by a space without water, which is further enclosed by a cage of water molecules linked together by hydrogen bonds.[13]

When the polyion has nonpolar side residues, the special water structure formation is weakened because these nonpolar groups hinder the smooth arrangement of water molecules. The larger the nonpolar group, the greater the change in the water structure. Therefore, in the vicinity of the large alkyl residue, as in the case of C_4H_9, the special water structure is never formed. Accordingly the alkyl side residues are supposed to interact with the quaternary ammonium ions through this space.

On the other hand, on the basis of potentiometric titration results Strauss et al.[14] concluded that the electrostatic attractive force between negative charged sites and positive cations increases because of the decrease of the local dielectric constant due to the nonpolar groups. This conclusion is contrary to the result that the larger the alkyl side chain, the more the amount of bound quaternary ammonium ions. From the standpoint of thermodynamics, Chen et al.,[2] using microcalorimetry, concluded that bulky tetraalkylammonium ions are never bound to the ionized sites on a protein but should be bound to the hydrophobic area on the protein due to hydrophobic interactions. With regard to transport phenomena, the quanitity $\partial(K_2 - K_1)/\partial C_s$ shows the increase of bound alkyl quanternary ammonium ions due to hydrophobic force in the course of adding quaternary ammonium ion; in other words, it shows the strength of the hydrophobic interactions. Especially, in the low added salt concentration region, it may indicate the strength of hydrophobic interaction per polyion and per counterion. When a nearly equivalent amount of quaternary ammonium ion is added, it may show the degree of bound quaternary ammonium ions in the stoichiometrically saturated region. In Table I, the quantity $\partial(K_2 - K_1)/\partial C_s$ is shown as a function of the length of the alkyl side chain in the low and high salt concentration regions. Obviously, the above results show that the amount of quaternary ammonium ions bound to the polyion increases in the order Bu_4NBr > Pr_4NBr >

Table I
Values of $\partial(K_2 - K_1)/\partial C_s \times 10^3$ in (S·L)/(cm·mol) for Various Polyions and Various Quaternary Ammonium Ions[a]

	Me_4N^+	Et_4N^+	Pr_4N^+	Bu_4N^+
p(Et-co-MA)	8	12	5	7
	2	0	0.7	0.5
p(MVE-co-MA)	22	22	29	32
	12	6.5	7	12
p(EVE-co-MA)	30	32	34	38
	6	10	13	16
p(iBVE-co-MA)	36	38	38	42
	16	12	16	11
p(nBVE-co-MA)	56	48	48	46
	11	10	12	15

[a] For each copolymer, the first row of values is for the low salt region ($C_s < 0.0008$ mol/L) and second row is for the saturated region.

$Et_4NBr > Me_4NBr$ and the strength of hydrophobic interaction per alkyl side chain follows the order n-$C_4H_9 \sim i$-$C_4H_9 > C_2H_5 > CH_3$. Accordingly, it can be concluded that tetraalkylammonium ions must be bound to the polyion due to hydrophobic interactions.

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Sodium Ion Diffusion in Aqueous Salt-Free Heparin Solutions

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ABSTRACT: Sodium ion tracer diffusion coefficients have been determined in aqueous salt-free heparin solutions at 25 °C over a concentration range of 4×10^{-5} to 8×10^{-2} N. With increasing dilution, the Na^+ diffusion coefficients decreased, reached a shallow minimum, and then increased. The data are discussed in light of the Manning and Yoshida theories. Condensation of multivalent ions onto heparin is demonstrated in aqueous salt solutions of excess polyelectrolyte to salt concentration.

It has been demonstrated[1,2] that the charge fraction of polyelectrolyes, i.e., the fraction of stoichiometric charge on the polyelectrolyte uncompensated by bound counterions, of high charge density can be determined by using the Manning theory[3-5] together with the experimentally determined counterion and coion tracer diffusion coefficients in aqueous solution containing simple salt. With Na^+ as the counterion, rN_p equivalents of Na^+ of the total $N_p + N_s$ equivalents are condensed on the polyion and the fraction of Na^+ in the solution which is condensed, $f^c{}_{Na^+}$, is[1,2]

$$f^c{}_{Na^+} = rN_p/(N_p + N_s) = rX/(X + 1) \qquad (1)$$

where $X = N_p/N_s$, N_p and N_s are the equivalent concentrations of polyelectrolyte and salt, respectively, and r is the fraction of condensed or bound Na^+ ions originally on the polyelectrolyte and $1 - r$ is the charge fraction of the polyelectrolyte. From the Manning theory we have shown[1,2] that

$$f^c{}_{Na^+} = 1 - (D_{Na^+}/D_{Na^+}{}^\circ)(D_{Cl^-}/D_{Cl^-}{}^\circ)^{-1} \qquad (2)$$

where D_i and $D_i{}^\circ$ are the small-ion i diffusion coefficients in the presence of polyelectrolyte and at infinite dilution of salt in the absence of polyelectrolyte, respectively. Plots of eq 1 as $f^c{}_{Na^+}(X + 1)$ vs. X for several polyelectrolytes were found to be linear over the range of X studied.[1,2] The agreement for the experimental r values with the Manning theory predictions for monovalent counterions, $r = 1 - \xi^{-1}$, was excellent for the sodium salts of dextran sulfate, alginate, and poly(styrenesulfonate) and was good for DNA and heparin.[1,2] The fraction of Ca^{2+} ions condensed on heparin[1] and on DNA[2] determined from calcium and coion diffusion coefficients was found to be in excellent agreement with that predicted for divalent counterions from the Manning theory, $r = 1 - (2\xi)^{-1}$. Thus, the general experimental accord with the Manning theory for highly charged polyelectrolytes showed that the charge fraction in salt solutions was found to be constant, independent of ionic strength, independent of the ionic group of the polyelectrolyte, and dependent only on the charge density parameter ξ

$$\xi = e^2/\epsilon kTb \qquad (3)$$

where e is the protonic charge, ϵ is the dielectric constant of the solvent, T is the temperature, b is the average distance between charge groups on the polyelectrolyte, and k is the Boltzmann constant.

The salt-free limit of eq 1 gives $f^c{}_{Na^+} = r$, which according to the Manning theory is $r = 1 - (Z_1\xi)^{-1}$, indicating that the fraction of counterions dissociated from a polyion is constant and dependent only on its charge density[6] and Z_1, the valence of the counterion. Since this is inherent in the Manning theory, the salt-free limiting laws for the counterion diffusion coefficient D_{Na^+}, the counterion activity coefficient γ_{Na^+}, and the activity osmotic coefficient

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