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Interaction of calcium ion and maleic acid copolymer

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Abstract

Interaction between Ca^{2+} ion and poly(styrene-alt-maleic acid) was studied by using a Ca^{2+} ion sensitive electrode. The Ca^{2+} activity had a peak at a degree of neutralization of 0.5 and decreased with increasing $Ca(OH)_2$ concentration beyond it when the polymer solution was neutralized with $Ca(OH)_2$. The decrease in the Ca^{2+} activity was not observed when the polymer concentration was very low. The counter ion condensation theory did not hold for this solution except in the case of an extremely dilute solution. The additivity rule for Ca^{2+} was confirmed for this solution. When the maleic acid copolymer was neutralized with both $Ca(OH)_2$ and KOH, the Ca^{2+} activity had a peak at a degree of neutralization of 0.5 when neutralization with KOH was less than 0.3 and the Ca^{2+} activity decreased more drastically than that neutralized with only $Ca(OH)_2$. The appearance of the peak of the Ca^{2+} activity at a degree of neutralization of 0.5 was independent of the ratio of Ca^{2+} concentration to polymer concentration or absolute Ca^{2+} concentration, but depended on the degree of ionization, i.e., linear electric charge density on the polymer because of ionization of the carboxyl groups. Interpretations of the behavior of the Ca^{2+} activity are discussed.

Keywords: Maleic acid copolymer; Ca^{2+} activity; Counter ion condensation; Additivity rule; Ca^{2+} binding

1. Introduction

The counter ion condensation and the additivity rule of counter ion activity are important properties of polyelectrolytes and are derived from simple theories [1–6]. Most experiments in polyelectrolyte solutions have been performed with monovalent counter ions although these theories are also applicable to divalent counter ions. Only a few results have been reported concerning interaction between divalent counter ions and polyelectrolytes [7–9]. Many polyelectrolytes in aqueous solutons are precipitated in the presence of divalent counter ions. Divalent ions, such as alkaline earth metals or transition metals, are believed to be bound to carboxyl or sulfonate groups on polyelectrolytes, resulting in their precipitation. Divalent ions such as Ca^{2+} or Mg^{2+} play an important role in biological systems, and it is desirable to elucidate their interaction with biopolymers. For this reason, in this study the interaction of Ca^{2+} with an alternating copolymer of maleic acid and styrene (STMA) has been investigated because this copolymer does not precipitate in the presence of Ca^{2+} ions at low concentrations.

Ion activity in polyelectrolyte solutions was usually measured at a monomolar concentration of about 10^{-2}. Here, we have measured the Ca^{2+} ion activity by using a Ca^{2+} ion sensitive electrode at a low polymer concentration (0.1 mN) such that interpolymer interaction is negligible. We measured a very low Ca^{2+} activity of less than 10^{-7} M by improving the reference electrode. The pinhole at the tip of the reference electrode was made very small and the KCl concentration eluted from it was less than 10^{-5} M.

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Maleic acid copolymer does not bind Ca^{2+} chemically [7] as reported previously. Hydrogen ion titration of maleic copolymers suggested a conformational transition from a compact form at a low degree of ionization to a coiled conformation concomitant with the increase in the ionization [10–12]. It is interesting to know how a conformational change affects the interacton between Ca^{2+} ions and macro ions, if it happens.

2. Materials and methods

Poly(styrene-alt-maleic acid) (STMA) was purchased from Scientific Polymer Products, Inc. (New York). A stock STMA solution was prepared by dissolving STMA in dilute KOH. The solution was dialyzed against 10 mM HCl and finally dialyzed against pure water. The concentration of the stock solution was determined by titrating with $Ba(OH)_2$ solution. The 99.9% $Ca(OH)_2$ and $CaCl_2$ were purchased from Wako Pure Chemical Industries, Ltd., and $Ca(OH)_2$ was used in a saturated solution (contaminating carbonate was eliminated by centrifugation). Carbonate free KOH solution was prepared according to Powell and Miller [13] using a Chelex 100 (Bio Rad) column. The concentration of the alkaline solution was determined by titration with standard HCl solution (1.000 M; Wako Pure Chemical Industries). Ultrapure (Suprapur) KCl was purchased from Merck.

The Ca^{2+} activity was measured by a PHM85 pH meter using a F2112Ca Ca^{2+} ion sensitive electrode (Radiometer, Copenhagen) and a handmade silver–silver chloride reference electrode as described previously [14]. A small, thin pinhole with an electric resistance of ca. 400 KΩ was used. The concentration of KCl eluting from the reference electrode was measured using a K^+ ion sensitive electrode and was found to be less than 10^{-5} M under the experimental conditions used. The asymmetric potential was less than 0.5 mV in the presence of 1 mM KCl. The pH was measured using a G202B glass electrode (Radiometer). Nitrogen gas saturated with water vapor was flushed continuously over the titration solution. The cell was immersed in a temperature controlled bath (25 ± 0.02°C).

The degree of neutralization, β, of the STMA solution is defined as

$$\beta = [BOH]/[C_p] \tag{1}$$

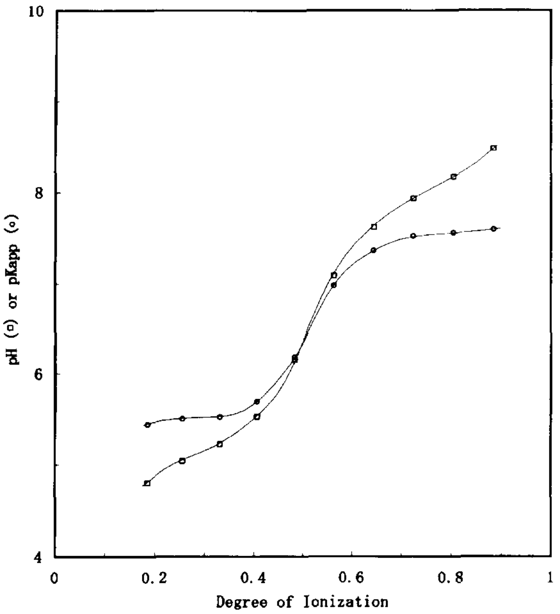

Fig. 1. Hydrogen ion titration of STMA. STMA solution was titrated in the absence of KCl with $Ca(OH)_2$: pH (□) and pK_{app} (○) are plotted against degree of ionization. pK_{app} is defined as, $pK_{app} = pH - \log(\alpha/(1-\alpha))$, where α is the degree of ionization. The degree of ionization at complete neutralization is defined as unity. The polymer concentration was 1 mN.

and the degree of ionization, α, is defined as

$$\alpha = ([BOH] + [H^+] - [OH^-])/[C_p] \tag{2}$$

where [BOH] is the normality of added base, $[H^+]$ and $[OH^-]$ are the molarities of free hydrogen and hydroxyl ions, respectively, and $[C_p]$ is the normality of the STMA. The degree of ionization at complete neutralization is defined as unity, and this is different from those of Strauss and co-workers [10,15] or Ohno et al. [11,12] who defined α to be 2 at complete ionization.

3. Results

The copolymer STMA has pairs of adjacent carboxyl groups and the carboxyl groups are believed to chelate the Ca^{2+} ion. The hydrogen ion titration curve of STMA with $Ca(OH)_2$ (Fig. 1) shows a two step ionization of carboxyl groups having two distinct pK-values corresponding to the first and the second ionization of the paired carboxyl groups, which is similar to the titration with KOH. Simizu et al. reported that the

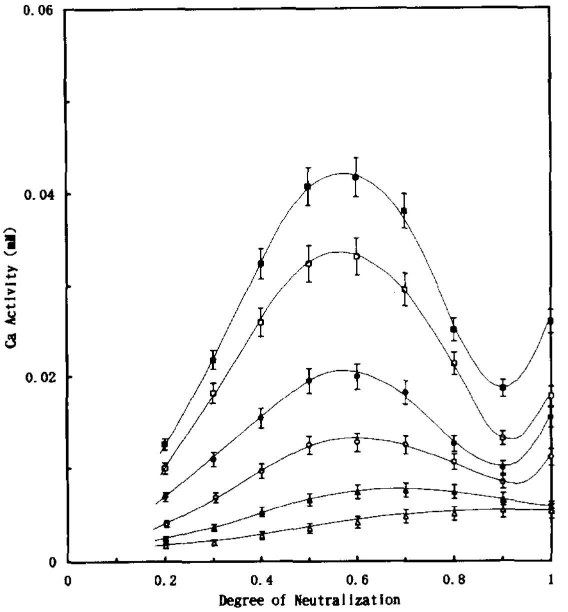

Fig. 2. Ca^{2+} activity vs. degree of neutralization. STMA solutions were neutralized with $Ca(OH)_2$ in the absence of salt ion. The STMA concentrations were 0.05 (△), 0.1 (▲), 0.2 (○), 0.4 (●), 0.6 (□), and 1 mN (■).

hydrogen ion titration curve of a copolymer [maleic acid and ethyl vinyl ether titrated with $Ca(OH)_2$] exhibited a two step dissociation behavior [9]. The pK-values titrated with $Ca(OH)_2$ were almost constant above $\alpha = 0.5$, showing that the ionization of a paired carboxyl group was not influenced by the ionization of the other carboxyl groups. The pK-values less than $\alpha = 0.5$ were greater than those reported by Shimizu et al. It was reported that STMA underwent a conformational transition from a compact form to an extended random conformation below $\alpha = 0.4$ [11,12], and the plateau-like high pK-values at $\alpha = 0.2$ to 0.4 may be a result of this conformational transition.

The Ca^{2+} activity of STMA solution neutralized with $Ca(OH)_2$ is shown in Fig. 2. When the STMA solution was neutralized with $Ca(OH)_2$ in the absence of salt ions, the Ca^{2+} activity increased with increasing β when $\beta \leq 0.5$, and decreased above $\beta = 0.5$ except when the polymer concentration was 0.05 mN. When $\beta > 0.5$ the Ca^{2+} activity was not constant as shown in Fig. 2, but decreased with increasing Ca^{2+} concentration. The steepness of the peaks decreased with decreasing STMA concentration and there was no peak at $C_p = 0.05$ mN. The minimum of the Ca^{2+} activity appeared at around $\beta = 0.8$ at $C_p \geq 0.4$ and the value of β at which Ca^{2+} activity was minimum approached unity at very low concentration.

If the counter ion condensation theory holds for this solution, the activity should increase linearly with increasing degree of neutralization until the ξ-value (defined below) reaches 1/2 and then remains constant:

$$\xi = e_0^2 / \epsilon kTd \tag{3}$$

where e_0, ϵ, and d is the proton charge, the dielectric constant of the solvent, and the distance between charges on the polymer, respectively. The value of α at which $\xi = 1/2$ is 0.18. The experimental result shows the counter ion condensation theory is apparently not applicable to Ca-STMA solutions except for an extremely dilute (0.05 mN) solution.

It is difficult to measure the Ca^{2+} activity precisely in salt free solution because the diffusion potential of the reference electrode is unstable in the absence of salt ions. The presence of salt ions, even as dilute as 1 mM, facilitates measurement of the Ca^{2+} activity. The curves of the Ca^{2+} activity versus the degree of neutralization in the presence of 1 mM KCl at different polymer concentrations were similar to those in the absence of KCl, although the peak values of the Ca^{2+} activity in the presence of 1 mM KCl were increased by ca. 50% of those in the absence of KCl. Therefore, we measured the Ca^{2+} activity in the presence of 1 mM KCl in the following experiments. This change in the conditions provided reasonable results.

The activity coefficient of Ca^{2+}.in the STMA solutions decreased with increasing degree of neutralization when the polymer concentration was less than 0.4 mN and was constant or rather increased slightly with increasing degree of neutralization for $0.2 \leq \beta \leq 0.5$ when the polymer concentration was > 0.4 mN as shown in Fig. 3. When the polymer concentration was > 0.4 mN, the Ca^{2+} activity coefficient at low degree of neutralization (i.e., 0.2) decreased remarkably as compared with that at low polymer concentration, (e.g., 0.05 mN).

In order to study whether the peak of the Ca^{2+} activity around $\beta = 0.5$ depends mainly on the ratio of the Ca^{2+} concentration to the carboxylate concentration or on the degree of ionization of the polymer, the maleic acid copolymer was partially neutralized with KOH, prior to titration with $Ca(OH)_2$. The degree of neutralization, β, titrated both with KOH and $Ca(OH)_2$ is defined as: $\beta = \beta_{KOH} + \beta_{Ca(OH)_2}$, where β_{KOH} or $\beta_{Ca(OH)_2}$ is the degree of neutralization with KOH or $Ca(OH)_2$, respectively. When the STMA solution was neutralized with KOH first and then with $Ca(OH)_2$, the Ca^{2+} activity decreased markedly compared to the Ca^{2+} activity titrated with $Ca(OH)_2$ only. This is because K^+ ions were not bound to the macro ion leading to a larger increase in the electric charge on it. The electric charge on the polymer increased compared to that titrated with $Ca(OH)_2$ only, and hence the interaction between Ca^{2+} and the polymer was strengthened. The Ca^{2+} activity has a maximum at $\beta = 0.5$, irrespective of the amount of KOH added at first when the degree of neutralization with KOH was less than 0.3 (Fig. 4). The result shows that the degree of ionization of the polymer was essential for the peak at $\beta = 0.5$ to occur. The absolute concentration of Ca^{2+} or the concentration ratio of Ca^{2+} to the polymer was not essential to the decrease in Ca^{2+} activity.

The activity coefficient of Ca^{2+} titrated with mixed alkali increased slightly with increasing degree of neutralization for $0.2 \leq \beta \leq 0.4$ similar to the titration with $Ca(OH)_2$, as shown in Fig. 5.

In order to confirm that the interaction between Ca^{2+} and the macro ion is mainly electrostatic, we examined whether or not the additivity rule for Ca^{2+} holds for

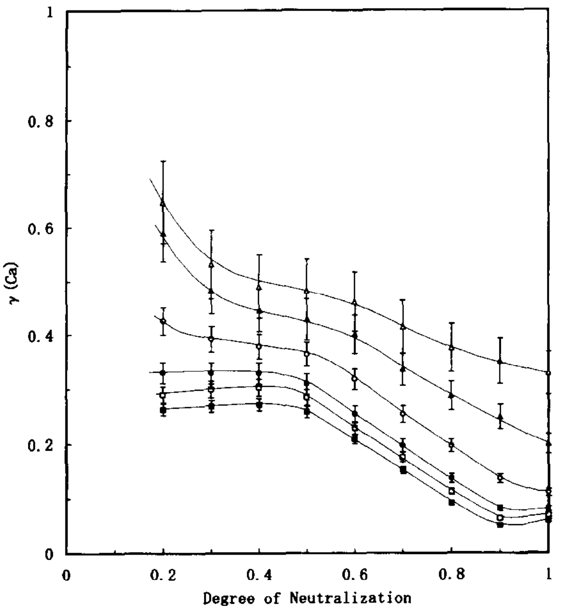

Fig. 3. Activity coefficient of Ca^{2+} when the STMA solution was neutralized with $Ca(OH)_2$ in the presence of 1 mM KCl. STMA concentrations were 0.05 (△), 0.1 (▲), 0.2 (○), 0.4 (●), 0.6 (□), and 1 mN (■).

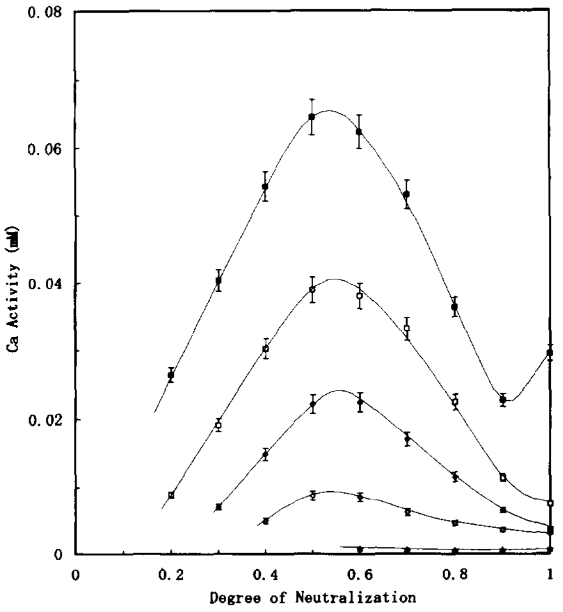

Fig. 4. Ca^{2+} activity in the presence of KOH. STMA solutions were neutralized at first with KOH until the degree of ionization became 0 (■), 0.1 (□), 0.2 (◆), 0.3 (◇), and 0.5 (▲), in the presence of 1 mM KCl and then neutralized with $Ca(OH)_2$. The STMA concentration was 1 mN.

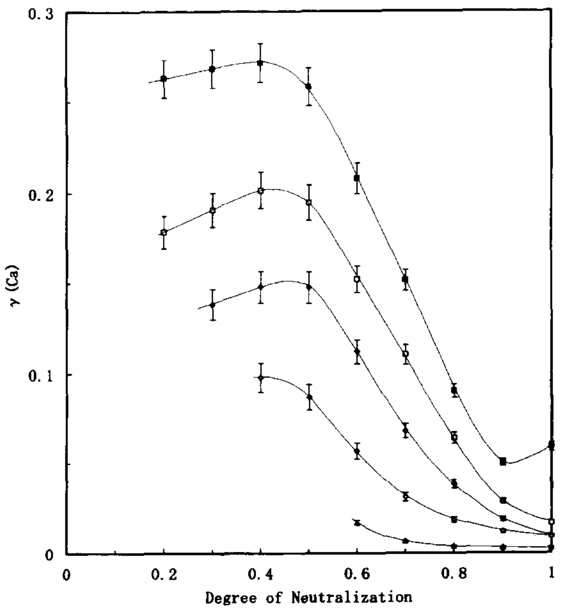

Fig. 5. Activity coefficient of Ca^{2+} is replotted from Fig. 4. Symbols are the same as in Fig. 4.

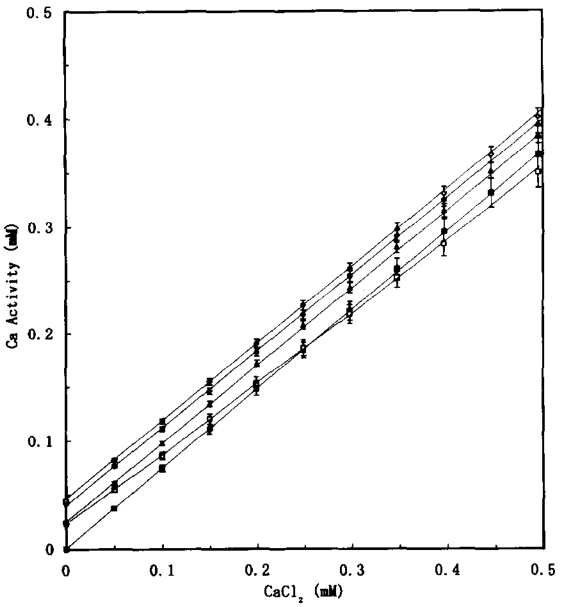

Fig. 6. Additivity rule for Ca^{2+} ion. The Ca^{2+} activity is plotted vs. added $CaCl_2$. The STMA solution was neutralized with $Ca(OH)_2$ and $CaCl_2$ was added successively. The degree of neutralization was: (■) pure solvent, (□) 0.3, (◆) 0.5, (◇) 0.7, and (▲) 0.9. The STMA concentration was 1 mN.

the Ca-STMA solution. The additivity rule of Ca^{2+} activity is expressed as follows:

$$A_{Ca} = \gamma_p C_p + A_s \qquad (4)$$

where A_{Ca}, γ_p, C_p, and A_s are the Ca^{2+} activity , the activity coefficient of Ca^{2+} in the absence of added $CaCl_2$, the concentration of ionized groups on the polymer, and the activity of Ca^{2+} due to added $CaCl_2$, respectively. As shown in Fig. 6 the Ca^{2+} activity linearly increased with added $CaCl_2$ and parallel to that of polymer-free solution except at $\beta = 0.3$. The Ca^{2+} activity of the STMA solution in the presence of $CaCl_2$ is a simple sum of the Ca^{2+} activity of the STMA solution in the absence of $CaCl_2$ and that of the $CaCl_2$ solution. When β is less than 0.3, the activity is not parallel to that of STMA-free solution and the additivity rule is not satisfied. Under these conditions, the assumption that the polymer is rod-like may not be valid.

The Ca^{2+} activity profile was not changed when STMA solution was titrated with $Ca(OH)_2$ in the presence of $CaCl_2$ as shown in Fig. 7. Titration curves in the presence of $CaCl_2$ were parallel to that in the absence of $CaCl_2$, and this result is consistent with the additivity rule mentioned above. This result also reinforces the fact that the absolute Ca^{2+} concentration does not effect the appearance of the peak of the Ca^{2+} activity.

4. Discussion

The Ca^{2+} activity decreased with increasing Ca^{2+} concentration in the degree of ionization range of 0.5 to unity when the STMA solution was neutralized with $Ca(OH)_2$ as shown in Fig. 2. This phenomenon is not consistent with the condensation theory. One of the probable interpretations of this phenomenon is to assume local strong binding of Ca^{2+} to a pair of carboxyl groups of the STMA molecule when both of the paired carboxyl groups are ionized. We calculated free Ca^{2+} concentration and free hydrogen ion concentration according to the following scheme:

$$A^{2-} + H^+ \leftrightharpoons AH^- \quad [AH^-] = K_1[A^{2-}][H^+]$$

$$AH^- + H^+ \leftrightharpoons AH_2 \quad [AH_2] = K_1K_2[A^{2-}][H^+]^2$$

$$A^{2-} + Ca^{2+} \leftrightharpoons ACa \quad [ACa] = K_{Ca}[A^{2-}][Ca^{2+}]$$

where A is a pair of carboxyl groups and K_1 and K_2 are the first and second association constants for H^+,

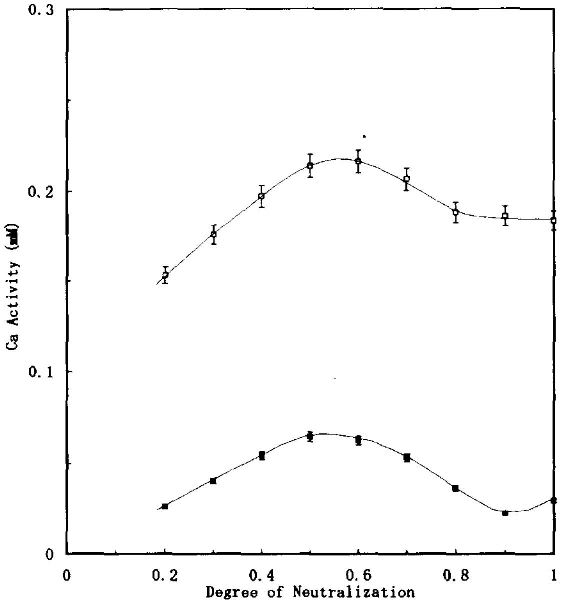

Fig. 7. Ca^{2+} activity of STMA solution in the presence of 0.25 (□) and 0 mM (■) $CaCl_2$, in the presence of 1 mM KCl. The STMA concentration was 1 mN.

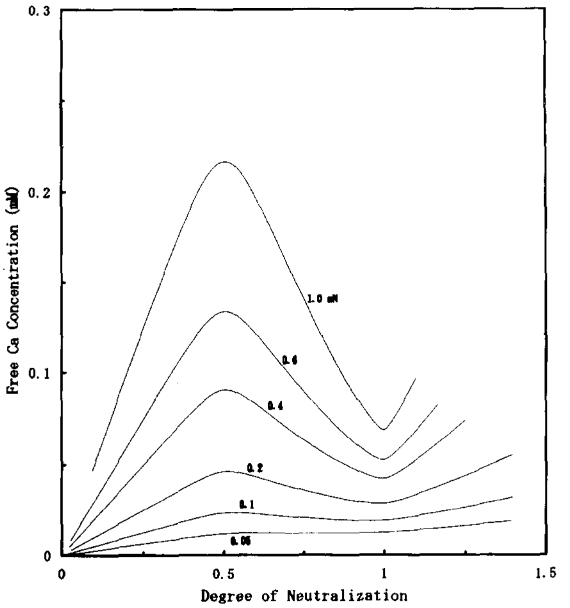

Fig. 8. Dependence of free Ca^{2+} concentration on polymer concentration calculated according to the scheme in the Discussion section. The numbers give the polymer concentration in mN. pK_1, pK_2, and pK_{Ca} are assumed to be 4.5, 8.5, and 5, respectively.

respectively, and K_{Ca} is the association constant for Ca^{2+}. Interaction between paired groups was neglected. Fig. 8 shows the free Ca^{2+} concentration, which corresponds to the Ca^{2+} activity in the experiment, calculated under the assumption mentioned above. The free Ca^{2+} concentration has a peak at $\beta = 0.5$ and a minimum at $\beta = 1$. The polymer concentration dependence of the free Ca^{2+} concentration is also shown in Fig. 8. As may be seen, it is possible to reproduce the Ca^{2+} binding to paired carboxyl groups, which gives qualitative agreement with the experimental binding curves if the Ca^{2+} binding constant, K_{Ca}, is properly assumed, i.e., 10^5 M^{-1}. But the activity coefficient of Ca^{2+} in the region $\beta \leq 0.5$ was small and constant as shown in Fig. 3, and the simulation mentioned above cannot reproduce the experimental results. The low value of the activity coefficient in the low β region cannot be explained by assuming a local strong Ca^{2+} binding to a carboxyl group. The K_{Ca}-values for simple dicarboxylic acids such as maleic acid are less than 10^5 M^{-1}; e.g., $10^{2.43}$, $10^{2.00}$, and $10^{3.0}$ for maleic acid, formalic acid, and oxalic acid, respectively [16]. The Ca^{2+} binding constant is increased by the electrostatic field produced by the negative charges on the macro ion.

The free Ca^{2+} concentration was calculated under the same assumption in the presence of $CaCl_2$ to examine the additivity rule. The free Ca^{2+} concentration is parallel to that of polymer-free solution when the degree of ionization is less than 0.6, as shown in Fig. 9. When the degree of ionization approaches unity, the free Ca^{2+} concentration is depressed when $CaCl_2$ is added, and the calculated curves show a slight deviation from the experimental one.

The second possible interpretation of the cause of the decrease of Ca^{2+} activity at the degree of neutralization unity is *to assume* an assembly of STMA molecules in the range $0.5 \leq \beta \leq 1$. As the decrease of the Ca^{2+} activity was not observed when the polymer concentration was very low, it was suggested that this phenomenon is due to polymer–polymer interaction. According to the counter ion condensation theory, if several polymer molecules form an assembly, ion condensation takes place on the polymer assembly and the counter ion activity decreases in proportion to the degree of assembly [1]. Most experiments about counter ion interactions reported were studied in the presence of monovalent counter ions. A divalent counter ion, however, strengthens the electrostatic

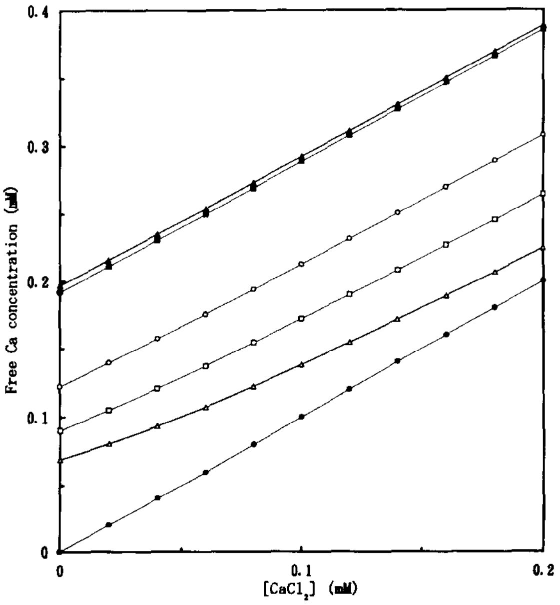

Fig. 9. Calculated free Ca^{2+} concentration calculated according to the scheme in the Discussion section in the presence of $CaCl_2$. Free Ca^{2+} concentration is plotted vs. $CaCl_2$ concentration. The degree of neutralization is 0 (●), 0.4 (■), 0.6 (▲), 0.8 (○), 0.9 (□), and 1 (△). The polymer concentration was 1 mN.

interaction between a counter ion and a macro ion and it also reduces the entropy of the counter ion distribution and, hence, reduces the repulsive force between macro ions because the number of counter ions is half that of monovalent ions. Concentrated STMA solution (for example 10 mN) precipitated when neutralized with $Ca(OH)_2$ at $\beta \geq 0.6$. However, there is no clear evidence for an attractive force between linear polyelectrolytes which have the same charges, and there is no theory to explain the peak of Ca^{2+} activity. The measurement of the molecular weight is essential to clarify the assembly of the polymer, but the measurement would be difficult because the polymer concentration is as low as 0.1 mN and the the polymer is polydisperse. Notwithstanding this, we need to study the molecular weight of the polymer in solution neutralized with $Ca(OH)_2$ by light scattering or other methods.

The third possible interpretation is to assume chelate binding between Ca^{2+} ions and carboxyl groups which are positioned distant from each other along a polymer chain or on other molecules. The formation of the chelate may or may not induce a conformational change in the polymer. But no gel-like structure was formed on chelation of Ca^{2+} with an STMA molecule because the specific viscosity, η_{sp}, of the STMA solution neutralized with $Ca(OH)_2$ at a degree of neutralization of unity was decreased to about 1% of the η_{sp} at a degree of neutralization of 0.4 (data not shown).

The same decrease in the Ca^{2+} activity by neutralization with $Ca(OH)_2$ was also observed in other maleic acid copolymers such as methyl or ethyl vinyl ether copolymers (data not shown), and this phenomenon is common to maleic acid copolymers. However, Shimizu et al. reported no such decrease in the Ca^{2+} activity when a copolymer of maleic acid and ethyl vinyl ether solution was neutralized with $Ca(OH)_2$ [9]. Judging from the steepness of the peak at $\beta = 0.5$, local strong Ca^{2+} binding to the paired carboxyl group contribute to the decrease of Ca^{2+} activity, at least to some extent, beyond $\beta = 0.5$. However, quantitative agreement between experimental data and calculation has not been obtained on the basis of simple strong binding. The three models mentioned above have advantages and disadvantages and we does not know which model is best to intepret the phenomenon. We need to study the Ca^{2+} activity in many polyelectrolyte solutions including polyacrylic acid and polymethacrylic acid.

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