Competitive counterion binding and dehydration of polyelectrolytes in aqueous solutions

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(Received 22 January 1990; revised 9 March 1990; accepted 12 March 1990)

Apparent molar volumes, V_{app}, have been estimated by measuring densities of aqueous solutions of polyelectrolytes, namely Na poly(styrene sulphonate) (PSSNa), Na poly(*L*-glutamate) (PLGNa) and Na polyacrylate (PAANa), in the presence of chloride salts of divalent cations, Mg^{2+}, Ca^{2+}, Ba^{2+}, and ethylenediamine hydrochloride (EDA^{2+}). Increases in the observed V_{app} on addition of divalent counterions have been found to be proportional to binding degrees estimated with a condensation model, implying that dehydration on counterion binding changes additively with binding degree. However, for PSS systems containing two species of divalent counterions, non-linear variations of V_{app} with the concentration fraction of M^{2+} have been observed. This suggests that dehydrations on atmospheric counterion binding are influenced by other counterion species, probably through a perturbation of hydration structure around the bound counterions.

(Keywords: counterion binding; hydration; apparent molar volume; polyelectrolyte)

INTRODUCTION

Competitive binding between counterions with different valences has been investigated for various polyion systems to examine the counterion condensation phenomenon[1-8]. Mattai and Kwak[5] studied the counterion binding of Mg^{2+} and Ca^{2+} onto several polyelectrolytes. They found that binding degrees of the divalent counterions were well predicted by the two-variable theory of Manning for polyelectrolytes having sulphonate or sulphate groups (i.e. poly(styrenesulphonate) and dextran sulphate). For poly(carboxylate)s, however, the observed higher degrees of counterion binding than estimated by theory suggested enhanced restriction of the counterions near the relevant polyions.

We have also found[8] that activity coefficients of Mg^{2+} and Ca^{2+} in aqueous solutions of Na poly(styrenesulphonate) (PSSNa) are quantitatively reproduced by our condensation model, intermediate model (IMM)[9], while those for Na poly(*L*-glutamate) (PLGNa)[8] and Na polyacrylate (PAANa)[10] are overestimated. These observations should be explained in terms of specific interactions of the divalent counterions with poly(carboxylate) anions. Such specificities in the counterion binding have been discussed in relation to dehydration degree accompanied by binding. For example, Strauss *et al.*[11] used the dehydration degree on counterion binding as a measure of the so-called site binding. According to their studies, Mg^{2+} and Ca^{2+} were much more dehydrated on the binding to poly(acrylate) than on binding to poly(styrenesulphonate). Thus the hydration changes accompanied by the counterion bindings seem to play an essential role in selective binding, especially in the case of multivalent counterions. However, until now most studies on competitive counterion binding have ignored the accompanying dehydration in discussing the binding. For example, degrees of counterion binding estimated by nuclear magnetic resonance (n.m.r.) line width measurements were compared with theoretical predictions from the Poisson–Boltzmann equation and counterion condensation model[12,13].

In a previous study[10], we reported that the degrees of dehydration of PAANa and PLGNa accompanied by exchange reaction of alkaline earth metal cations with Na^+ ions are in a proportional correlation with an excess binding energy, ΔE_{ex}, estimated by IMM through the observed depression in the counterion activity coefficients. In the present report, we investigate dehydration behaviour and correlation with the degree of divalent counterion binding, namely whether the dehydration occurs additively or not, in competitive binding systems of monovalent and divalent counterions and also between two species of divalent counterions.

Changes in the apparent molar volume, V_{app}, of polyions (PSSNa, PLGNa, PAANa) were measured with varying concentration of a divalent counterion (Mg^{2+}, Ca^{2+}, Ba^{2+}, ethylenediamine (EDA^{2+})). These polyion systems were chosen because PSSNa, PLGNa and PAANa show different binding strengths for alkaline earth metal cations as suggested by the activities[8,10]. The V_{app} values obtained are plotted against the degree of counterion condensation, θ_2, estimated by IMM. For PLGNa and PAANa/Mg^{2+} systems, the excess interaction energies (ΔE_{ex}) estimated from the counterion activities have been incorporated into the theoretical estimation of θ_2.

The present paper shows that the dehydration degrees accompanied by the 1–2 valent competitive binding increase additively with θ_2, while in the 2–2 valent competition the dehydration behaviour of PSSNa exemplifies a breakdown of the additivity.

0032-3861/91/050892-05

EXPERIMENTAL

Preparation and purification of the polymer samples were described in our preceding papers[9,10]. The molecular weights of PAANa, PLGNa and PSSNa were 9.9 × 10^4, 6.9 × 10^4 and 9.3 × 10^4, respectively. $MgCl_2$, $CaCl_2$ and $BaCl_2$ of analytical grade were purchased from Nakarai Chemical Co. Ethylenediamine hydrochloride (EDA2HCl) from Kanto Chemical Co. was used as a salt of a divalent counterion having two singly charged sites for comparison with the alkaline earth metal cations. This salt was added only to the PSSNa system. All the sample solutions were gravimetrically prepared with deionized and distilled water. The polymer concentrations were adjusted to ≈ 0.01 base mol dm^{-3} and the concentration ratio of the divalent counterions to the polymers was varied from 0 to 0.8 (0 to 0.4 for PAANa/$MgCl_2$, $CaCl_2$, $BaCl_2$).

The density measurements were made at 25 ± 0.005°C with a precision density meter DMA-02C (Anton Paar). The detail of the calibration has been described elsewhere[14]. The apparent molar volume, V_{app}, of polymers is given by the following equation:

$$V_{app} = \{M + 1000(d_0 - d)/C_p\}/d_0 \qquad (1)$$

where M is molecular weight of monomer unit, C_p polymer concentration (base mol dm^{-3}) and d density (g cm^{-3}), and suffix 0 means solvent salt solution. Although the experimental errors of d, d_0 and C_p are estimated to be ± 0.0002, 0.02, and 0.01%, respectively, V_{app} values were reproducible within ± 0.3 cm^3 mol^{-1} irrespective of polymer species and systems.

Binding selectivity in mixed divalent counterion systems was estimated by an ultrafiltration method[15]. Aqueous solutions (40 ml) of PSSNa or PLGNa containing both $MgCl_2$ and $CaCl_2$ were ultrafiltrated through an ultrafilter UH-1 (Toyo Roshi Co.), which fractionates molecules of $MW < 1000$. The concentration ratios of Mg^{2+} to Ca^{2+} were 3:7, 5:5 and 7:3, and the total concentration was constant ($= 0.8C_p$). The concentrations of the divalent counterions in the feed, in the filtrate (0–5, 5–10, 10–15, 15–20 ml) and in the residue were measured with an atomic absorption spectrophotometer (Shimadzu AA-640-12). Polymer leakage in the filtrate was found to be negligible ($\approx 2\%$). The same experiment was also performed for the PLGNa/Mg^{2+}/Ba^{2+} system.

RESULTS AND DISCUSSION

When divalent counterions are added to a polyelectrolyte solution which originally contains monovalent counterions, the former counterions will be favourably bound to the polyion to replace the pre-condensed monovalent counterions. This phenomenon has been demonstrated by many experimental studies which have used counterion activity measurements[2,7,8,13,16], conductometry[6], dye spectrophotometry[3–5], n.m.r.[17–19] and so on. If any hydration changes are accompanied by the divalent counterion binding, V_{app} will vary as a function of C_2/C_p.

Figure 1 a–c shows V_{app} *versus* C_2/C_p plots for PSSNa, PLGNa and PAANa, respectively, in the aqueous salt solutions. For comparison, the figures also contain theoretical curves for θ_2 estimated by IMM. The ordinate scales for θ_2 were adjusted to those of V_{app} for Mg^{2+}, Ba^{2+} and Mg^{2+}, respectively. In all the polyion systems studied here, V_{app} increases with C_2/C_p. Since water molecules in the ionic or electrostrictive hydration have smaller molar volume than in bulk, the increases in V_{app} mean that dehydration of the polyions occurs on divalent counterion binding. For PSSNa (*Figure 1a*), the increase in V_{app} is saturated above $C_2/C_p \approx 0.4$–0.5, except for the $BaCl_2$ system, in which polymer precipitation was observed (therefore, the results on PSSNa/$BaCl_2$ are omitted from the discussion below). The saturation suggests that in the high C_2/C_p region the binding degrees of the divalent counterions (Mg^{2+}, Ca^{2+}, EDA^{2+}) are almost constant. Theoretical estimates of divalent counterion binding seem to correspond to such variations in V_{app}.

A similar dependence of V_{app} on C_2/C_p is also found for PLGNa systems (*Figure 1b*). The C_2/C_p values above which the V_{app} values are saturated are ≈ 0.4 for $MgCl_2$ and ≈ 0.5 for $CaCl_2$ and $BaCl_2$. Since the activity coefficients of Mg^{2+} and Ca^{2+} in PLGNa solution have been reproduced by IMM with $\Delta E_{ex} = -1.5$ kcal mol^{-1} (Reference 8), the corresponding θ_2 values estimated with this excess interaction energy are plotted in the figure. The θ_2 values seem to be saturated above $C_2/C_p \approx 0.5$, consistent with the variation of V_{app} for Ca^{2+} and Ba^{2+}.

For PAANa/Mg^{2+} systems, the divalent counterions were added up to $C_2/C_p = 0.4$, because PAA precipitates at $C_2/C_p > 0.4$. V_{app} increases almost linearly in the low C_2/C_p region (*Figure 1c*). The theoretical θ_2 curve in the figure was drawn for Mg^{2+} by using IMM with $\Delta E_{ex} = -1.2$ kcal mol^{-1} (Reference 10). For the Ca^{2+} system, ΔE_{ex} has been estimated as -3.9 kcal mol^{-1} (Reference 10). With the latter value, IMM predicts that almost all of the added divalent counterions are bound to PAA, i.e. $\theta_2 \approx C_2/C_p$. This is consistent with the linear increase in V_{app} for Ca^{2+} and Ba^{2+} systems.

For a detailed comparison of the three polyion systems,

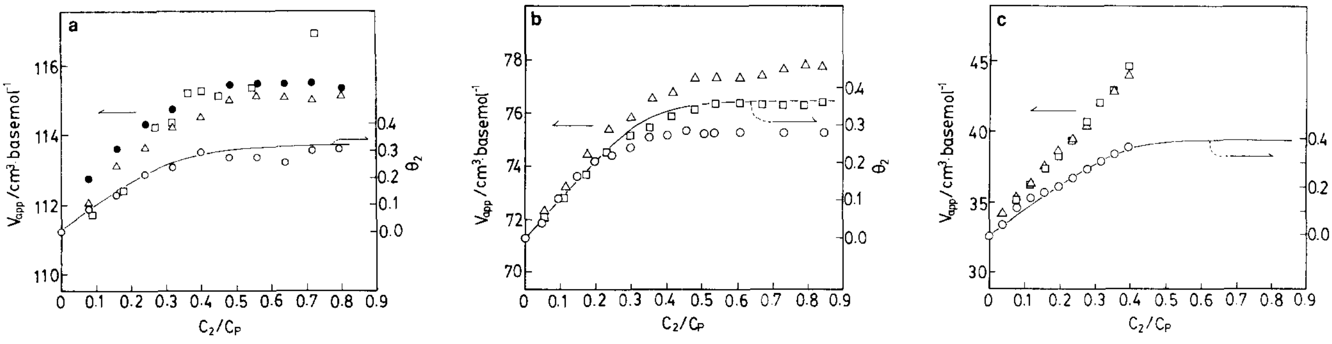

Figure 1 Dependence of V_{app} on C_2/C_p for: (a) PSSNa; (b) PLGNa; (c) PAANa. ——, Theoretical θ_2 values estimated by the intermediate model with ΔE_{ex} = (a) 0, (b) −1.5 kcal mol^{-1} and (c) −1.2 kcal mol^{-1} (Mg^{2+}). ○, $MgCl_2$; △, $CaCl_2$; □, $BaCl_2$; ●, EDA·2HCl

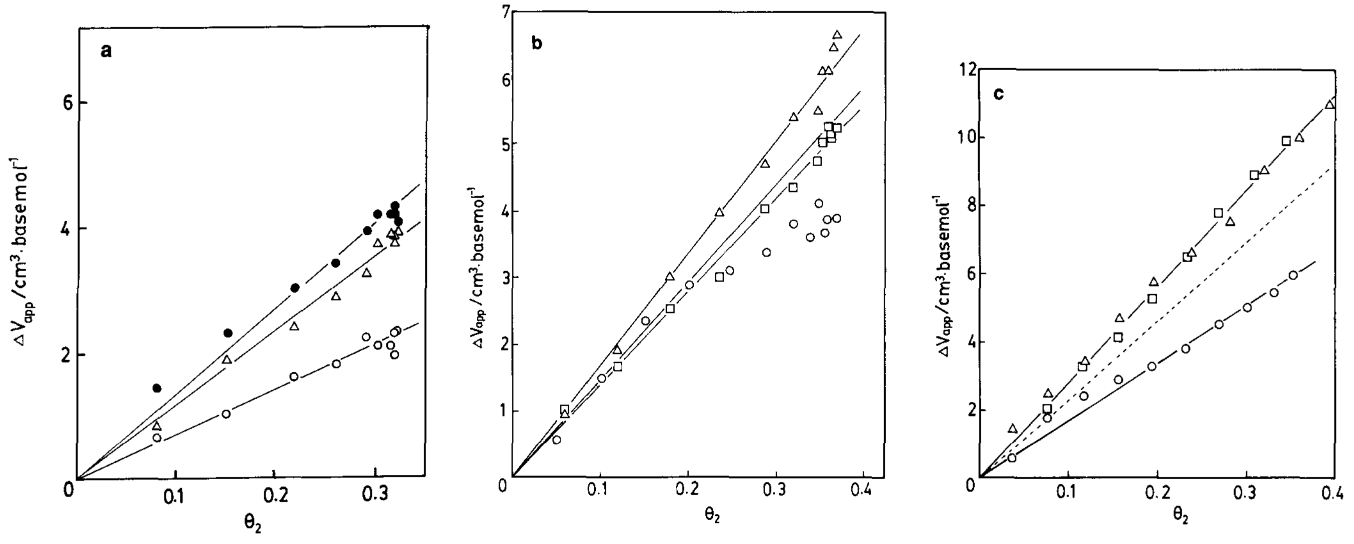

Figure 2 Dependence of V_{app} on θ_2 for: (a) PSSNa; (b) PLGNa; (c) PAANa. (----, Linear correlation assumed only at low θ_2; see text). θ_2 values were estimated with the same ΔE_{ex} values as in *Figure 1*. ($\Delta E_{ex} = -3.9$ kcal mol⁻¹ for Ca^{2+} and Ba^{2+} in (c)). Symbols as in *Figure 1*

ΔV_{app} (i.e. $V_{app} - V_{app}$ at $C_2/C_p = 0$) is plotted against theoretical θ_2 values in *Figure 2*. In PSSNa/Mg^{2+} systems (*Figure 2a*), ΔV_{app} increases linearly with θ_2. This result indicates that the dehydration of PSSNa due to the divalent counterion binding is additive on binding degree. The charge density of the counterion appears not to affect the linearity, as seen in the PSSNa/EDA·2HCl system.

For PLGNa/Mg^{2+} systems (*Figure 2b*), ΔV_{app} for Ca^{2+} and Ba^{2+} shows linear dependence on θ_2, although that for Mg^{2+} deviates downward from the linearity above $\theta_2 \approx 0.2$. According to the counterion activity measurements for the PLGNa system, the activity coefficients of Mg^{2+} and Ca^{2+} agree within experimental error, so θ_2 values for the two divalent cations were estimated by IMM with the same ΔE_{ex} value. Therefore, the downward deviation from linearity for Mg^{2+} suggests that the dehydration degree decreases with increasing θ_2. This may be explained as follows. Dehydrative counterion binding occurs only when the dehydration energy can be compensated by a sufficient electrostatic stabilization. Since the Mg^{2+} ion has a strong hydration shell (the hydration energy is higher than that of Ca^{2+} and Ba^{2+}), carboxyl anions may require a more restrictive configuration for coordination onto a Mg^{2+} at an optimum binding condition. Such a restrictive binding mode would become less plausible with the decrease in the free sites of carboxyl groups. For Mg^{2+} with lower hydration energies, the restriction will be weaker. A similar explanation was given by Ikegami *et al.* for dehydration on Mg^{2+} binding to PAA[20]. They assumed that the binding mode changes from $(-COO^-)_2Mg^{2+}$ to $-COO^-Mg^{2+}$ with increasing θ_2. Thus the upward deviation at $\theta_2 \approx 0.1$ from the linear plot observed for PAANa/Mg^{2+} (*Figure 2c*) may be only apparent; in fact, ΔV_{app} or the degree of dehydration may deviate downward from the dashed line. With the exception of Mg^{2+}/PLG and PAA systems, the ΔV_{app} values are linearly correlated with the theoretical θ_2 values irrespective of binding modes; the dehydration degrees on divalent counterion binding are additive on binding degrees for 1–2 valent counterion competition.

Based on additivity, dehydration numbers were estimated from the slope of the ΔV_{app} *versus* θ_2 plots (*Figure 2*) by assuming 2.7 cm³ mol⁻¹ for the reduction of the molar volume of electrostrictly hydrating water[21]. Dehydration numbers are summarized in *Table 1*. Agreement with values previously obtained from compressibility data[13] seems to be reasonable. Special attention should be paid to the results for PSSNa/Mg^{2+} systems. The atmospheric binding mode has been assigned to the Mg^{2+} and Ca^{2+} binding to PSSNa in aqueous solution (i.e. $\Delta E_{ex} = 0$[8]). Therefore, the non-negligible discrepancy for the dehydration numbers for Ca^{2+}/PSS and for Mg^{2+}/PSS suggests that dehydration degrees cannot be used as a measure of counterion binding strength in such atmospheric binding systems. This point will be discussed in the discussion on 2–2 valent competition systems below.

Table 1 Dehydration numbers for divalent counterion binding onto polyanions

Polymer	Divalent counterion	Dehydration number[a]
PSSNa	Mg^{2+}	2.6
	Ca^{2+}	4.5
	$EDA \cdot 2H^+$	5.0
PLGNa	Mg^{2+}	3.9–5.6[b](4.6)
	Ca^{2+}	6.2
	Ba^{2+}	5.2
PAANa	Mg^{2+}	6.1–7.8[c](8.3)
	Ca^{2+}	10.2(12)
	Ba^{2+}	10.2(13)

[a] Values in parentheses are dehydration numbers (n^{i}_{DH} of Reference 10) estimated from compressibility data

[b] The lower value shows the decrease in the dehydration number at the highest θ_2 value ($C_2/C_p = 0.8$)

[c] The lower and the higher values were estimated from the slopes of the solid and the dashed lines, respectively, in *Figure 2c*

To examine the additivity in competitive binding systems of 2–2 valent counterions, we obtained ΔV_{app} values as a function of the composition of two species of divalent counterions added in the relevant polyion systems. *Figure 3* shows hydration changes due to competitive binding for PSSNa/Mg^{2+}/Ca^{2+} or EDA^{2+} and PLGNa/Mg^{2+}/Ca^{2+} or Ba^{2+} systems in terms of plots of ΔV_{red} *versus* $C_x/(C_{Mg} + C_x)$. Here ΔV_{red} is a

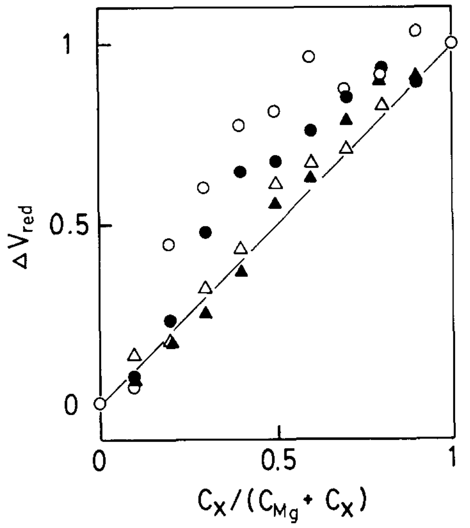

Figure 3 Dependence of ΔV_{red} on the mole fraction of divalent counterions. Total concentrations of the divalent counterions are constant at $0.8C_p$. ○, PSSNa/Mg^{2+}/Ca^{2+}; ●, PSSNa/Mg^{2+}/EDA^{2+}; △, PLGNa/Mg^{2+}/Ca^{2+}; ▲, PLGNa/Mg^{2+}/Ba^{2+}

normalized value for the apparent molar volume change, given by

$$\Delta V_{red} = \{V_{app} - V_{app}(C_{Mg}/C_p = 0.8)\}/\{V_{app}(C_x/C_p = 0.8) - V_{app}(C_{Mg}/C_p = 0.8)\} \quad (2)$$

where x means Ca^{2+}, Ba^{2+} or EDA^{2+}. Since the total divalent counterion concentration is $0.8C_p$, sufficient to repel monovalent counterions out of the condensation region, it is assumed that the presence of Na^+ ions does not influence the V_{app} values. Although the ΔV_{red} values contain large errors (± 0.16 for PSSNa/Mg^{2+}/Ca^{2+} to ± 0.12 for PLGNa/Mg^{2+}/Ca^{2+}), we consider that these two polyion species exhibit distinct patterns in the plots. Namely, ΔV_{red} values for PSSNa systems deviate upward from a linear dependence on concentration fraction, while almost linear changes can be seen for PLGNa systems. If hydration changes involved with counterion binding in the pertinent 2–2 valent counterion competition systems change additively with binding degree, the dependence of ΔV_{red} on concentration ratio should reflect the binding fraction of Ca^{2+} (or Ba^{2+}, EDA^{2+}) ions. Then the results shown in *Figure 3* suggest that a PSS anion prefers Ca^{2+} and EDA^{2+} to Mg^{2+}, and that a PLG anion binds equally Mg^{2+} and Ca^{2+} or Ba^{2+}. However, according to the binding selectivity estimated by the ultrafiltration method, neither polyion significantly discriminates Mg^{2+} and Ca^{2+} ions; it was found that Mg^{2+} compositions in the filtrate solutions were enriched by ≈ 2 and 10% for PSS and PLG systems, respectively. This means that the binding affinity of Ca^{2+} is only slightly higher than that of Mg^{2+} for the polyions. Since a selectivity for Ca^{2+} more than seven times that for Mg^{2+} must be assumed to interpret the convex shape of the dependence in *Figure 3* in terms of additive dehydrations, these experimental results on dehydration behaviour and binding selectivities indicate that additivity does not hold for 2–2 valent counterion competition in PSS systems. This contrasts with PLG systems.

The facts and their interpretation discussed above can be summarized as follows:

(1) In 1–2 valent counterion competition, dehydration degrees estimated by ΔV_{app} additively increase with theoretical θ_2 values, irrespective of the binding mode, i.e. atmospheric (PSS) or site binding (-like) (PLG, PAA).

(2) In 2–2 valent counterion competition, additivity is observed for PLG systems but not for PSS systems.

What explanation can be proposed for such a difference in behaviour in the dehydration accompanied by counterion binding? As a counterion approaches a polyion, the total electrostatic energy due to long range interactions (between polymer charges) and short range interactions (between counterion and polymer charge(s)) decreases, while the positive dehydration energy almost counterbalances the electrostatic stabilization. When stabilization by the inner sphere (contact ion pair) or the outer sphere (solvent shared ion pair) complex formation with polyion charges overcomes the unfavourable dehydration, so-called site binding will dominate. In this case, degrees of dehydration will be determined by short-range rather than long-range interaction. This has actually been observed for PLG and PAA systems, as shown by the linear correlation between ΔE_{ex} and dehydration degree. Since the dehydration is caused mainly by short range interaction between counterions and polymer charges, it seems reasonable that dehydration with Ca^{2+} binding is independent of that of Mg^{2+} in the PLG system. On the other hand, when the specific interaction is not favoured, the dehydration degree will be determined mainly by the energy balance between the long range interaction and the dehydration. Therefore, Ca^{2+} ions with lower (de)hydration energies are able to get closer to a polyion such as PSS than Mg^{2+} ions of higher (de)hydration energies, up to the point that the lowering of the long range electrostatic energy is cancelled by the inevitable increase in the total dehydration energies. This mechanism may explain the equal binding strength ($\Delta E_{ex} = 0$) and different dehydration degrees for PSS/Mg^{2+} and PSS/Ca^{2+} systems. Furthermore, since counterions in atmospheric binding systems are attracted not by any specific charged site(s) but by the whole polyion, dehydration of a counterion species may be affected through perturbation of the water structure by nearby counterions of different species. Therefore, the positive deviations from the linear additivity observed for PSS systems in *Figure 3* may result from the perturbation in the hydration structure, which allowed enhancement of dehydration of Mg^{2+} ions. On the other hand, in 1–2 valent counterion competition, the additivity of dehydration on θ_2 is observed for PSSNa/Mg^{2+} systems, too, as shown in *Figure 2a*. This may be because divalent counterions are attracted closer to polyions than monovalent counterions: monovalent Na^+ ions are bound outside the perturbation by the bound divalent counterions or because the perturbation to the hydration of Na^+ ions is too weak to be detected. Since dehydrations of Na^+ upon the binding to PSS are small, the latter reason seems more probable.

In conclusion, the interrelation between counterion binding and dehydration behaviour in competitive binding systems implicitly includes and sometimes explicitly provides information on the binding mode, namely atmospheric or site binding, and also on the stoichiometry. We have extracted and shown some examples by using IMM as a reference for the atmospheric binding mode. An analysis along the same lines would seem to be useful for examination of more complex counterion binding systems, in which hydrophobic interactions and hydrations are both involved.

REFERENCES

1 Magdelenat, H., Turr, P., Tivant, P., Chemla, M., Menez, R. and Drifford, M. *Biopolymers* 1979, **18**, 187

2 Shimizu, T., Minakata, A. and Imai, N. *Biophys. Chem.* 1981, **14**, 333

3 Joshi, Y. M. and Kwak, J. C. T. *Biophys. Chem.* 1981, **13**, 65

4 Mattai, J. and Kwak, J. C. T. *J. Phys. Chem.* 1982, **86**, 1026

5 Mattai, J. and Kwak, J. C. T. *Macromolecules* 1986, **19**, 1663

6 de Jong, H. G., Lyklema, J. and van Leeuwen, H. P. *Biophys. Chem.* 1987, **27**, 173

7 Yonese, M., Tsuge, H. and Kishimoto, H. *J. Phys. Chem.* 1987, **91**, 1971

8 Satoh, M., Kawashima, T. and Komiyama, J. *Biophys. Chem.* 1988, **31**, 209

9 Satoh, M., Kawashima, T., Komiyama, J. and Iijima, T. *Polym. J.* 1987, **19**, 1191

10 Satoh, M., Hayashi, M., Komiyama, J. and Iijima, T. *Polymer* 1990, **31**, 501

11 Strauss, U. P. and Leung, Y. P. *J. Am. Chem. Soc.* 1965, **87**, 1476

12 Braunlin, W. H., Drakenberg, T. and Nordenskiold, L. *Biopolymers* 1987, **26**, 1047

13 Quian, C., Asdjodi, M. R., Spencer, H. G. and Savitsky, G. B. *Macromolecules* 1989, **22**, 995

14 Satoh, M., Hayashi, M., Komiyama, J. and Iijima, T. *Polym. Commun.* 1988, **29**, 49

15 Boyd, G. E. and Wilson, D. P. *Macromolecules* 1982, **15**, 78

16 Manning, G. S. *Q. Rev. Biophys.* 1978, **11**, 179

17 Westra, S. W. T. and Leyte, J. C. *Ber. Bunsenges. Phys. Chem.* 1979, **83**, 678

18 Karenzi, P. C., Meurer, B., Spegt, P. and Weill, G. *Biophys. Chem.* 1979, **9**, 181

19 Paulsen, M. D., Anderson, C. F. and Record, M. T. Jr. *Biopolymers* 1988, **21**, 1249

20 Ikegami, A. and Imai, N. *J. Polym. Sci.* 1962, **56**, 133

21 Conway, B. E., Desnoyers, J. E. and Smith, A. C., *Phil. Trans. Roy. Soc. Lond.* 1964, **256**, 398