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THE BINDING OF DIVALENT METAL IONS TO POLYELECTROLYTES IN MIXED COUNTERION SYSTEMS.

II. DEXTRANSULFATE–Mg^{2+} AND DEXTRANSULFATE–Ca^{2+} IN SOLUTIONS CONTAINING ADDED NaCl OR KCl

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Measurements of magnesium and calcium ion activities in solutions of the polyelectrolyte dextransulfate, with added sodium chloride or potassium chloride are presented. A two wavelength dye spectrophotometric method is used. Dextransulfate concentrations C_p (expressed as moles sulfate ion/litre) vary between 0.001 and 0.007, total ionic strengths between 0.005 and 0.08 mole/ℓ. Divalent metal ion concentrations are varied between 0 and 1.2 C_p. The results for the metal ion activities are expressed in the form of parameters $\theta_2 = C_2/C_p$ (C_{2b} = bound divalent metal ion concentration) and $K_2 = \theta_2/(C_2 - C_{2b})$. For each divalent/univalent counterion pair the values obtained for θ_2 and K_2 as a function of C_2, C_p, and ionic strength are compared to predictions of the "two variable theory" developed for these mixed counterion systems by Manning. This comparison shows that the observed decrease in θ_2 with increasing ionic strength at fixed C_2 and C_p is generally well predicted by the two variable theory. The extent of divalent ion binding at a given C_2, C_p, and ionic strength is largest for the Ca/Na counterion combination, and lowest for the Mg/K combination.

1. Introduction

The interactions between polyions and counterions are particularly strong in the case of multivalent counterions. The binding of divalent metal ions by negatively charged biopolymers is of considerable importance in many biological processes. The influence of added electrolyte on this binding process, and especially on that part of the binding process which can be described by long range electrostatic interactions, has recently been the topic of theoretical attention [1–6]. Experimental data in such systems, studying a variety of polyions, of divalent metal ions, and of added electrolytes are in fact rather scarce. In the preceding paper [7] we discussed a dye spectrophotometric method to determine the activity coefficient of the divalent metal ion in polyelectrolyte solutions with added salt, and compared the results to data in corresponding systems obtained with Donnan equilibrium and EMF methods. In this paper we will apply the two wavelength dye spectrophotometric method ("ratio method") to investigate the binding of Ca^{2+} and Mg^{2+} to dextransulfate in mixed counterion systems containing dextransulfate, $MgCl_2$ or $CaCl_2$, and excess NaCl or KCl. Of the theoretical approaches mentioned above, both Manning and Iwasa [1–4] make use of the counterion condensation concept. In systems with a single counterion condensation theory predicts that counterions of charge Z_i will be bound by ("condensed on") the polyion when the net charge density ξ of the polyion, defined by $\xi = e^2/DkTb$, e = proton charge, D = dielectric constant, k = Bolzmann constant, T = temperature, b = average linear charge separation on polyion, exceeds Z_i^{-1} [8]. Manning's limiting laws [9], combining the condensation criterion with the Debye–Hückel approximation for the potential around the polyion have been remarkably successful in describing the colligative properties of such polyelectrolyte systems, especially osmotic coefficients and mean activity coef-

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ficients of the added salt. Although recently it has been shown that the Debye–Hückel approximation may not be sufficiently accurate to describe the long range interactions between polyions and small ions in systems where the added salt is not in excess [10–13], this complication is not expected to exist in solutions containing excess salt, i.e. in solutions where the polyion to co-ion concentration ratio X is smaller than 1. In mixed counterion systems the predictions from condensation theory are more complicated because the condensation criterion is different for counterions of different charge. In such systems, depending on the value of ξ_{net}, we can recognize three regions [14,15]:

1) $\xi_{\mathrm{net}} = 1$. This represents the region where C_2/C_p (1 = univalent counterion, 2 = divalent counterion, p = polyion) is small. The general condition is that when $C_2/C_\mathrm{p} < \frac{1}{2}(1 - \xi^{-1})$ all divalent counterions will be bound, and sufficient univalent counterions will be bound to make $\xi_{\mathrm{net}} = 1$.

2) $\frac{1}{2} < \xi_{\mathrm{net}} < 1$. This is the intermediate case, where $\frac{1}{2}(1 - \xi^{-1}) \leqslant C_2/C_\mathrm{p} \leqslant \frac{1}{2}(1 - \frac{1}{2}\xi^{-1})$. Depending on C_2/C_p ξ_{net} ranges between 0.5 and 1. All divalent counterions are bound, all univalent counterions are free.

3) $\xi_{\mathrm{net}} = \frac{1}{2}$. When $\frac{1}{2}(1 - \frac{1}{2}\xi^{-1}) < C_2/C_\mathrm{p}$ divalent counterions will be bound until $\xi_{\mathrm{net}} = \frac{1}{2}$. All univalent counterions are free.

The derivation of the colligative properties of mixed counterion solutions based on these simple condensation considerations is fairly straight forward [14,15]. In systems NaCl + $\mathrm{CaCl_2}$ + Na polystryrenesulfonate the mean NaCl activity coefficient was shown to follow these predictions closely, however for $\mathrm{CaCl_2}$ the results were in serious disagreement with the theoretical expressions in that they did not show the expected strong lowering of the $\mathrm{CaCl_2}$ activity as the calcium to polyion concentration ratio was decreased [15]. The same effect had already been observed by Krakauer [16] who used a dye spectrophotometric method to measure $\mathrm{Mg^{2+}}$ binding to polynucleotides. Manning [14] used Krakauer's data to show that the quantity θ_2, defined as $\theta_2 = C_{2\mathrm{b}}/C_\mathrm{p}$, the ratio of the bound $\mathrm{Mg^{2+}}$ concentration to the total polyion concentration, is closest to the simple condensation predictions

$$C_2/C_p < \tfrac{1}{2}(1 - \tfrac{1}{2}\xi^{-1}), \qquad \theta_2 = C_2/C_p, \tag{1}$$

$$\tfrac{1}{2}(1 - \tfrac{1}{2}\xi^{-1}) < C_2/C_p, \qquad \theta_2 = \tfrac{1}{2}(1 - \tfrac{1}{2}\xi^{-1}), \tag{2}$$

in mixtures of the lowest total ionic strength. In other words, in the region where $C_2/C_\mathrm{p} < \frac{1}{2}(1 - \frac{1}{2}\xi^{-1})$ the activity of the divalent ion is not zero as predicted by simple condensation considerations, but it does decrease as the total ionic strength of solutions of constant C_2/C_p is lowered. This is in agreement with the observations of Kwak et al. [15]. We will express our results for the magnesium and calcium ion activities in polyelectrolyte solutions in the presence of added univalent electrolyte in terms of the parameters θ_2 (defined above) and K_2 given by:

$$K_2 = \theta_2/C_{2\mathrm{f}} \tag{3}$$

where $C_{2\mathrm{f}}$ is the concentration of free (i.e. non-bound) divalent counterions. K_2 and θ_2 can be directly calculated from the measured activity or activity coefficient of the divalent ion:

$$a_2 = a_{2\mathrm{f}}, \tag{4}$$

where a_2 is the measured activity of 2, here equated to the activity of free 2. Thus:

$$\gamma_2^{\mathrm{exp}} C_2 = \gamma_2^0 C_{2\mathrm{f}}, \tag{5}$$

γ_2^{exp} is the experimentally observed activity coefficient defined by the left hand sides of eqs. (4) and (5), γ_2^0 is the activity coefficient of 2 in a solution of the same ionic strength without polyelectrolyte present. With $C_2 = C_{2\mathrm{b}} + C_{2\mathrm{f}}$, and combining eqs. (4) and (5), we find:

$$C_{2\mathrm{b}} = C_2(1 - \gamma_2^{\mathrm{c}}) \tag{6}$$

since [7]:

$$\gamma_2^{\mathrm{c}} = \gamma_2^{\mathrm{exp}}/\gamma_2^0. \tag{7}$$

Thus:

$$\theta_2 = (C_2/C_\mathrm{p})(1 - \gamma_2^{\mathrm{c}}) \tag{8}$$

and:

$$K_2 = (1/C_\mathrm{p})\{(\gamma_2^{\mathrm{c}})^{-1} - 1\}. \tag{9}$$

In essence, in comparing the procedure to determine γ_2^{c} [7] and the derivation given above, we realize that $C_{2\mathrm{f}}$ simply equals the divalent metal ion concentration in a reference solution of e.g. NaCl + $\mathrm{MgCl_2}$ of ionic strength I which has the same $\mathrm{Mg^{2+}}$ activity as the $\mathrm{Mg^{2+}}$ containing polyelectrolyte solution. This procedure would be valid for the dye spectrophotometric measurements reported here but equally so e.g. for metal ion activity determinations with selective ion electrodes.

The equality (5) makes it clear that this procedure is valid only when the total ionic strength sufficiently exceeds the polyion concentration, so that the influence of the polyion on the small ion activities through long range interactions (e.g. Debye–Hückel type) can be neglected. If this last condition is not met, either method still measures the divalent metal ion activity, $a_2 = a_{2f}$ as before, but C_{2f} can no longer be obtained from a simple ionic strength consideration. Manning, in his theory for the binding of multivalent ions by polyions [2], precisely considers the limiting case that $C_p/C_s \ll 1$ (C_s = total co-ion concentration, $C_s = C_1 + 2C_2 - C_p$), and also $C_s \ll 1$. This last condition is not strictly met in our or in Krakauer's [16] measurements, but is conveniently and reasonably circumvented by using the γ^0 procedure [17–19, 14]. The major difference between Manning's recent binding theory and expressions (1) and (2) derived from a simple condensation model, is that now the entropy of the condensed ions is not supposed to be zero, but rather equal to the entropy of an ion free to move in a volume V_p around the polyion, designated as the "free volume". In the "two variable theory" [2] the total free energy of charging the polyion (in the Debye–Hückel approximation) and the entropy of mixing of the small ions in both the bound and the free states is minimized with respect to θ_1 ($\theta_1 = C_{1b}/C_p$) and θ_2 to obtain the following expressions for our case of univalent counterions 1 and divalent counterions 2:

$$\ln(10^3 e\, \theta_1/C_1 V_p) = 2\xi(1 - \theta_1 - 2\theta_2)\ln\{1 - \exp(-\kappa b)\}, \tag{10}$$

$$\ln K_2 = 2\ln(\theta_1/C_1) - \ln(V_p e/10^3), \tag{11}$$

$$V_p = 10^3 e(1 - \xi^{-1})(\kappa b)^2/C_1, \tag{12}$$

κ is the Debye–Hückel parameter,

$$\kappa^2 = (4\pi e^2/DkT)\sum_i C_i Z_i^2. \tag{13}$$

All other quantities appearing in eqs. (10)–(11) have been defined earlier. Manning solves eqs. (10)–(11) as follows. For a given value of θ_2, eq. (10) is solved by interation for θ_1, and subsequently K_2 is obtained from eq. (11). From the definition of K_2, eq. (3), we find the value for C_2 at which θ_2 is reached:

$$C_2 = \theta_2(C_p + K_2^{-1}). \tag{14}$$

These equations yield values of θ_2 at a given C_2 which are always lower than the condensation results eqs. (1)–(2). The dependence of θ_2 and K_2 on the ionic strength will be discussed later; when $C_1 \gg C_2$ and $C_1 \gg C_p$ the ionic strength will simply equal C_1.

2. Experimental

Sodium dextransulfate, average molecular weight 500,000 was obtained from Pharmacia, Uppsala, Sweden. Purification by ultrafiltration, analysis, and conversion to the potassium salt by dialysis have all been described earlier [20]. Vacuum dried reagent grade NaCl, KCl, $MgCl_2$, and $CaCl_2$ were used without further purification. The concentrations of $MgCl_2$ and $CaCl_2$ stock solutions were determined by complexometric titration to ±0.1%. Eriochrome Black T (G. Frederick Smith Co., Columbus, Ohio) was further purified according to the method of Diehl and Lindstrom [21]. Purified tetramethylmurexide (TMM) was a gift from Dr. R. Kohn [22]. Reagent grade tris(hydroxymethyl)aminomethane neutralized with HCl to pH 8.00 ± 0.02 was used as buffer (buffer concentration 2×10^{-3}M) in the magnesium activity determinations with EBT. All solutions were made up by weight using deionized water, all concentrations as shown are in moles/litre (M).

The two wavelength dye spectrophotometric method ("ratio method") described in the previous communication [7] was used throughout. The EBT concentration used for Mg^{2+} activity determinations was 3×10^{-5} M, the TMM concentration for Ca^{2+} activity determinations was 4×10^{-5} M. Experimental procedures, instruments and calculations were the same as described before [7]. Needed $MgCl_2$ or $CaCl_2$ activity coefficients were interpolated from literature values [23].

3. Results and discussion

Experimental results for the systems dextransulfate – $MgCl_2$ – NaCl (which we will call the Mg/Na system), Mg/K, Ca/Na, and Ca/K are given in the figs. 1–6. In these figures the experimental results are expressed in the form of θ_2 calculated from the measured activities via eqs. (7) and (8). A polyion concentration (expressed as moles of sulfate groups per litre), C_p, of 0.001 mole/ℓ is used in all four systems. For the Mg/Na system a study of the polyion concentration dependence was performed, with additional C_p values of 0.002, 0.004 and 0.007. Total solution ionic strengths of 0.005, 0.008 and 0.01 were used in all four systems, in the Mg/Na system at $C_p = 0.001$ additional series at higher ionic strengths 0.02, 0.03 and 0.045 were carried out. In Mg/Na at $C_p = 0.002$ ionic strengths used were 0.01, 0.02, 0.04, 0.06 and 0.08. At each C_p and ionic strength the Mg or Ca concentration was varied to cover C_2/C_p values between 0.02 and 1.2. The ionic strength of each individual solution is adjusted to the desired value by adding the required amount of NaCl or KCl, taking into account the contributions from the buffer and from the free divalent ions. This procedure ensures the validity of the experimental procedure to obtain a_2 from a comparison of the absorbance ratio with the calibration curve for reference solutions of identical ionic strength, however it leads to an ambiguity in the comparison to the theoretical θ_2 curves, since theoretical θ_2 values (eqs. (10–11)) depend on the value of C_1. This difficulty would be most pronounced in the series with the lowest ionic strength and in the solutions with the highest C_2 values. We will further discuss this problem in a forthcoming publication [24], it is sufficient here to state that only the theoretical curves for $I = 0.005$, $C_p = 0.001$ and $I = 0.01$, $C_p = 0.002$ are noticeably affected, and then only for C_2/C_p values larger than 0.4. Adjustment of the theoretical curves would in fact slightly improve the qualitative agreement with the experimental data in that the theoretical curves would not level off quite as much at higher C_2/C_p values. We should note that in spite of this problem in comparing the experimental results to the theoretical expression, the excess salt condition, $C_s \gg C_p$, which allows Manning to neglected the effect of Debye–Hückel type polyion–small ion interactions on the total free energy, is still quite reasonably satisfied even at the lowest ionic strengths and highest C_2/C_p values used. The theoretical curves in figs. 1–6 are calculated from simple condensation theory (eqs. (1)–(2), straight lines in graphs) and from the two variable theory (eqs. (10)–(14)). The theoretical curves in these figures are calculated for the same ionic strengths as used in the various experimental series, as indicated by the arrows.

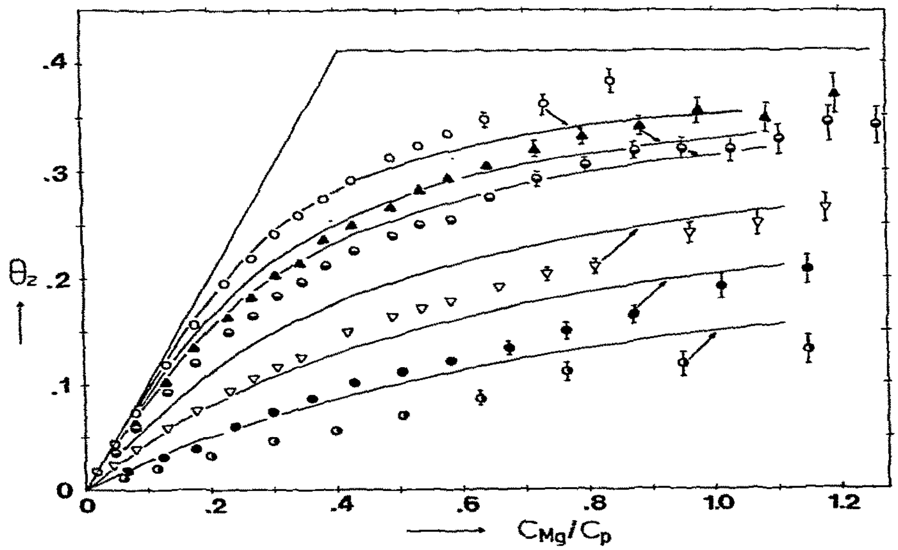

Fig. 1. θ_2 versus C_2/C_p for the system $MgCl_2$, NaCl, dextransulfate. $C_p = 0.001$ mole/ℓ, ○: $I = 0.0052$; ▲: $I = 0.008$; ◉: $I = 0.010$; ▽: $I = 0.020$; ●: $I = 0.030$; ◑: $I = 0.0455$. Error bars indicate estimated errors. Full lines: eqs. (1)–(2), upper line; eqs. (10)–(11), others. Arrows indicate the theoretical curves calculated for the ionic strength of a given series of data.

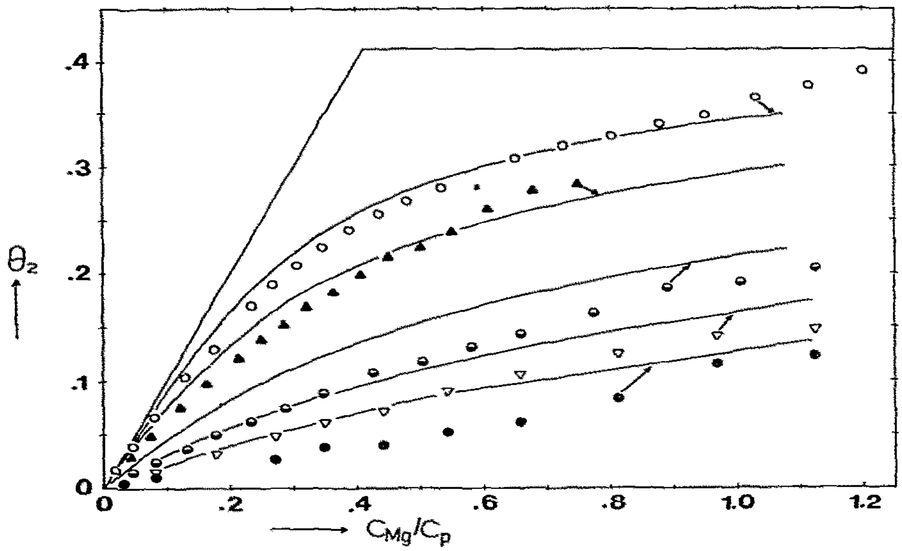

Fig. 2. θ_2 versus C_2/C_p for the system $MgCl_2$, NaCl, dextransulfate. $C_p = 0.002$ mole/ℓ. ○: $I = 0.0107$; ▲: $I = 0.020$; ⊖: $I = 0.040$; ▽: $I = 0.060$; ●: $I = 0.080$. Estimated errors and theoretical curves (full lines) as in fig. 1.

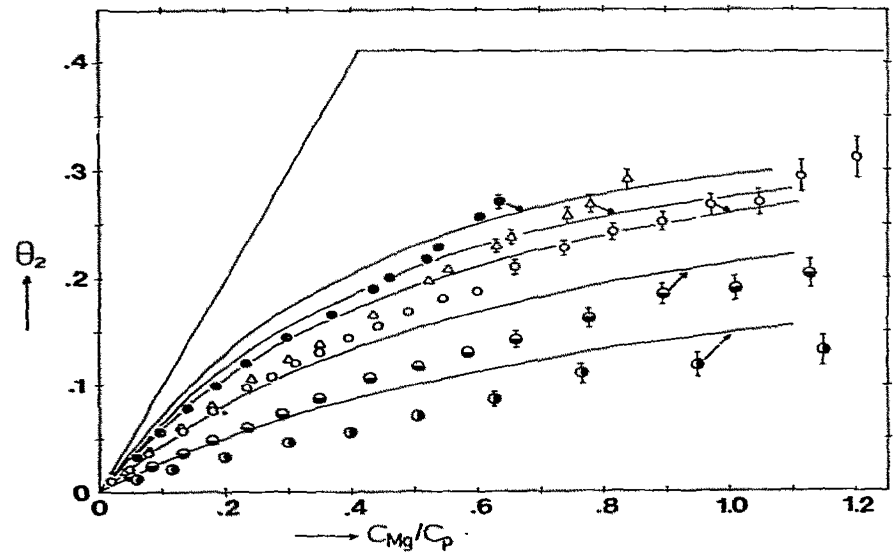

Fig. 3. θ_2 versus C_2/C_p for the system $MgCl_2$, NaCl, dextransulfate, at varying C_p. ◑: $C_p = 0.001, I = 0.0455$; ⊖: $C_p = 0.002, I = 0.040$; ○: $C_p = 0.004, I = 0.040$; △: $C_p = 0.007, I = 0.0495$; ●: $C_p = 0.010, I = 0.048$. Estimated errors and theoretical curves (full lines) as in fig. 1.

We will first consider the experimental data in the Mg/Na system (figs. 1–3). The estimated experimental errors in the determination of θ_2 are indicated by the error bars in fig. 1, where no error bars are drawn in this figure the estimated error in θ_2 is within the size of the data points as plotted. The estimated errors in θ_2 in the other data (figs. 2–6) are similar to the ones for corresponding points in fig. 1. The most distinctive feature of the data in figs. 1–3 is the strong dependence of θ_2 on the ionic strength. Clearly, at fixed values of C_p and C_2/C_p, θ_2 increases as the ionic strength decreases and at low ionic strength it tends to approach the condensation value (eqs. (1)–(2)). This is identical to what was observed by Krakauer [16] as calculated by Manning [14], and can also be seen from a number of other binding experiments [2, 25–28]. Apparently the Na^+ ion competes with the Mg^{2+} ion for binding by the polyion, thus as the Na^+ concentration increases, θ_2 decreases, eventually to very small values. These observations are compatible with an explanation which views the binding process as governed by the entropy gain accompanying the release of about 2 Na^+ ions when a Mg^{2+} ion is bound. This entropy gain is caused by the fact that the concentration of the various ions in the bound state (the "free volume" V_p in Manning's theory) is much higher than in the bulk of the solution. Therefore, at constant C_p, the higher the bulk ionic strength the lower the entropy gain when Na^+ ions are released from V_p to the bulk of the solution, leading in turn to lower θ_2 values.

In the other mixtures studied (Mg/K, Ca/Na, Ca/K, figs. 4–6) θ_2 shows a similar dependence on the ionic strength. However, when these data for the different divalent counterion/univalent counterion combinations are compared, some interesting features are revealed. θ_2 values for M^{2+}/K^+ combinations at a given C_2, C_p, and I are lower than the corresponding values for the M^{2+}/Na combinations. Also, θ_2 values for Mg^{2+}/M^+ combinations for both Na^+ and K^+ as M^+ are lower than the corresponding values for Ca^{2+}/M^+, again at the same C_2, C_p and I. In the absence of univalent salt, there is evidence that sulfonated or sulfated polyions do not show specificity for either Mg^{2+} or Ca^{2+} [18]. Similarly, in the absence of divalent salt there is no different in the colligative properties of added NaCl or KCl in dextrasulfate solutions, at least not in the concentration range of interest here [20]. Thus the observed difference in θ_2 for either NaCl or KCl as added salt may

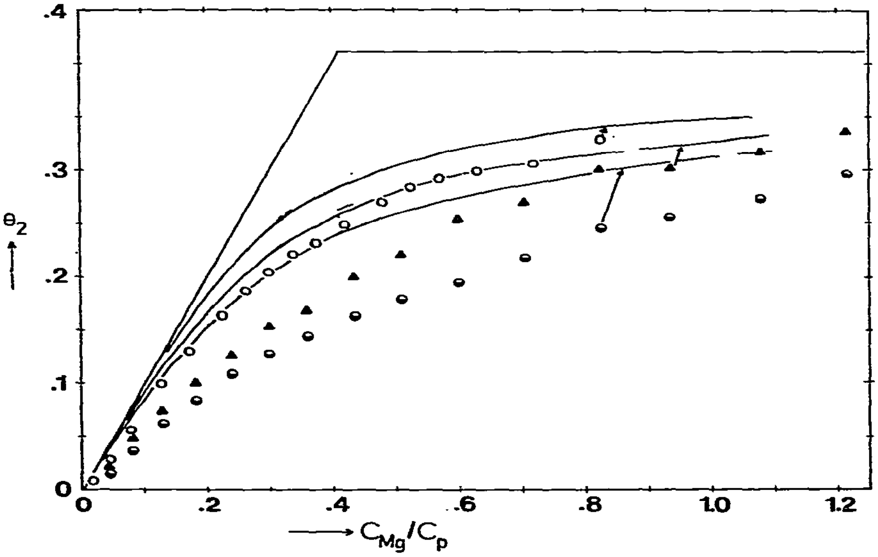

Fig. 4. θ_2 versus C_2/C_p for the system $MgCl_2$, KCl, dextransulfate. C_p = 0.001 mole/ℓ. ○: I = 0.005; ▲: I = 0.0075; ⊖: I = 0.010; Estimated errors and theoretical curves as in fig. 1.

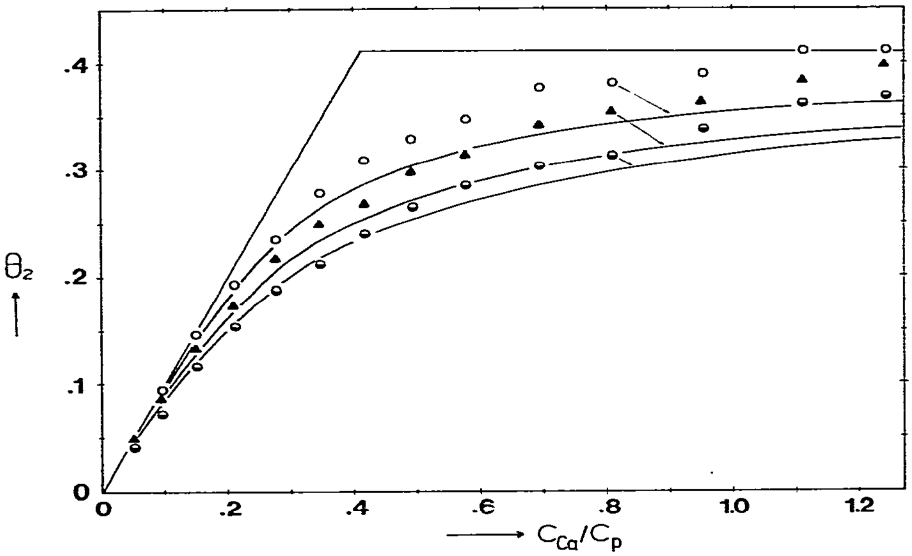

Fig. 5. θ_2 versus C_2/C_p for the system $CaCl_2$, NaCl, dextransulfate. C_p = 0.001 mole/ℓ. ○: I = 0.005; ▲: I = 0.008; ⊖: I = 0.010. Estimated errors and theoretical curves as in fig. 1.

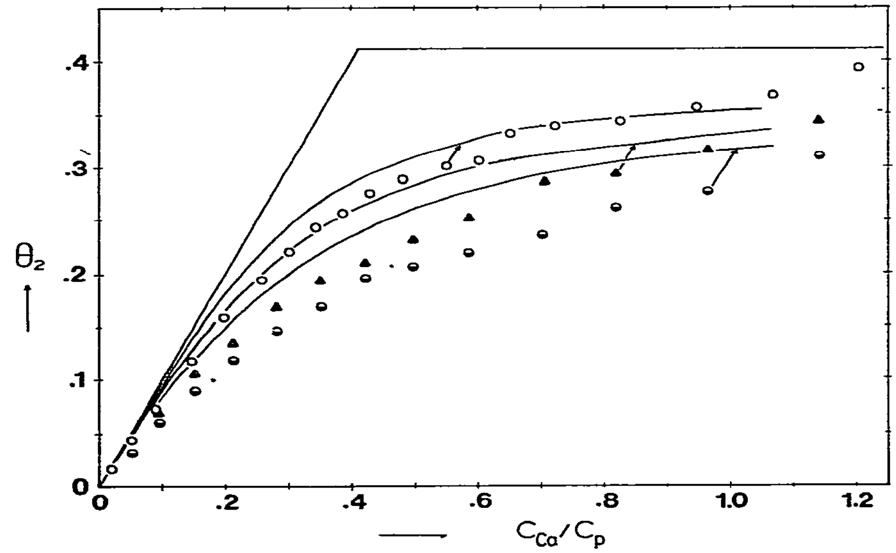

Fig. 6. θ_2 versus C_2/C_p for the system $CaCl_2$, KCl, dextransulfate. C_p = 0.001 mole/ℓ. ○: I = 0.005; ▲: I = 0.008; ⊖: I = 0.010. Estimated errors and theoretical curves as in fig. 1.

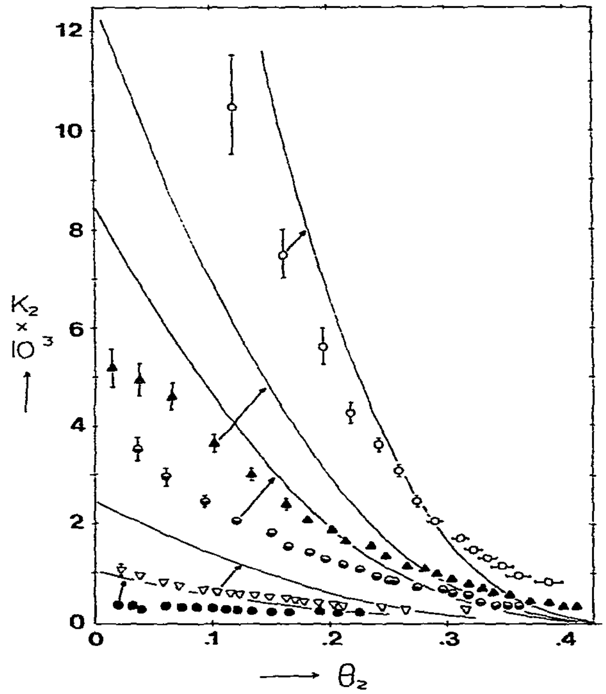

Fig. 7. Scatchard curves, K_2 (ℓ/mole, eq. (9)) versus θ_2, in the system $MgCl_2$, NaCl, dextransulfate. C_p = 0.001 mole/ℓ. Estimated errors indicated by error bars. Symbols and theoretical curves (full lines) as in fig. 1.

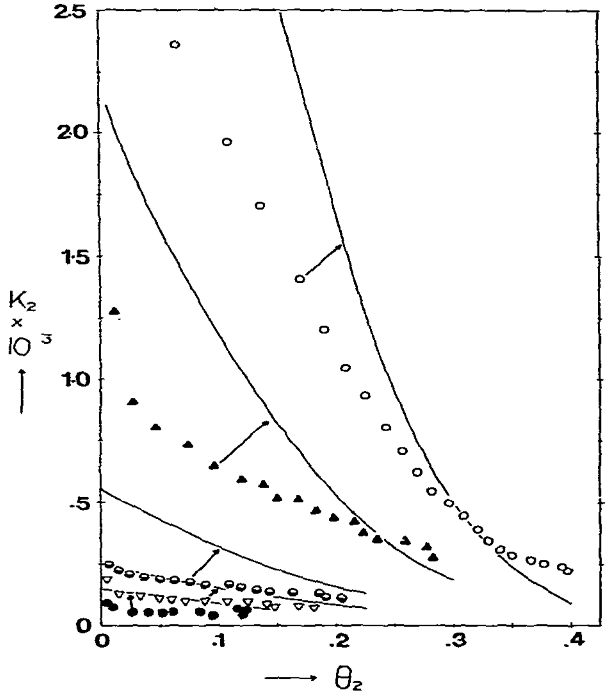

Fig. 8. Scatchard curves, K_2 (ℓ/mole, eq. (9)) versus θ_2, in the system $MgCl_2$, NaCl, dextransulfate. C_p = 0.002 mole/ℓ. Symbols as in fig. 2, estimated errors and theoretical curves (full lines) as in fig. 7.

be due to the ease with which Mg^{2+} or Ca^{2+} displaces Na^+ or K^+, even though the extent of binding of either Na^+ or K^+ the absence of divalent ions is the same and well described by condensation theory. It may be expected that Ca^{2+} because of its smaller hydrated size can remove any M^+ ion more easily than Mg^{2+} can, and hence the observed order in θ_2, $\theta_2(Ca^{2+}/M^+) > \theta_2(Mg^{2+}/M^+)$. Similarly, K^+, because of its smaller hydrated size is less easily displaced than Na^+, and thus $\theta_2(M^{2+}/Na^+) > \theta_2(M^{2+}/K^+)$.

Of course Manning's two variable theory does not consider these specific effects at all, and all theoretical curves drawn in figs. 1–6 are identical for given C_p and I. The data clearly indicate that compared to the ionic strength dependence of θ_2 in all systems the specific differences between the various ion pairs mentioned above are relatively small, and the decrease in θ_2 with increasing I is remarkably well described by eqs. (10)–(14). We notice that the agreement between the two variable theory and the experimental data is best when the radius of the hydrated ion is large for the univalent ion and small for the divalent ion, i.e. the agreement is best for Ca^{2+}/Na^+ and worst for Mg^{2+}/K^+. In any case it is remarkable how well the two variable theory describes the general shape, ionic strength and C_p dependence of the θ_2 versus C_2/C_p curves. If we consider the C_p dependence of θ_2 at constant ionic strength, as is done in fig. 3, we see that θ_2 increases slightly as C_p increases. This increase is predicted by the two variable theory and its magnitude is well described by it.

We will now consider the K_2 versus θ_2 plots which are the well known Scatchard plots. We recall that K_2 is a quantity proportional to the equilibrium constant for the transfer of a divalent ion from a free state (the

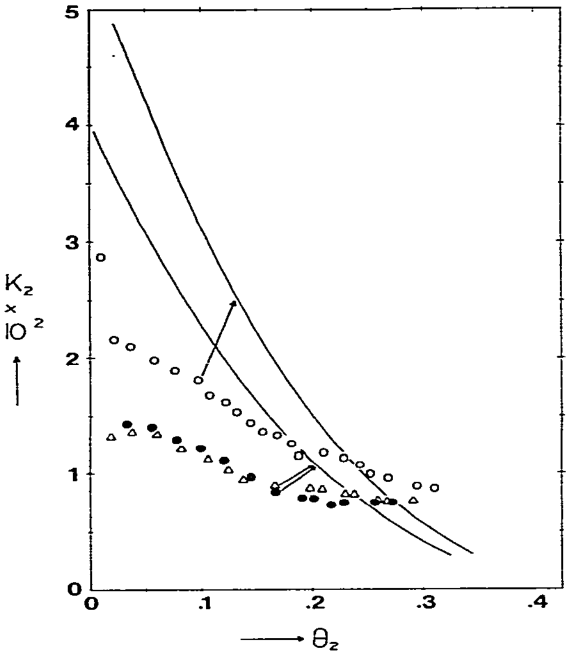

Fig. 9. Scatchard curves, K_2 (ℓ/mole, eq. (9)) versus θ_2, in the system $MgCl_2$, NaCl, dextransulfate, the C_p dependence. ○: $C_p = 0.004, I = 0.040$; △: $C_p = 0.007, I = 0.0495$; ●: $C_p = 0.010, I = 0.048$. Symbols as in fig. 3, estimated errors and theoretical curves (full lines) as in fig. 7. Additional data at $C_p = 0.001$ and $C_p = 0.002$ and comparable ionic strength are represented in figs. 7 and 8. These data have been deleted here to avoid confusion due to overlap with the data presented (see text).

bulk of the solution) to a bound state (a region within V_p). The experimental points in the Scatchard plots (figs. 7–12) are the same as in figs. 1–6, and the theoretical K_2 versus θ_2 curves are calculated from eqs. (10–(14) as are the theoretical curves in figs. 1–6. Thus the Scatchard curves are simply a different representation of experimental data and theoretical curves, but they will exhibit the same trends as before. In figs. 7 and 11 typical estimated errors are indicated by error bars. Where no error bar is indicated on these figures the estimated error is within the size of the data points. No error bars are given the in the other Scatchard plots, the estimated errors there are comparable to what is indicated in fig. 7 (Mg systems) or fig. 11 (Ca systems). The data at low ionic strengths clearly show the upward curvature generally described as "anti-cooperative" behaviour. This is in complete agreement with the expected behaviour for polyelectrolytes, where the binding process would become less and less favourable as more divalent ions are bound. Moreover, since K_2 decreases as the ionic strenght decreases, we notice again that the binding process becomes less and less favourable as the ionic strenght increases. It was noticed earlier that θ_2 increases as C_p increases at constant ionic strenght (fig. 3). Interestingly enough if such a comparison is done for the Scatchard plots (figs. 7–9), the observed values of K_2 are very similar (notice the different scales used for K_2 in these figures). Again this is also predicted by the two-variable theory. Indeed, the observed Scatchard plots are very closely predicted by Manning's binding theory. As before the best agreement is for the Ca^{2+}/Na^+ combination (fig. 11), the worst agreement is for the Mg^{2+}/K^+ combination (fig. 10) where the observed K_2 values are considerably below the predicted values. In comparing these figures we should note the different scales used for K_2 in order to represent the data with the required sensitivity. It is remarkably enough that the two variable theory, without any adjustable parameters and using only the structurally determined average linear charge separation as a polyion determined quantity, is able to describe both

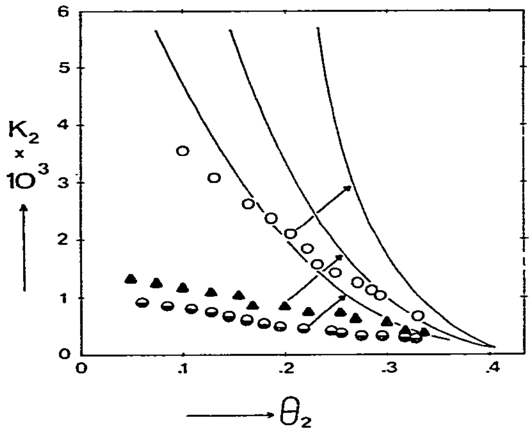

Fig. 10. Scatchard curves, K_2 (ℓ/mole, eq. (9)) versus θ_2, in the system $MgCl_2$, KCl, dextransulfate. C_p = 0.001 mole/ℓ. Symbols as in fig. 4, estimated errors and theoretical curves as in fig. 7.

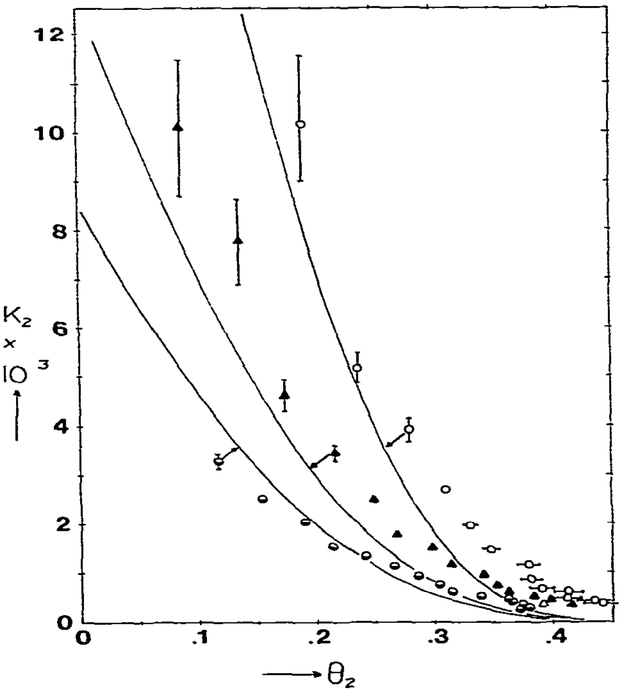

Fig. 11. Scatchard curves, K_2 (ℓ/mole, eq. (0)) versus θ_2, in the system $CaCl_2$, NaCl, dextransulfate. C_p = 0.001 mole/ℓ. Error bars indicate estimated errors. Symbols and theoretical curves as in fig. 5.

the absolute K_2 values and their ionic strength and θ_2 dependence so well. This agreement clearly corroborates the view that the "anti-cooperative" behaviour in these Scatchard plots is a typical polyelectrolyte effect largely determined by non-specific electrostatic interactions [2]. No notions of "site binding" or "specific binding" are needed. There are individual variations between ions and various combinations of ions, but these variations are relatively small compared to the primary polyelectrolyte effect. It is possible to force a close agreement between theory and data by using V_p as a variable rather than taking V_p as fixed by the free energy minimization procedure [1]. All experimental data can be well predicted by using a value of V_p which is at most three times (log Mg/K) the value given by eq. (12). Such a procedure is unjustified without additional theoretical considerations.

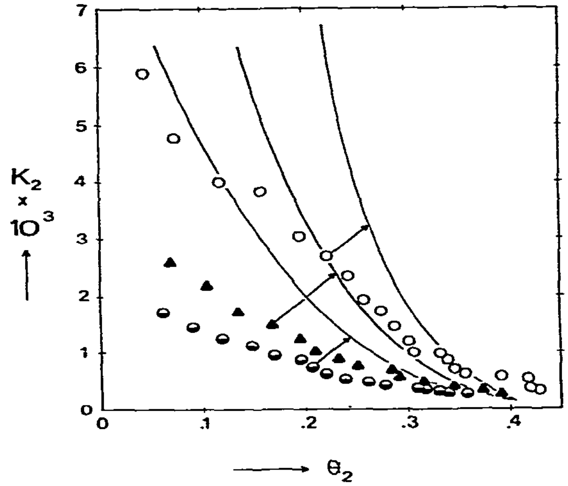

Fig. 12. Scatchard curves, K_2 (ℓ/mole, eq. (9)) versus θ_2, in the system $CaCl_2$, KCl, dextransulfate. C_p = 0.001 mole/ℓ. Symbols as in fig. 6. Estimate errors and theoretical curves as in fig. 11.

Finally, we focus our attention on the variation of K_2 with C_1. This is most easily expressed in the form of the dependence of $d\ln K_2/d\ln C_1$ on θ_2, since for low θ_2, θ_1 becomes constant and independent of C_1, thus $d\ln K_2/d\ln C_1$ at low θ_2 (and thus low C_2/C_p) should become equal to −2, and the magnitude of this quantity is predicted to decrease slightly at high θ_2 (eq. (11)). The values of $d\ln K_2/d\ln C_1$ calculated from the data for the four ion pair combinations studied here generally

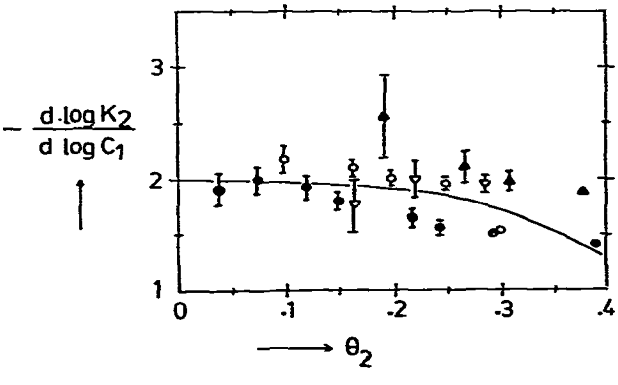

Fig. 13. Check of eq. (11). Data points were obtained from the systems: ●: Mg/Na; ○: Mg/K; ▲: Ca/Na; △: Ca/K. Full line calculated from eq. (11). For explanation see text.

show such a behaviour (fig. 13). It should be pointed out that at low θ_2 the calculation of this derivative can be based on only very few data points. Because of the generally somewhat lower accuracy of the calcium data at very low C_2 [7], large uncertainties in the values of $\mathrm{dln}K_2/\mathrm{dln}C_1$ in the calcium systems result at low θ_2, and these data have been omitted here. In an upcoming publication we will show that the calcium systems also adhere closely to the behaviour predicted by theory, as do a number of other divalent metal ion systems [24].

We conclude that Manning's two variable theory gives a surprisingly accurate description of the degree of divalent metal ion binding in the systems studied, both with respect to the dependence on the divalent metal ion concentration and the dependence on the ionic strength. Because the polyelectrolyte's effects are most noticeable at lower ionic strengths it becomes important to generalize Manning's two variable treatment to cases where the condition $C_p/C_s \ll 1$ is no longer satisfied. Also, we have shown that there are differences between the results with different combinations of divalent and univalent counterions. Refinement of the theoretical treatment to allow for such differences is necessary. Finally, it is desirable to extend the measurements to different types of polyions and to a range of uni- and divalent counterions. At the same time there is a considerable need for experimental data based on different measurement techniques to allow critical comparisons of the data obtained.

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References

[1] G.S. Manning, Biophys. Chem. 7 (1977) 141.
[2] G.S. Manning, Quart. Rev. Biophysics 2 (1978) 179.
[3] K. Iwasa, J. Phys. Chem. 81 (1977) 1829.
[4] K. Iwasa, Biophys. Chem. 9 (1979) 397.
[5] M. Gueron and G. Weisbuch, Biopolymers 19 (1980) 353.
[6] J.A.L.I. Walters, H.A.M. Geerdes and C.H. Hilbers, Biophys. Chem. 7 (1977) 147.
[7] J.C.T. Kwak and Y.M. Joshi, Biophys. Chem. 13 (1981) 55.
[8] N. Imai and T. Ohnishi, J. Chem. Phys. 30 (1959) 1115.
[9] G.S. Manning, J. Chem. Phys. 51 (1969) 924.
[10] K. Iwasa and J.C.T. Kwak, J. Phys. Chem. 81 (1977) 408.
[11] K. Iwasa, D.A. MacQuarrie and J.C.T. Kwak, J. Phys. Chem. 82 (1978) 1979.
[12] Y.M. Joshi and J.C.T. Kwak, J. Phys. Chem. 83 (1979) 1978.
[13] K. Iwasa, Y.M. Joshi and J.C.T. Kwak, in: Dynamic aspects of biopolyelectrolytes and biomembranes, ed. F. Oosawa (Kodansha, Tokyo and Elsevier, Amsterdam, to be published).
[14] G.S. Manning, in: Polyelectrolytes, ed. E. Sélègny (Reidel, Dordrecht, Holland, 1974) p. 9.
[15] J.C.T. Kwak, N.J. Morrison, E.J. Spiro and K. Iwasa, J. Phys. Chem. 80 (1976) 2753.
[16] H. Krakauer, Biopolymers 10 (1971) 2459.
[17] J.D. Wells, Proc. R. Soc. London Ser. B, 183 (1973) 399.
[18] J.C.T. Kwak, M.C. O'Brien and D.A. MacLean, J. Phys. Chem. 79 (1975) 2381.
[19] K. Iwasa and J.C.T. Kwak, J. Phys. Chem. 80 (1976) 215.
[20] Y.M. Joshi and J.C.T. Kwak, Biophys. Chem. 8 (1978) 191.
[21] H. Diehl and F. Lindstrom, Anal. Chem. 31 (1959) 414.
[22] R. Kohn and I. Furda, Collect. Czech. Chem. Comm. 32 (1967) 1925.
[23] R.A. Robinson and R.H. Stokes, Electrolyte solutions, 2nd Ed. (Butterworth's, London, 1970).
[24] J. Mattai and J.C.T. Kwak, to be submitted.
[25] G. Zubay and P. Doty, Biochem. Biophys. Acta 29 (1958) 47.
[26] J. Skerjanc and U.P. Strauss, J. Am. Chem. Soc. 90 (1968) 3081.
[27] J.T. Shapiro, B.S. Stannard and G. Felsenfeld, Biochemistry 8 (1969) 3233.
[28] R.M. Clement, J. Sturm and M.P. Daume, Biopolymers 12 (1973) 405.