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A DYE SPECTROPHOTOMETRIC METHOD FOR BINDING STUDIES OF Zn^{2+} AND Mn^{2+} BY BIOPOLYELECTROLYTES

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A dual-wavelength dye spectrophotometric method is reported for measuring zinc and manganese activities using the dyes tetramethylmurexide (TMMX) and murexide (MX) respectively. The method is applied to the measurement of the activities of these metal ions in solutions of the polyelectrolyte dextransulfate with added sodium chloride. Polyion concentrations, C_p (expressed as moles sulfate ion/litre) of 0.001 and 0.002 are studied at total ionic strengths 0.005, 0.0075, 0.01, 0.02 and 0.03 mole/l. Divalent metal ion concentrations are varied between 0 and 1.0 C_p. The results for the metal ion activities are expressed in the form of binding isotherms, θ_2 versus C_2/C_p ($\theta_2 = C_{2b}/C_p$; C_{2b} = bound divalent metal ion concentration) and Scatchard plots, K_2 versus $\theta_2/(C_2 - C_{2b})$, at different ionic strengths. The experimental data are correlated with the "two-variable theory" developed for these mixed counterion systems by Manning. This comparison shows that the observed decrease in θ_2 and K_2 with ionic strength at fixed C_2 and C_p is generally well predicted by the two-variable theory. Both Zn and Mn bind to the same extent to dextransulfate. This observation, and the reasonable agreement of the data with the "two-variable theory" may be interpreted as indicating a delocalized form of binding of these metal ions to the polymer.

1. Introduction

Divalent metal ion binding to polyelectrolytes in mixed counterion systems, i.e. systems containing both uni–univalent and di–univalent electrolytes, is of considerable practical and theoretical interest. For example, in systems of physiological importance polyions such as DNA, RNA, proteins, polysaccharides are present in solutions containing Na^+, K^+, Mg^{2+} and Ca^{2+} as major counterions, and Cl^- as co-ion. The extent of divalent metal ion binding to polyions has been qualitatively followed by methods such as dilatometry [1,2], viscosity [1], ultrasonic absorption [3], index of refraction measurements [4], and circular dichroism [5]. However the binding can be quantitatively monitored by determining the free divalent metal ion activity or concentration after binding has occurred. Techniques applied in the past for determining this quantity are electrochemical methods (EMF and Donnan) [6], ESR [7] and spectrophotometry [8–12].

In the first paper of this series, Kwak and Joshi [13] have shown the limitations of the electrochemical and ESR methods for determining divalent ion activities in the physiological concentration range, i.e. below 10^{-3} M. Following the principle of Kohn et al. [8–11], these authors described a dual-wavelength spectrophotometric method for determining free Mg^{2+} and Ca^{2+} activities in polyelectrolyte solutions. In the second paper of this series [14], the method was applied to the binding of Mg^{2+} and Ca^{2+} to the polyelectrolyte dextransulfate (DS), a highly sulfated polymer of α-D-glucose.

To further study the interaction between polyions and divalent ions in mixed counterion systems, we have utilized the dual-wavelength spectrophotometric method to examine its applicability to the determination of activities of the transition metal ions Zn^{2+} and Mn^{2+}. The binding of these metal ions to DS, using the spectrophotometric method, will be discussed quantitatively. It is of

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particular interest to compare the results of binding studies of Zn^{2+} and Mn^{2+} to similar results for the binding of Mg^{2+} and Ca^{2+} to the same biopolyelectrolyte.

The binding of multivalent counterions to polyions in the presence of an excess of uni–univalent salt has been the topic of a considerable number of theoretical efforts recently. In particular, the methods of Manning and of Iwasa [15–18] make use of a free energy minimization procedure for a two-phase model of polyelectrolyte solutions. In this paper, as in the previous papers in this series [13,14], we will compare our experimental results to the theoretical predictions of Manning's "two-variable theory" [16], a theory which has proven to be particularly successful in describing the experimental results. It should perhaps be pointed out that the name "two-variable theory" derives from the fact that the degrees of binding of both univalent and multivalent counterions are variables in the free energy minimization procedure. Thus in fact the two-variable theory as used in this paper does not contain any adjustable parameters since the so-called "condensation volume", which is the volume around the polyion available to bound (condensed) ions, is fully determined by the charge density parameter ξ of the polyion and the ionic strength [19].

2. The dye spectrophotometric method

In this study, Zn^{2+} and Mn^{2+} activities are determined by using as an "activity probe" a dye which binds relatively weakly to these ions. Consider a typical dye H_nD^{m-}, which releases n H^+ ions when binding a metal ion M^{z+} (n will depend on the dye and the pH range, in some cases n may equal 0):

$$M^{z+} + H_nD^{m-} \rightarrow MD^{(m+n-z)-} + nH^+ . \qquad (1)$$

It can be shown [13] that at constant pH, i.e. constant a_H, and constant ionic strength, i.e. constant activity coefficient ratio $\gamma_{MD}/\gamma_{H_nD}$, the activity of the metal ion is given by

$$\log a_M = \log C_{MD}/C_{H_nD} + \text{constant}. \qquad (2)$$

Thus the sensitivity of the method depends on having a dye for which the apparent binding constant $K_{app} = C_{MD}/C_M C_{H_nD}$ is not too large, typically between 500 and 10000. Under these conditions, with dye concentrations around 10^{-5} M and metal ion concentrations between 10^{-5} M and 10^{-3} M only a small fraction of the metal ions are bound by the dye, and thus addition of the dye does not influence the metal-polyion equilibrium. By employing a two-wavelength method problems of dye instability are largely overcome and sensitivity is enhanced. The use of reference solutions of identical ionic strength and a calibration curve procedure allows for the direct determination of the fraction of free metal ions [13,14].

In this work, Zn activities are determined using the dye tetramethylmurexide (TMMX), and Mn activities are determined using the dye murexide (MX). TMMX was used by Kohn et al. [8–11], Ohnishi [20], Kwak and Joshi [13,14] for calcium activity determinations. MX was used by Ohnishi [20] for other divalent metal ions. Other conditions to be satisfied by the dye, i.e. large extinction coefficients and adequate separation of the absorbance maxima of complexed and uncomplexed forms, were previously discussed [13]. For TMMX, n in eq. (1) equals zero, and the solutions do not need to be buffered at a given pH. For MX, the pH can be used as a variable to optimize K_{app}.

3. Experimental

Sodium dextransulfate, average molecular weight 500000, was obtained from Pharmacia Uppsala, Sweden. Its purification, concentration and analysis have been described elsewhere [21]. Zn^{2+} and Mn^{2+} concentrations were determined by complexometric titration with EDTA, using Eriochrome Black T (EBT) as indicator. These determinations were accurate to ±0.1%. Purified tetramethylmurexide (TMMX) was a gift from Dr. R. Kohn [8]. Murexide (MX) (Fisher Scientific) was used without further purification. Reagent grade tris (hydroxymethyl) aminoethane neutralized with HCl to a pH of 8.00 ± 0.02 was used as buffer (buffer concentration 2×10^{-3} M) in the manganese activity determinations with MX. 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 as applied to Zn^{2+} and Mn^{2+} activity determinations was used throughout. The TMMX concentration used for Zn^{2+} activity determinations was 2.4×10^{-5} M, and the MX concentration for Mn^{2+} activity determinations was 4.0×10^{-5} M. Experimental procedures and instrumentation were described previously [13].

Activity coefficients of the metal ions in reference solutions were calculated with the MacInnes convention:

$$\gamma_{M^{2+}}(I) = \gamma_{\pm}^{2}(MCl_2)/\gamma_{\pm}^{2}(KCl),$$

where $\gamma_{\pm}(MCl_2)$ and $\gamma_{\pm}(KCl)$ are the mean activity coefficients of MCl_2 and KCl in pure salt solutions at the same ionic strength. Mean activity coefficient data for $ZnCl_2$ and $MnCl_2$ are scarce at low ionic strength. We have elected to use the values for $CaCl_2$ provided in Robinson and Stokes [22]. Because ratios of activity coefficients are used in the calculation of the final results [14], these results are not affected by slight variations in the choice of $\gamma_{\pm}(MCl_2)$.

4. Results and discussion

The determination of Zn^{2+} activities using TMMX is an excellent example of the sensitivity and the precision possible with the dual-wavelength dye spectrophotometric method, due to the particularly favourable experimental parameters of the Zn–TMMX system (no pH dependence, stable dye, high extinction coefficients, good peak separations, favourable K_{app} value). Fig. 1 shows the spectra of free TMMX (A), the Zn–TMMX complex (B), and a typical Zn–polyelectrolyte–TMMX mixture where the TMMX is partly free and partly Zn–TMMX (C). The Zn–TMMX complex absorbance maximum is at 460 nm, the free TMMX absorbance maximum is at 530 nm. If we call ϕ the absorbance ratio A''/A' at wavelengths λ'' and λ', it follows from eq. (2) [13]:

$$\phi = \left(\epsilon''_{H_nD} + \epsilon''_{MD} K a_M\right) / \left(\epsilon'_{H_nD} + \epsilon'_{MD} K a_M\right). \quad (3)$$

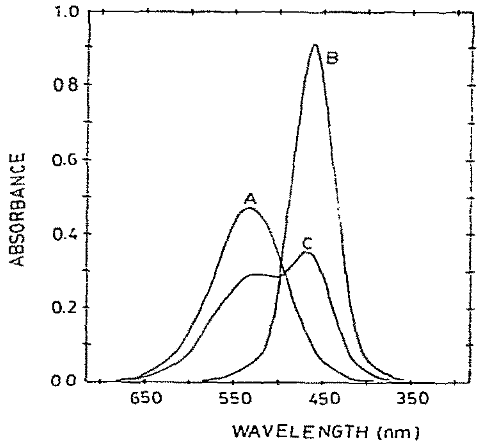

Fig. 1. Absorption spectra of (A) TMMX (3.023×10^{-5} M), (B) Zn–TMMX complex (excess Zn) and (C) mixture of TMMX (3.023×10^{-5} M), Zn (3.428×10^{-4} M) and dextransulfate (0.001 mole/l).

where ϵ' and ϵ'' are extinction coefficients of free dye H_nD and metal–dye complex MD at λ' and λ'' respectively, and K is the constant in eq. (2). For Zn–TMMX, the H_nD and MD absorbance peaks are well enough separated to make ϕ linear in the Zn activity range $0–3 \times 10^{-4}$ (fig. 2). This linear response occurs over a much larger activity range for Zn with TMMX than for Mg with EBT [13], Ca^{2+} with TMMX [8], or Mn^{2+} with MX (see below). The calibration curve is independent of the ionic strength of the solution, i.e. the absorbance at a given concentration of Zn^{2+} is determined only by the Zn^{2+} activity coefficient. This is as expected since Zn forms a similar complex with TMMX as Ca, and Kohn [8] has shown that ϕ is independent of ionic strength for Ca–TMMX.

The K_{app} value for the Zn–TMMX complex, determined by the method of Diehl and Lindstrom [23], was found to be 8500. This is approximately the upper limit for a "good dye". This large K_{app} value and the large extinction coefficients lead to a large increase in the absorbance of the complex and a corresponding large decrease in the dye absorbance as the zinc activity increases. ϕ will change rapidly for even small changes in activity, and the calibration curve suggests that zinc activi-

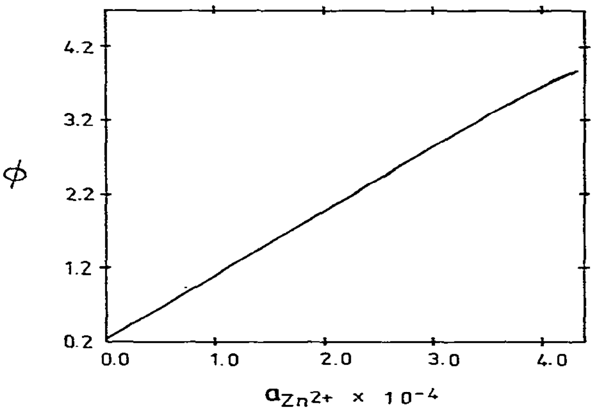

Fig. 2. Calibration curve for Zn activity determinations (absorbance ratio ϕ versus Zn activity in moles/l), at $I = 0.005$, 0.0075, 0.01, 0.02 and 0.03. All individual points, regardless of ionic strength, coincide with the curve shown within the accuracy of the graph.

ties can be determined quite accurately in the activity range $(0.5 \times 10^{-6} - 5 \times 10^{-4}$ M).

Manganese cannot be determined with the dye TMMX because its K_{app} value has been found to be 180 [20] at pH 7.00. This would make ϕ (Mn^{2+}) insensitive to small changes in Mn^{2+} activity. However, murexide (MX) is known to behave similarly to TMMX [8], but unlike TMMX, MX is sensitive to pH changes. K_{app} values of MX with divalent metal ions are known to increase with pH [24]. At pH ≈ 8.00, using the method of Diehl and Lindstrom [23] we found K_{app} for the Mn^{2+}–MX complex to be 2400. At pH 8.0, the Mn^{2+} activity can therefore be determined by the dual-wavelength spectrophotometric method, but with less accuracy than the Zn^{2+} activity determination.

λ_{max} of MX was found to be at 530 nm, in agreement with Kohn [8], while λ_{max} of the Mn^{2+}–MM complex occurred at 480 nm. The separation of the peaks here is not as good as for the Zn–TMMX system. However, it was found that by varying λ' and λ'', using wavelengths larger than λ_{max} of free MX, and smaller than λ_{max} of Mn–MX complex increases the sensitivity of the method. In this work, we have selected λ'_{MX} at a value of 540 nm instead of 530 nm. Fig. 3 shows that the ϕ values increased by ≈ 20%.

On comparing figs. 2 and 3, it can be seen that over an identical activity range, ϕ changes by ≈ 4.0 units for Zn–TMMX, while the change for Mn–MX is only ≈ 0.70 units. The small change in ϕ for Mn–MX is attributed to peak overlap of the dye and complex and also a relatively low K_{app} value. The calibration curve for Mn–MX, fig. 3, which is also ionic strength independent, is seen to be non-linear, as opposed to the Zn–TMMX system (fig. 2). In principle, both Zn and Mn activities can be determined in the range $(0.5–5.0) \times 10^{-4}$ M. However, because of the smaller rate of change of ϕ with Mn^{2+} activity, the uncertainty in the latter determination, particularly in the lower activity range, is greater than that for Zn. Table 1 summarizes the important parameters for Zn and Mn activity determinations.

For both Zn and Mn, ϕ was found to be independent of dye concentration indicating that the fraction of dye taking part in the metal–dye equilibrium was constant. In addition, in all systems studied the activity of the metal ion was found to be independent of dye concentration, indicating that the dye does not affect the polyion–metal ion equilibrium.

It should be noted that MX is known to be

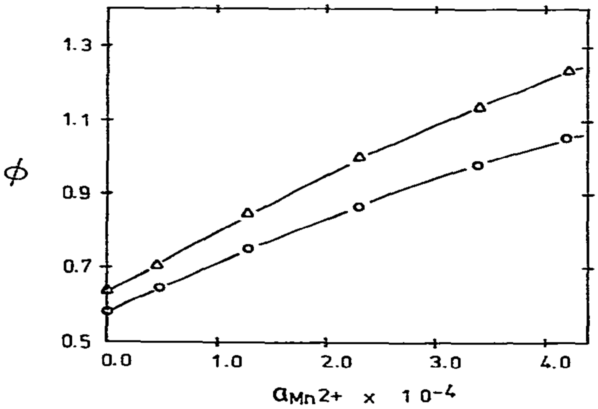

Fig. 3. Calibration curve of Mn activity determinations (absorbance ratio ϕ versus Mn activity in moles/l) at $I = 0.01$. ○: $\phi(A_{480}/A_{530})$; △: $\phi(A_{480}/A_{540})$.

Table 1

Important parameters for Zn and Mn activity determination

Metal ion	Dye	Dye concentration (moles/l)	λ′ (nm) (free dye)	λ″ (nm) (complex)	K_{app}	Changes in ϕ a_M: $0-5\times10^{-4}$ M
Zn^{2+}	TMMX	2.4×10^{-5}	530	460	8500	0.200–4.50
Mn^{2+}	MX	4.0×10^{-5}	540	480	2400	0.600–1.36

unstable in solution [20] and the absorbances of free dye and of the metal–dye complex decrease with time. However, one of the advantages of the ratio method, as opposed to the extrapolation method [12], for divalent metal ion activity determinations is its insensitivity to dye or dye–metal complex decomposition. With the unknowns "sandwiched" between reference solutions, ϕ was found to be approximately constant in the 1.5 h required for a measurement series.

Using the two-wavelength dye spectrophotometric method, and the experimental parameters as given above, activities of Zn^{2+} and Mn^{2+} were determined in solutions containing the polyanion dextransulfate (DS) and NaCl. Experimental data for the system NaDS–$ZnCl_2$–NaCl (which we will call Zn/Na), and for the corresponding system Mn/Na are presented in figs. 4–7. The data are expressed as "binding isotherms" showing a quantity θ_2,

$$\theta_2 = C_{2b}/C_p, \tag{4}$$

as a function of C_2/C_p. C_{2b} is the concentration of bound divalent metal ions, C_2 the total divalent metal ion concentration, and C_p the polyion concentration in moles of sulfate groups per litre. As described in ref. [14], the free divalent metal ion concentration, $C_{2f} = C_2 - C_{2b}$, simply equals the divalent metal ion concentration in a reference solution of e.g. NaCl + $ZnCl_2$ or NaCl + $MnCl_2$ of identical ionic strength as the polyelectrolyte solution, which has the same metal ion activity as the Zn^{2+} or Mn^{2+} containing polyelectrolyte solu-

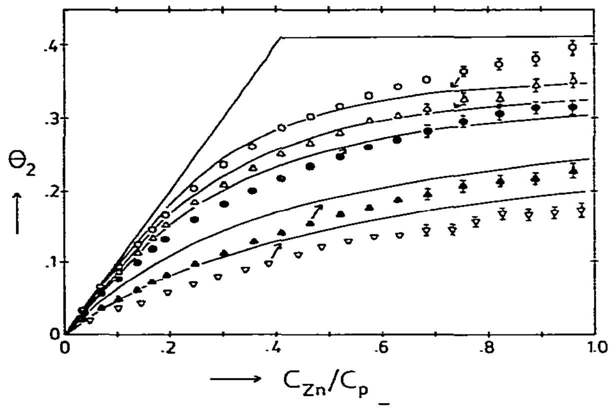

Fig. 4. θ_2 versus C_2/C_p for the system $ZnCl_2$, NaCl, dextransulfate. C_p = 0.001 mole/l. ○: I = 0.005; △: I = 0.0075; ●: I = 0.010; ▲: I = 0.020; ▽: I = 0.030. Error bars indicate estimated errors. Upper line condensation model, lower curves: two-variable theory. Arrows indicate the theoretical curves calculated for the ionic strength of a given series of data.

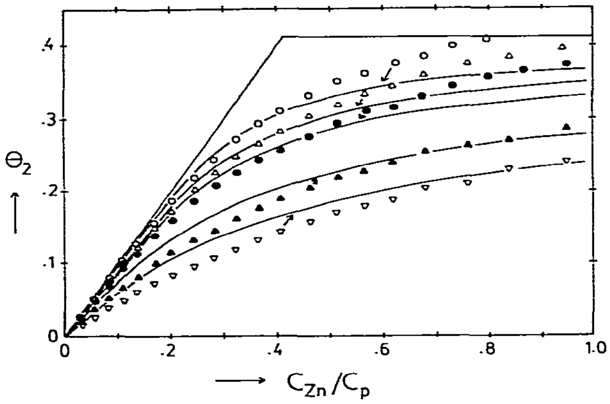

Fig. 5. θ_2 versus C_2/C_p for the system $ZnCl_2$, NaCl, dextransulfate. C_p = 0.002 mole/l. ○: I = 0.005; △: I = 0.0075; ●: I = 0.010; ▲: I = 0.020; ▽: I = 0.030. Estimated errors and theoretical curves as in fig. 4.

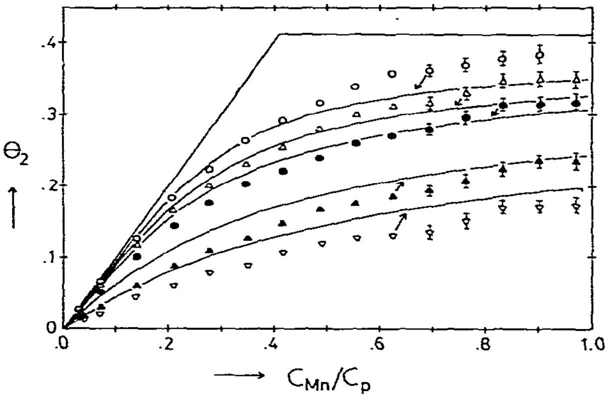

Fig. 6. θ_2 versus C_2/C_p for the system MnCl, NaCl, dextransulfate. $C_p=0.001$ mole/l. ○: $I=0.005$; △: $I=0.0075$; ●: $I=0.010$; ▲: $I=0.020$; ▽: $I=0.030$. Error bars indicate estimated errors. Theoretical curves as in fig. 4.

tion. In other words, the measured value of ϕ in the polyelectrolyte solution combined with the calibration curve of ϕ versus a_M at a given ionic strength for $ZnCl_2$ or $MnCl_2 + NaCl$ mixtures immediately yields a value for the free metal ion concentration in the polyelectrolyte solution. Alternatively, the results can also be expressed in the form of activity coefficients [14].

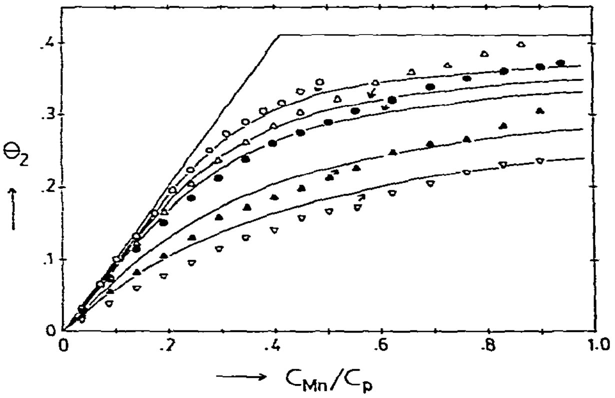

Fig. 7. θ_2 versus C_2/C_p for the system $MnCl_2$, NaCl, dextransulfate. $C_p=0.002$ mole/l. ○: $I=0.005$; △: $I=0.0075$; ●: $I=0.010$; ▲: $I=0.020$; ▽: $I=0.030$. Estimated errors and theoretical curves as in fig. 6.

Polyion concentrations of 0.001 and 0.002 moles/l are used for both the Zn/Na and Mn/Na systems, at total ionic strengths of 0.005, 0.0075, 0.01, 0.02 and 0.03 moles/l. C_2/C_p ratios were varied between 0.02 and 1.0 for each C_p and ionic strength value. The full lines in figs. 4–7 were calculated from a simple condensation model (single upper line) and from the predictions of the two-variable theory (solid lines for various ionic strengths) following the procedure outlined before [14]. Figs. 4 and 6 show the estimated experimental errors in θ_2 for respectively the Zn/Na and Mn/Na systems; where no error bars are drawn the estimated error in θ_2 is within the size of the data points as drawn. Estimated errors for the data presented in figs. 5 and 7 are similar to those for the corresponding points in figs. 4 and 6. The estimated errors for the Mn activity determination are larger than for the Zn case. Noticeable in these figures is the strong dependence of θ_2 on ionic strength, predicted by the two-variable theory, but not by the simple condensation equations. With a decrease in ionic strength, θ_2 increases and at low ionic strength, it tends to approach the condensation equation. This θ_2 dependence on ionic strength, I can be attributed to an entropy factor. The binding of a divalent ion M^{2+} to the polyion is known to lead to a release of about two M^+ ions [12]. However, the release of an M^+ ion will be dictated by the concentration difference of M^+ and M^{2+} between the bound region, V_p, and the bulk volume. When the concentration difference is larger, at low I, the entropy gain is large and hence M^+ is released and more M^{2+} is bound. An increase in I causes a smaller entropy gain, less M^+ is released and hence there is a decrease in M^{2+} binding leading to smaller θ_2 values. For identical I values, and at double C_p, θ_2 values are higher, figs. 5 and 7. This behaviour also is as predicted from the two-variable theory.

A close examination of the binding isotherms of Zn/Na and Mn/Na (figs. 4 and 6, 5 and 7) shows that the degree of binding of these two metal ions to dextransulfate is identical, when consideration is taken of the limits of error. The results are also essentially identical to the data for the Mg/Na system reported by Joshi and Kwak [14], but the degree of binding of Ca to dextransulfate was found to be greater than for Mg. Kwak and Nelson [25] have shown that Mg^{2+} and Zn^{2+} interactions with polystyrenesulfonate are identical and apparently non-specific. It is therefore not surprising that the Zn^{2+} and Mg^{2+} binding curves to another sulfonated polyelectrolyte, DS, are identical. Very little information is available for Mn^{2+} binding to a polyion with one kind of charged group. However, Clement et al. [5] in their investigation of the binding of Mg^{2+} and Mn^{2+} to DNA concluded that Mg^{2+} binds to the phosphate group through "site-binding" while Mn^{2+} binds by "chelation" of Mn^{2+} between the phosphate group and N_7 of guanine. Leroy and Gueron [7], also found that the affinity of Mg^{2+} for tRNA is about half that of Mn^{2+}. In their interaction with DS, there appears to be little difference between Mg^{2+}, Zn^{2+} and Mn^{2+}.

Figs. 8–11 show alternative representations of the binding data in terms of Scatchard plots. In

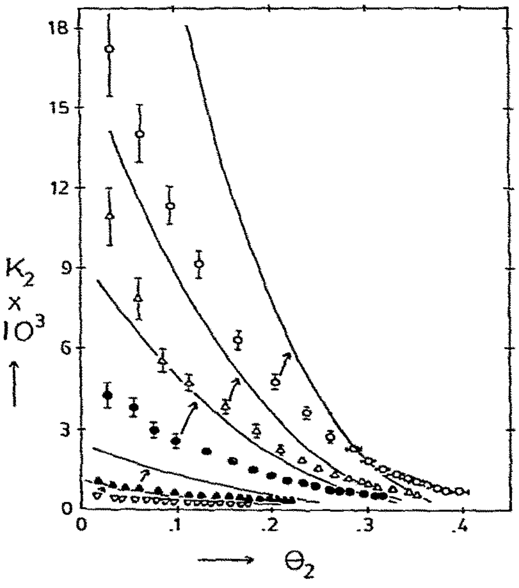

Fig. 8. Scatchard curves, K_2 (l/mole) versus θ_2 in the system $ZnCl_2$, NaCl, dextransulfate. $C_p = 0.001$ mole/l. Estimated errors indicated by error bars. Symbols as in fig. 4, theoretical curves (solid lines) as calculated from the two-variable theory for each ionic strength.

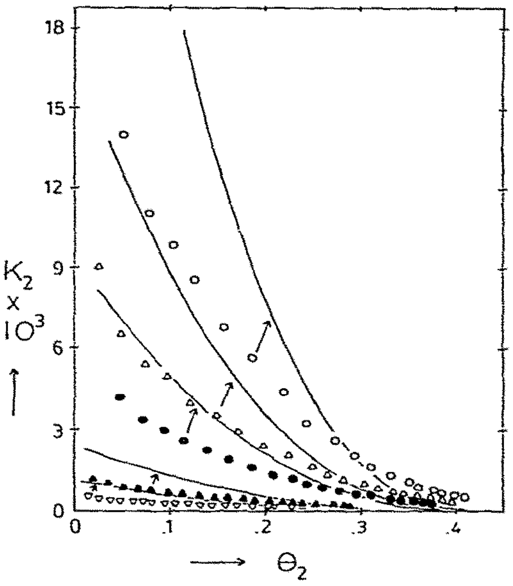

Fig. 9. Scatchard curves, K_2 (l/mole) versus θ_2 in the system $ZnCl_2$, NaCl, dextransulfate. $C_p = 0.002$ mole/l. Symbols, estimated errors and theoretical curves (solid lines) as in fig. 8.

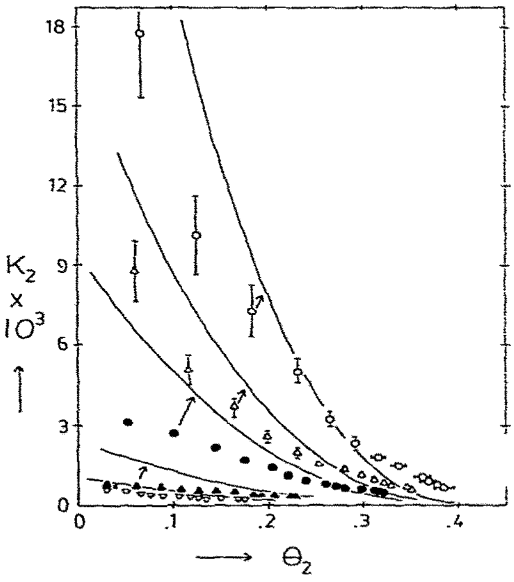

Fig. 10. Scatchard curves, K_2 (l/mole) versus θ_2 in the system $MnCl_2$, NaCl, dextransulfate. $C_p = 0.001$ mole/l. Estimated errors indicated by error bars. Symbols and theoretical curves (solid lines) as in fig. 8.

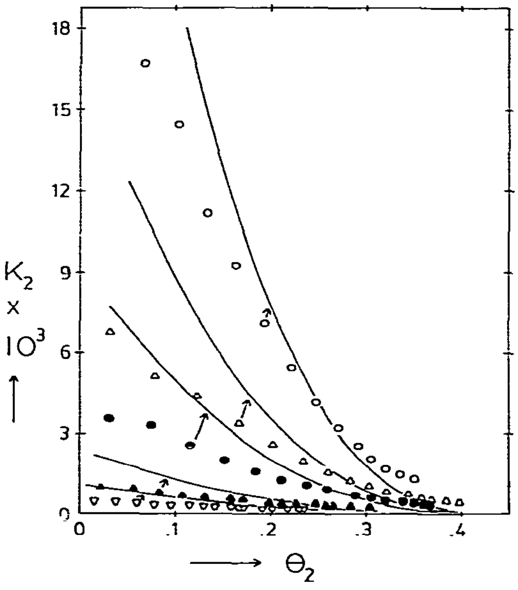

Fig. 11. Scatchard curves. K_2 (l/mole) versus θ_2 in the system $MnCl_2$, NaCl, dextransulfate. $C_p = 0.002$ mole/l. Symbols, estimated errors and theoretical curves (solid lines) as in fig. 10.

these plots a quantity K_2, defined as

$$K_2 = \theta_2 / C_{2f}, \tag{5}$$

is plotted versus θ_2. The experimental points in these Scatchard plots are the same as in figs. 4–7. The theoretical curves are as calculated from the two-variable theory [14,16]. In figs. 8–11, estimated errors are indicated by error bars, where no error bar is indicated in these figures the estimated error is within the size of the data points as plotted.

The Scatchard plots are curved for both Zn/Na and Mn/Na at both polyion concentrations: apparently the affinity of the polyion for the metal ion decreases as more Zn^{2+} or Mn^{2+} binds to the polyion. This is the typical anti-cooperative behaviour expected for polyelectrolytes where, on a qualitative basis, one can see that binding of metal ions decreases the electrostatic potential and makes further binding less and less favourable. This "anti-cooperativity" is more pronounced at low Na^+ concentrations, i.e. at low I, and binding decreases with increase in I. Both the anti-cooperative behaviour, and ionic strength dependence of this anti-cooperative behaviour, are predicted by the two-variable theory, as shown by the theoretical curves. Increasing the polyion concentration will increase θ_2, but the theory predicts that K_2 is independent of C_p; comparison of figs. 8 and 9 for Zn/Na at C_p 0.001 and 0.002 shows that the two sets of curves are identical within the limits of experimental error; similar behaviour is seen for the Mn/Na system, figs. 10 and 11. When compared to the theoretical curves, one can see reasonable agreement with the two-variable theory. For both systems at different I and C_p the experimental and calculated values differ by only a factor of two or less. This difference should not be considered critical since the theoretical calculations were based on I and the charge density parameter, ξ, alone.

For θ_2 less than 0.30, one can use eq. (29) of ref. [16] to obtain K_2 values. This equation was obtained by the "one-variable approach" for low binding levels, i.e. small θ_2. Substitution of $V_p = 1200$ cm^3 as determined from eq. (7), and $\xi = 2.80$ for DS, into eq. (29) of ref. [16] gives for dextransulfate:

$$\log K_2 = -0.035 + 2\log(1 - 3.1\,\theta_2) - 2\log C_1. \tag{6}$$

By extrapolating to $\theta_2 = 0$, one can determine "intrinsic binding constants" K_M^0. Values of these theoretical intrinsic binding constants at different ionic strengths can be used for further correlation with the experimental data. Table 2 shows a comparison of the theoretical and experimental values of $\log K_M^0$ at ionic strengths 0.005, 0.0075, 0.01, 0.02 and 0.03 for Zn/Na and Mn/Na systems at C_p 0.001 and 0.002. In all cases, the experimental values are somewhat lower than the theoretical values, but the agreement is quite good. Included in the table are $\log K_{Mg}^0$ values for the system Mg/Na at $C_p = 0.001$ and at identical ionic strengths as Zn/Na and Mn/Na systems, obtained from Joshi and Kwak [14]. Within the limits of experimental error, one can see that the $\log K_M^0$ values at a particular ionic strength are identical

Table 2

Comparison of theoretical and experimental values of K_M^0

C_1	log K_M^0 (theor.)	log K_{Zn}^0 (exp.) ($m_p = 0.001$)	log K_{Zn}^0 (exp.) ($m_p = 0.002$)	log K_{Mn}^0 (exp.) ($m_p = 0.001$)	log K_{Mn}^0 (exp.) ($m_p = 0.002$)	log K_{Mg}^0 (exp.) [a] ($m_p = 0.001$)
0.03	3.01	2.78±0.05	2.73±0.05	2.80±0.05	2.72±0.05	2.64±0.05
0.02	3.36	3.09±0.05	3.12±0.05	2.99±0.05	3.05±0.05	3.05±0.05
0.01	3.97	3.65±0.05	3.70±0.05	3.62±0.05	3.68±0.05	3.61±0.05
0.0075	4.21	3.93±0.05	3.92±0.05	4.01±0.05	3.90±0.05	3.83±0.05
0.005	4.75	4.32±0.04	4.23±0.04	4.34±0.05	4.37±0.05	4.29±0.04

[a] Calculated from data in ref. [14].

for Zn, Mn and Mg. This further supports the idea of non-specific electrostatic binding for these divalent ions to the sulfate groups of DS.

The variation of K_2 with ionic strength can be expressed in the form of the dependence of dln K_2/dln C_1 on θ_2; at low θ_2, θ_1 becomes constant and independent of C_1, and hence dln K_2/dln C_1 at low θ_2 (and thus low C_2/C_p) should be equal to -2. At higher θ_2, this value is predicted to decrease slightly. Fig. 12 shows a plot of $-$dlog K_2/dlog C_1 versus θ_2 for the systems Zn/Na and Mn/Na at $C_p = 0.001$ and 0.002. The size of the data points represents the limits of error. In all the systems studied, the experimental points follow the trend of the theoretical curve, i.e. values are close to 2 for low θ_2. A similar behaviour was observed for Krakauer's data [12,16].

In conclusion we note that Kwak and Joshi [13] have indicated a number of uncertainties associated with the dual-wavelength dye spectrophotometric method for activity determinations. Despite these limitations, they have shown the method to be an excellent tool to study metal binding, often superior to classical techniques like Donnan equilibrium or EMF methods.

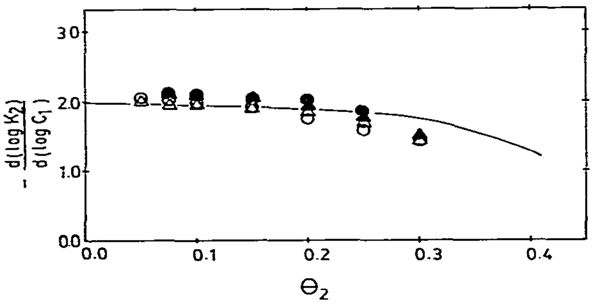

Fig. 12. Dependence of $-$dlog K_2/dlog C_1 on θ_2. Data points obtained from the systems: ○: Zn/Na, $C_p = 0.001$ mole/l; △: Zn/Na, $C_p = 0.002$ mole/l; ●: Mn/Na, $C_p = 0.001$ mole/l; ▲: Mn/Na, $C_p = 0.002$ mole/l. Solid line: two-variable theory.

We have shown the method to be further applicable to activity determinations of Zn and Mn. We have found the experimental binding data in the systems Zn/Na and Mn/Na with dextransulfate to be in good agreement with predictions of the two-variable theory of Manning [16]. Without the use of adjustable parameters the two-variable theory is able to predict the shape of binding isotherms, Scatchard plots and their ionic strength dependence. Clearly, the two-variable theory gives a surprisingly accurate description of the degree of divalent metal ion binding to the polyion in the systems studied. Identical results found for the systems Zn/Na, Mn/Na, and Mg/Na [14] present a strong case for delocalized binding of these metal ions to the polyelectrolyte dextransulfate. Additional work is necessary with polyions with other charged groups, e.g. carboxylate and phosphate, and with different charged groups on the same polymer backbone, for further correlation with available theories.

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