Ion Binding in Polyelectrolyte Systems with or without Added Salt

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The activity of sodium ion in the pure sodium salts of polystyrenesulfonic acid, polyvinylsulfate (PVS), polyacrylic acid, polyphosphoric acid (Kurrol's salt), and deoxyribonucleic acid (DNA) and mixtures of these with sodium chloride has been determined by measurements of ion-exchange membrane potentials. Similarly, the activity of magnesium ion in aqueous solutions of DNA and PVS with and without magnesium chloride has been determined. The data are used as follows: (1) an experimental method is suggested to distinguish qualitatively between site binding vs. nonspecific binding of counterions by polyions; (2) the empirical rule of additivity of activities of counterions in polyelectrolyte–simple salt mixtures is critically re-examined; (3) the anomalous behavior of the system DNA–$MgCl_2$ with regard to nature of the deviation from the rule of additivity is interpreted in terms of a charge reversal phenomenon.

It is now well established that a considerable fraction of the counterions surrounding a highly polymerized polyion in aqueous solutions are in some manner bound by the polyion as shown by thermodynamic and transport measurements.[2] A determination of an osmotic or activity coefficient does not in itself distinguish among the several ways by which the counterions interact with the polyion; *i.e.*, they may be (1) covalently bound to the dissociable sites, (2) involved in a specific interaction at such a site in an ion pair of the Bjerrum type, or (3) held by the long-range forces owing to the electrostatic potential of the polyion. We exclude from the present discussion any other types of interactions that might occur such as, for example, charge-transfer complexes[3a] or coordination compounds[3b] (Werner complexes).

We wish to make clear at the outset our definitions of the types of ion binding with which we are dealing. "Site binding" is here applied to interactions between counterions and specific dissociable groups on the polymer; these interactions can be described by intrinsic and over-all equilibrium constants. The participating species obey the law of mass action. Covalently bonded counterions, such as the protons of a partially neutralized, weak polyacid, and species in Bjerrum-type ion pairs fall into this category. On the other hand, we use the term "diffuse binding" to include all nonspecific electrostatic interactions due only to the potential of the polyion. No provision need be made for the discreteness of the dissociable sites in diffuse binding—they enter only through their obvious effect on the magnitude of the potential of the polyion. We emphasize that we are dealing here only with ion binding; the effects of the distribution of dissociable sites and the discreteness of the sites on the total free energy of a polyelectrolyte system are considerable but are not of concern in the present discussion.

We make a thermodynamic definition of binding in terms of measured single-ion activity coefficients for the counterions, γ_+, such that $1 - \gamma_+$ is the fraction of bound ions. A single measurement of this quantity is not particularly meaningful; however, a systematic study of the dependence of γ_+ on concentration and ionic environment can be informative. In this report we wish to deal with two aspects of the binding problem: (1) the concentration dependence of binding in pure polyelectrolyte solutions and (2) the proposed rule of the additivity of activities of counterions in systems containing polyelectrolyte and added salt. From an examination of the concentration dependence of the binding we will show how, in many cases, it is possible to obtain information as to which type of binding occurs. From the study of the additivity rule, we will show how deviations from the rule can be used to obtain further information on interactions between polyelectrolyte and added salt. Further comment on the additivity rule is appropriate before proceeding to the experimental details.

The empirical rule of the additivity of counterion activities in mixtures of polyelectrolyte and simple electrolyte was first posed as a result of measurements of the activity of hydrogen ion in polystyrene–vinyltoluene copolymer–hydrochloric acid mixtures.[4] In this same study it was noted that the counterion activity coefficient in the absence of simple electrolyte is constant, or nearly so, over a broad range of concentration of polyelectrolyte. These phenomena were subsequently studied in detail in the sodium polyvinylsulfate–sodium chloride system,[5,6] and the same conclusions were reached, namely, that the activities of the cations appeared to be additive and that the cation activity without added salt was but little effected by dilution.

The additivity rule can be written for polyanions; with cations as counterions, as

$$a_+^{\text{obsd}} = a_+^{\text{p}} + a_+^{\text{s}} \qquad (1)$$

where a_+^{p} is the counterion activity of cations from the polyelectrolyte in pure polyelectrolyte solution, and a_+^{s} is the counterion activity of the salt cations in pure salt solution. An equivalent statement can be written for the osmotic pressure

$$\pi_{\text{obsd}} = \pi_{\text{p}} + \pi_{\text{s}} \qquad (2)$$

(1) Monsanto Co., St. Louis, Mo. 63166.

(2) See, for example, the review by S. A. Rice and M. Nagasawa, "Polyelectrolyte Solutions," Academic Press Inc., New York, N. Y., 1961.

(3) (a) R. S. Mulliken, *J. Am. Chem. Soc.*, **72**, 600 (1950); **74**, 811 (1952); (b) J. C. Bailar, Ed., "The Chemistry of the Coordination Compounds," Reinhold Publishing Corp., New York, N. Y., 1956.

(4) R. A. Mock and C. A. Marshall, *J. Polymer Sci.*, **13**, 263 (1954).

(5) M. Nagasawa and I. Kagawa, *ibid.*, **25**, 61 (1957); **31**, 256 (1958).

(6) M. Nagasawa, M. Izumi, and I. Kagawa, *ibid.*, **37**, 375 (1959).

It is clear that a sufficient thermodynamic condition for these equations to be valid is simply that the polyelectrolyte and the simple salt components constitute separate noninteracting phases. That the phases do interact (*e.g.*, by ion exchange) is evident. The question is then whether there are compensating effects which could lead to the additivity result. What such counterbalancing forces might be is not at the outset obvious, and the issue has not been resolved as to how this seemingly odd behavior of the counterions can be explained. If, on the other hand, the additivity rule is not valid, we should not be lacking for explanations; *e.g.*, those which explain nonideal behavior in the mixing of simple electrolytes.

There have been two recent theoretical treatments of polyelectrolyte systems with added salt which were specifically directed to the additivity rule. One treatment[7] used no model for the polyion but assumed that the activity coefficient of counterions in the absence of added simple salt is independent of concentration and that the activity coefficient of the co-ions from the added salt in the mixed system is unity. The result of this treatment is a prediction that the total counterion activity should be the sum of a_+^P and m_+^s; *i.e.*, the theory predicts that the simple salt will behave ideally. This result is not identical with eq. 1, and a total activity greater than that given by eq. 1 is predicted. The two assumptions of the theory are approximate, and the second one, regarding the co-ions, is questionable in light of experimental evidence of the effect of polyions on co-ion activities.[6] In any case, even reasonable approximations may not be sufficient for a theoretical treatment since, as we will show, deviations from eq. 1 need not be more than a few per cent before an entirely new interpretation must be placed on the behavior of the system.

A treatment based on the rod model for the polyion and using the Donnan approximation for the potential has shown the limits in which eq. 2 would be expected to apply.[8] This theory in essence assumes that the number of counterions bound by the polyions is independent of added salt, and, hence, the objective is to predict the osmotic behavior of the added salt with varying amounts of salt and polyelectrolyte present. The assumption made about the polymer counterion is open to question for the reasons cited in the previous paragraph: namely, it is approximate and may be inadequate for accounting for small but significant variations. In any case, Oosawa's theory predicts that eq. 2 will be followed when either polyelectrolyte or simple salt is present in excess, but it also shows that there will be appreciable deviations from eq. 2. We will see that this prediction is borne out experimentally.

By examining the behavior of both synthetic and natural polyelectrolytes in the presence and absence of added simple salt, we will show the extent to which the additivity rule applies with either sodium or magnesium ions as counterions. The distinctive behavior of polyelectrolytes will be discussed with particular attention to the concentration dependence of the additivity relation or of the activity coefficient in salt-free solutions. Finally, a new phenomenon is postulated involving possible charge reversal of the polyion in the magnesium salt of DNA in the presence of excess $MgCl_2$.

(7) A. Katchalsky and Z. Alexandrowicz, *J. Polymer Sci.*, A1, 2093 (1963).

(8) F. Oosawa, *ibid.*, 23, 421 (1957); A1, 1501 (1963).

Experimental

Activity Coefficients. The experimental apparatus consists of a cation-exchange membrane clamped between two cell compartments. The compartments are provided with miniature stirrers next to the membrane surfaces and with a magnetic stirrer in the bottom of one chamber to permit thorough mixing of increments of added salt. The compartments are blanketed with water-saturated nitrogen and are kept at 21.5 ± 0.5°. Contact with the compartments is made with removable salt bridges (agar-agar gel saturated with KCl). The bridge tips are curved upward and drawn to a fine point to minimize flow of KCl solution into the compartment. Matched pairs of these bridges were used in order that duplicate measurements could be made. The tips were renewed in saturated KCl solution between measurements. The e.m.f. is detected with a Leeds and Northrup K-3 potentiometer and 2430-D galvanometer.

The membrane[9] is first converted to the desired form by exhaustive dialysis and washing. Solutions are placed in each compartment, and stirring is continued for 30 min. at which time two measurements of the e.m.f. are made with the matched pairs of bridges. Equilibrium was shown to be attained within 20 min. for a pair of $MgCl_2$ solutions differing in concentration by a factor of 10. The e.m.f. held steady to ±0.1 mv. for 18 hr. indicating negligible water transport through the membrane.

The membrane potential, E_m, is composed of three terms[10]—two opposed Donnan potentials at the membrane surfaces and a diffusion potential across the membrane phase. If the membrane excludes co-ions owing to high concentration of fixed charge in the membrane phase, then the diffusion potential vanishes, and E_m reduces to the Nernst equation

$$E_m = \frac{RT}{z_+F} \ln \frac{a_{+(I)}}{a_{+(II)}} \qquad (3)$$

where $a_{+(I)}$ and $a_{+(II)}$ are the cation activities on the left and right, respectively, z_+ is the cation valency, F is the Faraday constant, and RT has its usual meaning. When saturated calomel half-cells are used, E_m is the total e.m.f. measured. An ideal membrane will give a slope of 0.0588 for a plot of E_m *vs.* log a_+ at 22° for univalent counterions in the concentration range of interest. Deviations from ideal behavior occur at high salt concentrations in the external solutions owing to failure of the membrane to exclude co-ions leading to a change in the Donnan boundary potentials and to the establishment of a diffusion potential across the membrane phase. At very low concentrations in the external solution deviations occur because of contributions from other counterions, namely, protons from the solvent and potassium ions from the salt bridges. These contributions not only modify the Donnan potentials but also establish transient potentials across

(9) AMF C103C kindly supplied by American Machine and Foundry Co., Springdale, Conn.

(10) For a detailed analysis of membrane phenomena see F. Helfferich, "Ion Exchange," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 8. Also see J. W. Lyons, Doctoral Dissertation, Washington University, St. Louis, Mo., Aug. 1964.

the membrane phase owing to different rates of movement of the counterions as equilibration is slowly approached. For this reason the membranes are useful only down to about 2×10^{-5} *m* in magnesium ion or about 5×10^{-5} *m* in sodium ion. The pH of the solutions must be kept as close to 7 as possible when working at low concentrations. Protection of the bridge tips by appropriate shields is helpful in reducing contamination by KCl. The operating range of the C103C membrane used in this work was from the lower limits just given up to 0.1 *m*. The slopes of the two membrane electrodes at 22° are 0.058 for sodium and 0.030 for magnesium ion (*cf.* 0.0588 and 0.0294 theoretical). Experiments with the magnesium membrane using chloride, nitrate, and sulfate as co-ion in the external solutions showed no detectable effect on the measured activity when the latter was calculated according to the Debye–Hückel theory.

The membrane electrode gives directly the ratio of single-ion activities in the two compartments. To obtain the absolute values of the single-ion activities[11] the following assumptions have been made: (1) the Debye–Hückel theory can be applied to the simple salts in the range of concentration studied; (2) the activity of the chloride ion in the calibrating solutions is a function only of the ionic strength; (3) the activity of K^+ is equal to that of Cl^- in pure KCl solutions.[12] Thus, $\gamma_{K^+} = \gamma_{Cl^-} = \gamma_{\pm KCl}$. We then compute single-ion activity coefficients in the following manner.

(1) The ion size parameter, $\mathring{a}$, in the Debye–Hückel equation is calculated for KCl, NaCl, and $MgCl_2$ from the reported values of $\gamma_\pm$ for each of these salts at 0.1 *m*.[13] Then $\gamma_\pm$ for each salt at any concentration can be computed.

(2) The following simple relations are then used to obtain the single-ion activity coefficients

$$\gamma_{Na^+} = \frac{(\gamma_{\pm NaCl})^2}{(\gamma_{\pm KCl})} \qquad \gamma_{Mg^{2+}} = \frac{(\gamma_{\pm MgCl_2})^3}{(\gamma_{\pm KCl})^2} \qquad (4)$$

where it is understood that the mean molal coefficients in each relation correspond to the same ionic strength.

(3) The single ion activity is finally obtained from $a_+ = \gamma_+ m_+$. After establishing the values of $\mathring{a}$ the remaining computations are routinely performed on an IBM 704 computer.

Materials. The deoxyribonucleic acid (DNA) was isolated from calf thymus tissue by standard methods[14] and converted to the pure NaDNA or MgDNA by dialyzing at high DNA concentration in ice water and then recrystallizing repeatedly from ice-cold water–ethanol solutions. The properties of the materials so produced have been described in detail in a previous report.[15] For the work described here it suffices to say that the DNA is in the "native" state and has a molecular weight of about 7×10^6. Potassium polyvinylsulfate (KPVS) was obtained from the Eastman Kodak Co. and purified by recrystallization from potassium hydroxide–ethanol–water solution. The sodium form was obtained both by dialysis against NaCl followed by dialysis against water and alternatively by ion exchanging the KPVS to the free acid (≈0°) and titrating in ice to pH 7 with NaOH. The MgPVS was made by dialysis with final adjustment to pH 7 with $Mg(OH)_2$ solution. PVS solutions were held in the refrigerator prior to use to minimize hydrolysis.

Sodium polystyrenesulfonate (NaPSS) was obtained from the Dow Chemical Co., Midland, Mich., recrystallized three times from 10:1 ethanol–water, washed in ether, and vacuum dried. Sodium polyphosphate was obtained from Dr. E. J. Griffith of the Monsanto Co., St. Louis, Mo. The compound, in the crystalline state known as sodium Kurrol's salt, was washed briefly with water, and the remaining solid was washed with alcohol, then ether, and then dried *in vacuo*. Aqueous solutions of the polyphosphate, $(NaPO_3)_x$, were prepared by stirring the dried salt in pure water for 24 hr., and removing insolubles by centrifuging at 20,000*g* for 1 hr. Polyacrylic acid (PAA) was obtained as an aqueous solution from Rohm and Haas Co., Philadelphia, Pa. The solution was dialyzed against deionized water to remove low molecular weight species and impurities. Solutions were prepared at 80% neutralization by titrating one of a pair of solutions of identical concentration to the end point at pH 10, calculating the amount of NaOH required for 80% neutralization, and adding this to the other solution. The pH of the most concentrated solution was 7.9. The solution was diluted to two lower concentrations, and the pH increased to 8.5 and 8.9, respectively, owing to hydrolysis of the COO^- groups. These pH changes were not large enough to have an appreciable effect on the accuracy of membrane electrode measurements.

Assay of the DNA for phosphorus and for magnesium has been described.[15] Assay of the PVS was done in two ways: ion exchange to the acid followed by titration to the end point at pH 7 (0°) gives the dissociable groups; ignition to Na_2SO_4 or $MgSO_4$ gives the counterion concentration. By igniting carefully dried KPVS the degree of esterification was determined to be 83%. Agreement between the ion-exchange and ignition methods was about ±1.5%. The NaPSS

Table I

Polymer	Temp., °C.	Solvent	$\bar{M}_v$
NaPVS[a]	20	0.5 *M* NaCl	6.2×10^4
$(NaPO_3)_x$[b]	25	0.415 *M* NaBr	$>1.2 \times 10^6$
PAA[c]	30	Dioxane	7.3×10^4
NaPSS[d]	30	0.5 *M* NaCl	$1\text{–}1.1 \times 10^6$

[a] F. Patat and K. Vogler, *Helv. Chim. Acta*, **35**, 128 (1952). [b] U. P. Strauss and P. L. Wineman, *J. Am. Chem. Soc.*, **80**, 2366 (1958). [c] S. Newman, W. R. Krigbaum, C. Langier, and P. J. Flory, *J. Polymer Sci.*, **14**, 451 (1954). [d] Private communication from Dr. R. A. Mock, Dow Chemical Co., Midland, Mich. This determination is made in a single bulb No. 100 Cannon–Fenske viscometer. The calibration is such as to give $\bar{M}_w$ rather than $\bar{M}_v$.

(11) For discussion of the various aspects of single-ion activities see E. A. Guggenheim, *J. Phys. Chem.*, **33**, 842 (1929); **34**, 1540 (1930); H. S. Frank, *ibid.*, **67**, 1554 (1963).

(12) We have compared this assumption for the chloride ion activity coefficient with the Bates–Guggenheim convention (*Pure Appl. Chem.*, 1, 163 (1960)), and we find a difference in γ_{Cl^-} of only 0.8% at an ionic strength of 6×10^{-2}. This difference will decrease with decreasing ionic strength, and hence the values of γ_+ will be virtually uneffected over the concentration ranges studied. Any reasonable extrathermodynamic assumption as to single-ion activity coefficients thus will not materially alter the results of this work.

(13) R. A. Robinson and R. H. Stokes, "Electrolytic Solutions," 2nd Ed., Butterworth and Co. Ltd., London, 1959, pp. 492, 494, 497.

(14) E. R. M. Kay, N. S. Simmons, and A. L. Dounce, *J. Am. Chem. Soc.*, **74**, 1724 (1952).

(15) J. W. Lyons and L. Kotin, *ibid.*, **86**, 3634 (1964).

Table II. Counterion Activities in the NaPVS–NaCl System

m_+^P	a_+^P	m_+^S	a_+^S	a_{obsd}	Δa_+
8.48×10^{-2}	2.27×10^{-2}	8.76×10^{-3}	7.97×10^{-3}	2.95×10^{-2}	+0.7
8.39×10^{-2}	2.24×10^{-2}	1.74×10^{-2}	1.54×10^{-2}	3.61×10^{-2}	+4.8
8.14×10^{-2}	2.17×10^{-2}	4.27×10^{-2}	3.58×10^{-2}	5.37×10^{-2}	+7.0
4.73×10^{-2}	1.25×10^{-2}	4.29×10^{-3}	4.00×10^{-3}	1.56×10^{-2}	+5.7
4.71×10^{-2}	1.24×10^{-2}	8.56×10^{-3}	7.80×10^{-3}	1.87×10^{-2}	+8.0
4.64×10^{-2}	1.22×10^{-2}	2.12×10^{-2}	1.85×10^{-2}	2.88×10^{-2}	+6.6
4.57×10^{-2}	1.20×10^{-2}	3.37×10^{-2}	2.87×10^{-2}	3.80×10^{-2}	+7.0
1.73×10^{-2}	4.41×10^{-3}	2.75×10^{-3}	2.60×10^{-3}	6.58×10^{-3}	+6.5
1.72×10^{-2}	4.40×10^{-3}	5.49×10^{-3}	5.08×10^{-3}	8.84×10^{-3}	+7.2
1.71×10^{-2}	4.33×10^{-3}	1.46×10^{-2}	1.30×10^{-2}	1.63×10^{-2}	+6.5
7.64×10^{-3}	1.84×10^{-3}	1.09×10^{-3}	1.05×10^{-3}	2.74×10^{-3}	+5.5
7.56×10^{-3}	1.82×10^{-3}	2.17×10^{-3}	2.06×10^{-3}	3.67×10^{-3}	+6.0
7.34×10^{-3}	1.76×10^{-3}	5.32×10^{-3}	4.93×10^{-3}	6.23×10^{-3}	+7.4
3.09×10^{-3}	7.10×10^{-4}	5.47×10^{-4}	5.33×10^{-4}	1.22×10^{-3}	+1.9
3.07×10^{-3}	7.04×10^{-4}	1.09×10^{-3}	1.05×10^{-3}	1.70×10^{-3}	+3.3
3.04×10^{-3}	6.96×10^{-4}	2.20×10^{-3}	2.09×10^{-3}	2.67×10^{-3}	+4.5
1.55×10^{-3}	3.39×10^{-4}	3.29×10^{-4}	3.22×10^{-4}	6.31×10^{-4}	+4.9
1.55×10^{-3}	3.38×10^{-4}	6.57×10^{-4}	6.38×10^{-4}	9.33×10^{-4}	+4.7
1.53×10^{-3}	3.35×10^{-4}	1.74×10^{-3}	1.67×10^{-3}	1.92×10^{-3}	+4.0

was assayed after drying by igniting to Na_2SO_4. The degree of sulfonation was calculated to be 82%. The stock solution pH was 8.7. The $(NaPO_3)_x$ was checked for low molecular weight species by the solution pH and by end group titration. No weak acid functions could be detected, and the solution pH was 6.2. The phosphate concentration in both the stock $(NaPO_3)_x$ and diluted solutions was determined by boiling several hours in *ca.* 1 *m* HNO_3 followed by double end point titration. The sodium concentration was taken equal to the phosphorus concentration since no low molecular weight species were detectable.

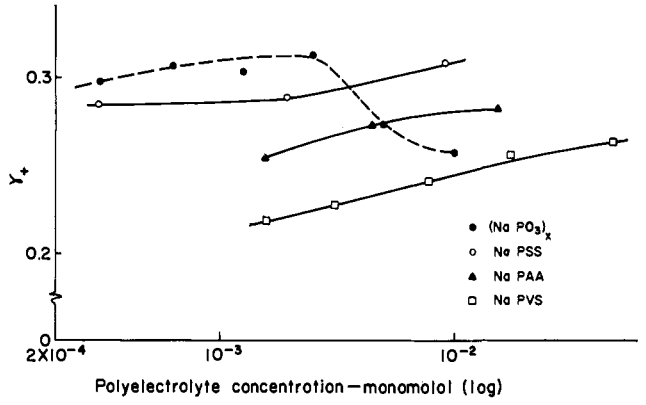

Figure 1. Concentration dependence of the counterion activity coefficient in solutions of sodium polyelectrolytes.

The sodium concentration in PAA and the degree of neutralization were simply determined by recording the solution weight during the solution preparation described above and by use of a standardized NaOH titrant.

Molecular weight determinations were carried out viscometrically using a multibulb, low-shear capillary viscometer and the relation $[\eta]_{\sigma=0} = KM^a$, where the values of K and a were obtained from the literature or from the supplier. The conditions and results are given in Table I.

All simple salts used in the work were recrystallized ACS reagent grade chemicals. Solution pH values (measured with a Beckman Zeromatic pH meter) were maintained above 6 by storing under N_2. Deionized water having a specific conductance less than 10^{-6} ohm^{-1} cm.$^{-1}$ was used throughout.

Results

The concentration dependence of the counterion activity coefficient, γ_+, for some sodium and magnesium polyelectrolytes is shown in Figures 1–3. In Figure 1, γ_+ decreases (increasing binding) for all the

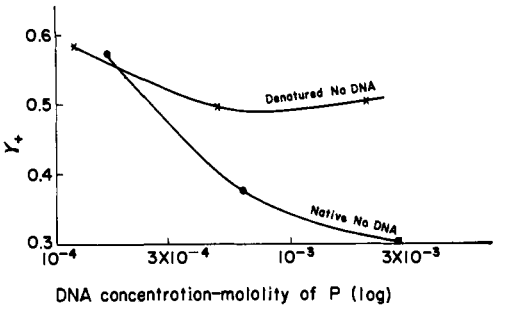

Figure 2. Concentration dependence of the counterion activity coefficient in solutions of native and denatured NaDNA. No simple salt added.

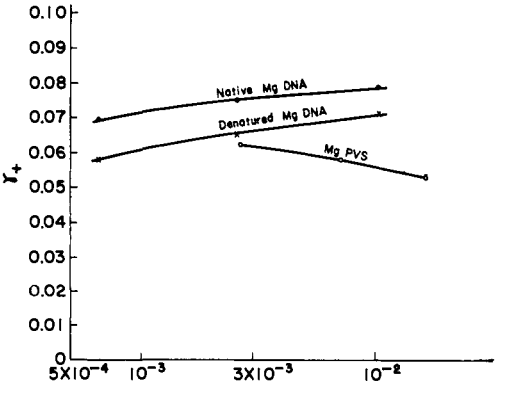

Figure 3. Concentration dependence of the counterion activity coefficient in solutions of MgPVS and MgDNA. No simple salt added.

Table III. Counterion Activities for Sodium in Polystyrenesulfonate (NaPSS) and Polyphosphate $(NaPO_3)_x$ Solutions with Added NaCl

m_+^p	a_+^p	m_+^s	a_+^s	a_{obsd}	Δa_+
		NaPSS			
9.11×10^{-3}	2.81×10^{-3}	3.34×10^{-3}	3.14×10^{-3}	5.79×10^{-3}	+2.8
1.97×10^{-3}	5.70×10^{-4}	6.73×10^{-4}	6.53×10^{-4}	1.19×10^{-3}	+2.4
2.93×10^{-4}	8.64×10^{-5}	1.07×10^{-4}	1.05×10^{-4}	1.87×10^{-4}	+2.1
		$(NaPO_3)_x$			
9.75×10^{-3}	2.50×10^{-3}	1.53×10^{-3}	2.40×10^{-3}	4.69×10^{-3}	+4.5
4.93×10^{-3}	1.34×10^{-3}	1.38×10^{-3}	1.33×10^{-3}	2.53×10^{-3}	+5.5
2.48×10^{-3}	7.77×10^{-3}	7.94×10^{-4}	7.69×10^{-4}	1.49×10^{-3}	+3.8
1.25×10^{-3}	3.77×10^{-4}	3.98×10^{-4}	3.90×10^{-4}	7.57×10^{-4}	+1.2
6.13×10^{-4}	1.88×10^{-4}	1.96×10^{-4}	1.93×10^{-4}	3.82×10^{-4}	−0.3
3.10×10^{-4}	9.19×10^{-4}	9.90×10^{-5}	9.79×10^{-5}	1.87×10^{-4}	+1.4

Table IV. Counterion Activities in NaDNA–NaCl Systems

m_+^p	a_+^p	m_+^s	a_+^s	a_{obsd}	Δa_+
		"Native" NaDNA			
2.91×10^{-3}	8.82×10^{-4}	6.51×10^{-4}	6.33×10^{-4}	1.49×10^{-3}	+1.5
2.90×10^{-3}	8.77×10^{-4}	1.30×10^{-3}	1.25×10^{-3}	2.10×10^{-3}	+1.5
2.86×10^{-3}	8.66×10^{-4}	2.58×10^{-3}	2.45×10^{-3}	3.25×10^{-3}	+2.0
6.31×10^{-4}	2.38×10^{-4}	1.19×10^{-4}	1.18×10^{-4}	3.47×10^{-4}	+2.5
6.23×10^{-4}	2.35×10^{-4}	2.37×10^{-4}	2.33×10^{-4}	4.53×10^{-4}	+3.4
6.09×10^{-4}	2.30×10^{-4}	4.68×10^{-4}	4.56×10^{-4}	6.63×10^{-4}	+3.6
1.68×10^{-4}	9.60×10^{-5}	6.39×10^{-5}	6.33×10^{-5}	1.64×10^{-4}	−3.4
1.67×10^{-4}	9.54×10^{-5}	1.27×10^{-4}	1.26×10^{-4}	2.35×10^{-4}	−5.6
1.65×10^{-4}	9.43×10^{-5}	2.53×10^{-4}	2.49×10^{-4}	3.70×10^{-4}	−7.9
		"Denatured" NaDNA			
2.17×10^{-3}	1.09×10^{-3}	6.56×10^{-4}	6.37×10^{-4}	1.67×10^{-3}	+3.5
2.16×10^{-3}	1.09×10^{-3}	1.31×10^{-3}	1.26×10^{-3}	2.24×10^{-3}	+4.9
2.14×10^{-3}	1.07×10^{-3}	2.60×10^{-3}	2.46×10^{-3}	3.36×10^{-3}	+5.1
4.79×10^{-4}	2.39×10^{-4}	1.12×10^{-4}	1.11×10^{-4}	3.54×10^{-4}	−1.2
4.74×10^{-4}	2.36×10^{-4}	2.24×10^{-4}	2.20×10^{-4}	4.68×10^{-4}	−2.5
4.64×10^{-4}	2.31×10^{-4}	4.42×10^{-4}	4.32×10^{-4}	6.60×10^{-4}	−4.2
1.18×10^{-4}	6.84×10^{-5}	6.07×10^{-5}	6.01×10^{-5}	1.32×10^{-4}	−2.7
1.17×10^{-4}	6.80×10^{-5}	1.21×10^{-4}	1.19×10^{-4}	1.91×10^{-4}	−1.7
1.16×10^{-4}	6.72×10^{-5}	2.41×10^{-4}	2.36×10^{-4}	3.08×10^{-4}	−1.4

Table V. Counterion Activities in the System MgPVS–$MgCl_2$

m_+^p	a_+^p	m_+^s	a_+^s	a_{obsd}	Δa_+
8.16×10^{-3}	4.35×10^{-4}	8.29×10^{-4}	6.72×10^{-4}	9.13×10^{-4}	+22
8.07×10^{-3}	4.32×10^{-4}	1.65×10^{-3}	1.24×10^{-3}	1.41×10^{-3}	+18
7.90×10^{-3}	4.24×10^{-4}	3.26×10^{-3}	2.23×10^{-3}	2.33×10^{-3}	+13
3.63×10^{-3}	2.16×10^{-4}	3.84×10^{-4}	3.31×10^{-4}	4.50×10^{-4}	+21
3.61×10^{-3}	2.15×10^{-4}	7.67×10^{-4}	6.26×10^{-4}	7.02×10^{-4}	+20
3.57×10^{-3}	2.13×10^{-4}	1.53×10^{-3}	1.16×10^{-3}	1.19×10^{-3}	+15
1.34×10^{-3}	8.38×10^{-5}	1.59×10^{-4}	1.44×10^{-4}	1.98×10^{-4}	+15
1.34×10^{-3}	8.36×10^{-5}	3.17×10^{-4}	2.77×10^{-4}	3.14×10^{-4}	+15
1.33×10^{-3}	8.33×10^{-5}	6.33×10^{-4}	5.26×10^{-4}	5.39×10^{-4}	+13

sodium polymers studied over the entire concentration range except for the most concentrated $(NaPO_3)_x$ samples. The results in Figure 2 are reproduced from ref. 14 and are included because the data are required in the experiments with added salt. In Figure 3, the values of γ_+ for MgDNA decrease with dilution as for the materials in Figure 1; the values for MgPVS, on the other hand, increase with dilution (decreased binding). This latter behavior is analogous to that of the concentrated $(NaPO_3)_x$ solutions.

In Tables II–IV results of experiments with added salt are reproduced for sodium ion systems. Tables V and VI contain similar data with the magnesium ion as counterion. The data are presented so that direct comparison with eq. 1 can be made. The quantity a_+^p is obtained from Figures 1–3 and suitable dilution factors arising from the addition of small increments of concentrated simple salt solutions (usually the factors are 0.98–0.995). The quantity a_+^s is calculated from the known molal concentration of added salt and eq. 4. The sum of a_+^p and a_+^s equals a_+^{calcd}. We measure the activity of counterions, a_+^{obsd}, and define the deviation from additivity as

$$\Delta a_+ (\%) = \frac{a_+^{calcd} - a_+^{obsd}}{a_+^{obsd}} \times 100 \qquad (5)$$

The most sensitive test of additivity occurs when the activities of counterions from each component are equal. Therefore, the tables are first examined under

Table VI. Counterion Activities in the System MgDNA–$MgCl_2$

m_+^p	a_+^p	m_+^s	a_+^s	a_{obsd}	Δa_+
		"Native" MgDNA			
5.20×10^{-3}	4.08×10^{-4}	8.45×10^{-4}	6.84×10^{-4}	9.63×10^{-4}	+13.3
5.15×10^{-3}	4.04×10^{-4}	1.67×10^{-3}	1.25×10^{-3}	1.50×10^{-3}	+10.7
4.98×10^{-3}	3.90×10^{-4}	4.05×10^{-3}	2.67×10^{-3}	2.76×10^{-3}	+14.6
1.31×10^{-3}	9.82×10^{-5}	1.07×10^{-4}	9.87×10^{-5}	1.87×10^{-4}	+5.0
1.30×10^{-3}	9.73×10^{-5}	2.13×10^{-4}	1.91×10^{-4}	2.85×10^{-4}	+1.1
1.28×10^{-3}	9.57×10^{-5}	5.21×10^{-4}	4.40×10^{-4}	5.17×10^{-4}	+3.6
3.25×10^{-4}	2.25×10^{-5}	2.11×10^{-5}	2.03×10^{-5}	4.32×10^{-5}	−0.7
3.22×10^{-4}	2.23×10^{-5}	4.18×10^{-5}	3.97×10^{-5}	5.35×10^{-5}	+16
3.12×10^{-4}	2.16×10^{-5}	1.01×10^{-4}	9.34×10^{-5}	9.50×10^{-5}	+21
3.05×10^{-4}	2.11×10^{-5}	2.20×10^{-4}	1.96×10^{-4}	1.66×10^{-4}	+31
		"Denatured" MgDNA			
5.23×10^{-3}	3.72×10^{-4}	4.57×10^{-4}	3.89×10^{-4}	7.01×10^{-4}	+8.7
5.20×10^{-3}	3.70×10^{-4}	9.08×10^{-4}	7.29×10^{-4}	1.01×10^{-3}	+9.7
5.11×10^{-3}	3.63×10^{-4}	2.23×10^{-3}	1.61×10^{-3}	1.85×10^{-3}	+6.9
1.30×10^{-3}	8.47×10^{-5}	6.59×10^{-5}	6.18×10^{-5}	1.38×10^{-4}	+6.2
1.29×10^{-3}	8.40×10^{-5}	1.30×10^{-4}	1.19×10^{-4}	1.79×10^{-4}	+14
1.23×10^{-3}	7.97×10^{-5}	3.75×10^{-4}	3.24×10^{-4}	3.53×10^{-4}	+15
3.27×10^{-4}	1.90×10^{-5}	1.07×10^{-5}	1.04×10^{-5}	2.72×10^{-5}	+8.0
3.23×10^{-4}	1.87×10^{-5}	3.17×10^{-5}	3.03×10^{-5}	4.03×10^{-5}	+22
3.13×10^{-4}	1.81×10^{-5}	2.17×10^{-4}	1.94×10^{-4}	1.61×10^{-4}	+31

these conditions. For NaPVS, Δa_+ is about +6% and but little affected by dilution. For NaPSS, similar results are observed except Δa_+ is smaller. For $(NaPO_3)_x$, Δa_+ decreases with dilution leveling off at the three lowest values. For NaDNA the values are erratic and, in fact, change sign at high dilution. Observe that Δa_+ is greatest when the contributing activities are about equal in the sodium system and decreases when the salt is present in excess. Also, note that Δa_+ is greater for those polyelectrolytes that have the lowest value of γ^+ in pure polyelectrolyte solution, γ_+^p. This latter correlation is striking in the polyphosphate case where Δa_+ falls as γ_+^p increases.

In the magnesium ion systems, Δa_+ is large and behaves differently in PVS and DNA. In MgPVS, Δa_+ is greatest at equal activity contributions and decreases with excess salt; the case is different in MgDNA where Δa_+ increases as more and more $MgCl_2$ is added, especially at the lowest concentrations of DNA. Again in the magnesium ion systems, we observe an insensitivity of Δa_+ to dilution at a given ratio of salt to polymer and a correlation between γ_+^p and Δa_+ similar to that of the sodium ion systems.

Discussion

Before considering the results, it is instructive to inquire as to (1) the expected concentration dependence of γ_+ for polyelectrolytes in the absence of simple salt and (2) the behavior of mixtures of simple salts with respect to the additivity rule to provide a basis for comparison with polyelectrolyte–simple salt mixtures.

The activity of the cation can be written as $a_+ = \gamma_+^{obsd} m = \gamma_+^{el} \alpha m_+$ or $\gamma_+^{obsd} = \gamma_+^{el} \alpha$, where γ_+^{el} is the activity coefficient for diffuse electrostatic binding effects and α is the degree of dissociation of counterions that are in equilibria involving ion pairs or covalently bound species. For the rod model at infinite dilution, theory[16] predicts that for $\alpha \geq$ *ca.* 0.8, γ_+^{el} is very nearly proportional to $1/\alpha$. If this be so, then $\gamma_+^{obsd} \approx$ constant $\times\ 1/\alpha \times \alpha \approx$ constant. Thus, little or no information as to the degree of site binding can be obtained from the magnitude of γ_+^{obsd}. We therefore look to the concentration dependence of γ_+^{obsd} as a possible means of determining the mode of binding.

The concentration dependence of γ_+^{obsd} is simply

$$\frac{\partial \gamma_+^{obsd}}{\partial m} = \gamma_+^{el} \frac{\partial \alpha}{\partial m} + \alpha \frac{\partial \gamma^{+el}}{\partial m} \qquad (6)$$

Both γ_+^{el} and α are positive, and $\partial\alpha/\partial m$ is expected to be negative; *i.e.*, ion pairs or sites with covalently bound counterions will tend to dissociate more with dilution. Thus, the first term in eq. 6 is negative. The sign of $\partial\gamma_+^{el}/\partial m$ is less easily determined. In solutions of polyelectrolytes, the individual polyions are coiled at high concentrations and the coils may, in fact, penetrate each other. In such a situation, the effect on a counterion of the potential of one polyion may be partially offset by that from a second polyion or from a segment of the same polyion which is close by owing to its coiling back on itself. On dilution the coils separate, and each coil expands (electroviscous effect) such that they become more rod-like. During this process the sign of $\partial\gamma_+^{el}/\partial m$ would be positive. It is well known from studies of the electroviscous effect[17] that disentanglement and coil expansion are occurring over the concentration range studied in the present work. We therefore assume the sign of the second term in eq. 6 to be positive in the concentration ranges studied.

Obviously, if $\alpha = 1.0$ over the entire range of concentration studied (no site binding), then $\partial\gamma_+^{obsd}/\partial m = \partial\gamma_+^{el}/\partial m$, and the slope of a plot of γ_+ *vs.* m will be positive according to the preceding discussion. If the slope is negative, the first term in the equation predominates, and there is appreciable site binding. Note that a positive slope does not rule out site binding; it merely indicates that the second term is overriding.

To see whether the proposed additivity rule (eq. 1) is peculiar to polyelectrolyte–simple salt mixtures, we have examined the behavior of mixtures of simple salt. The quantity Δa_+ was calculated for NaCl from the

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Debye–Hückel theory in the following manner. The NaCl was divided into two portions—one considered as "polymer," the other as "simple salt." Then the quantities a_+^p and a_+^s were calculated from eq. 4 for NaCl. These two quantities were then summed to give a_+^{calcd}. For the total molality of counterions present, the activity was computed and designated a_+^{obsd}. From these calculations, Δa_+ is obtained from eq. 5. The computations were carried out for various ratios of "polymer" NaCl to "simple salt" NaCl and at various total concentrations. The results (along with results for mixtures of $MgCl_2$ with $MgCl_2$) are shown in Table VII and an experimental check in sodium ion systems in Table VIII. From the tables

Table VII. Deviation from Additivity in Simple Salt Solutions as Calculated from the Debye–Hückel Theory

Ratio[a]	NaCl–NaCl: Final Na^+ concn., m^+	NaCl–NaCl: Δa_+, %	$MgCl_2$–$MgCl_2$: Final Mg^{2+} concn., m_+	$MgCl_2$–$MgCl_2$: Δa_+, %
1:1	2×10^{-4}	+0.5	2×10^{-5}	+1.0
	4	0.7	4	1.4
	10	1.0	10	2.2
	2×10^{-3}	1.3	2×10^{-4}	3.1
	4	1.8	4	4.3
	10	2.6	10	6.4
	2×10^{-2}	3.4	2×10^{-3}	9.2
	4	4.2	4	11.0
	10	5.2	10	14.6
	20	5.7	20	17.2
4:1	5×10^{-4}	0.5	5×10^{-4}	1.1
	5×10^{-3}	1.3	5×10^{-3}	3.2
	5×10^{-2}	3.1	5×10^{-2}	8.2
9:1	10^{-3}	0.4	10^{-3}	0.9
	10^{-2}	1.1	10^{-2}	2.6
	10^{-1}	2.2	10^{-1}	6.4

[a] Ratio of "polymer salt" to "simple salt."

Table VIII. Experimental Test of Additivity in Simple Salt Solutions

Electrolyte[a]	Total concn. of Na^+, m_+	Δa_+, %
NaCl only	3.4×10^{-3}	+2.9
Na_2SO_4 only	3.4×10^{-3}	+2.9
$Na^+_{(NaCl)}$–$Na^+_{(Na_2SO_4)}$	3.4×10^{-3}	+3.1
NaCl only	1.0×10^{-2}	+4.5
Na_2SO_4 only	1.0×10^{-2}	+5.7
$Na^+_{(NaCl)}$–$Na^+_{(Na_2SO_4)}$	1.0×10^{-2}	+6.1

[a] All compositions measured at 1:1 ratio of contributing components. In the pure NaCl or Na_2SO_4 cases, measurements were made at both the final concentration and half that concentration.

the following observations can be made: (1) the deviation from additivity, Δa_+, is greatest at equal contributions to the total activity from each component and decreases when either component is present in excess; (2) Δa_+ is large at high concentrations and tends to zero with dilution; (3) Δa_+ is greater for 2:1 than for 1:1 electrolytes; in the mixed system Na_2SO_4–NaCl, Δa_+ is greater than for either Na_2SO_4–Na_2SO_4 or NaCl–NaCl. These are merely restatements of certain aspects of the nonideality of mixing of simple salt solutions. However, when examined in this way, the results are useful for the polyelectrolyte case. Note from the tables that Δa_+ is only 5–6% for 0.1 *m* NaCl or 0.01 *m* $MgCl_2$; *i.e.*, simple salts obey the additivity rule rather well.

The Binding of Sodium Ion. The magnitude of γ_+ for sodium ion depends on linear charge density in large measure. This is best shown by denatured NaDNA (Figure 2) where the linear charge density is very much lower than for the polymers in Figure 1 (1 per 7 Å. *vs.* 1 per 3 Å.), and the value of γ_+ is about twice that for the synthetic polymers. The diameter of the polyion can at times become a major effect; *e.g.*, in the vinyl series, the thickest polyion, PSS, has the highest value of γ_+.[18] Diameter (or surface charge density) can be overriding in extreme cases; *e.g.*, NaDNA in its native form has a linear charge density of 1 per 1.7 Å., but the diameter of the double helix is about 26 Å. The value of γ_+ for native NaDNA is comparable to that for NaPSS which has half the linear charge density but an effective diameter of only *ca.* 5 Å. The molecular weight or degree of polymerization of the various polymers has little, if anything, to do with the magnitude of γ_+. This point has already been clearly shown within a series of NaPVS samples.[19]

The decrease in γ_+ with dilution shown in Figure 1 appears to be characteristic of most sodium polyelectrolytes. Such behavior has been found for sodium salts of carboxymethylcellulose, cellulose, NaPSS, NaPVS,[19] and polymethacrylic acid[20] in the same concentration range studied here. The experimental results then indicate the positive value of $\partial\gamma_+^{obsd}/\partial m$ is typical for sodium polymers (which we expect will not, for strongly dissociating polymers at least, form covalent bonds or ion pairs with the counterion). The results for NaPSS and NaPVS give us greater confidence in our assertion that $\partial\gamma_+^{el}/\partial m$ is positive in this concentration range.

The plots for $(NaPO_3)_x$ above about 2×10^{-3} *m* and for NaDNA show the opposite behavior. The DNA results are due to changes in secondary structure caused by dilution and will not be further discussed here[21] except to re-emphasize the connection between disruption of the double helix and reduction in charge density, which in turn is correlated with the magnitude of γ_+.[15] For the concentrated $(NaPO_3)_x$ solutions the negative slope in Figure 1 is interpreted as meaning there is extensive site binding in this region; at higher dilutions diffuse binding predominates. In the concentrated region 0.01–0.02 *m*, some success has been achieved in calculating over-all binding equilibrium constants.[22] The failure to obtain a value which is truly independent of concentration indicates that even at high concentrations there is a fair amount of diffuse binding which is not accounted for in the theory used.[23]

(18) It is not strictly proper to intercompare various polymers even within the vinyl series. This is due to configurational differences established by the difference in side groups and to differences in acid strength (*cf.* NaPAA *vs.* NaPVS).

(19) M. Nagasawa and I. Kagawa, *J. Polymer Sci.*, **25**, 61 (1957).

(20) Z. Alexandrowicz, *ibid.*, **43**, 337 (1960).

(21) Dilution denaturation has been extensively explored: R. B. Inman and D. O. Jordan, *Biochim. Biophys. Acta*, **42**, 427 (1960); **43**, 206 (1960).

(22) U. P. Strauss and P. D. Ross, *J. Am. Chem. Soc.*, **81**, 5295, 5299 (1959).

(23) That the negative slope of γ_+ *vs. m* plot is likely associated with site binding is perhaps most clearly shown by plots for the silver salt of carboxymethylcellulose. These plots have a definite negative slope down to 10^{-3} *N* in counterion concentration. One expects a considerable degree of site binding in such a compound: I. Kagawa and K. Katsuura, *J. Polymer Sci.*, **27**, 365 (1955).

The experiments with added salt (Tables II–IV) show that the additivity rule is not followed closely by polyelectrolyte–simple salt systems, and, by comparison with Tables VII and VIII, we see that mixtures of simple salts, in fact, obey the additivity rule better than the polyelectrolyte–simple salt systems. The data show that adding simple salt in excess decreases the deviation from additivity, Δa_+, as predicted for simple salt systems. However, in contrast to Tables VII and VIII, Δa_+ for the polyelectrolyte systems does not decrease markedly with dilution. This failure of the deviation to tend to zero over the range studied is essentially a failure to approach ideal mixing behavior. This behavior is undoubtedly connected with the essential feature of the polyions, *i.e.*, the fact that the charge is nearly undilutable in contrast to simple salts. Each polyion retains somewhat similar properties in its immediate environment despite addition of large quantities of solvent.

There are signs that the stronger the binding (the lower γ_+) in pure polyelectrolyte solution, the larger is the interaction between polyelectrolyte and added salt (larger Δa_+). Note, in particular, the values for $(NaPO_3)_x$—as γ_+ goes up, Δa_+ goes down.

Table IV shows that changes in secondary structure can confuse the picture considerably. The values obtained for NaDNA cannot be meaningfully interpreted because no reasonable assumption about the value of a_+^P can be made since denaturation and renaturation occur with dilution followed by addition of simple NaCl.

We conclude that eq. 1 is not applicable to the sodium systems since deviations from it are greater than for simple salt mixtures. What about the earlier reports which gave rise to this relation? If one examines the literature and computes Δa_+ when the contributions from the polyelectrolyte and simple salt to the *activity* (not the molality) are equal, it is found that eq. 1 is not followed in the earlier work either. For polystyrene–vinyltoluenesulfonic acid–hydrochloric acid,[4] Δa_+ is about +10%; for NaPVS–NaCl, about +5%.[6] The osmotic coefficients, φ, for polymethacrylic acid neutralized with NaOH[24] are even more interesting. Defining $\Delta\varphi$ in a manner analogous to Δa_+, the data show that (1) $\Delta\varphi$ is relatively constant with dilution at fixed degree of neutralization and polymer–salt ratio and (2) $\Delta\varphi$ increases with increasing degree of neutralization (increasing charge density) while φ^P decreases (decreasing γ_+^P in our notation). For degrees of neutralization of 0.8, 0.5, 0.3, and 0.1, $\Delta\varphi$ is about +6, +5, +3, and <+1%, respectively, at equal contributions to the osmotic pressure from the two components. The swamping effect of excess salt or excess polyelectrolyte is clearly apparent in all the earlier work, and Δa_+ or $\Delta\varphi$ go to zero under these circumstances. It is probably this effect which led to the initial suggestion of the additivity rule.

The Binding of Magnesium Ion. In Figure 3 the magnitudes of γ_+ for magnesium ion are seen to be 0.1 or less. Values for sodium ion are about three times as large, and the question arises as to whether the low value of γ_+ for magnesium ion implies a different mode of binding than for sodium ion. The present state of polyelectrolyte theory does not permit us to make precise statements as to the magnitude of activity coefficients for sodium and magnesium at finite concentrations of polyelectrolyte. However, the results of two theories[16,25] predict that the ratio $\gamma_{Na^+}/\gamma_{Mg^{2+}}$ is expected to be 2/1. Within the confines of the assumption of the theories and the assumptions used in calculating the single-ion activities in the calibrating solutions for the membrane electrode, the experimental ratio of 3/1 is not unreasonable. Therefore, one need not invoke new modes of binding or large amounts of site binding simply because $\gamma_{Mg^{2+}}$ is small.

The concentration dependence of $\gamma_{Mg^{2+}}$ shown in Figure 3 is interpreted, in terms of our discussion of eq. 7, to mean that there is considerable site binding in solutions of MgPVS but that diffuse binding predominates in MgDNA solutions without added salt. In PVS the functional groups are only 3 Å. apart, and it is easy to see how magnesium ion could be bound to two sulfate groups at the same time. In DNA, on the other hand, the distance of closest approach between phosphates is about 7 Å. In pure MgDNA solutions there is but one magnesium ion for every two phosphates; thus, simultaneous interaction between the magnesium ion and two phosphates is not probable. If one estimates the depth of the potential well at each phosphate site along a line 4 Å. away from the phosphorus atoms, it is found that the wells are very shallow—only about 2% of the total potential. Therefore, the magnesium ion can move along the polymer chain jumping with little difficulty from site to site, and on the average behaves as though it were not site bound to any large extent. We have already[15] discussed the possible reasons for the small differences in binding of magnesium ion by native and denatured MgDNA.

The experiments in which $MgCl_2$ was added to MgPVS and MgDNA show that the additivity rule does not apply in these systems. For PVS and DNA, when the contributions to the activity are equal ($a_+^P \approx a_+^s$), the quantity Δa_+ is insensitive to dilution, in sharp contrast to the simple $MgCl_2$–$MgCl_2$ system. Note that Δa_+ is larger for the PVS which has the smaller γ_+^P, just as was observed in the sodium ion systems in Table II. In the PVS system, when $MgCl_2$ is added in excess, Δa_+ decreases as for simple salt mixtures and for the sodium polyelectrolyte–salt systems. The behavior of MgPVS–$MgCl_2$ systems is thus qualitatively the same as the sodium ion systems in that additivity of counterion activities is not observed, and the concentration dependence of Δa_+ is very different from simple salt systems.

When excess $MgCl_2$ is added to MgDNA, the system does not respond with a swamping effect; rather Δa_+ increases. The data for both forms of MgDNA at the highest dilutions show this most strikingly since the $MgCl_2$ was present in greater excess than in most of the other cases. The MgDNA appears to be binding magnesium ions from the added $MgCl_2$ in large measure. As the *molality* of added magnesium ion approaches the *molality* of magnesium from the MgDNA, deviations from eq. 1 of over +30% are observed. This could mean that the magnesium ion is being bound to individual sites in 1:1 ion pairs.

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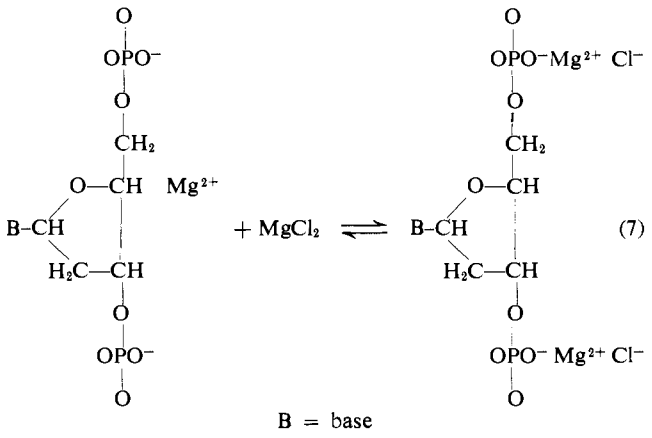
(25) F. Oosawa, N. Imai, and I. Kagawa, *ibid.*, **13**, 93 (1954).

In the discussion of magnesium binding in the absence of simple salt, the unfavorable spacing of the phosphate sites in DNA was cited to explain the failure to observe appreciable site binding in the pure polyelectrolyte solution. When additional magnesium ions are added, the requirement that one magnesium ion simultaneously is to satisfy two phosphate sites no longer applies. It is suggested that the following may occur to some extent.



(7)

B = base

In the above representation the two magnesium ions in the product can be treated as site bound with the result that the charge on this segment of the DNA is reversed; *i.e.*, viewed from the solvent, the segment now appears to be a polycation with negative univalent counterions (Cl^-). Reports of charge reversal are by no means rare, the phenomenon having been invoked to explain reversal in sign of the electrophoretic mobility of polyvinylpyridinium bromides in excess KBr[26] and for colloidal ferric hydroxide in base.[27] Recently, a reversal of charge on the surface of AgI or AgBr colloids by addition of aluminum ions hydrolyzed to varying extents has been thoroughly studied.[28] Thus, eq. 7 is not unreasonable.

In a current paper,[29] we discuss the effect of excess $MgCl_2$ on the secondary structure of DNA and show *via* additional experimental techniques that eq. 7 is the most plausible explanation of the binding by DNA of magnesium ions from $MgCl_2$.

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Catalysis of the Dehydration Reaction of Carbonic Acid by Poly-N-vinylimidazole

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The rate of the dehydration reaction of carbonic acid at 25° was found to be catalyzed by poly-N-vinylimidazole (PVI) as measured by the Roughton thermometric continuous-flow method. The first-order rate constant in 0.001 M PVI was found to be 36.8 sec.$^{-1}$ compared with 26.2 sec.$^{-1}$ for the uncatalyzed reaction. The catalytic coefficient for this reaction was found to be 400 for PVI as compared with 1.5 for imidazole and 12 for 2,4-dimethylimidazole. Solutions of PVI in the presence of Zn(II) with and without nitrilotriacetic acid showed no further enhancement over that due to the polymer alone, probably owing to the low extent of complex formation in the dilute solutions investigated.

The kinetics of the reaction between carbon dioxide and water has long been of interest; it is of fundamental importance in processes of respiration and was one of the first "rapid" reactions to be studied quantitatively. This system is often used as a standard for the testing of new apparatus for measuring fast reactions.[2] Rate studies show that the rates of the hydration and dehydration reactions are strongly affected by buffers,[3,4] with anionic activation the predominant effect.[3,5] More recent studies by Eigen, Kustin, and Maass[6] on a more general reaction scheme have been followed by the work of Ho and Sturtevant[7] and Gibbons and Edsall,[8] who evaluated these rate constants. No known synthetic catalyst compares in effectiveness with the enzyme carbonic anhydrase, a zinc-containing protein.[9-12] The structure of the complex is unknown; a $4s4p^3$ complex of Zn(II) in carbonic anhydrase has been postulated.[13] Edsall and Wyman[14] have written an excellent review of the chemistry, particularly of the kinetics of the reactions of carbon dioxide and carbonic acid.

(1) Taken in part from the dissertation of K. J. Liu, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in chemistry at the Polytechnic Institute of Brooklyn, N. Y., 1963.

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