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ION BINDING IN CHARGED POLYMERS

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CONTENTS

A. INTRODUCTION

The quantitative examination of ion-binding equilibria in charged polymer systems is of considerable importance to many disciplines of chemistry. The compilation of such information for metalloenzymes, proteins, polynucleotides and polynucleosides, for example, would provide much needed insight to the bio-oriented investigator for the interpretation of metal-induced phenomena in such biological organizations. In the case of the soil chemist a knowledgeable interpretation of the binding of ions to the charged polymeric constituents of various agricultural media would lead to a better understanding of a number of important problems encountered by the agronomist.

The literature abounds with studies purporting to develop this kind of information. Unfortunately, the treatment of ion-binding data obtained with such systems has for the most part been inadequate. As a consequence false estimates of the nature of the binding phenomena have resulted, to the disadvantage of the investigator and the investigation.

There are two very important complicating factors that must be resolved to affect a quantitative dissection of the equilibria involved in these systems [1]. The first of these arises from the high and variable electric field at the surface of the charged macromolecule. The electric field which determines the effective concentration of mobile counter ions at the polyion surface is a function of the degree of ion binding and the ionic strength of the medium. The extent and nature of ion binding may lead as well to conformational changes in the molecule which complicate further accurate estimate of the effect of the field on the number distribution of the mobile counter ions at the surface of the macromolecule, i.e. at the site of the reaction,

The second troublesome aspect arises from difficulties encountered in the accurate assessment of the concentration, at equilibrium, of the dissociated and variously bound ligand species that occur along each polymer strand. In a simple molecule the concentration a of free ligand can be evaluated from stoichiometric considerations and a consideration of the equilibrium of a competitive reaction. For example, in studying the complexation of a metal ion by a simple monobasic acid, e.g. acetic acid, the concentration of acetate ion is available from a pH measurement of the system. With knowledge of the dissociation constant of the acid at the ionic strength of the system and by accounting for the amount of standard base added the evaluation of a is unambiguous and the various ligand species concentrations can be easily and accurately deduced [2]. In these complicated systems, however, such an analysis is difficult because of the inability to assess the effective concentration of the mobile counter ions at the site of reaction.

Even should the concentration of free ligand be available its representation by an appropriata concentration unit is difficult. The repeating functional units of the polymerized molecule occur as ensembles and their concentration representation on a monomer basis must be expressed as moles per effective volume of the macromolecule, existing as a separate phase. To express their concentrations as moles (on a monomer basis) per liter of solution not only overlooks the polymer existence as a separate phase but also provides a false picture as uniform distribution of each functional unit throughout the solution is implied. The effective volume of the polymer domain, which can vary in the course of an experiment because of conformational changes, is not accessible, however; it therefore does not permit an absolute expression of concentration units and concentrations are most often based on the solution volume. Results obtained with this concentration unit when more than one ligand unit is attached to the metal are influenced by the volume of the polymer [1].

The expression of concentration units on a polymer basis provides a proper description of the uniform distribution of each assembly of functional units. The volume of the polymer is not a factor either. Expression of concentration units on this basis may be considered quite appropriate in numerous instances.

Expression of concentrations on a mole fraction basis would be acceptable but the possibility of unsymmetric binding of metal to ligand, e.g. as MA* and MA, in the M^{2+}—$(HA)_p$ system, makes data resolution by this approach excessively difficult for such systems. The use of equivalent fractions as a concentration unit to overcome this problem leads to an incorrect application of the mass-action iaw to eliminate consideration of this approach. In both instances volume changes in the molecule are not a complicating factor.

In the following review the means for effective resolution of these problems that have been developed by the author and his co-workers are examined in detail. Unambiguous experimental verification of the validity of the various steps taken to overcome these complexities are presented. Examples in the literature of the inadequate treatment of metal-binding studies of charged polymers and the consequence of such treatment are also presented to emphasize the importance and value of the approach that is recommended for meaningful analysis of these complex systems,

B. THE EVALUATION OF FREE LIGAND CONCENTRATION AND AVERAGE LIGAND NUMBER IN CHARGED POLYMERS FOR THE DETERMINATION OF FORMATION CONSTANTS

It was pointed out in Sect. A that one very important factor which interferes with the quantitative analyses of equilibria between mobile counter ions and the ionized groups fixed on a polymer is the high and variable electric field at its surface. There is considerable deviation from ideality of the mobile counter ions at the reaction sites of the charged polymer as a consequence of this field and it is extremely difficult to estimate the free ligand concentration a in a particular sequence of experiments.

No such complication is encountered with simple monomeric systems where non-ideality either can be kept invariant by the use of an inert neutral electrolyte such as $NaClO_4$ at a sufficiently high concentration [3] or can be accurately calculated with the Debye—Hückel equation or some well-tested semiempirical modification [4] by keeping the system sufficiently dilute. Because of such experimental control of non-ideality encountered in these systems the formation constants of a sequence of equilibria can be uniquely resolved [2].

For example, in the case of a monobasic acid HA analysis of complexation of metal ion by the anionic ligand can be facilitated simply by very accurate and precise potentiometric measurement of the pH of the system as a function of the standard base added. With the material balance equations listed below for the total initial acid concentration and the total initial ligand concentration the average number of ligands attached to the metal ion can be determined at each experimental condition.

$$H_T = h + b + [HA] - K_w/h \tag{1}$$

$$A_T = a + [HA] + [MA] + 2[MA_2] + \ldots + n[MA_n] \tag{2}$$

where a is the free ligand concentration, h is the free hydrogen ion concentration, b is the base concentration added and K_w is the dissociation constant of the water at the temperature and ionic strength of the experiment. An earlier measurement of the dissociation constant K_a of HA under the same experimental conditions but in the absence of metal ion permits precise assessment of a for use in eqn. (2). With these data, the average number $\overline{Z}$ of ligands attached to the metal M is calculable:

$$H_T - h - b + (K_w/h) = [HA] \tag{1b}$$

$$A_T - a - [HA] = [MA] + 2[MA_2] + \cdots + n[MA_n] \tag{2b}$$

$$\overline{Z} = \frac{[MA] + 2[MA_2] + \ldots + n[MA_n]}{M_T} \tag{3}$$

$$\overline{Z} = \frac{[MA] + 2[MA_2] + \ldots + n[MA_n]}{m + [MA] + [MA,] + \ldots + [MA,]} \tag{3b}$$

$$\overline{Z} = \frac{\beta_1 a + 2\beta_2 a^2 + \cdots + n\beta_n a^n}{1 + \beta_1 a + \beta_2 a^2 + \cdots + \beta_n a^n} \tag{3c}$$

where m is the concentration of free metal ion and β_n is the formation constant of the nth complex.

As

$$\int_0^a \overline{Z}\, d \log a = \log(1 + S) \tag{4}$$

where

$$S = \beta_1 a + \beta_2 a^2 + \cdots + \beta_n a^n \tag{5}$$

graphical integration of a plot of $\overline{Z}$ versus log *a can* be employed to obtain the value of $\log(1 + S)$ for the ultimate eval-uation of the sequence of stability constants characterizing the formation of complex species in the system under investigation.

Use of this approach without proper modification for electric field effects for the analysis of ion-binding data obtained with charged polymers is of course unacceptable. Any attempt to interpret such ion-binding data without consideration of perturbations of the system arising from electric field effects is of little value. In spite of this a number of investigators have neglected to consider this factor in their research with charged polymers.

For example, in a recently reported study of the binding of alkaline earth metal ions to alginate, the reaction between MCl_2 and the Na^+ ion form of alginate as well as the reverse reaction was investigated by the equilibrium dialysis technique [S]. Estimates of deviation from ideality at the site of reaction that could have been provided by monitoring the neutralization of the acid during the addition of standard NaOH in the presence of MCl_2 were not accessible with their experimental approach. On analyzing their data they neglected to consider even qualitatively the effect of such perturbation of their system.

They proposed, on the basis of volume changes in the course of reaction and the stoichiometry of the reactions that there were two kinds of sites available for the Na^+ ion, one less strongly binding than the other. The mass-action equation $K_s = 2[M_b][Na_f^+]/[Na_b][M_f^{2+}]$ seemed to describe the observed exchange with the more numerous, less firmly bound Na^+ ions. It was deduced from this result that most of the uronic residues of the alginate molecule were arranged in divalent ion-binding sites which could hold one divalent ion or one monovalent ion. Gel formation and contraction were presumed to involve binding to residues not so arranged.

Because the possibility of perturbation due to the electric field at the surface of the polyion was not considered in this estimate these conclusions are highly suspect. By analogy with the PAA and PMA systems that are discussed fully later it is strongly suspected that the Na^+ ion form of the alginate is fully dissociated and that the postulate of Na, bound weakly or strongly, is incorrect. Instead, the amount of Na presumed to be bound to the alginate is believed to correspond to the concentration a of free ligand in these systems. With values of a thus accessible the most appropriate analysis of their data should have proceeded through the expression of $\overline{Z}$ (eqn. (3)), calculable on this basis by the evaluation of A_b/M_t where $A, = A_t - [Na]_b = A, - a$. For the formation of two species, MA^+ and MA_2, eqn. (3), modified by incorporation of a correction *term* f and rearranged relates $\overline{Z}, \beta_1, \beta_2$ and a *as* follows:

$$\frac{\overline{Z}}{1-\overline{Z}af} = \beta_1 + \frac{2-\overline{Z}}{1-\overline{Z}}\beta_2 a \tag{3d}$$

Without a reasonable estimate of *fit* is apparent that meaningful resolution of the binding data that have been reported is very unlikely. A much more careful scrutiny of the alginate system than has been made needs to be conducted before the interpretations that have been proposed can be accepted.

The experimental procedure for the accurate estimate of the deviation term f that is essential for effective study of these highly charged systems unfortunately interferes with the assessment of a. The analysis of complexation of metal ions in these systems by the method outlined above for the analysis of metal-monomer ligand interactions is thus not straightforward. With a weakly acidic polyacid (HA)„ the increasing deviation f from ideality of the mobile counter ions ($H^+, M^{\nu+}$) with increasing dissociation of the acid is measured during controlled neutralization with standard base. The concentration of HA is still calculable with eqn. (lb) from the pH measurement and the amount of standard base added. The assignment of a reasonable correct value for a to permit the estimate of bound ligand with eqn. (2b) for evaluation of $\overline{Z}$ with eqn. 3 is not. The measured ph is related to the ph at the site of reaction by eqn. (6).

$$2.303\ ph_m - 2.303\ ph_s = -\epsilon\Psi_s/kT = 2.303\ pf \tag{6}$$

where Ψ_s (the potential at the surface of the charged macromolecule) is a primary function of the degree of neutralization α and can be significantly affected by conformational changes in the molecule that arise from concurrent metal-ion binding. Only the value of the product a $\exp(-\epsilon\Psi_s/kT)$ can be accurately determined from the potentiometric data in such metal-containing systems [1,6].

By using a modified form of the Henderson-Hasselbach equation

$$\mathrm{p}h_\mathrm{m} + \log[\mathrm{HA}] - \mathrm{p}K_\mathrm{a} = \log \mathrm{a} - 0.4343\ \epsilon\Psi_s/kt \tag{7}$$

In this equation K_a corresponds to the intrinsic dissociation constant of the repeating monomer unit of the polyacid that is evaluated by a potentiometric study of the metal-free but otherwise equivalent system.

In an attempt to overcome this problem Gregor and coworkers [73 used the standard Henderson-Hasselbach equation

$$\mathrm{p}h_\mathrm{m} = \mathrm{p}K_\mathrm{a} - n\ \log\{(1-\alpha)/\alpha\} \tag{8}$$

This form of the equation has been shown to give an empirical representation of the titration curve for many polyacids, the constant corrective parameter n accounting for deviation from ideality at a selected acid concentration and ionic strength as the degree of dissociation of the acid is varied, By determining K_a and n for the polyacid in the absence of the complexing metal ion the value of a for the metal-polyacid interacting system was calculated by an iterative procedure with eqn. (9).

$$K_\mathrm{a}\left(\frac{a}{A_\mathrm{t}-a}\right)^{1-n} = \frac{h\,a}{[\mathrm{HA}]} \tag{9}$$

where the term $a/(A_\mathrm{t}-a)$ is substituted for $\alpha/(1-\alpha)$ in eqn. (8) to correct for the functional group bound to the metal ion, A_T representing as before the total polyacid concentration (dissociated, associated and metal-bound).

Mandel and Leyte [8] used a different approach to attempt the evaluation of a. A reference titration curve of the polyaeid (without *metal ion) was* transformed to a plot of $\mathrm{p}(h/[\mathrm{HA}])$ against pa. After determining $\mathrm{p}(h/[\mathrm{HA}])$ for a solution containing the same total amount of polyacid and salt, but with the compiexing ion present, the concentration of a in the solution was conveniently determined from the reference graph. This method is applicable even when eqn. (8) is not. In a number of polyacids (eg. PMA) the value of n is not unique.

The assumptions implicit in the evaluation of a by these two methods are that (1) the interaction of metal ion with individual functional groups, rather than the polyion as a whole, reduces the negative charge of the polyion and that (2) conformational changes that occur during such an interaction do not strongly influence the potentiometric characteristics of the polyacid.

Even though the first assumption is most probably valid there is one possible mode of multivalent metal-ion complexation that negates its applicability in the two methods described for estimating the concentration of a. Let us assume that the valence of the metal ion is two. With the formation of MA^+, there is a need to neutralize this extra positive charge to conserve electroneutrality of the system. The most likely path to such charge neutralization is via additional dissociation of the polyacid as shown below:

$$M^{2+} + 2HA \rightleftharpoons MA^+ + A^- + 2H^+ \qquad (10)$$

Charge neutralization by the co-ion is resisted by the net negative charge of the polymer surface, increasingly as the dissociation of the polyacid is increased. The only alternative path requires the decomposition of water. As a consequence, even though only one functional group is chemically bound the effective charge of the matrix may be reduced by a factor approaching two in order to prevent the potentiometric duplication of the reference system when the ratio of $h/[HA]$ is identical. Unless there is some contraction of the molecule to compensate for this extra loss of charge, the estimate of pa will tend to be high.

In our studies where the presence of MA^+ as the dominant species in PAA and PMA systems was unambiguously proved [9], it was shown that, as the concentration of M^{2+} relative to the polyacid increased, the removal of iigand predicted by the method of Mandel and Leyte became increasingly greater than could be accounted for by the binding of the metal on a one to one basis with the repeating functional unit [10]. In fact Mandel and Leyte in their studies [8] found that the apparent ligand number predicted by use of their reference graph approached a value of 2 no matter whether the dominant species, identified positively by our studies, was present as MA^+ (Co, Zn, Ni) or MA_2 (Cu) [9—11].

The second assumption is highly questionable even if the MA_2 species is dominant. Any perturbation of the polyelectrolyte configuration as a consequence of such metal-ion binding must modify the potentiometric characteristics, thereby leading to an incorrect estimate of a. It is necessary to keep the concentration of metal ion at a very low level relative to that of the polyelectrolyte to minimize this source of error with either approach.

On the basis of the above it is suggested that a sufficiently accurate evaluation of a in weakly acidic polymers with the two methods may be possible by potentiometric measurements of pH only when the valence of the metal ion is unity or equal to the valence of the repeating functional unit. Otherwise the above methods can be employed with limited success only to systems where the existence of well-defined species has been unambiguously characterized by an independent approach.

To demonstrate this we have re-examined below the data resolved by Mandel and Leyte from their potentiometric studies of several M^{2+}—PMA systems [8]. This re-analysis of their data, made possible only through knowledge about these systems that has been obtained from the author's more recent studies [9—11], is shown to support the above prognosis by yielding information about the complexed species of these systems which is in reasonable agreement with the more accurate assessment of these systems by the author and his coworkers.

Leyte and Mandel [8], like Gregor et al. [7], have expressed $\bar{Z}$ as a function of [HA], h and β_n^R, the constant for the reaction $M^{2+} + nHA \rightleftharpoons MA, + nH^+$ rather than as a function of a and the formation constant β_{MA_n}, as described earlier. With their approach

$$\bar{Z} = \sum_{n=1} n\,\beta_n^R([HA]/h)^n / \sum_{n=1} \beta_n^R([HA]/h)^n \tag{11}$$

and

$$\beta_n = \frac{[MA_n]\,h^n \exp(-ne\Psi_s)/kT}{[HA]^n\ m \exp(-2\epsilon\Psi_s/kT)} \tag{12}$$

These equations are applicable to systems containing divalent metal ions only when $n \geqslant 2$. The corrective term accounting *for* the deviation from ideality of the competing counter ions appears in both the numerator and the denominator of eqn. (12) and cancels when $n = 2$

$$\beta_2^R = \frac{[MA_2]\ h^2}{[HA]^2\ m} \tag{13}$$

Mow $[MA_2] = \beta_{MA_2}\, m\, a^2$ and $[HA] = \beta_a\, k\, a$ and such substitution in eqn. (13) yields the following relationship between β_2^R and β_{MA_2}.

$$\beta_2^R = \beta_{MA_2}/\beta_a^2 \tag{14}$$

With β_a available from the potentiometric study of the polyaeid acid the value of β_{MA_2} is resolvable, the need for accurate assessment of the exponential term that would be required in an approach paralleling that described earlier for the evaluation of the stability constants in simple metal-ligand systems having been avoided.

In the analysis of their potentiometric data Leyte and Mandel plotted $\bar{Z}$ as a function of $p([HA]/h)$. One flat region in the formation curve was obtained at $\bar{Z} = 2$ for all the systems studied. We have stated that, in these systems, this result can indicate the presence of one dominant species, either MA^+ *or* MA_2. When MA^+ is *the* dominant species as it is in the Co^{2+}-, Ni^{2+}- and Zn^{2+}-PMA systems the reaction is represented by eqn. (10); $\bar{Z}$ approaches a value of 2 rather than 1 because that fraction of free ligand whose charge is neutralized by MA^+ is undetectable by the use of the reference potentiometric titration plot.

Equation (11) is not applicable with these systems (n < 2). Here, from eqn. (10),

$$\beta_1^R = \frac{[MA+]\ h^2\ a \exp(-2\epsilon\Psi_s/kT)}{m\ [HA]^2 \exp(-2\epsilon\Psi_s/kT)} \tag{15}$$

At $\overline{Z}_{\mathrm{app}} = 1$, $[\mathrm{MA}^+] = m$ in these systems and the exponential terms cancel once again so that

$$\log \beta_1^{\mathrm{R}} = 2p\left(\frac{[\mathrm{HA}]}{h}\right) - pa \tag{16}$$

Now

$$\log \beta_1^{\mathrm{R}} = \log \frac{\beta_{\mathrm{MA}^+}}{\beta_{\mathrm{a}}^2} \tag{17}$$

and β_{MA^+} for CoA^+, NiA^+ and ZnA^+ can be computed. The results of this computation are compared in Table 1 with the respective stability constants resolved by the author and his coworkers for the MA^+ species that is dominant in these systems. Sufficient agreement between the two sets of numbers is obtained to support the validity of this treatement of Mandel and Leyte's data.

With the Cu^{2+}—PMA system the interpretation by Mandel and Leyte of their data is correct subject to the limitations of their method for the estimate of a. The dominant species is CuA_2 and $\log \beta_2^{\mathrm{R}} = 2\mathrm{p}([\mathrm{HA}]/h) = \log(\beta_{\mathrm{MA}_2}/\beta_{\mathrm{a}}^2)$.

In the comparison in Table 2 of β_{CuA_2} computed from Leyte and Mandel's data with β_{CuA_2} resolved from the author's examination of this system only one PMA concentration level is considered. The important problem of proper representation of concentration terms for the computation of β_{MA_2} has been noted in the introduction, and a more detailed comparison of the β_{CuA_2} values obtained by Leyte and Mandel and by the author and his coworkers as a function of PMA concentration appears in Sect. D. which is devoted to this aspect.

The discrepancy between the two values for β_{CuA_2} is not too unreasonable since the extra interaction of the mobile Cu^{2+} ion with SO_4^{2-} as compared with NO_3^- has not been considered in these computations. Also there is, in the region where $\overline{Z} = 1$, $(a < 0.3)$Cu—Cu interaction [12] (n-merization) to

TABLE 1

A comparison of formation constant values derived for the species MA^+ in M^{2+}—PMA systems

From Mandel and Leyte as interpreted by the author					From Marinsky and co-workers		
System	2p ([HA]/h)	pa	$\log \beta_1^{\mathrm{R}}$	β_{MA^+}	β_{MA^+}		
Co^{2+}—PMA	-5.7	3.75	9.45	1.6 [a]	0.9 [b]	6.6 [c]	3 [d]
Ni^{2+}—PMA	-5.6	3.88	9.48	1.5	0.9		
Zn^{2+}—PMA	-5.2	3.95	9.15	3		19	8

[a] 2.35×10^{-3} M PMA, 0.05 M NaNO_3.
[b] 1.0×10^{-2} M PMA, 0.05 M $\mathrm{Na}_2\mathrm{SO}_4$.
[c] $2.0\text{-}5.0 \times 10^{-2}$ M PMA, 0.1 M NaClO_4.
[d] 3.0×10^{-2} M PMA, 0.4 M NaClO_4.

TABLE 2

A comparison of the value of the formation constant of CuA_2 in PMA at one concentration level

From Mandel and Leyte as interpreted by the author				From Marinsky and co-workers
System	2p([HA]/*h*)	$\log \beta_2^R$	β_{CuA_2} [a]	β_{CuA_2} [b]
Cu^{2+}—PMA	-4.2	-4.2	2.9×10^5	7.85×10^4

[a] 1.2×10^{-2} *M* PMA, 0.05 M $NaNO_3$.
[b] 1.0×10^{-2} *M* PMA, 0.05 *M* Na_2SO_4.

complicate the analysis of the data from Mandel and Leyte [8]. In addition data obtained by the author with a PMA gel are compared with data obtained with the linear polyacid by Leyte and Mandel. As we shall see from Sect. D the volume differences of the gel and linear analog can also contribute to the observed discrepancy [6].

The method used by the author and his coworkers to demonstrate the existence of a dominant species, MA^+ or MA_2, and to determine the stability constants reported for the dominant species in these systems analyzed the complex formation reactions as

$$M^{2+} + A^- \rightleftharpoons MA^+$$
$$M^{2+} + 2A^- \rightleftharpoons MA_2$$

by correcting for the deviation from ideality of the mobile metal ion. This more generally applicable approach is described in the following sections. The utility of eqns. (13) and (14) and eqns. (15) and (17) for the same purpose when one dominant species exists has been claimed above. Further verification of this claim is presented below. The data that were obtained with Ni^{2+} and PMA gel in 0.05 *M* Na_2SO_4 are employed in eqns. (15) and (17) to evaluate β_{NiA^+} for comparison with the value obtained from our more direct analysis. These data are summarized in Table 3. The agreement between the β_1 values obtained by the two approaches is exact and provides a full testimony for the validity of our estimate of the proper use of this approach. Use of eqns. (13) and (14) for the Cu^{2+}—PMA gel--M Na_2SO_4 system not detailed here also yields a value for β_{CuA_2} in exact agreement with the value for this term derived from our more direct approach.

It is of interest to note here that Mandel and Leyte considered the interacting free ligand in the stepwise formation-of CuA_2 as non-equivalent. They preferred to designate the ratio $h/[HA]$ at $Z = 1$ as β_n^R (ave). This number was suggested as differing from the true constant by $h/(HA)$ and a constant parameter f which they introduced to correct for difference in the effective concentration of the second interacting ligand once its neighbor has been bound. It is our contention that the ligand groups are kinetically equivalent and that

TABLE 3

Comparison of the value of β_{MA^+} computed by two different methods for the system 0.00468 M $NiSO_4$—0.01 M PMA gel-0.05 M Na_2SO_4

b	$\log[MA^+]$	$2 \log h$	$\log m$	$\log a$	$2 \log[HA]$	$\log \beta_1^R$	$\log \beta_{MA^+}$	β_{MA^+} (reaction)	β_{MA^+} (direct)
0.1	-3.882	-9.314	-2.325	-3,031	-4.092	-9.810	-0.150	0.71	0.71
0.2	-3.253	-10.106	-2.366	-2.835	-4.194	-9.634	+0.026	1.06	1.06
0.3	-3.030	-10.800	-2.406	-2.683	-4.310	-9.797	-0.137	0.73	0.73
0.4	-2.887	-11.148	-2.448	-2.567	-4.444	-9.710	-0.050	0.89	0.88
0.5	-2.779	-11.572	-2.495	-2.476	-4.602	-9.730	-0.070	0.86	0.84
0.6	-2.687	-11.940	-2.552	-2,404	-4.796	-9.683	-0.023	0.86	0.84
0.7	-2.651	-12.406	-2.581	-2,321	-5.046	-9.751	-0.091	0.81	0.80
0.8	-2.655	-13.068	-2.683	-2,283	-5.398	-9.825	-0.165	0.68	0.68
0.9	-2.609	-13.628	-2.755	-2,229	-6.000	-9.611	+0.049	1.12	1.11

Gregor et al. [7], who first employed $\overline{Z}$ data as we have in order to compute β_n^{R}, were conceptually correct in spite of the criticism by other workers. To support this estimate we refer to the studies of Gottlieb [13] who has shown that the exchange between free and bound counter ions in polyelectrolyte solutions is rapid, correcting the erroneous observation by Wall et al. [14] that exchange rates are extremely slow. In addition, results of a temperature jump relaxation study by Weiss et al. [15] of the kinetics of proton transfer between polyacrylic acid and polymethacrylic acid and the pH indicator phenol red in the pH range 6-8.5 support this as well. A single, well-defined relaxation process with a time constant between 20 and 250 Ms, depending on polyacid concentration and pH, was observed in their experimental study and demonstrated that the carboxylic acid groups of each polyacid are kinetically equivalent, under the conditions of their study. This result is obtained in spite of the fact that some of the acidic groups are undoubtedly more exposed than others which are buried within the polymer's coiled matrix, a situation expected to be most severe with the PMA system which undergoes a transformational change during the binding of protons.

With the dissociation of hydrogen ions from the polyacid, the several concurrent combinations of the dissociated side-chain carboxyl groups with Cu^{2+} is thus with identical sites. The equilibrium constant for each kind of reactions can be related to a single intrinsic constant for each reaction type as a consequence. It is true that the effect of binding at any site on the binding affinity of other sites must differ, even though they are identical in their intrinsic properties. Because of their position with respect to other sites, there must be a different response to charge [16,17] as evidenced by a change in the relaxation time constant, but this is automatically accounted for in the exponential terms which cancel in the above treatment.

We have seen that the methods proposed in the literature'for the reasonably accurate assessment of the free ligand concentration of charged polymers in the course of their complexation by metal ion have limited applicability. With these methods, it is difficult to distinguish between the formation of species such as $MA^{(\nu-1)+}$, $MA_2^{(\nu-2)+}$ --- MA,. The tendency is always to indicate the presence of MA, as the dominant species. As a consequence, only when MA, is the dominant species is it even likely that the computed value of a may be accurate.

In addition the expression for $\overline{Z}$ (eqn. (11)) is not correct. For the sequence of reactions that may arise from interaction of a ν-valent metal ion with a repeating univalent functional unit of a polymer the correct expression based upon electroneutrality conservation considerations is

$$\overline{Z} = \frac{\sum_{n=1}^{n} n\beta_n^{\mathrm{R}} \left([\mathrm{HA}]/h\right)^{n} a^{(\nu-n)}}{\Sigma\beta_n^{\mathrm{R}}\left([\mathrm{HA}]/h\right)^{n} a^{(\nu-n)}} \qquad (18)$$

If several species co-exist the great advantage derived from the fact that the exponential non-ideality term is cancelled is of little value as an accurate value of a and consequently $\overline{Z}$ has not been affected by the several methods so far devised for this purpose. We have therefore turned to alternate experimental avenues in order to achieve a correct analysis of complexation reactions in charged polymer systems. With the development of such alternatives, the additional fundamental information about such systems allow successful analysis either by providing, as described above, the opportunity for a more correct interpretation of the experimental data via the two methods outlined for the computation of free ligand concentration or by permitting other avenues for analysis as described in Sect. C. In the course of development of these latter approaches an experimental technique for the accurate evaluation of a has been forthcoming and allows us to compute $\overline{Z}$ and thus to evaluate,,&, β_n as well

C. A MORE USEFUL APPROACH TO THE DETERMINATION OF STABILITY CONSTANTS OF COMPLEX SPECIES FORMED IN CHARGED POLYMERS

We have shown that it is not possible with the methods so far proposed, to evaluate the concentration a of free ligand in metal—polyacid systems in order to obtain $\overline{Z}$; therefore computational methods based on $\overline{Z}$ as a function of a cannot be employed (except in the special cases that we have described) for the evaluation of meaningful formation constants in these systems. Only the combined term $a \exp(-\epsilon\Psi_s/kT)$, as we have already pointed out (eqn. (‘I)), is measured in such systems. We must, therefore, determine directly and accurately an experimental quantity which can be related to the complex formation constants through this term. The measurement of free metal ion concurrently with the measurement of pH has been employed to facilitate such an analysis [6,9—11].

By measuring the concentration m of free metal ion we determine the fraction α_0 of metal ion added that is free and mobile. In simple metal-ligand systems

$$\alpha_0 = \frac{m}{M_T} = \frac{m}{m + \sum\limits_{n=1} \beta_n a^n} = \frac{1}{1 + \sum\limits_{n=1} \beta_n a^n} \tag{19}$$

Here $\beta_0 = m/m = 1$. In charged polymer systems the effective concentration of free metal ion at the reaction site is expressed as $m \exp(-\nu\epsilon\Psi_s/kT)$, ν representing as before the valence of the metal ion. For these systems

$$\alpha_0 = \exp(-\nu\epsilon\Psi_s/kT)/(1 + \sum_{n=1} \beta_n a^n) \exp(-\nu\epsilon\Psi_s/kT) \tag{19a}$$

By equating β_0 to $\exp(\nu\epsilon\Psi_s/kT)$

$$\alpha_0 = \frac{1}{1 + \sum\limits_{n=1} \beta_n a^n \exp(-\nu\epsilon\Psi_s/kT)} \tag{19b}$$

Rearranging eqn. (19b)

$$\frac{1-\alpha_0}{\alpha_0 a \exp(-\epsilon\Psi_s/kT)} = \sum_{n=1} \beta_n a^{(n-1)} \exp\{-(\nu-1)\epsilon\Psi_s/kT\} \tag{19c}$$

It is reasonable to assume that in metal-containing polyacidic (or polybasic) systems $M^{\nu+}$, at equilibrium, is exposed to the same potential Ψ_s as the H^+ ion in the system, The a $\exp(-\epsilon\Psi_s/kT)$ term extracted from the potentiometric measurement of pH is applicable on this basis to an estimate of the electrostatic work $\exp(-\nu\epsilon\Psi_s/kT)$ in bringing the $M^{\nu+}$ ion to the site of reaction.

In order to examine the application of eqn. (19) for the evaluation of the formation constants of a sequence of complexed species that may form in a particular system it is appropriate to consider first the possibility that the number of separate and independent species that can form in these unique systems may be limited. If the number is smaller than 3, the analysis will be much less complicated.

Each polyacid strand in the presence of excess neutral salt provides an independent, equivalent ligand environment. These strands repel each other increasingly as the degree of neutralization increases and the tendency for a random orientation of the molecules becomes less. As a consequence the close approach of flexible segments of the same molecule becomes unlikely. A cylindrically symmetrical geometry in which the strands are extended parallel to each other and as far apart as possible is the net conformational result. Ligand interaction with the metal ion is thus sterically limited. The opportunity for metal attachment to more than one strand is unlikely to happen also, especially at higher degrees of neutralization, in the normal polymer concentration range employed.

Because the binding of the metal ion is confined to individual polymer strands its complexation behavior is essentially unaffected by increasing the polymer concentration and is to a first approximation only a function of the degree of neutralization. The net result is that the effective range of ligand concentration for complexation is approximately *one* order of magnitude ($0.05 \leqslant \alpha \leqslant 1.0$). Even if steric factors were not operable to limit the coordination of ligand to metal ion this effective concentration range of ligand, by analogy with simple ligand-metal ions systems, is insufficient to yield more than two complex species concurrently. Equation (19) reduces to

$$\frac{1-\alpha_0}{\alpha_0\, a \exp(-\epsilon\Psi_s/kT)} = \beta_1 \exp\{-(\nu-1)\epsilon\Psi_s/kT\} + \beta_2 a \exp\{-(\nu-1)\epsilon\Psi_s/kT\} \tag{19a}$$

The graphical representation of experimental data by plotting the left-hand side of the equation against $a \exp(-\epsilon\Psi_s/kT)$ leads to a line with an intercept of $\beta_1 \exp\{-(\nu-1)\epsilon\Psi_s/kT\}$ and a slope of $\beta_2 \exp\{-(\nu-2)-\epsilon\Psi_s/kT\}$. When the valence of the metal ion is 2, only the intercept value is variable and resolution of β_1 and β_2 is a reasonably straightforward exercise. With $\nu \geqslant 3$, the intercept and slope are both variable and complicate appreciably the evaluation of formation constants with this approach. In addition the hydrolytic chemistry of these multivalent ions becomes an extra source of complexity as the pH of the system is continuously varied during the experimental program. We therefore limit the detailed discussion of this approach which follows to the analysis of complex formation of divalent ions with charged polymers.

The only possible way to treat the complexation of higher valent metal ions by charged polymers quantitatively requires the evaluation of the free ligand concentration which, as we have seen, has not been possible with the methods so far proposed. In the course of the author's experimental studies to demonstrate the validity of the approach a method to achieve this objective with accuracy is suggested.

(i) The effective concentration of mobile counter ions in charged polymers

The first fundamental insight into the analysis of non-ideality in charged polymeric systems, such as proteins, was due to Linderstrøm-Lang [18]. His complicated treatment was restated much later in a more convenient form by Scatchard [19] in order to facilitate the study of ion binding in complicated protein systems. The more rigorous derivations of Scatchard have been essentially duplicated by Tanford [20] with the simplified version of the Linderstrøm-Lang treatment that is presented below.

All ionizable groups of a protein are assumed to be completely independent of one another and to obey the ionization equation. The equilibriumconstant K' is related to the free energy of ionization

$$\Delta F^{\circ}_{\text{ion}} = -RT \ln K' = 2.303 \; RT \; \text{p}K' \tag{20}$$

Part of this free energy is electrostatic work which must be done to increase the charge of the conjugate base molecule by one. The ionizable group is considered to be on the surface of the protein molecule which is taken to be a sphere with a charge of Z uniformally distributed about its surface. The electrostatic free energy required to increase the charge to $Z + 1$ is then

$$\frac{(Z+1)^2 N\epsilon^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) - \frac{Z^2 N\epsilon^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) = (\Delta F^{\circ}{}_{\text{ion}})_{\text{elec}}$$

and

$$\frac{(2Z+1)N\epsilon^2}{\text{W}}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) = -(\Delta F^{\circ}{}_{\text{ion}})_{\text{elec}} \tag{21}$$

where b is the radius of the sphere, a is its radius of exclusion, κ has the usual meaning in the Debye—Hückel theory, D is the dielectric constant of the medium and ϵ is the protonic charge.

The subscript zero is used to denote that the protein sphere has zero net charge when the ionizing group under consideration is in the form of its conjugate base.

Then

$$-\{(\Delta F^{\circ}_{\text{ion}})_{\text{elec}}\}_0 = \frac{N\epsilon^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right)$$

and

$$\{\Delta F^{\circ}_{\text{ion}}\}_Z - \{\Delta F^{\circ}_{\text{ion}}\}_0 = \frac{2ZN\epsilon^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) \tag{22}$$

We assume that the difference in ionization constant of a particular group on the protein is the same whether neighboring groups are ionized or not except in so far as the electrostatic free energy changes with the charge and

$$2.303RT(\mathrm{p}K_Z' - \mathrm{p}K_0) = -\frac{2ZN\epsilon^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right)$$

$$(\mathrm{p}K_Z' - \mathrm{p}K_0) = \frac{2ZN\epsilon^2}{2.303RT\,2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) \tag{23}$$

It is customary to equate

$$\frac{N\epsilon^2}{2DRT}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right) \text{ to } w$$

so that at any charge Z

$$\mathrm{p}K = \mathrm{p}K_0 - \frac{2Zw}{2.303}$$

and

$$\mathrm{pH} - \log\frac{\alpha}{1-\alpha} = \mathrm{p}K_0 - \frac{2Zw}{2.303} \tag{24}$$

From eqn. (7)

$$\epsilon\Psi_s/kT = 2Zw \tag{25}$$

to demonstrate the equivalence of the approach the author has adapted by employing directly the modified form of the Hasseibach-Henderson equation for this purpose.

Arnold and Overbeek [21] in their pioneer demonstration of this approach plotted the potentiometric titration data obtained with polymethacrylic acid as pH $-$ log $\{\alpha/(1-\mathrm{a})\}$ versus α. Ideally pH $-$ log$\{\alpha/(1-\alpha)\} = \mathrm{p}K_a$ and any deviation from a straight line of zero slope in such a plot is presumed to be a quantitative measure of the deviation from ideal behavior of the system as the polyacid is progressively dissociated. A representative plot of such data obtained with PMA is presented in Fig. 1. The extrapolation of the upper curve of the figure is by a straight-line extension of the data as it should be in this region of low charge density where the Debye—Hückel approximation is valid. The long extrapolation of the lower line must be slightly curved to reach the

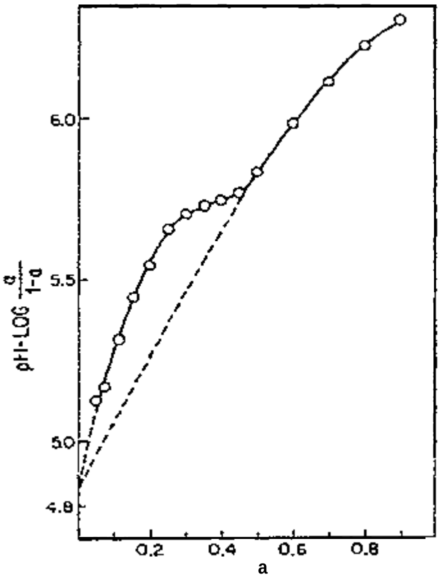


Fig. 1. Potentiometric study of polymethacrylic acid; system 0.01 *M* PMA—0.05 M Na_2SO_4.

same intercept value [22]. Such curvature is to be expected in the higher charge density region. The K_a value of 1.48×10^{-5} that is determined from this analysis of the potentiometric data is in good agreement with the dissociation constant reported for isobutyric acid, the repeating monomer unit of this polymer. Since there is no ionization of the polymer at $\alpha = 0$ this number should correspond to the negative logarithm of the intrinsic dissociation constant of the repeating acidic group of PMA, as it does, if the source of deviation is exclusively electrostatic in nature. Thus the change (ΔpK) in the value of pK with a is attributable to the change in the electrostatic free energy of the molecule as a consequence of the group-group interactions accompanying the ionization process. Such a deviation from ideality at the polymer surface is expressed as $0.4343\epsilon\Psi_s/kT$ (see eqns. (6) and (7)), the starting point of the Poisson-Boltzmann equation for a uniformally charged cylinder in a uniform dielectric [23].

(ii) A test of the general applicability of the non-ideality term

In the development of eqn. (19) it was assumed that the fundamental deviation term $\exp(-\epsilon\Psi_s/kT)$ derived from the study of the H^+ ion in polyacid systems provides an accurate basis for an estimate of the deviation from ideality of the mobile $M^{\nu+}$ counter ions also present in these complicated systems. To demonstrate that this is indeed the case, a study of the complexation of trace-level concentrations of Ca^{2+}, Co^{2+} and Zn^{2+} by polymethacrylic and polyacrylic acid as a function of *a* was made by the author and a co-worker [9]. The distribution of trace-level concentrations of these respective metal ions between a cation-exchange resin (Na ion form) and a solution (M NaClO$_4$) in the absence and presence of various concentrations of the respective polyacids was measured at different degrees of neutralization to facilitate this objective. The partition coefficient D_0 (absence of polyacid) and D (presence of ligand) bear the following relationship to β and a for M^{2+}—polyligand systems.

$$\frac{D_0 - D}{Da} = \beta_1 \exp(-2\epsilon\Psi_s/kT) + \beta_2 a \exp(-2\epsilon\Psi_s/kT) \tag{26}$$

Analysis of the distribution results, with $\exp(-\epsilon\Psi_s/kT)$ and a values directly available from pH measurements (binding of trace metal ion does not affect the stoichiometry of the system) made concurrently with the distribution measurements provided unambiguous verification of the general applicability of the non-ideality term so obtained in these systems. The experimental values of $(D_0 - D)/Da$ were plotted versus the a $\exp(-\epsilon\Psi_s/kT)$ term. The data were extrapolated and the intercept of the ordinate defined the value of β_1. A plot of $\log\{(D_0 - D)/Da\beta_1\}$ versus $\text{pH} - \log\{\alpha/(1-\alpha)\} -$ pka then yielded a line with a slope of 2 which intersected the origin. The ordinate $\log\{(D_0 - D)/Da\beta_1\}$ is equal to

$$\frac{2\epsilon\Psi_s/kT}{2.3} + \log\left(1 + \frac{\beta_2 a}{\beta_1}\right)$$

while the abscissa $\text{pH} - \log\{\alpha/(1-\alpha)\} - \text{p}K_a$ is equal to $\epsilon\Psi_s/2.3kT$; the observed result demonstrates that (1) essentially only the MA+ species exists in these systems (i.e. $\beta_2 < 0.1\,\beta_1$) and (2) the non-ideality of the divalent ion is defined by $2\epsilon\Psi_s/kT$. These results are presented graphically in Figs. 2 and 3.

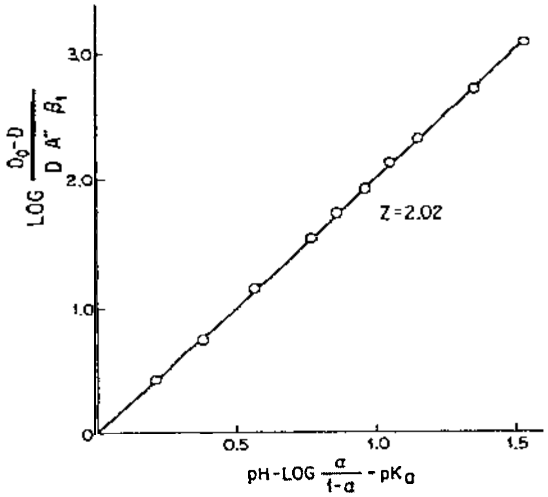
These results show unambiguously that the above approach to a quantitative estimate of the non-ideality of multivalent metal ion competing with the H^+ ion in their interaction with the repeating ligand unit of polyelectrolytes is valid. A bonus of this result is that a means of accurate analysis of the free ligand concentration a in $(HA)_p$—$M^{\nu+}$ systems is also provided. If instead of using trace-level concentrations of Zn^{2+} in these systems macroscopic concentrations are employed, the concurrent measurement of pH and pZn with a glass electrode and a Zn amalgam electrode, respectively, will yield, after appropriate consideration of stoichiometry, the terms a $\exp(-\epsilon\Psi_s/kT)$ and a $\exp(-2\epsilon\Psi_s/kT)$. If we express equilibrium in these systems by

$$\frac{[HA]}{h\,\beta_a} = a \exp(-\epsilon\Psi_s/kT) \tag{7c}$$

and since ZnA^+ is the dominant species in these systems,

$$\frac{[ZnA^+]}{[Zn^{2+}]\,\beta_{ZnA^+}} = a \exp(-2\epsilon\Psi_s/kT) \tag{7d}$$

Division of $[HA]/h\,\beta_a$ by $[ZnA^+]/[Zn^{2+}]\beta_{ZnA^+}$ yields the value of $\exp(-\epsilon\Psi_s/kT)$ and thus allows computation of a in every experimental situation,



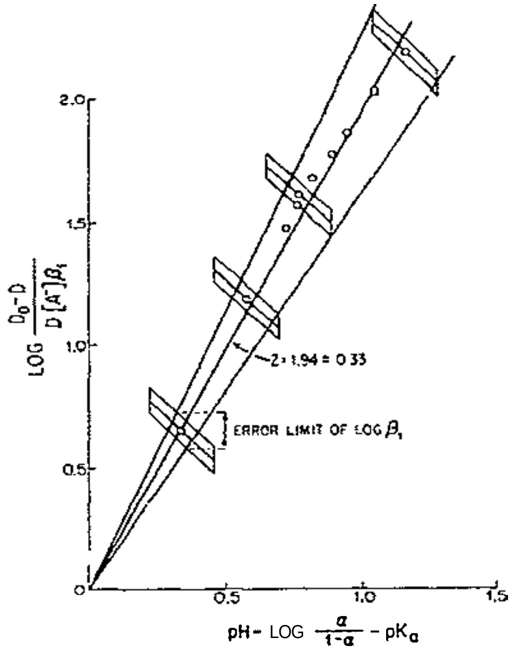


Fig. 2. Plot of non-ideality relationships; system ""Co-0.03 M PAA—0.1 M $NaClO_4$.

Fig. 3. Plot of non-ideality relationship; system ^{60}Co—PMA—0.1 M $NaClO_4$.

With this approach, a Zn^{2+} ion can be used as a probe to evaluate a during the study of the complexation of other metal ions of valence not less than 2. The measurement of free mobile $M^{\nu+}$ concurrently with the measurement of pH and pZn is desirable for greatest accuracy but is not essential in this procedure since with the a available an accurate assessment of $\overline{Z}$ is possible. With a, $\exp(-\epsilon\Psi_s/kT)$ and Z or α_0 available, proper resolution with eqn. (3), (18) or (19) (if the concentration of free $M^{\nu+}$ is measured) is susceptible only to the extra complication which arises from the expression of the concentration, in these polymeric systems, of all species in molarity units based upon the solution volume. The nature of this problem with its advantages and disadvantages is fully detailed in Sect. D.

D. THE IMPORTANCE OF CONCENTRATION UNITS

Only the accurate resolution of β_1 is possible in M^{2+}—polyacidic (or polybasic) systems where two complex species (MA^+ and MA_2) are formed if the concentration of the various species are expressed in the standard way by molarity units based on the solution volume V. Its value will be uniquely obtained from the intercept of the ordinate axis of a plot of $(1 - \alpha_0)/\alpha_0 a \exp(-\epsilon\Psi_s/kT)$ versus $a\exp(-\epsilon\Psi_s/kT)$. The straight line of slope equal to β_2 that is expected from this plot, however, may instead be curved with only segments linear. If a straight line is obtained over the complete range. of the experimental study its slope will be inversely proportional to the initial concentration of polyacid employed, i.e. $\beta_2 = k/M_{HA}$.

Such failure of eqn. (19) to resolve uniquely the stability constant of MA_2 species using molarity units based on the solution volume is very well illustrated from the results obtained in a detailed study by the author and co-workers of the binding of Cu^{2+} by polyacrylic acid [6]. In this study, the PAA contained at fixed concentrations and degrees of neutralization in 0.05 M Na_2SO_4 was titrated with $CuSO_4$. The pH and pCu were measured concurrently. Graphical representation of a typical set of data is shown in Fig. 4. The best line that can be drawn through the experimental points is linear in two $a\exp(-\epsilon\Psi_s/kT)$ regions: at the very lowest values of the abscissa where the $CuSO_4$ to PAA concentration ratio is largest and in the low $CuSO_4$ to PAA concentration ratio range. The linear portion of the curve at, low values of $a\exp(-\epsilon\Psi_s/kT)$ is magnified in the figure insert through the use of a much expanded coordinate scale to show this result clearly. Experimental points obtained at the same PAA concentration but at different fixed a values ($\alpha = 0.23$ to 0.90) in the two separate $CuSO_4$ to PAA concentration ratio regions of Fig. 4, where linear-

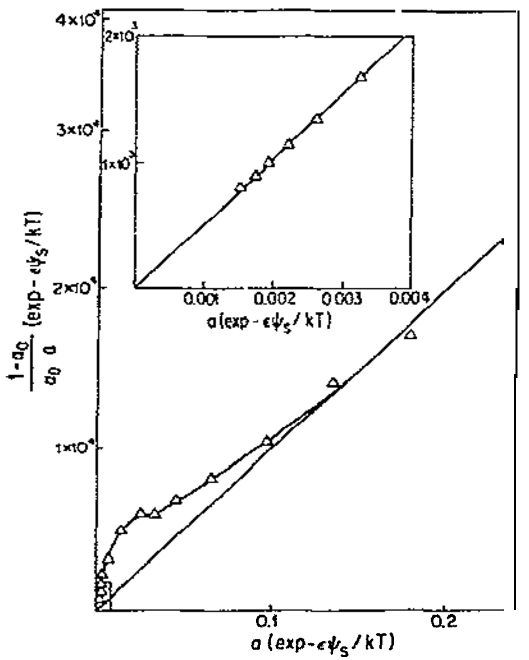


Fig. 4. The variation of $(1-\alpha_0)/\alpha_0\, a\exp(-\epsilon\Psi_s/kT)$ versus $a\exp(-\epsilon\Psi_s/kT)$ with varying $CuSO_4$ concentration in PAA at a fixed neutralization value; system 0.0146 M PAA—0.05 M Na_2SO_4; $\alpha = 0.75$.

ity is observed, are plotted in Fig. 5 and Fig. 6 and continue to yield straight lines with essentially the two respective slopes observed in Fig. 4. The magnitude of the respective slopes in the separate concentration ratio regions is shown to be inversely proportional to the initial concentration of PAA by plots of β_2 versus M_{PAA}^{-1} in Fig. 7. The results obtained at the low $CuSO_4$ to PAA concentration ratio were reproduced in a potentiometic study, during neutralization with standard base, of a PAA system containing Cu^{2+} and PAA fixed at the appropriate concentration levels.

The failure of eqn. (19) to resolve β_2 uniquely *is* a direct consequence of the use of molarity units based on the solution volume. This concentration unit does not properly represent the discrete distribution of polymer species as a separate phase of these systems. For this the concentration of each polymer species must be based upon the total volume v of the polymer contained in the solution volume. With this expression of polymer species concentration $V \gg v$ and

$$\begin{aligned}\beta_{1(v)} &= \frac{[\mathrm{MA}^+]/v}{\{m/(V-v)\}\exp(-2\epsilon\Psi_s/kT)\ (a/v)} \\ &= \frac{[\mathrm{MA}^+](V-v)}{ma\exp(-2\epsilon\Psi_s/kT)} \\ &\approx \frac{[\mathrm{MA}^+]V}{\mathrm{ma}\exp(-2\epsilon\Psi_s/kT)}\end{aligned} \tag{27}$$

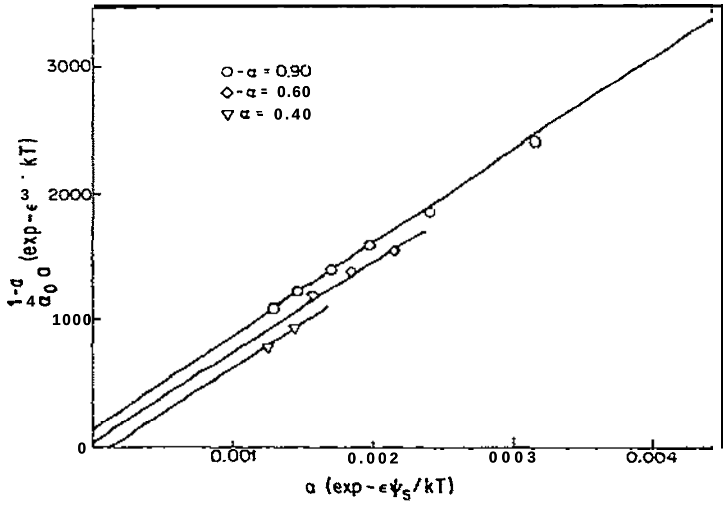
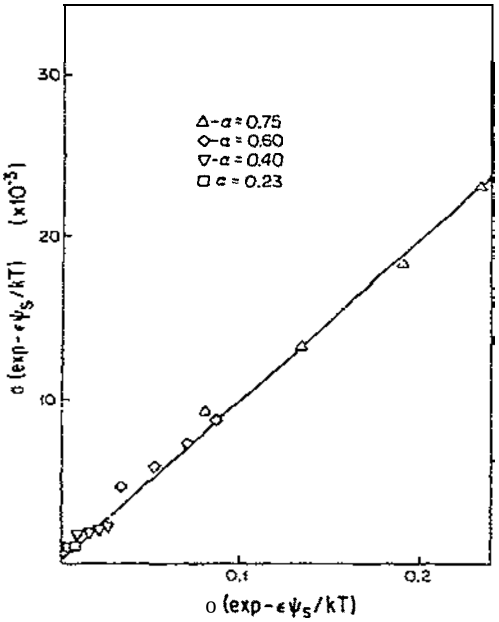


Fig. 5. A potentiometric study of PAA in Cu^{2+}-crowded systems as a function of PAA neutralization and Cu^{2+} concentration; system 0.0146 M PAA–0.05 *M* Na_2SO_4.



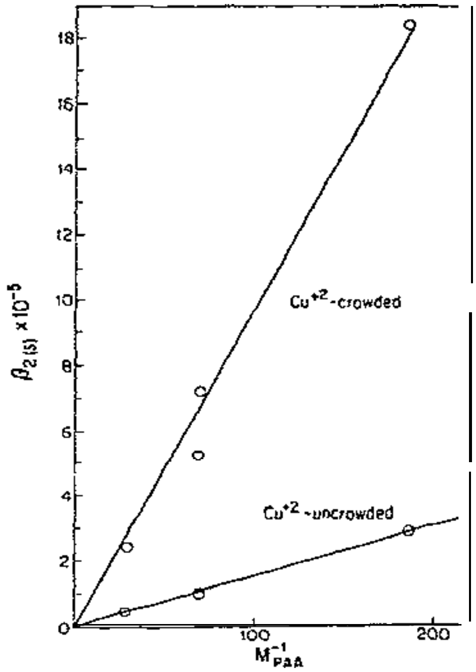


Fig. 6. A plot of $(1-\alpha_0)/\alpha_0 \exp(-\epsilon\Psi_s/kT)$ versus a $\exp(-\epsilon\Psi_s/kT)$ at several different fixed and low Cu^{2+} to PAA ratios in one concentration of PAA at different fixed neutralization values; system 0.0146 *M* PAA—0.05 *M* Na_2SO_4.

Fig. 7. A plot of $\beta_{2(s)}$ versus the inverse of the PAA concentration.

while

$$\begin{aligned}\beta_{2(v)} &= \frac{[MA_2]/v}{\{m/(V-v)\}\,(a/v)^2 \exp(-2\epsilon\Psi_s/kT)}\\ &= \frac{[MA_2]\,(V-v)v}{ma^2 \exp(-2\epsilon\Psi_s kT)}\\ &\approx \frac{[MA_2]\,vV}{ma^2 \exp(-2\epsilon\Psi_s kT)}\end{aligned} \tag{28}$$

'In the standard representation

$$\begin{aligned}\beta_{1(s)} &= \frac{[MA^+]V}{ma \exp(-2\epsilon\Psi_s kT)}\\ \beta_{2(s)} &= \frac{[MA_2]V^2}{ma^2 \exp(-2\epsilon\Psi_s kT)}\end{aligned} \tag{29}$$

so that

$$\beta_{1\,(v)} \approx \beta_{1\,(s)} \text{ and } \beta_{2\,(v)} \approx \beta_{2\,(s)} \frac{v}{V} \qquad (36)$$

Since the definition of $\beta_{n(v)}$ is believed to represent correctly the equilibria operative in these systems, only the representation of β_n where $n > 1$ can be expected to yield unique resolvable formation constants for the $\Sigma_{n=1}$ [MA,] complex species that may form. Misrepresentation of the concentration of polymer species in the standard expression for β_1 does not lead to noticeable error in the evaluation of this formation constant because the dimension of v in the numerator and denominator is the same and the v terms cancel in defining $\beta_{1\,(v)}$ so that $\beta_{1\,(s)} \approx \beta_{1(v)}$. However, in the case of MA_2, for example, incorrect representation of the concentration of polymer species in the standard expression leads to the prediction that $\beta_{2\,(s)}$ will not be constant but will vary inversely with any change in volume of the polymer domain during an experiment in which V is maintained constant. Even if the polymer is dimensionally stable, it is predicted that $\beta_{2(s)}$ will vary inversely with the concentration of polyelectrolyte employed in a series of experiments conducted at constant V.

On the basis of the above examination of eqn. (19) the observed distribution of the experimental points of Fig. 4 suggest that the polymer molecule exists as CuA_2 in a dimensionally stable but expanded form at the first addition of $CuSO_4$. Eventually a progressively greater contraction of the molecule with each additional increment of Cu^{2+} occurs until at the highest concentration levels of $CuSO_4$ a constant minimal volume of the molecule is reached. At the point where rapidly increasing contraction of the molecule is indicated, turbidity is observed in the solution. The precipitated polymer is believed to correspond to the dimensionally stable contracted form of the molecule.

It is of interest at this point to return to the study by Mandel and Leyte of the binding of Cu^{2+} by PMA [8]. With a sixfold increase in the concentration of PMA from 0.01 to 0.06 M the binding constant derived from their data is approximately one-sixth the value obtained by them at the lower PMA concentration. This result is anticipated by our analysis.

To test further the validity of the above interpretation of the Cu^{2+} binding data, the $CuSO_4$ interaction with a PMA gel in 0.05 A4 Na_2SO_4 was studied potentiometrically and polarographically as a function of its neutralization with a standard base. It was known that the volume change of the gel, constrained by cross-linking with divinylbenzene, would be small over a large α range and this dimensional stability of the molecule was expected to be reflected in the linearity of the line resolved from graphical representation of the data with eqn. (19). This was indeed the case at α values of not less than 0.35. The divergence from a straight line below this α value could be explained by a Cu—Cu interaction that is known to prevail in PMA in the low α region [12].

It is appropriate to refer at this time to the report by Felber et al. [24] on their study of metal complexes in the alternating 1 :1 copolymer of ethylene and maleic acid. Unaware of the fact that concurrent study of the reaction of H^+ competitively with the M^{2+} would provide an accurate estimate of the deviation of ideality of the mobile counter ions at the site of reaction they estimated stability constants in these systems neglecting this correction term. Instead they attempted to apply the interionic attraction theory of electrolytes for this purpose, badly underestimating the high field due to the charge on the polymer by assuming a uniform distribution of the charged polymer sites throughout the solution. In their evaluation of the pK value of the bicoordinated complex formed they used molar concentration units based on the solution volume to repeat this mistake.

The discrepancy of a factor of six orders of magnitude between their pK value and the pK value reported for the corresponding metal—monosuccinate complex was discussed in terms of the enthalpy and entropy of complex formation in these polymeric systems, It is quite apparent, however, that the extra magnitude of their β_{MA} is a consequence of their low assessment of (1) the electrostatic deviation term by a factor of 10—100 and (2) their use of molarity units based on the solution volume which magnifies their answer by at least another factor of 1000.

(i) An interesting and important observation

In studies of the Cu^{2+}—PMA system in the author's laboratory [25] it has been observed from concurrent polarographic and pH measurements made during progressive neutralization with a standard base of PMA containing a constant concentration level of Cu^{2+} that conformational stability of the CuA_2 species in these systems occurs over a fixed neutralization range. The extent of this neutralization range appears to be a function of the concentration ratio of Cu^{2+} to PMA; the larger the ratio the more extended is the range of dimensional stability.

Representative data and the method employed for their interpretation are presented below tc illustrate this observation. The fundamental equation derived by DeFord and Hume [26] for the evaluation of formation constants through the polarographic examination of stepwise equilibria between successively formed complexes in solution was employed. This equation, modified to correct for the deviation from ideality of the metal ion at the surface of the reaction site of the polymer, as described earlier, is

$$\frac{\operatorname{antilog}\left(\frac{0.4343nF}{RT}\,\Delta E_{1/2} + \log\frac{i_c}{i_s}\right) - 1}{a\exp(-\epsilon\Psi_s/kT)} = \beta_1\exp(-\epsilon\Psi_s/kT) + \beta_2\, a\exp(-\epsilon\Psi_s/kT) \qquad (31)$$

where $\Delta E_{1/2}$ is the difference in the half-wave potential of the experimental solution and the polymer-free but otherwise equivalent system, i_c is the diffusion current measured in the experimental system and i_s is the diffusion current of the polymer-free but otherwise equivalent system; n represents the electron change in the reduction of the metal ion and F is Faraday's constant. A plot of the left-hand side of eqn. (31) versus $a \exp(-\epsilon\Psi_s/kT)$ will, as before, yield a line with a dope of β_2 and an intercept of $\beta_1 \exp(-\epsilon\Psi_s/kT)$. The linearity of the line will depend upon the conformational stability of the molecule.

In the sequence of experiments performed, the effect of the presence of Cu^{2+} on the conformation of PMA was studied. For this purpose, a constant initial concentration of PMA (0.0109 M) was used while varying the concentration of copper tenfold (1.0×10^{-4} M to 1.0×10^{-3} M) in the presence of 0.30 M $NaClO_4$. The data, analyzed using eqn. (31) are presented in Fig. 8. As the Cu^{2+} to PMA ratio is increased, the negative deviation of the points from the initial straight line that was obtained over the α range ($0.3 \leqslant \alpha \leqslant 0.5$) in earlier studies (in sulfate media) diminishes until, at the highest ratio, no deviation of the points (other than experimental) from the straight line is observed, even at $\alpha \approx 0.8$. The evidence is strong for conformational stabilization of the compact form of PMA by Cu^{2+}, which increases with increasing amounts of copper.

The straight lines resolvable at the lower values of α for each set of data have somewhat different slopes, with the steepest slope being obtained from the system with the highest Cu^{2+} content. This result suggests that both dimensional stability and compactness are a function of the metal ion concentration level in this system.

The relationship between bound $(1 - \alpha_0)$ and free copper (α_0) is

$$\frac{1-\alpha_0}{\alpha_0\, a \exp(-\epsilon\Psi/kT)} = (\beta_1 + \beta_2 a) \exp(-\epsilon\Psi_s/kT) \tag{19}$$

the right-hand side of this equation being equal to the term calculated from the potentiometric and polarographic data with eqn. (31). Thus,

$$\frac{1-\alpha_0}{\alpha_0} = \text{antilog}\left(0.4343\, \frac{nF}{RT} + \log \frac{i_c}{i_s}\right) - 1 \tag{32}$$

With α_0 calculable in this way the amount of bound Cu, Cu_b, where

$$(1-\alpha_0)[Cu_t] = [Cu_b] \tag{33}$$

can be determined at every experimental point. To a first approximation, all bound copper exists as the CuA_2 species. The accurate value of a, in each equilibrated sample, can then be obtained by correcting for the amount bound to the Cu^{2+} ion:

$$a = [HA] - [HA_{,,}] - 2[Cu_b] \tag{2}$$

to permit evaluation of pK_{app} as a function of a. The results of these calculations, performed on the system containing 0.0109 M PMA and 0.001 M $CuSO_4$ *in* 0.30 M $NaClO_4$, where the greatest range of dimensional stabilization is observed for the Cu^{2+}-bound species are compiled in Table 4 and presented graphically in Fig. 9 together with the potentiometric data for the Cu^{2+}-free

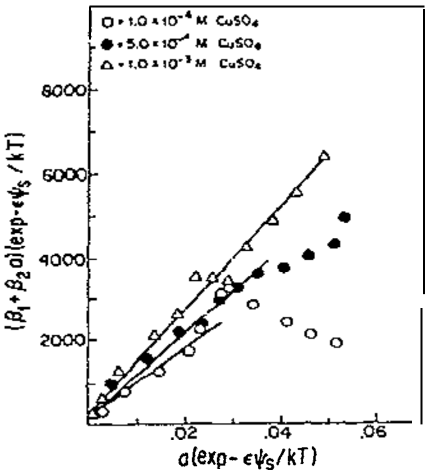


Fig. 8. Graphical analysis of a **polarographic** study of Cu^{2+}**-binding** by PMA; system 1.09×10^{-2} *M* PMA—0.3 *M* $NaClO_4$.

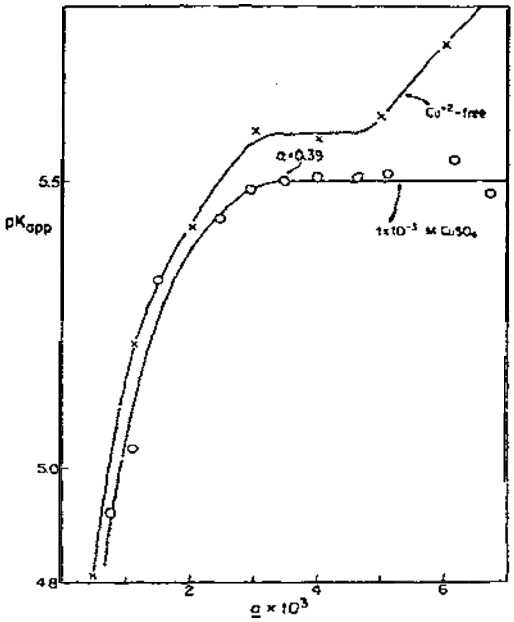


Fig. 9. Apparent p*K* of PMA in the presence and absence of Cu^{2+}; system 1.09×10^{-2} *M* PMA—1×10^{-3} *M* $CuSO_4$—0.3 *M* $NaClO_4$.

TABLE 4

The pK_{app} of PMA in the presence of Cu^{2+} for the system 1.09×10^{-2}*M* PMA-1.0×10^{-3}*M* $CuSO_4$—0.30 *M* $NaClO_4$

b	$[Cu_b]/[Cu_f]$ $(1-\alpha_0)/\alpha_0$	$[Cu_t]/[Cu_f]$ $(\alpha_0 - 1)$	$[Cu_f]$	*a*	$\alpha = a/(a \cdot [HA])$	pH $-\log\frac{\alpha}{1-\alpha}$
0.10	0.3670	1.367	7.315×10^{-4}	7.710×10^{-4}	0.0696	4.918
0.20	1.454	2.454	4.074	1.093×10^{-3}	0.1125	5.035
0.30	8.410	9.410	1.062	1.511	0.1658	5.323
0.40	27.72	28.72	5.482×10^{-5}	2.483	0.2756	5.435
3.45	47.38	48.38	2.067	2.960	0.3311	5.486
0.50	77.36	78.36	1.276	3.483	0.3902	5.500
0.55	89.84	90.84	1.101	4.022	0.4508	5.504
0.60	98.73	99.73	1.003	4.564	0.5117	5.504
0.65	141.7	142.7	7.007×10^{-6}	5.102	0.5724	5.511
0.70	184.9	185.9	5.379	5.643	0.6333	5.537
0.75	239.3	240.3	4.161	6.259	0.6968	5.560
0.80	310.8	311.8	3.207	6.727	0.7553	5.477

but otherwise equivalent system. When $\alpha > 0.4$, the ordinate value is essentially constant to within ±0.02 pK units, the range of the experimental precision of the potentiometric measurements in all but one instance.

This constancy in pK_{app}, and consequently the $\epsilon\Psi_s/kT$ term, is characteristically observed in the potentiometric titration of PMA (Fig. 1) at $0.3 < a < 0.5$ and has been identified with the transformation of a compact form of PMA to a random coil conformation. It is proposed that this phenomenon is closely analogous to the critical point in the case of a Pure substance [27]. At this critical point since the non-ideality curve is horizontal

$$\frac{\partial y\pm}{\partial a} = 0 \text{ and } \frac{\partial F}{\partial a} = 0$$

As the critical point is not a simple maximum or minimum the second derivatives are also zero. As $\partial F/\partial a$ and $\partial^2 F/\partial a^2$ equal zero the partial molar volume of the molecule must remain constant to explain the observed constancy of β_2.

The concept of ion condensation [28—30] has recently been introduced to analyze the conformational transition of rod-like palyelectrolytes possessing high charge density [31]. According to this theory, some counter ions must "condense" on the surface of the rod when the charge density reaches a critical value, specifically during the random coil → helix transition. With such condensation to keep the charge density constant one might suspect that the dimensions of the molecule are also maintained constant. Extension of this concept to the Cu^{2+}-contained PMA provides a logical explanation of the observed dimensional stability of the PMA molecule.

(ii) An exploration of molarity-based computations for detection of conformational change in charged polymers

From the above we have noted that with the formation of MA_2 species in M^{2+}—$(HA)_p$ systems information with respect to the conformational stability of the polymer molecule as a function of experimental conditions is derived from the use in eqn. (19) of molar concentrations based upon the solution volume. With PAA, for example, we observed dimensional stability of the macromolecule when the ratio of Cu^{2+} to PAA was, respectively, relatively high and relatively low. The volume of the dimensionally stable molecule at the higher Cu^{2+} to PAA concentrations was approximately one-sixth the volume of the dimensionally stable molecule at the lower Cu to PAA concentrations.

This interpretation of the data is further supported by the following analysis. The dominant complex species in the PAA—$CuSO_4$—Na_2SO_4 systems is CuA_2 when the concentration of Cu^{2+} relative to PAA is small. The free ligand concentration a can as a consequence be accurately determined (eqn. (2b)) in these instances. With the value of a thus available the apparent pK (pK_{app}) of the PAA as a function of a during addition of $CuSO_4$ can be computed with the Henderson—Hasselbach equation for comparison with its value in the initially Cu-free but otherwise equivdent system neutralized with a standard base. A typical representation of such analysis of the potentiometric data is presented in Fig. 10 for the 0.0146 *M* PAA—0.05 *M* Na_2SO_4—*x* *M* $CuSO_4$ system initially at a fixed neutralization value of 0.9. We note that with the metal-*free* system the plot of pK_{app} versus *a* is linear. Since $pK_{app} - pK_a = (-\epsilon\Psi_s/kT)(0.4323) = -2Zw(0.4323)$ this linearity is a reliable indication of the conformational stability of the Cu^{2+}-free PAA during neutralization. The electrical work term *w* is constant. With the addition of $CuSO_4$, however, linearity of the pfot is soon disturbed to indicate conformational change in the molecule beyond this point as $CuSO_4$ is added to the system. In this region the deviation from ideality is due to the relatively larger electrostatic field at *the* surface of the macromolecule as *a* decreases in value. The relatively higher charge density is due to the more compact conformation of the PAA as Cu is increasingly bound to the PAA. We have by *this* analysis of the data corroborated the earlier deductions.

The eventual exponential increase in pK_{app} in the Cu-crowded region is an artifact of the inaccurate estimate of free ligand concentration. By assuming the exclusive presence of CuA_2 in this experimental region for the evaluation of pK_{app} the value of *a* is increasingly underestimated to produce this result. Correction for this oversimplification is attempted in the following paragraphs.

In these systems there is, with the addition of Cu^{2+}, transformation from a less compact to a more compact form of the Cu-bound polymer. It seems reasonable to consider that in the Cu-crowded region of these systems a "critical solution" phenomenon equivalent to thatsuggested by the results of our earlier analysis of the Cu^{2+}—PMA system may prevail *as well.* To test this possibility, pK_{app} values were employed in the Henderson-Hasselbach equation together with the experimental pH and HA concentration values obtained for the seven points that make up the first linear portion (the insert) of the curve in Fig. 4. With the value of a redetermined in this way the concentration of the species CuA^+ and CuA_2 is resolvable by simultaneous solution of the following equations.

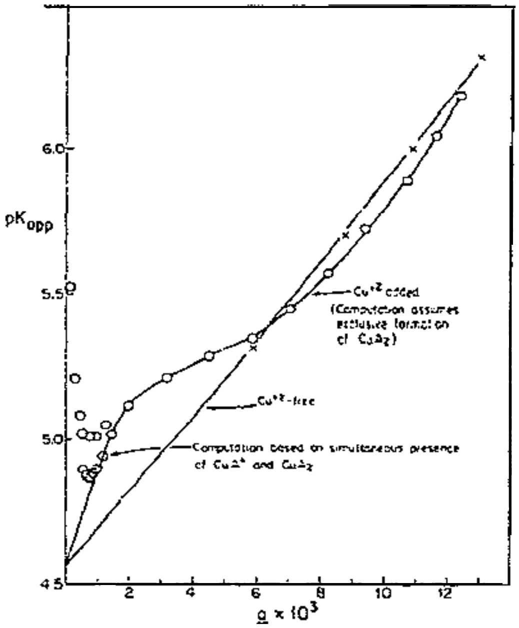
$$[Cu_b] = [Cu_T] - [CuA^+] + [CuA_2] \tag{3}$$

$$[CuA] + 2[CuA_2] = A_T - a - [HA] \tag{2}$$

With CuA, CuA_2 and a available by these operations the appropriate selection of pK_{app} for each experimental point could be tested by examining the constancy of β_2/β_1, calculable from each such reduction of the data thus

$$\frac{[CuA_2]}{[CuA]a} = \frac{\beta_2}{\beta_1}$$

A single pK_{app} value of 4.88 ± 0.01 (4.89, 4.87, 4.87, 4.875, 4.895) assigned to the first five points does indeed yield a constant value for β_2/β_1 (2.88, 2.&J, 2.84, 2.895, 2.74 X 10^4). The pK_{app} values that need to be assigned to the



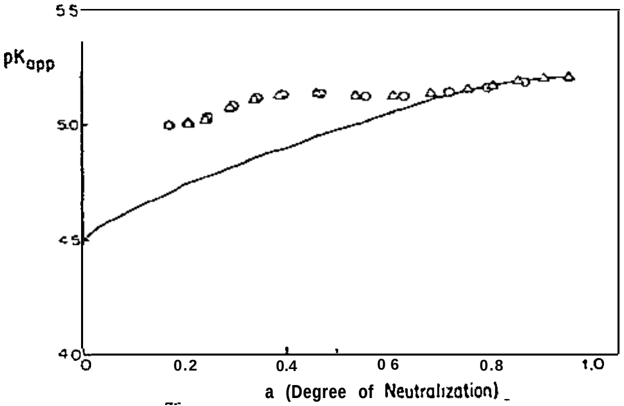


Fig. 10. Apparent p*K* of PAA in presence and absence of Cu^{2+}; system 0.0146 *M* PAA—0.05 *M* Na_2SO_4.

Fig. 11. Potentiometric study of poly-1-glutamic acid in 0.05 *M* Na_2SO_4.

sixth and seventh point to yield this constant ratio (2.88 and 2.70 X 10^4) are 4.935 and 5.017.

These seven points are replotted in Fig. 10. The straight line that can be drawn through the sixth and seventh point-s is a smooth extension of the rest of the curve defined by the other experimentally based points whose computation are not sensitive to neglect of minimal formation of CuA^+. It is interesting also to note that the line drawn in this manner intercepts the ordinate at $pK_{app} = 4.56$, the intrinsic p*K* of PAA at the experimental ionic strength.

These results do indeed corroborate formation of the dimensionally stable, compacted form of the Cu-bound FAA at the seventh point. Evidence for this is the straight line that can be drawn through the readjusted sixth and seventh points; the sought-for transition seems to occur in the dimensionally stable molecule at the fifth point.

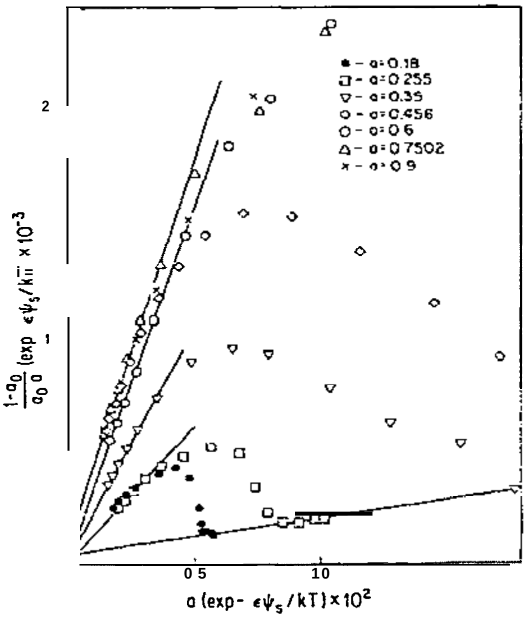
The value of β_1 is approximately 2.5 x 10 ($\beta_2 = 7.25 \times 10^5$); after correction for competitive eomplexation of Cu^{2+} by SO_4^{2-} as described later the β_1 value of approximately 3.25 x 10^2 that is resolved is in reasonable agreement with the β_1 value of 1.5 X 10^2 that is reported in the literature for the formation of $Cu(acetate)^+$ at zero ionic strength.

We have, with this approach, examined the conformational behavior of polyglutamic acid (PGA) in the presence of Cu^{2+} [32]. This molecule has been demonstrated to exist as a double-stranded helix [33] at low degrees of neutralization. From potentiometric and optical rotatory dispersion studies a transformation from helix to random coil has been shown to occur in PGA with its increased dissociation. The point of transformation from one form to another is a function of temperature and simple neutral electrolyte concentration.

In our experimental study a potentiometric titration curve of PGA in 0.05 *M* Na_2SO_4 was first obtained to fix the range of this transformation. These data are presented in Fig. 11. The same concentration of PGA in 0.05 *M* Na_2SO_4 fractionally dissociated by controlled addition of standard base to present helix, helix-random coil and random coil configurations were titrated with $CuSO_4$. After the addition of each increment of $CuSO_4$ the pH and pCu at equilibrium were measured. Plots of the data, $\{(1-\alpha_0)/\alpha_0 a\}\exp(-\epsilon\Psi_s/kT)$ versus a $\exp(-\epsilon\Psi_s/kT)$, are shown in Fig. 12.

Straight lines are obtained in the Cu-crowded region as was the case with PAA. Eventually the points deviate downwards from these straight lines in the presence of smaller amounts of $CuSO_4$. With all the PGA systems a Cu concentration is reached where the observed points become smaller than the preceeding ones obtained with more $CuSO_4$ to produce a maximum in the eventual curve. The maxima obtained with the more highly dissociated PGA samples occur outside the $a\exp(-\epsilon\Psi_s/kT)$ range examined in these plots. A second region of linearity in the lowest Cu^{2+} to PGA concentration ratio region, as was observed with PAA, is barely detectable. These data suggest that a dimensionally stable configuration of the molecule is formed only in the presence of relatively high concentrations of Cu^{2+}. When only a little Cu^{2+} is present, the expanded PGA molecule does not readily yield a second dimensionally stable configuration. Also, unlike the PAA, the volume of the dimensionally stable molecule which forms in the Cu-crowded region is not completely independent of the initial degree of neutralization of the polyacid. When $\alpha \geqslant 0.45$ the linear portion of the curves obtained do have the same slope to indicate constancy in volume of the product molecule. At $\alpha \leqslant 0.255$ the linear portions of the curves obtained are also characterized uniquely, but by a smaller slope to indicate constancy in the volume of a larger product molecule under these experimental conditions. At $\alpha = 0.35$, the slope of the straight line in the Cu-crowded region is intermediate in value between the slope characterizing the dimensionally stable product formed in the Cu-crowded regions at $\alpha \geqslant 0.45$ and $\alpha \leqslant 0.255$. These **results seem to indicate that, when only** the helix form of PGA exists initially, the dimensions of the CuA_2 product formed in the Cu-crowded system is defined by the double-stranded helical geometry which is preserved. As soon as a small amount of random coil is present initially, the tendency is to form an even more compact single-stranded molecule with Cu^{2+} ion binding. This is quite apparent from a more detailed examination of the Cu^{2+}—PGA binding data, corresponding to that performed for the Cu^{2+}—PAA system earlier.

The Cu^{2+} ion binding data obtained during titration with $CuSO_4$ of 7.5



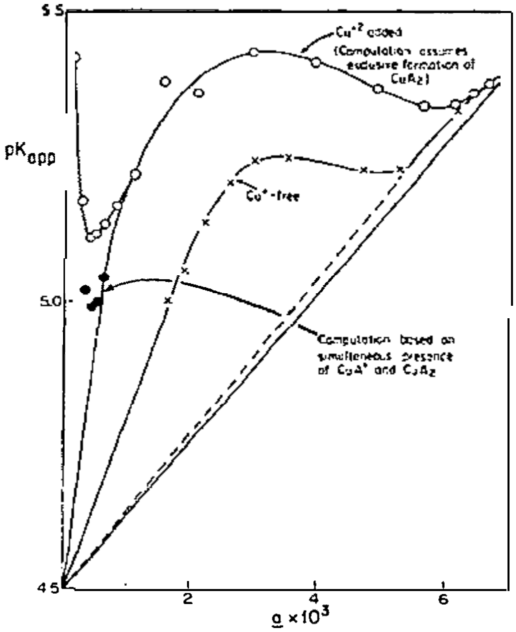


Fig. 12. The variation of $(1-\alpha_0)/\alpha_0 a \exp(-\epsilon\Psi_s/kT)$ versus $a \exp(-\epsilon\Psi_s/kT)$ with varying $CuSO_4$ concentration in PGA at fixed neutralization values; system 0.0075 M PGA-0.05 M Na_2SO_4.

Fig. 13. Apparent pK of PGA in the presence and absence of Cu^{2+}; system 0.0075 M PGA-0.05 M Na_2SO_4; α = 0.9.

$\times 10^{-3}$ M PGA, initially 18% and 90% dissociated, are analyzed as before to provide plots of pK_{app} versus a and are presented in Figs. 13 and 14. A parallel plot of the equivalent system, free of $CuSO_4$ and titrated with standard base, is presented once again in each figure for comparison. Both sets of points in each figure deviate upwards from the straight line drawn from the extreme experimental point to intercept the ordinate at pK_{int}. This line is drawn to correspond to the pK_{app} that should be obtained in the absence of conformational change in the PGA molecule. (In the figure representing the data obtained with the 18% dissociated PGA, where PGA already exists in the helical configuration, neutralization-based data are not accessible because of the spontaneous protonation of the PGA to this dissociation value.)

The shape of the neutralization curve and the curve corresponding to the potentiometric property of the Cu-bound PGA, initially 90% dissociated, is similar. Although transformation from random coil to helix seems to be indicated for the Cu-contained PGA, the absence of a linear segment in the plot presented in Fig. 12 in this apparent transitional region suggests otherwise. The higher degree of compactness transmitted to the molecule is responsible

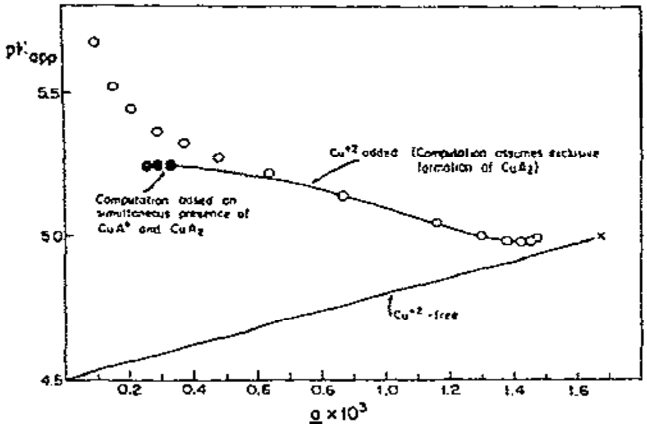


Fig. 14. Apparent pK of PGA in the presence and absence of Cu^{2+}; system: 0.0075 M PCA-0.06 M Na_2SO_4; α = 0.18.

for the maximum in the plot (Fig. 12) of $\{(1-\alpha_0)/\alpha_0\}$ a $\exp(-\epsilon\Psi_s/kT)$ versus a $\exp(-\epsilon\Psi_s/kT)$ not noticeable in the PAA system.

With the 18% dissociated sample the increased compactness of the helical molecule with addition of Cu^{2+} is dramatic. The somewhat higher pK_{app} value eventually reached by the dimensionally stable molecule in this instance, even though approximately three times larger in volume than the dimensionally stable single-stranded assemblage obtained from the initially randomly coiled PGA molecule, is believed to be a consequence of the higher charge density emanating from its double-stranded configuration.

The experimental points obtained in the Cu-crowded region where the respective dimensionally stable CuA_2 species are eventually formed have been analysed as before to yield corrected pK_{app} values which correspond to concentration values for CuA^+, CuA_2 and a leading to a constancy of β_2/β_1 in this region of the plots. These corrected pK_{app} values are included in Figs. 13 and 14.

With the 90% neutralized sample a pK_{app} value of 5.005 ± 0.015 (5.02, 4.99, 4.99) yields a constant&J&, ratio of 4.81 X 10^4 (4.81, 4.76 and 4.85 X 10^4) with the fist three of the four points that fall on the linear portion of the curve in Fig. 12. A pK_{app} value of 5.04 for the fourth point is needed to maintain the β_2/β_1 ratio constant. As with the PAA before, the natural extension of the line from this point is linear. Its slope is determined within narrow limits by the pK_{app} plateau formed by the other three points so that it intercepts the ordinate at 4.50, the intrinsic pK value for PGA at the experimental ionic strength, to duplicate the observations with the Cu^{2+}—PAA system.

In the 18% dissociated sample a pK_{app} value of 5.25 (5.25, 5.25, 5.25) yields a constant β_2/β_1 ratio of 1.61 X 10^4 (1.56, 1.65, 1.63 X 10^4) with the three points that are associated with the dimensionally stable molecule eventually formed in the Cu-crowded system. Once again there is evidence for the "critical solution" phenomenon with formation of the compact dimensionally stable complex compound.

The β_1 values computed from the ratios obtained in the two separate samples are 7.1 ($\beta_2 = 3.4 \times 10^5$) and 7.2 ($\beta_2 = 1.16 \times 10^5$). The agreement as to this number is excellent and provides strong support for the validity of this analysis of these complex systems.

E. THE DETERMINATION OF INTRINSIC FORMATION CONSTANTS OF COMPLEX SPECIES IN CHARGED POLYMERS

In the computation of stability constants, correction for the non-ideality of the mobile metal ion that is introduced to account for disturbance due to the variable electric field at the surface of the polyion is based upon a comparison of the effective H^+ ion concentration at the reaction site with its effective concentration in a region of the solution where Debye-like long-range interaction between the H^+ ion and co-ions of the added neutral electrolyte is dominant. A similar basis for computation must be applied to the free M^{2+} metal ions as well. Estimate of such non-ideality is made by reducing the concentration of the free metal ion that is experimentally measured to its effective value.

The β_{MA^+} values determined experimentally as described above have in practice been corrected by multiplying the experimental concentration of the free metal ion by the published mean molar activity coefficient of MX_2 at the effective ionic strength I of the system, where X is the co-ion of the neutral electrolyte employed in the experimental program. As the intrinsic stability constant of the acid, measured at the ionic strength of the experimental program, is the reference base for evaluating the non-ideality term $\exp(\epsilon\Psi_s/kT)$ at each experimental condition a correction for the effect of ionic strength on pK_a must be made. For this purpose the value of β_{MA^+} at I must be multiplied by the ratio of β_{HA} at $I = 0$, identified with the experimentally reported value of the intrinsic dissociation constant of the repeating monomer unit at infinite dilution (isobutyric acid in the case of PMA) and the experiment&y measured value of β_{HA} at the experimental I. With this treatment of data obtained by the author and his coworker with PMA and PAA using tracer-level concentrations of Ca, Co and Zn in 0.1 *M* and 0.4 M $NaClO_4$ the stability constants of CaA^+ CoA^+ and ZnA^+ at infinite dilution are less than 13, 13 and 37 for both PMA and PAA [9]. The formation constants at infinite dilution reported for these metal ions combined with acetate, MAc^+, are 13, 28 and 39 respectively [34].

Measurement of the stability constant of CoA^+ and NiA^+ during the study of the interaction of macroscopic quantities of $CoSO_4$ and $NiSO_4$ with a PMA gel in 0.05 *M* Na_2SO_4 also yield a formation constant of 13 at infinite dilution for the CoA^+ and NiA^+ species formed in this system. With this system, however, the resolution of the activity of Co^{2+} and Ni^{2+} at the measured concentration of these ions required prior consideration of the following competing reaction:

$$M^{2+} + SO_4^{2-} \rightleftharpoons MSO_4$$

in 0.05 M Na_2SO_4. The stability constant of $CoSO_4$ and $NiSO_4$ in H_2O at 25°C has been reported to be 2.95×10^2 and 2.5×10^2, respectively [35]. It can be assumed that the activity coefficient of the neutral ion pair is unity and that the mean activity coefficient of the dissociated electrolyte $M^{2+}\,SO_4{}^{2-}$ is calculable with sufficient accuracy by the Davies modification [4] of the extended form of the Debye—Hückel equation at the ionic strength defined by the Na_2SO_4. With this approach $y_{\pm M^{2+}SO_4{}^{2-}} = 0.675$ and the ratio of MSO_4 to M^{2+} is given by

$$\frac{M_{MSO_4}}{m} = \beta_{MSO_4}(M_{SO_4{}^{2-}})(y_{\pm M^{2+}SO_4{}^{2-}})^2$$
$$= \beta_{MSO_4}(0.05)(0.675)^2 = 0.023\beta_{MSO_4} \qquad (34)$$

From this expression

$$M_{MSO_4} = 0.023\beta_{MSO_4}m \qquad (35)$$

As the sum of MSO_4 and free, mobile M^{2+} ion *(ΣM) was* measured in the polarographic analysis of the equilibrated solution phase the concentration of free metal ion in these systems is

$$0.023\beta_{MSO_4}m + m = \Sigma M$$

and

$$m = \frac{CM}{1 + 0.023\beta_{MSO_4}} \qquad (36)$$

Its effective concentration is

$$a_m = \frac{\Sigma M}{1 + 0.023\beta_{MSO_4}}\,y_{\pm M^{2+}SO_4{}^{2-}} \qquad (37)$$

With this analysis

$$a_{Co^{2+}} = \frac{\Sigma M_{Co}}{1 + 0.023\beta_{MSO_4}}\,y_{\pm Co^{2+}SO_4{}^{2-}}$$
$$= \frac{\Sigma M_{Co}}{7.985}(0.675) = 0.084\Sigma M_{Co} \qquad (38)$$

and $\beta_{CoA^+} = 0.9/0.084 = 11$; at. infinite dilution $\beta_{CoA^+} = 13$.

To estimate the intrinsic stability constant of MA_2 species in these polyacid systems not only must the concentration of free metal ion at equilibrium be converted to activity terms as described above but the volume of the polymer domain must be known as well. The polymer volume was accessible by use of a FMA gel in the author's study of its complexation of Cu^{2+} in 0.05 *M* Na_2SO_4 for this purpose and

$$\beta_{2(v)} = \frac{\beta_{2(s)}}{y_\pm}\frac{v}{V} = \frac{7.85 \times 10^4 \times 0.09}{0.084 \times 100} = 5.3 \times 10^2$$

At infinite dilution $\beta_{2(v)} = 9.85 \times 10^2$. This value is in reasonable agreement with the $\beta_{2\,(v)}$ value that is derived from the experimental studies of Gustafson and Lurio [36]. In their program the exchange of 4.63 mmol of $Cu(NO_3)_2$ in 100 ml of 1 M $NaNO_3$ with 9.26 mmol of PMA gel (a concentration level 9.26 times larger than above) was studied as a function of the degree of neutralization of the polyacid with standard base. At 50% neutralization an equilibrium pH of 4.06 was measured by them; the molar concentration of HA, Cu^{2+} and CuA_2 was approximately 0.045, 0.023 and 0.023 M, respectively. With the use of eqns. (13) and (14), $\beta_{2\,(s)} = 1.64 \times 10^4$ and at infinite dilution $\beta_{2\,(v)} \approx 400$.

A value of 4.4×10^2 was measured by Froneaus [37] for the $CuAc_2$ complex in 1 M $NaClO_4$. If we correct for the deviation from ideality of the Cu ion at this ionic strength, β_2 is approximately 7.8×10^2 by a parallel analysis, in good agreement with the above. The formation constant reported for $CuAc_2$ at zero ionic strength [34] is 4.25×10^3, however, and suggests that the estimate of the excess free energy of the metal ion in these systems is subject to some uncertainty as is expected when single ion activity coefficients are sought.

The fairly good agreement observed between the intrinsic stability constants computed for the complex species MA^+ and MA_2 and those observed in simple acetate systems is believed to be of fundamental importance. Because of the chemical similarity of the ligand groups in the polymer to acetate ($pK_{\text{isobutyric acid}} \approx pK_{\text{acetic acid}}$) such agreement is expected if the approach employed to evaluate these stability constants is correct. A basic assumption in this approach assigns chemical properties of the monomer which most closely resemble the repeating unit in the polymer. The observed agreement is supportive evidence for the validity of (1) this assumption and (2) the correctness of the method of computation.

F. A GENERAL METHOD FOR DIAGNOSTIC STUDY OF CONFORMATIONAL CHANGES IN CHARGED POLYMERS

On the basis of the detailed analysis that has been made of metal-ion binding reactions with PAA, PMA and PGA a general method for the quantitative study of the conformational behavior of weakly acidic (and weakly basic) polymers appears possible. For example, a detailed study of the effect of various experimental parameters such as temperature, solvent composition, and the nature and concentration of neutral salt on the effective volume of the charged carboxylate-bearing polymers in the presence of different coordinating metal ions can be accurately monitored by using the Cu^{2+} ion as a probe. A Cu^{2+} ion selective electrode used in such systems for the quantitative estimate of the concentration of free Cu^{2+}, initially present at a sufficiently low concentration level to have little or no affect on the physical chemical properties of the macromolecule dominated by the experimental variables under investigation, will provide such information. Analysis of the formation of CuA_2, the dominant Cu species at these Cu^{2+} concentration levels, with eqn. (19) provides a quantitative estimate of the affect of the experimental variables studied on the value of $\beta_{CuA_2(s)}$ which can, as has been shown, be related, using eqn.(33), to the relative volume v of the molecule during the course of such an investigation.

The use of the Zn^{2+} ion as an additional probe to evaluate as described earlier the exponential term and a concurrently with the estimate of the relative volume change of the macromolecule provides the means for testing on a theoretical basis the most appropriate models that describe the changing geometry of the charged polymer. In this way the variable conformation of the polyelectrolyte as a function of (1) its interaction with various metals ions, (2) the system temperature, (3) the nature of the solvent and (4) the concentration and neutral salt composition can be deduced.

G. ANOTHER EXAMPLE OF THE DIAGNOSTIC QUALITY OF THE ELECTROSTATIC MODEL FOR TILE INTERPRETATION OF POLYELECTROLYTE PHENOMENA

In a study undertaken by Conio and coworkers [38] their objective was to show that the KSCN-induced coil to helix transformation [39—41] observed at pH = 3 for the fully ionized (α = 0) (L-lysine) $(CONHCH(CH_2)_4NH_2)_n$ (Lys,) could be quantitatively described in terms of (1) the reduction of the fixed electric charge on the polymer by the binding of the SCN^- ion to the molecule and (2) the screening effects of the ionic atmosphere due to mobile K^+ and SCN^- ions.

The analysis of the conformational transition induced by such ion binding required experimental evaluation of the separate binding effects on the helical and random-coil conformers. Because conformational transition of the Lys_n—KSCN system occurs at very low salt concentration, the study of these effects was made with the closely related compound, poly (L-ornithine) $(CONHCH(CH_2)_3NH_2)_n$ (Orn_n), which retains a random-coil conformation up to 1 M KSCN at pH 3 because of the low intrinsic stability of its helix form [42]. Parameters determined in this way included the intrinsic equilibrium constants for the dissociation of hydrogen ion and slopes of the pK_{app} versus Z plots (w) measured potentiometrically. The very similar polyelectrolyte properties of these two polymers, as evidenced by the essentially equivaient values of their intrinsic ionization constants [40,42] and of their electrostatic free energies at every value of α, justified transfer of these data to the Lys, molecule.

On the basis of data previously obtained [39—45] it was assumed that (1) SCN^- binds to the side-chain amino groups of both Lys, and Orn_n and (2) no such binding of the Cl^- ion occurs with these polymers. It had been demonstrated also that no coil to helix transition occurs in the Orn_n—KSCN and the Orn_n—KCl system at pH 3 up to a salt concentration of 1 M

For appropriate correlation of the data the e-amino groups of Om, (and Lys_n) were considered to be involved both in the dissociation equilibrium of H^+ ions and in the association equilibrium of SCN^- according to the reactions

$$R{-}NH_3^+ \rightleftharpoons R{-}NH_2 + H^+ \tag{39}$$

$$R{-}NH_3^+ + SCN^- \rightleftharpoons R{-}NH_3^+SCN^- \tag{40}$$

The corresponding equilibrium constants were expressed as

$$K = [\text{—NH}_2][\text{H}^+]/[\text{—NH}_3^+] = n\alpha\ h/n(1-\alpha)(1-\gamma) \\ = \alpha h/(1-\alpha)(1-\gamma) \tag{41}$$

and

$$K_b = [\text{—NH}_3^+\text{SCN}^-]/[\text{—NH}_3^+][\text{SCN}^-] = n(1-\alpha)\gamma/n(1-\alpha)\ (1-\gamma)a_s \\ = \gamma/(1-\gamma)\ y \pm C_s \tag{42}$$

where n is the total concentration of e-amino groups (mole per liter), α is the degree of dissociation and γ is the degree of association (the concentration of $\text{NH}_3^+\text{SCN}^-$ divided by $n(1-\alpha)$, the concentration of sites available for SCN^- binding); a_s, C_s and $y_\pm$ are the activity, the molar concentration and the mean activity coefficient of salt, respectively.

By proceeding as described earlier

$$K_{app} = \alpha h/(1-\alpha)(1-\gamma) = K_{int}\exp(\epsilon\Psi_s/kT) \\ = K_{int}\exp(2wZ) = K_{int}\exp\{(2w)(1-\alpha)(1-\gamma)\} \tag{43}$$

and

$$K_{app}{}^b \doteq \gamma/(1-\gamma)C_s y_\pm = K_{int}{}^b\exp(\epsilon\Psi_s/kT) \\ = K_{int}{}^b\exp(2wZ) = K_{int}{}^b\exp\{(2w)(1-\alpha)(1-\gamma)\} \tag{44}$$

In the deployment of these equations the value of w was determined from the slope of experimental pK_{app} versus α plots that were obtained for Orn, and Lys, at several concentrations of salt (KCl, NaCl and KF) which do not bind. Slopes obtained in the random-coil conformation and the helical conformation were plotted separately to obtain the value of w as a function of C_s in the two conformational states. It was stipulated that the occurrence of ion binding only altered the values of Z, K_{int} and w (which depend upon salt concentration) having been shown to be unaffected by salt type at a given concentration.

In the case where binding is negligible (i.e. in the presence of KCl)

$$K'_{app} = \alpha h/(1-\alpha) = K_{int}\exp\{(2w)(1\ \text{-a})) \tag{45}$$

The difference in pK_{app} in the presence of KSCN and KC1 at a given α is then

$$\text{p}K_{app} - \text{p}K_{app}{}' = \text{pk} - \text{pk'} + \log(1-\gamma) \\ = 0.868w(1-\alpha) - 0.868w(1\ \text{-a}) - 0.868w(1-\alpha)\ (1-\gamma) \tag{46}$$

With potentiometric titration data obtained for Orn, at a given salt concentration with both KC1 and KSCN the pH—pH′ difference at a fixed α value was used to determine γ to permit computation of $K_{int}{}^b$ and $K_{app}{}^b$. At the given α it was required that Om, maintained the same conformation in KC1 and KSCN.

With this evaluation of $K_{int}{}^b$ through data obtained at finite a values the value of $K_{app}{}^b$ and γ at a = 0 could be determined as a function of C_s by the following equation:

$$K_{\mathrm{app}}^{\mathrm{b}} = \gamma/(1-\gamma)C_{\mathrm{s}}y_{\pm} = K_{\mathrm{int}}^{\mathrm{b}}\exp\{(2w)(1-\gamma)\} \tag{47}$$

With this equation $K_{\mathrm{app}}^{\mathrm{b}}$ values were computed as a function of C_{s} for both the random-coil and the helical conformation using the w values obtained experimentally for the two conformations. The value of $K_{\mathrm{int}}^{\mathrm{b}}$ determined for Orn_n in KSCN was also used for Lys, (random-coil and helical forms).

In order to predict with these results the KSCN-induced random-coil to helix transformation observed experimentally for Lys, at pH 3 the free energy change AG accompanying the transformation was computed as a function of C_{s} from the evaluation of W_{el} (at $\alpha = 0$) for both random-coil (RC) and helical (H) Lys, with eqn. (48).

$$W_{\mathrm{el}} = wRT(1-\gamma)^2 \tag{48}$$

and by assigning a value due to Hermans [46] of -80 cal mol^{-1} to the free-energy change ΔG_0 for the transition between the uncharged conformers in eqn. (49)

$$\mathrm{AG} = W_{\mathrm{el}}^{\mathrm{H}} - W_{\mathrm{el}}^{\mathrm{RC}} + \mathrm{AG}, \tag{49}$$

The effect of the change in AG with KSCN concentration on the corresponding variation of the helical fraction was considered to be described by a theoretically based equation developed by Zimm and Rice [47].

There was satisfactory agreement between the experimental data (direct determination of helical content by ORD) and the theoretical predictions to support strongly, once again, the validity of the model that has been developed during this article, In addition there was good agreement between the calorimetric data that were obtained to test the internal consistency of the binding model and the model-based predictions.

H. ION BINDING IN MULTI-SITED POLYMERS

Useful and valid approaches for the reliable estimate of thermodynamic quantities associated with the interaction of metal ions with charged polymers have been carefully documented in this presentation. Examples, however, have been limited to polymeric systems in which only one kind of interacting site is repeated in the molecule, the extent of repetition depending on the degree of polymerization. With biopolymers, to which successful application of these methods is sought, a multiplicity of potentially reactive sites are repeated throughout their structure and make employment of these methods extremely difficult. It becomes necessary to identify these potentially reactive sites and even with correct identification of these sites resolution of their competitive interaction with various metal ions is a complicated operation. An excellent example of the enormity of this problem is provided by a brief review of the research that has been performed over the last two decades in the study of the binding of a single metal ion Zn^{2+} by plasma albumin [48—54].

In this molecule (MW -65,000) there are available a number of ionizable groups for interaction with Zn^{2+}. In bovine serum albumin a hydrogen ion titration study and amino acid analysis indicates, within an uncertainty of about one group in each case, the presence of 1 α-carboxyl, 99 β,γ carboxyl, 17 imidazole, 1 e-amino, 57 e-amino, 18 phenolic, 22 guanidine and 0.7 sulphhydryl (free) groups [58].'

In spite of the availability of these various sites for interaction many researchers have concluded that Zn^{2+} is bound primarily to the imidazole residues of histidine in this molecule [48,49,53,56]. In the more quantitative examinations which have led to this conclusion attempts were made to evaluate the intrinsic constant for the formation of the proposed complex species.

Tanford [56], for example, corrected for the deviation $\exp(-2Z_pZ_mw)$ from ideality of the Zn^{2+} ion at the site of reaction in $0.15\,M$ KC1 by estimating the net charge Z_p on the protein molecule. For this computation account was taken of the bound and dissociated protons and the bound metal; estimates were made of the bound chloride ion using data from Scatchard and co-workers [59]. In the assessment of Z_p and Z_m it was assumed that the predominant species entering into the complexation reaction was $ZnCl^+$. The value of w was based on analysis of potentiometric data obtained with the metal-free protein [SO]. This value of w was presumed to be applicable to the metal-contained system as well

With such treatment of the binding data obtained polarographically a $\text{p}K_{\text{int}}$ value of about 2.8 was computed by assuming exclusive interaction of $ZnCl^+$ with the imidazole sites of the protein. This value was in excellent agreement with the first association constant that had been reported for the interaction of Zn^{2+} with imidazole [61] to influence assignment of this group as the interaction site in the protein.

Gurd and Goodman [48] used a dialysis equilibrium technique to study the binding of Zn^{2+} by human serum albumin in $0.15\,M$ $NaNO_3$ and varying amounts of sodium hydroxide and zinc chloride. The counter ions Zn^{2+} and H^+ were considered to compete for the imidazole groups on the basis of Tanford's earlier study and from experimental observation that the concentration value of deprotonated imidazole groups as deduced from the mass-action relation for k_{HIm^+} corresponded to the experimentally observed concentration of bound Zn^{2+} in a set of experiments designed to examine the possibility of such parallel behavior.

In computing the intrinsic stability constant of $ZnIm^{2+}$ by consideration of the competitive interaction of H^+ and Zn^{2+} for the imidazole group the exponential electrical deviation term $\exp\{2(Z_H - Z_m)Z_pw\}$ was employed. To estimate the protein charge at equilibrium the number of bound nitrate ions was deduced from the data of Scatchard and Black [62]. The value of w at the experimental ionic strength was computed using an equation due to Scatchard. The $\text{p}K_{\text{int}}$ value of 2.82 ± 0.1 that was resolved was in agreement with Tanford's.

A later examination by Rao and Lal [51] of Zn^{2+} interaction with bovine albumin serum in acetate buffer of pH 6.5 and at an ionic strength of 0.2 led to results in conflict with those of Tanford and of Gurd and Goodman. Their analysis of Zn-binding data obtained via equilibrium dialysis, polarography (diffusion current measurement), electrophoresis and spectroscopy was affected by assuming the competive reaction of H^+ and $ZnAc^+$ for imidazole sites. This equivalence in charge of the competing ions eliminated the electrostatic correction term. A pK_{int} value of 3.62 was obtained at low degrees of Zn binding. The pK_{int} value fell with increased Zn binding until a value of 2.8 was reached after interaction with approximately 50% of the imidazole sites.

Rao and Lal were compelled to conclude from these results that Zn was initially bound more strongly than could be inferred from combination with the imidazole sites of the protein molecule. They suggested that binding to compound sites involving an imidazole and a neighboring peptide oxygen seemed most likely for the first two Zn atoms bound. With saturation of these more reactive compound sites competitive binding of the remaining Zn by imidazole and carboxyl sites on a one to one basis was postulated. Configurational changes in the protein molecule were also presumed to occur.

The scope of the experimental programs employed in these earlier studies of the binding of Zn^{2+} by serum albumin was much too limited. Once the imidazole group had been proposed as the primary site of the interaction by Tanford on the basis of a rather cursory investigation much of the later effort was confined to the examination of this particular interpretation of Zn^{2+} interaction with serum albumin. As a consequence the possibility of alternative site interaction was not sufficiently considered.

In addition the use in some of these studies of acetate and chloride in place of nitrate or percblorate salts to fix the ionic strength of the Zn^{2+}—albumin systems studied introduced uncertainty in the computation of intrinsic stability constants. The computed pK_{int} value was the basis for judgement of the validity of the interaction model being tested. Uncertainty introduced into its evaluation must be transferred to the model.

Because of the complexation of Zn^{2+} by acetate and chloride in these systems a decision as to whether the hydrated ion or the ion pair ($ZnCl^+$ and $ZnAc^+$), more predominant in these mixtures, interacted with this particular site needed to be made. It was presumed in both instances that the singly charged species was involved at the binding site. This unproved assignment defined the electrostatic correction term and the concentration of the unbound Zn species to be used in the mass-action expression of pK_{int} to influence its value significantly. The disagreement between Tanford and Rao and Lal is understandable on the basis of the uncertainty introduced in the computation of pK_{int} by their choice of neutral electrolyte.

Experimental evidence has since been compiled to suggest that the imidazole sites of serum albumin do not play an important role in the binding of Zn^{2+}. The most informative experimental program of Perkins [55] led to this conclusion and supports the earlier estimate by Rao and Lal of this possibility. In experiments with protein modified to have different histidine contents the binding to Zn^{2+} was not noticeably affected. Results obtained in equilibrium dialysis studies to compare the binding of Zn^{2+} in pepsin (six histidine residues) and human albumin (sixteen histidine residues) could not be correlated with the histidine content of these molecules; the same relative change in binding could be correlated, however, with the difference in free carboxyl group content. When the carboxyl group of the protein was esterified without significantly altering the histidine content, the Zn^{2+} ion binding was markedly decreased. The binding values observed led to Perkin's suggestion that the thiol group was the binding site involved in the modified protein.

With the results of this thoughtful study by Perkins it became apparent that a number of protein sites are competitively involved in the binding of Zn^{2+} ion by serum albumin and that experiments to analyze quantitatively such simultaneous involvement of sites must employ the classical methods developed for investigation of the sequential appearance of complex species in metal—ligand systems. Österberg [57] was the first experimenter to apply such an approach in his study of Zn^{2+} binding by serum albumin.

In this investigation the equilibrium between bovine plasma albumin and Zn^{2+} was investigated by potentiostatic e.m.f. titrations using glass and zinc amalgam electrodes. The ionic medium was 0.5 M $NaClO_4$ and the pH was maintained constant at 6.78 while varying the concentration of free Zn generated electrically. The amount of electricity used to maintain the pH and to vary the Zn^{2+} ion content during constant current electrolysis was measured with a coulometer.

Two sets of primary data were obtained. In one set the total Zn^{2+} concentration M and the protein concentration A were varied while maintaining the hydrogen concentration h and the Zn^{2+} concentration m constant. In the second, M and m were varied keeping h and A constant. In analyzing the data it was assumed that the equilibria in the Zn^{2+}—albumin system could be described by the general reaction

$$pZn^{2+} + qH^+ + rA^{y-} \rightleftharpoons Zn_pH_qA_n{}^{(2p+q-ry)^+} \tag{50}$$

and the equilibrium constant β_{pqr}. For a constant value of h we obtain from the mass balances

$$\begin{aligned} A &= a + \sum_q \sum_r rh^q a^r \beta_{0qr} + \sum_p \sum_q \sum_r rm^p h^q a^r \beta_{pqr} \\ &= \sum_r a' F_{0r} + \sum_p \sum_r rm^p a^r F_{pr} \end{aligned} \tag{51}$$

$$\begin{aligned} M &= m + \sum_p \sum_q \sum_r pm^p h^q a^r \beta_{pqr} \\ &= m + \sum_p \sum_r pm^p a^r F_{pr} \end{aligned} \tag{52}$$

where

$$F_{pr} = \sum_{q} h^{q}\beta_{pqr} \tag{53}$$

For constant values of both h and m

$$A = \sum_{r} ra^{r}G_{r} \tag{51a}$$

$$M = m + \sum_{r} a^{r}S_{r} \tag{52a}$$

where

$$G_{r} = \sum_{p \geqslant 0} m^{p}F_{pr};\ S_{r} = \sum_{p \geqslant 1} pm^{p}F_{pr} \tag{54,55}$$

When r is equal to unity

$$A = aG_{1};\ M = m + aS_{1} = m + A\ \text{const.} \tag{51b,52b}$$

With this approach to the analysis of p and r the introduction of any assumptions regarding the compositions of the complexes that form, except that they conform to the general formula $\mathrm{Zn}_p\mathrm{H}_q\mathrm{A}_r^{(2p+q-ry)+}$, is avoided.

Österberg found with this analysis that monomeric albumin complexes appeared to predominate at low Zn^{2+} ion concentrations and that the albumin molecules seem to condense into polymers through Zn ions at higher concentrations of Zn. The data carefully examined using a graphical approach and refined with the second version of the Legatrop program [63] yielded correlations found to be most consistent with the formation of the monomers ZnL and $\mathrm{Zn_2L}$ and the series of polymers $\mathrm{ZnL(Zn_5L)}_n$ where n = 1, 3 and 5. In this notation the general formula $\mathrm{Zn}_p\mathrm{H}_q\mathrm{A}_r$ has been reduced to $\mathrm{Zn}_p\mathrm{L}_r$, with L including all different protonated forms that may enter the complex.

Attempts were then made to identify the individual protein sites involved in the formation of ZnL, $\mathrm{Zn_2L}$ and in the bridging through Zn ions of protein molecules to form the series of polymers. By presuming that the thiol group is involved in the complexation of Zn the pK value that is deduced from such an interpretation of the data is 9.6. This value is not very different from the pK of 9.9 that has been reported for Zn^{2+}—cystein [64]. It was found by considering the second zinc association step to involve a bidentate complex via the a-amino and β-carboxylate groups of the albumin aspartyl residue that a pK value of 5.8 is resolved for the formation of this species. This number is in reasonable agreement with the pK value of 5.5 reported for Zn^{2+} chelates of amino acids [65]. Finally, by assuming the bridging of two Zn ions by coordination through imidazole groups from each molecule in the polymers formed and by further assuming that the bridging of Zn to the imidazole is independent of polymer formation the pK of $ZnIm^{2+}$ is calculated to be 3.3 to 3.4 in the dimer, tetramer and hexamer formed. This value approaches the pK of 2.8 reported for the corresponding low molecular weight complex.

There is no question that Österberg's approach is potentially the most useful that can be employed for the elucidation of the complex spectrum of metal—site interactions that occur in these complicated biopolymer systems. However, there is a serious oversimplification introduced by Osterberg in the analysis of his data which undoubtedly has distorted his perception of the Zn^{2+}—albumin system. Even though the pH is kept constant in his examination of the system, the charge on the surface of the protein molecule does vary with the binding of Zn^{2+} to make the effective concentration h_s of H^+ at the site of reaction vary as well. In the region of study where the degree of binding is low the net change in the charge on the surface of the protein is sufficiently small so that the equations derived by Österberg are essentially valid. The resolution of the two dominant species ZnL and ZnL_2 and the evaluation of the constants $F_{11}F_{01}^{-1}$ and $F_{21}F_{01}^{-1}$ with these equations most probably provide a reasonably accurate assessment of the complexation behavior of the bovine albumin serum in this limited region of study, However, with the binding of additional Zn^{2+} the net charge on the protein is sufficiently affected to lower the effective concentration of H^+ and Zn^{2+} at the protein surface relative to the constant concentration level maintained for these ions in the solution. The observed concavity of the curves obtained in the region of greater Zn^{2+} binding by plotting A4 versus A could be a consequence of this phenomenon and need not be attributed to the appearance of polynuclear complexes. To demonstrate unambiguously the presence or absence of polynuclear species in this system it is the constancy of H^+ and Zn^{2+} at the site of reaction that must be controlled in the use of eqns. (56) and (57) to evaluate p and r for the species Zn_pL_r

$$p = (\mathrm{A} \log(M - m)/\Delta \log m\}_{h_s,a}\,{*} \tag{56}$$

$$r = \{\Delta \log(M - m)/\Delta \log a\}_{h_s,m_s} \tag{57}$$

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* For the preparation of normalized graphs the symbol a in eqns. (56) and (57) is related to A by

$$\begin{aligned}\log (a/A) = \log (aF'_{01}/A) &= \log(a \sum_{q \geq 0} h_s{}^q \beta_{0q}/A)\\ &= \log(a/a_0)\end{aligned} \tag{58}$$

and the quantity

$$\log(a/a_0) = \int_{-\infty}^{\log m} (\partial M/\delta A)_{m_s,h_s}\, \mathrm{d} \log m \tag{59}$$

at A, h_s constant

To obtain the value of h_s and m_s for such examination of the serum albumin the exponential electrostatic deviation term needs to be estimated by the methods already described in the article. With accurate h_s and m_s values computed for every experimental point graphical representation of A $\log(M - m)$ versus log m and log a at fixed values of h_s and m_s can be affected to achieve a correct analysis of the system.

With the identity of the ionizable groups and their number known for serum albumin and with the reasonably knowledgeable estimate of the identity of the dominant complexed metal species deduced from the analysis by Österberg of data obtained at the lowest degrees of binding the following estimate of the competitive interaction at each experimental condition studied by Österberg has been made. To affect this analysis the possibility of a Zn^{2+} interaction with the various groups at the pH employed by Österberg was first assesssed by the consideration of both the pK_{int} values characterizing each ionizabfe group and the formation constants reported in the literature for Zn^{2+} with each group. At pH 6.782 the carboxyl groups are almost completely dissociated and complexation of Zn^{2+} ($pK_{int} \approx 1.5$) is Iikely; the ImH^+ is also partially dissociated and competitive complexation of the Zn^{2+} by the imidazole residue ($pK_{int} \approx 2.8$) is also likely; the only other sites which can be considered to be potentially competitive for interaction with Zn^{2+} at this pH ate the α-amino group and the sulfhydryl group because of the high pK value reported for the $Zn{-}\alpha{-}NH_3$ complex ($pK \approx 5.5$) and the Zn-SH complex ($pK \approx 9.9$). The remaining ionizable groups, essentially undissociated at the experimental pH, interact too weakly with Zn^{2+}. By using the pK_{int} values reported for these ionizable groups and their respective Zn^{2+} complex and by applying the appropriate non-ideahty correction term through knowledge of the degree of deprotonation and metal binding at each equilibrium concentration of Zn^{2+} through the coulemetric addition of a base or acid and Zn^{2+}, predictions of the quantity of Zn bound by complexation with these four sites were made. The computation procedure is outlined below:

$$\frac{[ZnA]}{a} = \beta_{ZnA}[Zn^{2+}]\exp(-2wZ_pZ_{Zn}) = \beta_{ZnA}[Zn^{2+}]\exp(-4wZ_p) \qquad (60)$$

$$\frac{[HA]}{a} = \beta_{HA}h\exp(-2wZ_pZ_H) = \beta_{HA}h\exp(-2wZ_p) \qquad (61)$$

$$A_T = [ZnA] + [HA] + a = \{\beta_{ZnA}[Zn^{2+}]\exp(-4wZ_p) + \beta_{HA}h\exp(-2wZ_p) + 1\}a$$

162)

With the literature-based stability constants, appropriate estimates of the exponential electrostatic deviation term and the experimental concentration of Zn^{2+} and H^+, the concentrations of ZnA and HA in terms of a were computed. The experimental value of A,, defined below by

$$A_T = M_pn_A \qquad (63)$$

where M_p represents the concentration of the protein H_qA_r and n_A the number of times the ionizable group A is repeated in the protein molecule, was used in the mass balance equation (eqn. (62)) to evaluate α. With a available, the concentration of ZnA could be evaluated.

With this computation the predicted total binding of Zn^{2+} by bovine albumin serum is in as good agreement with experiment as the computer-based predictions of Österberg. From these computations it is also determined that Zn^{2+} is bound at low concentrations predominantly to the sulfhydryl site. As the Zn^{2+} concentration is increased, the binding of Zn^{2+} to the α-NH_3 group becomes more and more important. Eventually with greater *amounts* of Zn^{2+} present, the binding by carboxyl and imidazole groups becomes significant increasing at roughly the same rate, the sulfhydryl and aspartyl residue sites having been saturated.

The method of Österberg, *refined to correct for the deviation from ideafity of the counter ions at the reaction site*, provides a most effective approach to a definitive study of metal-ion interaction with the multiple ionizable sites of biopolymers. The complication due to the interaction of metal-ion on a greater than 1 : 1 basis that arises from the use of molar concentrations based on the solution volume is also avoided by treating the ensemble of ionizable sites as a single unit. However, should analysis by this approach indicate that the metal-ion is coordinated to more than one functional unit of a particular ionizable group that is multiply distributed throughout the structure of the molecule, a supplementary analysis of this particular equilibrium can be made to examine the conformational property of the molecule as well. For this purpose the concentration of the polymer would be multiplied by the number of times this reactive sites is repeated in the polymer (eqn, (63)).

I. SUMMARY

Problems unique to the study of equilibria in metal-charged polymer systems have been detailed. If has been demonstrated that inadequate accomodation and/or neglect of these problems in the literature fead to the compilation of seriously incorrect interpretations of ion binding data in such systems. Methods for the correction of perturbations arising in these systems have been fully detailed and a number of examples to demonstrate the validity of their application have been presented. Specifically, the quantitative evaluation of the formation of complex species in these systems is made possible by (1) correcting for the large and variable field at the surface of the polyion, (2) expressing the concentration of polymer species in terms of the polymer domain volume and (3) correcting for the effect on the metal ion of interionic attraction in the simple salt media employed. It has been shown that with the insight gained from (2) it is possible, with the formation of multi-coordinated species, to monitor conformational changes in the molecule as a function of ion binding. Finally it has been demonstrated that these methods are as applicable to the quantitative thermodynamic examination of the sequence of

equilibria encountered in the multi-functional biopolymers, such as proteins, as they are to such examination of the simpler polyelectrolytes.

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