A Two-Phase Model for Determining the Stability Constants for Interactions between Copper and Alginic Acid

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The two-phase model used previously to calculate the polymer-subphase volume of alginic acid was applied to interpret the dependence of apparent metal binding equilibrium on environmental conditions. In this model, the polymer subphase, a small aqueous region surrounding the polymer chain, was considered as a separate phase in the aqueous solution and as the protonation–deprotonation and metal binding reaction zone. Three factors were taken into account when treating experimental data: (1) the electric field due to the charged ligands on the polymer molecule, (2) the effective concentration of ligands based on the polymer-subphase volume, and (3) the competition from hydrogen ions for the metal binding sites. The data of base titration of alginic acid in the presence of trace amounts of copper at different alginic acid concentrations and ionic strengths yielded unique intrinsic stability constants for complexes formed between a cupric ion and one or two binding ligands.

Introduction

The strong affinity of group IIA metal ions such as Ca^{2+} and transition-metal ions such as Cu^{2+} for alginic acid (a weakly acidic biopolymer, consisting of guluronic acid and manuronic acid subunits, from kelp and some bacterial strains) has been well-documented.[1,2] The gel-forming properties of alginic acid in the presence of a threshold concentration of the bivalent metal ion have been attributed to the unique "egg box" structure in the sequence of polymer chain containing blocks of polyguluronic acid. The "cavity" that traps the divalent cations contains electron-donating hydroxyl and carboxyl groups from uronate residues of adjacent polymer chains. The divalent cations that fit into the cavity are held in place by interaction with electron-donating oxygen atoms of hydroxyl and carboxyl groups.

When the concentrations of both alginic acid and bivalent cations are well below the levels needed to form gels, alginic acid molecules behave more like individual colloids and can be treated as polyelectrolytes in an aqueous solution. To describe quantitatively the relationship among the polymer concentration, the amount of metal bound, and the amount of metal in solution under different environmental conditions, the use of several empirical approaches is required. In a review paper,[3] it has been pointed out that some workers used a correction factor, f, to interpret the difference between the measured (bulk phase) pH and the pH at the site of protonation–deprotonation reaction for a weakly acidic polyacid. This correction factor was also used to modify the relationship among Z (average number of ligands attached to a metal ion), formation stability constants of complexes formed between a metal ion and one or more binding ligands on the polymer chain, and concentration of unbound ligands. The correction factor, however, deviates increasingly from the ideal value of unity with increasing dissociation of the acid and/or with changing ionic strength of the solution. In another attempt to interpret the deviation from ideality in weakly acidic polyacid systems $(HA)_p$, a corrective parameter, n, was introduced to the Henderson–Hasselbach equation: $pH^{measured} = pK_{HA}^{intrinsic} - n \log [(1 - \alpha)/\alpha]$. Like the previous factor, f, this corrective parameter n remains constant only at a selected polymer concentration and ionic strength as the degree of dissociation α of the polyacid is varied.

The deviation from "ideality" (i.e., physicochemical properties of a solution containing free monomeric species with a chemical structure identical with the repeating subunits of the polyacid molecule) described above reflects the polyelectrolyte properties of a polyacid solution. According to Marinsky,[3] there are two complicating factors that must be resolved to accurately describe the equilibria in polyacid systems: (a) A high and variable electric field exists at the surface of a charged polyacid. This electric field which governs the partition of counterions between the surface of the polyacid and the bulk solution is a function of ionization, degree of ion binding, ionic strength, and possibly the conformation of the polyacid. (b) It is very difficult to evaluate the effective concentrations of the bound and unbound ligands along each polymer strand. This is because the ligands of a polyacid exist as ensembles and do not distribute uniformly throughout the solution, even though the solution may appear to be homogeneous and clear. It is more appropriate to express the concentration of ligands as moles of acid groups per unit volume of the region in which the ligands are restricted, i.e., the polymer "domain",[4] or more rigorously the polymer subphase defined in our previous work.[5] The problem of finding the volume of polymer subphase within the colloid formed by the polymer chain has only recently been resolved by using an iterative procedure[5] [Marinsky, J. A., private communication] based on Donnan equilibrium, osmotic properties of an polyelectrolyte solution,[6] and base titration data.

In the two-phase model used in our previous work,[5] the colloid (of the polymer) is assumed to be separated from the bulk solution by a semipermeable membrane, which is permeable to simple electrolytes (and counterions) but not to the polymer chain. This hypothetical membrane delineates two domains: a bulk liquid and a gel phase (i.e., the polymer colloid) which contains the polymer chain and any liquid trapped therein. A small aqueous region surrounding the charged polymer chain in which a strong electrostatic attractive force exists should be included with the polymer chain as a separate polymer subphase in the gel phase. Donnan equilibrium theory can then be applied to predict the counterion activity in this polymer subphase from the activity measured in the bulk liquid phase.

Unlike the conventional approach in which the solution pH and ionic strength are fixed in metal absorption experiments and the conditional stability constant(s) are obtained by Scatchard's approach,[7-9] we attempt to determine stability constants for inter-

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actions between alginic acid and cupric ions using potentiometric titration data collected at different pH's, polymer concentrations, and ionic strengths. It is known that, at lower pH, hydrogen ions compete strongly with metal ions for the carboxyl groups on the biopolymer. As standard base is added to a polyacid solution that contains a trace amount of the metal ion, not only do more ionized sites become available for metal binding but a stronger electric field around the polymer chain also develops. Thus, the binding "capacity", which is usually treated as a constant value in pH-stat absorption experiments, actually becomes a variable in an environment of changing pH.

It is the purpose of this article to attempt to overcome the aforementioned difficulties by using the two-phase approach which treats the polymer subphase as a separate phase in the solution. By using the five methematical models described below, we will show that it is not sufficient to just correct for electrostatic attraction of counterions to the charged ligands in solution when treating the potentiometric titration data of alginic acid in the presence of trace amounts of copper. When both the activities of counterions and the concentration of ligands are based on the conditions in the polymer subphase, the potentiometric data obtained at different polymer concentrations and ionic strengths permit determination of improved copper binding stability constants. We will also discuss the factors that may cause the deviation from a simple, ideal two-phase model as the polymer approaches the state of complete ionization.

Theory

Conditional Stability Constant (Model 1). Since the effective binding "capacity" of a polyacid changes with solution pH, a stability constant may be defined on the basis of the concentration of A^- (the dissociated, unoccupied ionized carboxyl groups, one on each uronate subunit, referred to as "ligand" hereafter in this article) rather than the weight of polymer per unit volume of solution as adopted in the Scatchard approach for pH-stat systems. When the electric field due to the charged ligands is not considered and the concentration of ligands is based on the volume of bulk solution, the conditional stability constant may be written as

$$M^{2+} + A^- \rightleftarrows MA^+; \quad \beta_{1s}^{app} \tag{1}$$

$$\beta_{1s}^{app} = \frac{(MA^+)}{(M^{2+})(A^-)} = \frac{\langle MA^+\rangle}{(M^{2+})\langle A^-\rangle} \approx \frac{\langle M_{sb}\rangle}{(M^{2+})\langle A^-\rangle} \tag{2}$$

where the moles of metal bound to ligands $\langle M_{sb}\rangle$ can be calculated as the total moles of metal minus the moles of free metal in the bulk solution.

In the equations above and hereafter, the parentheses ( ) denote the activity (molal concentration multiplied by molal single-ion activity coefficient) and the brackets ⟨ ⟩ denote the number of moles of a species. The mass of water (in kilograms, equivalent to volume in liters) is canceled from the denominator and numerator of eq 2. (M^{2+}) is the activity of free bivalent metal ion in the solution. (The molal single-ion activity coefficient $\gamma_{Cu^{2+}}$ is 0.224 and 0.501 at ionic strengths of 0.1 M (0.105 *m*) and 0.01 M (0.01 *m*) $NaNO_3$, respectively.) The ratio of the molal activity coefficients of bound ligands to unbound ligands has been proven to be unity in weakly acidic polyelectrolytes because their respective nonideality cancels.[10] In the above equations, the conditional stability constant is defined arbitrarily for the complex formed between a cupric ion and a binding ligand (referred to as type I complex in this article). Similarly, the conditional stability constant can also be defined for the complex formed between a cupric ion and two binding ligands (type II complex):

$$M^{2+} + 2A^- \rightleftarrows MA_2; \quad \beta_{2s}^{app} \tag{3}$$

$$\beta_{2s}^{app} = \frac{(MA_2)}{(M^{2+})(A^-)^2} = \frac{\langle MA_2\rangle V_s}{(M^{2+})\langle A^-\rangle^2} \approx \frac{\langle M_{sb}\rangle V_s}{(M^{2+})\langle A^-\rangle^2} \tag{4}$$

where V_s is the mass of water in kilograms (which is very close to the volume of a dilute solution in liters).

In reality, both type I and type II complexes could coexist (so, $\langle M_{sb}\rangle = \langle MA^+\rangle + \langle MA_2\rangle$), and the moles of dissociated, unoccupied ligands $\langle A^-\rangle$ can be calculated from the equation

$$\langle A^-\rangle = \langle A_t\rangle - \langle HA\rangle - \langle MA^+\rangle - 2\langle MA_2\rangle \tag{5}$$

where

$$\langle HA\rangle = \langle A_t\rangle - \left[m_{b,ac} + \frac{10^{-pH} V_s}{\gamma_{H^+}} \right] \tag{6}$$

$\langle A_t\rangle$ is the moles of total titratable ligands in the solution, which is taken as the moles of base added to reach the end point of titration of metal-free polyacid solution; $\langle HA\rangle$ is the moles of protonated, undissociated ligands; $m_{b,ac}$ is the moles of standard base added to the metal-containing solution at any point of titration; γ_{H^+} is the molal activity coefficient of hydrogen ion (= 0.914, 0.830, 0.835, and 0.893 at ionic strengths of 0.01, 0.1, 0.3, and 0.5 M $NaNO_3$ or 0.01, 0.105, 0.32, and 0.53 *m* $NaNO_3$, respectively). However, the ratio of moles of type I complex to type II complex can only be calculated through their respective stability constants, which are yet to be determined. No serious errors will be caused by assuming that type I complex is the dominant species when evaluating $\langle A^-\rangle$, as long as the metal ions exist in a trace amount (i.e., $\langle Cu^{2+}\rangle \leq 5\%$ of $\langle A_t\rangle$) in potentiometric titration experiments.[4] Thus, eq 5 can be simplified to

$$\langle A^-\rangle = \langle A_t\rangle - \langle HA\rangle - \langle M_{sb}\rangle \tag{7}$$

and the degree of ionization α is approximately equal to $\langle A^-\rangle / \langle A_t\rangle$.

Correction for the Electric Field of Charged Ligands (Model 2). When the conditional stability constants determined from model 1 vary with environmental conditions, such variation may be due to the electric field around the charged ligands. For a hydrophilic polymer, the ligands on the polymer chain are accessible to the various hydrated counterions (H^+, Na^+, and M^{2+}) in solution, so that the sensitivity of apparent stability constants to the ionic strength of the solution is anticipated. At low ionic strengths (e.g., 0.01 M $NaNO_3$), the electric field is not effectively screened by the low concentrations of neutral electrolytes. Thus, the partition coefficient for a counterion (the ratio of counterion activity in the polymer domain of alginic acid to that in the bulk solution) can be many times greater than unity.[5]

To correct for the effect of electric field in systems where apparent binding equilibrium is sensitive to ionic strength, the stability constants in model 1 may be redefined on the basis of the activity $(\overrightarrow{M^{2+}})$ in the polymer subphase while the ligand concentration is expressed in terms of the volume of the bulk liquid:

$$\beta_{1s}^{poly} = \frac{\langle M_{sb}\rangle}{(\overrightarrow{M^{2+}})\langle A^-\rangle} \tag{8}$$

$$\beta_{2s}^{poly} = \frac{\langle M_{sb}\rangle V_s}{(\overrightarrow{M^{2+}})\langle A^-\rangle^2} \tag{9}$$

The value of $(\overrightarrow{M^{2+}})$ can be estimated from the measured free metal activity (M^{2+}) in the bulk solution by using Donnan equilibrium relationship:

$$(\overrightarrow{M^{2+}}) = (M^{2+})10^{2\Delta pK} \tag{10}$$

where $\Delta pK = pK_{HA}^{apparent} - pK_{HA}^{intrinsic}$ (pK_{HA}^{int} was determined by the base titration of metal-free polyacid solutions[5] and $pK_{HA}^{apparent}$ of a polyacid containing a trace amount of bound metal was calculated in this work as $pH - \log\{\langle A^-\rangle/\langle HA\rangle\}$) and the arrow over the symbols in equations above and hereafter refers to polymer subphase. In our previous work,[5] $pK_{HA}^{apparent}$ was indirectly determined from the base titration data of the copper-free but otherwise equivalent alginic acid solution. This approach could cause a sizable uncertainty in the value of ΔpK if the polymer undergoes a significant change in the morphology (and in turn the free energy of ionization is changed) due to the presence of metal.

(10) Marinsky, J. A.; Miyajima, T.; Muhammed, M. *J. React. Polym.*, in press.

Once the titration data have been corrected for the electrostatic attraction of counterions toward charged ligands, it is possible to determine whether type I complex and type II complex coexist. Since $\langle M_{sb}\rangle = \langle MA^+\rangle + \langle MA_2\rangle$, eq 8 can be rearranged to

$$\beta_{1s}^{poly} = \beta_{1s} + \beta_{2s}\frac{\langle A^-\rangle}{V_s} = \beta_{1s} + \beta_{2s}\frac{\langle A_t\rangle}{V_s}\alpha \quad (11)$$

Likewise, eq 9 can be presented as

$$\beta_{2s}^{poly} = \beta_{2s} + \beta_{1s}\frac{V_s}{\langle A^-\rangle} = \beta_{2s} + \beta_{1s}\frac{V_s}{\langle A_t\rangle}\frac{1}{\alpha} \quad (12)$$

where

$$\beta_{1s} = \frac{\langle MA^+\rangle}{(\overrightarrow{M^{2+}})\langle A^-\rangle} \quad (13)$$

$$\beta_{2s} = \frac{\langle MA_2\rangle V_s}{(\overrightarrow{M^{2+}})\langle A^-\rangle^2} \quad (14)$$

If the proposed model is correct, a plot of β_{1s}^{poly} versus α should yield a straight line with an intercept of β_{1s} and a slope $\langle A_t\rangle\beta_{2s}/V_s$ that is proportional to the polymer concentration. Likewise, plots of β_{2s}^{poly} versus $1/\alpha$ should give an intercept of β_{2s} and a slope $\beta_{1s}V_s/\langle A_t\rangle$ that is inversely proportional to polymer concentration.

Correction for the Electric Field and the Polymer-Subphase Volume (Model 3). Equation in model 2 could be further modified by expressing the concentration of bound and unbound ligands based on the volume of polymer subphase V_p:

$$\beta_{1p}^{poly} = \frac{\langle M_{sb}\rangle}{(\overrightarrow{M^{2+}})\langle A^-\rangle} = \beta_{1p} + \beta_{2p}\frac{\langle A^-\rangle}{V_p} \quad (15)$$

$$\beta_{2p}^{poly} = \frac{\langle M_{sb}\rangle V_p}{(\overrightarrow{M^{2+}})\langle A^-\rangle^2} = \beta_{2p} + \beta_{1p}\frac{V_p}{\langle A^-\rangle} \quad (16)$$

where

$$\beta_{1p} = \beta_{1s} = \frac{\langle MA^+\rangle}{(\overrightarrow{M^{2+}})\langle A^-\rangle} \quad (17)$$

$$\beta_{2p} = \frac{\langle MA_2\rangle V_p}{(\overrightarrow{M^{2+}})\langle A^-\rangle^2} = \beta_{2s}\frac{V_p}{V_s} \quad (18)$$

In our previous work,[5] it was concluded that the volume of polymer subphase, V_p (or more accurately the mass of water in kilograms excluding electrolytes and the polymer chain on a molal basis), accounts for only a small fraction of the total solution volume. Figure 1 describes how V_p per mole of total titratable ligands on the alginic acid varies with polymer concentration and ionic strength. (In this work ΔpK was directly calculated as described in model 2.) The distinction between eq 11–14 and 15–18 is clear: In the former set of equations, monomeric ligands are assumed to distribute evenly throughout the solution, while in the latter set of equation, the ligands (bound and unbound) are restricted to the polymer subphase. Thus, even at low polymer and copper concentrations where gel formation is not favored, it is more reasonable to express the mass-action stability constants based on the concentration of ligands in the reaction zone (polymer-subphase volume).

Alternative Expressions Combining Acid Dissociation and Metal Binding Reactions (Model 4). Coupling the acid dissociation reaction

$$H^+ + A^- \rightleftarrows HA; \quad \beta_{HA}^{int} = [K_{HA}^{int}]^{-1} \quad (19)$$

with the type I complex forming reaction (eq 1) yields

$$M^{2+} + 2HA \rightleftarrows MA^+ + A^- + 2H^+; \quad D_1 \quad (20)$$

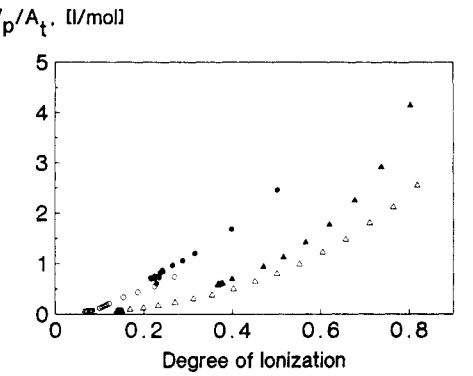

Figure 1. Relationship between polymer-subphase volume and degree of ionization in presence of trace amount of cupric ion: 0.83 mg of alginic acid/mL, I = 0.1 M $NaNO_3$ (▲); 0.83 mg/mL, I = 0.01 M (●); 4.00 mg/mL, I = 0.1 M (△); 4.00 mg/mL, I = 0.01 M (○).

Similarly, coupling the type II complex forming reaction (eq 3) with eq 19 yields

$$M^{2+} + 2HA \rightleftarrows MA_2 + 2H^+; \quad D_2 \quad (21)$$

where β_{HA}^{int}, D_1, and D_2 are all based on the conditions in the polymer subphase:

$$\beta_{HA}^{int} = \frac{(\overrightarrow{HA})}{(\overrightarrow{H^+})(\overrightarrow{A^-})} = \frac{\langle HA\rangle}{(\overrightarrow{H^+})\langle A^-\rangle} \quad (22)$$

$$D_1 = \frac{(\overrightarrow{H^+})^2}{(\overrightarrow{M^{2+}})}\frac{(\overrightarrow{MA^+})(\overrightarrow{A^-})}{(\overrightarrow{HA})^2} = \frac{(H^+)^2}{(M^{2+})}\frac{\langle MA^+\rangle\langle A^-\rangle}{\langle HA\rangle^2} = \frac{\beta_{1p}}{[\beta_{HA}^{int}]^2} \quad (23)$$

$$D_2 = \frac{(\overrightarrow{H^+})^2}{(\overrightarrow{M^{2+}})}\frac{(\overrightarrow{MA_2})}{(\overrightarrow{HA})^2} = \frac{(H^+)^2}{(M^{2+})}\frac{\langle MA_2\rangle V_p}{\langle HA\rangle^2} = \frac{\beta_{2p}}{[\beta_{HA}^{int}]^2} \quad (24)$$

The value of β_{HA}^{int}, the intrinsic stability constant for the acid-association reaction of alginic acid, was determined to be $10^{2.96}$ in our previous work.[5] If experimental data are expressed in the form of the pseudo-type I complex forming distribution constant D_1^{poly}, then

$$D_1^{poly} = \frac{(H^+)^2}{(M^{2+})}\frac{\langle M_{sb}\rangle\langle A^-\rangle}{\langle HA\rangle^2} = D_1 + D_2\frac{\langle A^-\rangle}{V_p} \quad (25)$$

Correspondingly, the pseudo-type II complex forming distribution constant D_2^{poly} may be described by

$$D_2^{poly} = \frac{(H^+)^2}{(M^{2+})}\frac{\langle M_{sb}\rangle V_p}{\langle HA\rangle^2} = D_2 + D_1\frac{V_p}{\langle A^-\rangle} \quad (26)$$

Equations 25 and 26 are alternative expressions for eq 15 and 16, respectively, by dividing the latter two equations by the factor $\{\beta_{HA}^{int}\}^2$ throughout. Equations 25 and 26 may prove to be a convenient means to treat experimental data. Unlike eq 15 and 16, which require an accurate estimation of $(\overrightarrow{M^{2+}})$ at every experimental point, the ratio $(\overrightarrow{H^+})^2/(\overrightarrow{M^{2+}})$ in eq 25 and 26 can be replaced by $(H^+)^2/(M^{2+})$[4,11] according to Donnan equilibrium theory and (H^+) and (M^{2+}), the activities of free hydrogen and metal ions, in the bulk liquid phase, can be easily measured electrochemically.

(11) Marinsky, J. A.; Lin, F. G.; Chung, K.-S. *J. Phys. Chem.* **1983**, *87*, 3139.

Existence of Electrovalent Chelation Bonds: The Effect of Competition from Hydrogen Ion for Metal Binding Sites (Model 5). In all of the four models above, mass-action equilibrium constants are used to describe the stability of complexes that involve bonding between metal ions and ionized ligands. Recently, the importance of carboxyl groups in cupric ion binding has been emphasized. In the case of cupric ion binding to fully protonated carboxyl groups on acidic polysaccharides, electron-rich oxygen atoms on the carboxyl group serve as the primary electron donor.[12] Since the alginic acid in the aqueous solution used in this work is partially ionized, we shall test in the following model whether cupric ions form bonds with both ionized and fully protonated carboxyl groups on alginic acid in aqueous solutions:

$$M^{2+} + 2HA \rightleftarrows MA^{+} + 2H^{+} + A^{-}; \quad D_1 \qquad (27a)$$

$$M^{2+} + 2HA \rightleftarrows MA^{+}\text{–}HA + H^{+}; \quad D_{2ph} \qquad (27b)$$

where

$$D_{2ph} = \frac{(\overrightarrow{H^+})}{(\overrightarrow{M^{2+}})}\frac{(\overrightarrow{MA^+\text{–}HA})}{(\overrightarrow{HA})^2} = \frac{(\overrightarrow{H^+})}{(\overrightarrow{M^{2+}})}\frac{\langle MA^+\text{–}HA\rangle V_p}{\langle HA\rangle^2} \qquad (28)$$

In this model, a cupric ion and an ionized carboxyl group form a bond in the type I complex. However, it is assumed that in the type II complex the cupric ion and the second carboxyl group (protonated form) form a chelate. The electron-donating oxygen atoms on the hydroxyl groups serve to stabilize the complexes formed between the cupric ion and the carboxyl group(s).

Following a procedure similar to that depicted in model 4, the distribution constants for the stability of pseudo-type I complex and pseudo-type II complex an be expressed by

$$D_1^{poly} = \frac{(H^+)^2}{(M^{2+})}\frac{\langle M_{sb}\rangle\langle A^-\rangle}{\langle HA\rangle^2} = D_1 + D_{2ph}\frac{\langle A^-\rangle(\overrightarrow{H^+})}{V_p} \qquad (29)$$

and

$$D_{2ph}^{poly} = \frac{(\overrightarrow{H^+})}{(\overrightarrow{M^{2+}})}\frac{\langle M_{sb}\rangle V_p}{\langle HA\rangle^2} = D_{2ph} + D_1\frac{V_p}{\langle A^-\rangle(\overrightarrow{H^+})} \qquad (30)$$

respectively, where $\langle M_{sb}\rangle = \langle MA^+\rangle + \langle MA^+\text{–}HA\rangle$ and $(\overrightarrow{H^+}) = (H^+)10^{\Delta pK}$. When eq 27 is coupled with eq 19, the stability constant for type II complex, β_{2ph}, can be defined by

$$M^{2+} + H^+ + 2A^- \rightleftarrows MA^+\text{–}HA; \quad \beta_{2ph} \qquad (31)$$

where $\beta_{2ph} = D_{2ph}\{\beta_{HA}^{int}\}^2$. Compared with model 4, this model not only considers the possible existence of the chelation bond but also suggests that a strong competition from hydrogen ion for metal binding sites may affect the apparent binding equilibrium.

Experiments and Methods

Experimental conditions and procedures are described in detail elsewhere.[5] Briefly, 1-μL volumes of a concentrated cupric sulfate solution (0.1 M) were added to a 0.83 and 4.00 mg/mL solution of alginic acid prepared at different ionic strengths (0.01 and 0.1 M $NaNO_3$) until total moles of cupric ion reached 5% total titratable ligands. Then the copper-containing alginic acid solution was continually titrated with a standard base (0.1 or 0.01 M NaOH). Free cupric ion concentration and solution pH were simultaneously monitored. During the addition of cupric sulfate, pH dropped slightly while free copper ion concentration increased. During the addition of NaOH, pH increased and the free cupric ion concentration decreased. At the lower ionic strength of 0.01 M, the solution pH was higher (lower free hydrogen ion concentration) and the free cupric ion concentration was lower compared to experiments conducted at the higher ionic strength of 0.1 M. The titration data were evaluated by the five models described above.

(12) Manzini, G.; Cesaro, A.; Delben, F.; Paoletti, S.; Reisenhofer, E. *Bioelectrochem. Bioenerg.* **1984**, *12*, 443.

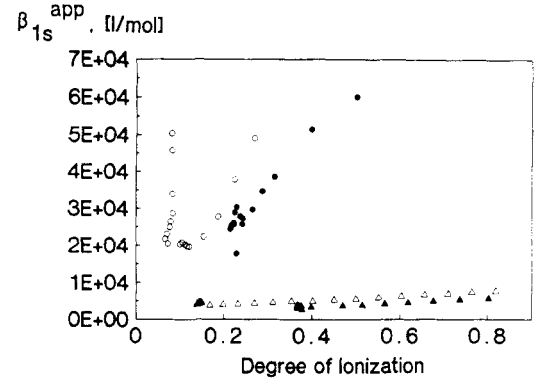

Figure 2. Conditional pseudo-type I stability constant versus degree of ionization. Symbols as in Figure 1.

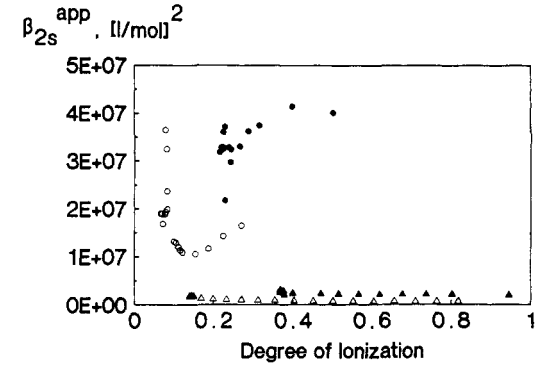

Figure 3. Effect of degree of ionization on the conditional pseudo-type II stability constant. Symbols as in Figure 1.

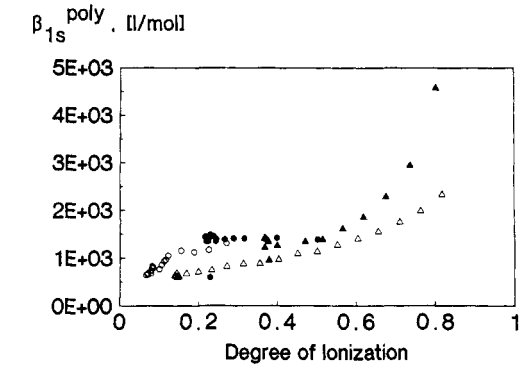

Figure 4. Variation in pseudo-type I stability constant after correction for the electric field due to charged ligands. Symbols as in Figure 1.

Results

Model 1. The conditional stability constants β_{1s}^{app} (eq 2) and β_{2s}^{app} (eq 4) for the interaction between cupric ion and alginic acid at different polymer concentrations 4.00 and 0.83 mg/mL) and ionic strengths (0.1 and 0.01 M $NaNO_3$) are plotted against α (Figures 2 and 3, respectively). When the solution has an ionic strength of 0.1 M, the values of β_{1s}^{app} at different polymer concentrations are very close and do not vary significantly with α (Figure 2). The β_{1s}^{app} values at the lower ionic strength of 0.01 M scatter over a wider range and appear to be more sensitive to α. As the two groups of data at 0.01 and 0.1 M ionic strengths are compared, the values of β_{1s}^{app} for the former are several times that of the latter. The observed dependence of apparent metal binding equilibrium on the ionic strength of solution suggests that the two-phase approach is needed to interpret metal binding data

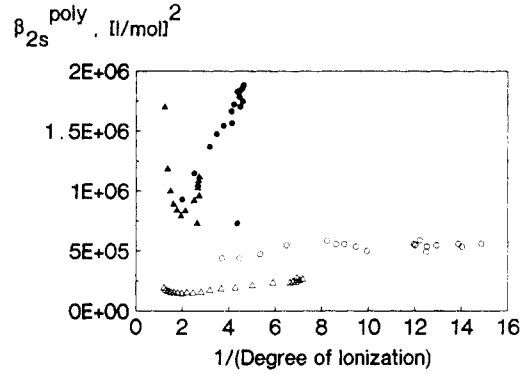

Figure 5. Variation in pseudo-type II stability constant after correction for the electric field due to charged ligands. Symbols as in Figure 1.

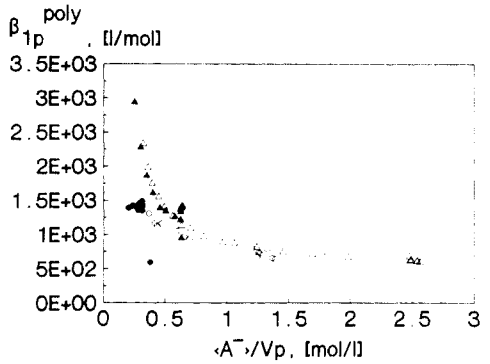

Figure 6. Pseudo-type I stability constant corrected for the electric field and the polymer-subphase volume. Symbols as in Figure 1.

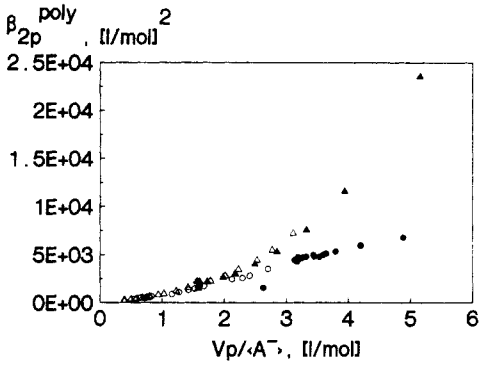

Figure 7. Pseudo-type II stability constant corrected for the electric field and the polymer-subphase volume. Symbols as in Figure 1.

of alginic acid. Similar conclusions can also be drawn when β_{2s}^{app} is plotted against α (Figure 3).

Model 2. When binding data are corrected for the electrostatic attraction of counterions toward charged ligands, the values of β_{1s}^{poly} (Figure 4) at different polymer concentrations and ionic strengths deviate to a lesser extent. The data of β_{1s}^{poly} do converge to a common value of β_{1s} at the ordinate as eq 11 predicts. However, the slopes of the data at different polymer concentrations are similar, thus violating an assumption of this model that the slope should be proportional to polymer concentration (i.e., the slope of the line for polymer concentration of 4.00 mg/mL should be approximately 5 times greater (4.0/0.83) than that for the polymer concentration of 0.83 mg/mL) assuming a uniform distribution of ligands in the solution. When β_{2s}^{poly} is plotted against $1/\alpha$ (Figure 5, eq 12), again the data points of titration at the higher polymer concentration (4.00 mg/mL) do not vary significantly with $1/\alpha$ at different ionic strengths. The data points at the lower polymer concentration (0.83 mg/mL) do not define a unique slope, and no comparison regarding the dependence of slope on $1/\alpha$ can be made. Nevertheless, it is clear that β_{2s}^{poly} values at different polymer concentrations do not converge to a common value of β_{2s} on the ordinate. These results suggest that

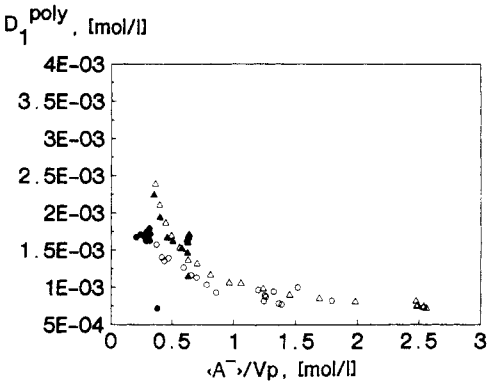

Figure 8. Distribution coefficient for the pseudo-type I complex. Symbols as in Figure 1.

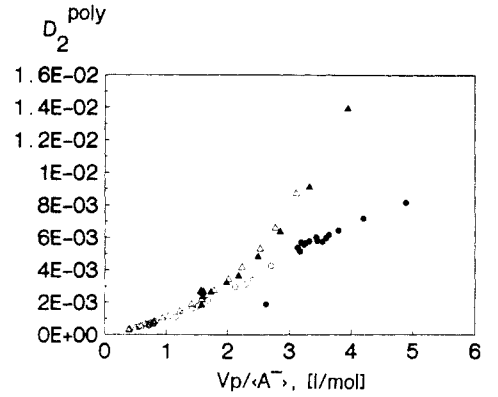

Figure 9. Distribution coefficient for the pseudo-type II complex. Symbols as in Figure 1.

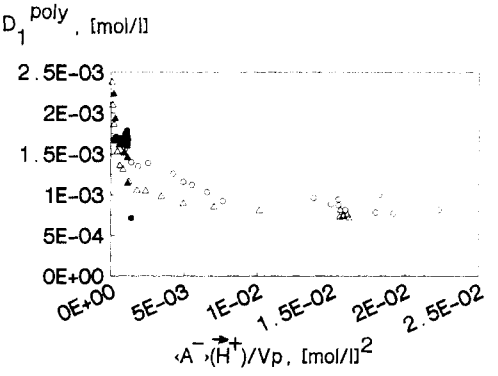

Figure 10. Effect of competition from hydrogen ion for binding ligands on the distribution coefficient for pseudo-type I complex. Symbols as in Figure 1.

the polymer subphase be considered as a separate phase in the solution.

Model 3. The pseudo-type I and pseudo-type II stability constants, β_{1p}^{poly} and β_{2p}^{poly}, corrected for both electrostatic attraction of counterions and the concentration of ligands (on the basis of polymer-subphase volume) are presented in Figures 6 and 7, respectively. There is no unique (curved) line that can be drawn from the data of β_{1p}^{poly} versus $\langle A^- \rangle / V_p$ (Figure 6) to yield the values of β_{1p} and β_{2p} by using eq 15. However, a straight line can be drawn through the data of β_{2p}^{poly} for $V_p/\langle A^- \rangle < 3.0$ L/mol (Figure 7). Model 3 improves over models 1 and 2 because metal binding data at different polymer concentrations and ionic strengths can now be described by a single function that converges at the ordinate giving an intercept of $\beta_{2p} \approx 4.0$ and a slope $\beta_{1p} \approx 1.4 \times 10^3$. Further improvements of the data are sought in model 4.

Model 4. When the experimental data are presented as D_1^{poly}, values ranged between 1×10^{-3} and 3.5×10^{-3} and scattered widely at $\langle A^- \rangle / V_p < 0.5$ (Figure 8), while a straight line describes

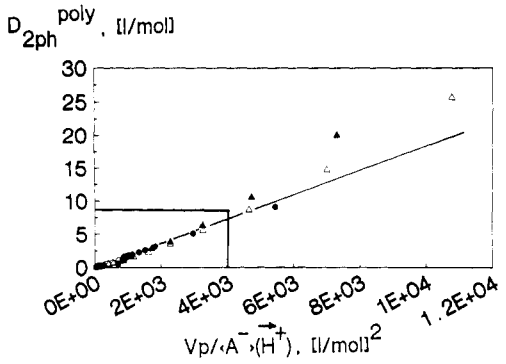

Figure 11. Effect of competition from hydrogen ion for binding ligands on the distribution coefficient for the pseudo-type II complex. Symbols as in Figure 1.

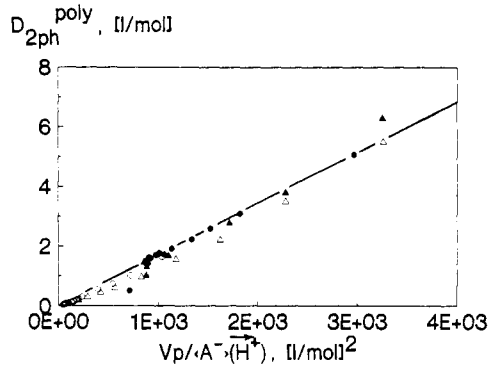

Figure 12. Enlargement of the enclosed area in Figure 11. Symbols as in Figure 1.

data of D_2^{poly} versus $V_p/\langle A^- \rangle$ very well up to $V_p/\langle A^- \rangle$ of 2.0, or approximately 70% of complete ionization (Figure 9). This best-fit straight line in Figure 9 has an intercept of 1.0×10^{-4} ($=D_2$) and a slope of 1.6×10^{-3} ($=D_1$). Multiplying D_1 and D_2 by $\{\beta_{HA}^{int}\}^2$ ($=\{10^{2.96}\}^2$) yields intrinsic stability constants of $\beta_{1p} = 1.33 \times 10^3$ (type I complex) and $\beta_{2p} = 8.0 \times 10^1$ (type II complex). The experimental values of D_2^{poly} at the higher ionic strength of 0.1 M are greater than those predicted with eq 26 (i.e., the corresponding points on the extrapolated straight line) by a factor of 1–2 in the range of $V_p/\langle A^- \rangle > 3$.

Model 5. When data are presented as D_1^{poly} versus $\langle A^- \rangle \langle \overrightarrow{H^+} \rangle / V_p$ (Figure 10), the values of D_1^{poly} range between 1.0×10^{-3} and 2.5×10^3. When the experimental data are presented as D_{2ph}^{poly} versus $V_p/\langle A^- \rangle \langle \overrightarrow{H^+} \rangle$ (Figures 11 and 12), a straight line with a slope of 1.82×10^{-3} ($=D_1$) and an intercept of 2.0×10^{-2} ($=D_{2ph}$) fits the whole range of data more satisfactorily than that in Figure 9 of model 4. Multiplying D_1 and D_{2ph} by $\{\beta_{HA}^{int}\}^2$ yields $\beta_{1p} = 1.51 \times 10^3$ and $\beta_{2ph} = 1.7 \times 10^1$.

Discussion

The results of model 1 (Figures 2 and 3) support our hypothesis that the high and variable electric field due to the charged ligands affects the apparent metal binding equilibrium. Since the electric field at the lower ionic strength of 0.01 M is not effectively screened by the neutral electrolyte, counterions are attracted electrostatically toward the charged ligands. According to mass-action law, more cupric ions are bound to the binding ligands at ionic strength 0.01 M than at 0.1 M. Since model 1 is based on the counterion activities in the bulk solution phase instead of the polymer subphase where binding reactions actually take place, the apparent stability constants at ionic strength 0.01 M are several times higher than at 0.1 M.

After correcting for the electrostatic attraction of counterions (model 2), the stability constants show less variation with ionic strength (Figures 4 and 5) than those obtained with model 1. Nevertheless, model 2 fails to yield a unique stability constant for type I complex and a unique stability constant for type II complex because it assumes the monomeric ligands distribute evenly throughout the solution. These results indicate that alginic acid and other acidic polysaccharides exist as colloids in aqueous solution and the ligands on the polymer act as if they reside in a phase separate from that of the bulk phase.

Both model 3 and model 4, which are equivalent approaches, take into account the electrostatic attraction of counterions by the charged polymer chain and that the polymer subphase exists as a separate phase in the solution. The results obtained from these two models are similar and can be accepted with confidence for degrees of ionization up to 60%.

When complexation reactions between cupric ions and the acidic polysaccharide alginic acid are evaluated by the five models described in this work, it was found that the best resolution of experimental data is obtained with eq 30 (model 5). The competition from hydrogen ions for metal binding sites is taken into account in this model.

It is noted that the variables on the ordinate and abscissa in Figure 11 (model 5) are obtained by dividing those in Figure 9 (model 4) by $\langle \overrightarrow{H^+} \rangle$ of each data point. In essence, Figure 11 is a "rescaling" of Figure 9. An improvement in data fit suggests that bonding between the cupric ions and the fully protonated carboxyl groups may contribute to the stability of the complex between cupric ion and alginic acid.

The assumption used in this work that type I complex is the dominant species is corroborated by the results of the following calculations: By dividing eq 23 by eq 24 in model 4 and rearranging, the ratio of moles of type I complex to type II complex at every experimental point up to 70% of complete ionization can be expressed by the values of D_1 and D_2 obtained from Figure 9:

$$\frac{\langle MA^+ \rangle}{\langle MA_2 \rangle} = \frac{D_1}{D_2} \frac{V_p}{\langle A^- \rangle} \tag{32}$$

The ratio $\langle MA^+ \rangle / \langle MA_2 \rangle$ at $V_p/\langle A^- \rangle = 0.4$ (lowest degree of ionization attained in these studies) is calculated to be $\{1.6 \times 10^{-3}/1.0 \times 10^{-4}\} \times 0.4 = 6.4$. As base is added to the alginic acid solution to about 70% degree of ionization when $V_p/\langle A^- \rangle$ is about 2.0, the ratio $\langle MA^+ \rangle / \langle MA_2 \rangle$ increases to 32. Similarly, the corresponding ratio can be expressed by D_1 and D_{2ph} in model 5

$$\frac{\langle MA^+ \rangle}{\langle MA^+\text{–}HA \rangle} = \frac{D_1}{D_{2ph}} \frac{V_p}{\langle A^- \rangle \langle \overrightarrow{H^+} \rangle} \tag{33}$$

for all experimental data collected at pH <6.0 (where about 90% ionization was attained). This ratio is calculated to be $\{1.82 \times 10^{-3}/2.0 \times 10^{-2}\} \times 10^2 \approx 9$ (when the lowest degree of ionization was attained) and increases with added base. This change in the type I/type II ratio is likely due to a change in the morphology of the polymer chain as the degree of ionization increases. As more carboxyl groups are ionized, the increased repulsion among the charged groups likely causes the polymer molecule to uncoil. Unfolding of the polymer chain may increase the distance between the functional groups with which cupric ions interact and destabilize or discourage the formation of type II complexes.

The deviation of data points from the best-fit straight lines in Figures 9 and 11 may represent some factors not taken into account in eq 26 (model 4) and eq 30 (model 5). First of all, the two-phase model proposed in this work assumes that there is only one type of binding site. In the case of alginic acid, the molecule contains regions composed of blocks of polyguluronic acid (GG), regions composed of blocks of polymanuronic acid (MM), and other regions composed of alternating sequences of guluronic acid and manuronic acid (GM). Among the three regions, the –GG– region is known to provide the greatest binding stability for copper. This is because the coillike morphology of the –GG– region (as opposed to the ribbonlike morphology of the –MM– region) offers a more favorable environment for copper bound.[1,2] Thus, expression of the binding reactions using a singlet set (monodentate and bidentate) of intrinsic stability constants may have limited accuracy.

A second reason for the deviation of data points from the best-fit straight lines in Figures 9 and 11 may be due to the fact that the

single-ion activity coefficient of counterions used in this work was estimated with the following equation

$$\gamma_{M^{2+}} = \{\gamma_{\pm MCl_2}\}^3/\{\gamma_{\pm KCl}\}^2 \quad (34)$$

at a given ionic strength. The use of eq 34 assumes $\gamma_{K^+} \approx \gamma_{Cl^-}$ because of the similarity in size (also charge density) of the K^+ and Cl^- ions. By dividing $\{\gamma_{\pm MCl}\}^3$ by $\{\gamma_{\pm KCl}\}^2$, γ_{Cl^-} is presumed to be canceled effectively to yield reasonable estimated values of the single-ion activity coefficient $\gamma_{M^{2+}}$ (primarily for ionic strength not exceeding 0.1 M). However, in the polymer subphase where the ionic strength is so much higher than the bulk liquid due to the electrostatic attraction between counterions and a highly charged polymer chain (with a small intercharge distance of only 4.71 Å/α),[6] other theories of ionic interactions[13] that permit a more reliable estimation of the single-ion activity coefficient need to be considered.

Finally, in our studies we observed that the "free" cupric ion concentration decreased quickly when approaching the end of potentiometric titration at pH > 6.5. The increase in the moles of ionized binding ligands alone cannot explain this drastic reduction in the free cupric ion concentration. The moles of copper bound to alginic acid was calculated by subtracting the moles of measured "free" cupric ions from the total moles of copper in the system without taking into account the $Cu(OH)_2$ that forms at pH > 6.0. Therefore, we present only the data collected at pH < 6.0 in this work.

Our two-phase model does not take into account the detailed spacial distribution of counterion, and it treats the region in which a strong electrostatic attractive force exists as a separate, homogeneous phase. (An inherient assumption made in the iterative procedure for computing the polymer-subphase volume[5] is that both monomeric ligands and counterions are evenly distributed within the region where they are restricted, i.e., the polymer subphase.) In fact, the thermal motion (vibration and oscillation) of the individual ligands could be hindered by the covalent bonds linking the ligands so that the ligands may not have an equal probability of existing at different locations in the polymer subphase. Furthermore, the concentrations of counterions are highest in the very vicinity of the polymer chain and gradually decrease with increasing distance from the polymer chain, as described by the Poisson-Boltzmann theory. However, our two-phase model, which emanates essentially from the experimentally proven phenomenological theory of Donnan equilibrium, improves over the conventional approach in the treatment of potentiometric data for determination of intrinsic stability constants that describe metal-acidic polysaccharide interactions.

(13) Pitzer, K. S. In *Activity Coefficients in Electrolyte Solutions*; Pytkowic, R. M., Ed.; CRC Press: Boca Raton, FL, 1979; Vol. 1, Chapter 7.

Conclusions

1. A molecule of a hydrophilic polyacid such as alginic acid which has a high ligand density should be considered as a separate phase in an aqueous solution when treating potentiometric titration data.

2. The apparent copper binding equilibrium of alginic acid is a strong function of the ionic strength in the solution. When the electrostatic attraction of counterions toward charged ligands and the existence of a separate polymer subphase are taken into account, the two-phase approach yields intrinsic stability constants. The best resolution of data is obtained when model 5 (the coupled protonation-deprotonation and copper binding reactions with the pH effects taken into account) is used for pH < 6. Model 5 suggests that the bidentate complex formed may involve both ionic bonding and chelation bonding.

3. Improvements on the present two-phase approach are possible if (a) the model can be modified to take into account the coexistence of more than one type of binding ligand, (b) the activity coefficients of both counterions and monomeric ligands in the polymer subphase can be more accurately assessed, and (c) the effect of morphology of the polymer chain on the copper binding equilibrium of alginic acid can be accurately described.

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Structure of Electron Tracks in Water. 1. Distribution of Energy Deposition Events

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The distributions of energy deposition events for electrons in gaseous and liquid water and in amorphous ice have been calculated. These calculations make use of an inelastic cross section that takes into account binding energy, exchange, and relativistic effects and that is based on experimentally determined dipole oscillator strength distributions. The most probable energy loss in a single event by a 1-MeV electron is 22.5 eV in the condensed phases and 13.5 eV in the gaseous phase, while the average energy loss is 34, 38, and 40 eV/event for gaseous and liquid water and amorphous ice, respectively. Very little dependence of the distributions on incident electron energy is found. At 1 MeV the differential or single event distribution is found to be the same as the integral or cumulative distribution. The fractions of the total energy lost in events that are less than 100 eV are 0.76, 0.75, and 0.69 for gaseous and liquid water and amorphous ice, respectively.

I. Introduction

The trajectory of a high-energy electron in water is marked by a sequence of energy deposition events. Although most of the energy deposition events involve small energy transfers, one can find secondary tracks, or δ-rays, showing the location of high-energy, less probable transfers. The amount of energy deposited in each energy loss event can have a profound effect on the subsequent chemical processes, and it is necessary to know the distribution of energy deposition events in order to model the overall chemical processes leading to observed product formation in the radiolysis of water. Theoretical studies of the physical

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