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An Iterative Procedure Based on the Donnan Equilibrium for Calculating the Polymer-subphase Volume of Alginic Acid

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Synopsis

A numerical iterative procedure is presented to predict the polymer-subphase volume that is formed when anionic polysaccharides such as alginic acid (polyuronic acid from kelp) are suspended in an aqueous solution. (The aqueous region surrounding the polymer chain where a strong electrostatic attractive force for counterions exists is defined as the separate polymer subphase within the colloidal phase enclosed by the polymer coil.) Based on the phase-partition model of Marinsky et al.[5] and Donnan equilibrium theory, this iterative procedure utilizes the base titration data of the acidic polysaccharide at different ionic strengths as well as the osmotic properties of the sodium form of the polysaccharide. No detailed structural information of alginic acid is required. The resulting calculations show that the polymer subphase, which accounts for a small fraction of the total solution volume, should be regarded as the reaction zone for acid dissociation and metal binding reactions. The volume of polymer subphase thus calculated may serve as an excellent index for the morphology of the polymer molecule at different ionic strengths, degrees of ionization, polymer concentrations, and extent of polymer-metal binding.

INTRODUCTION

Rigid, water-insoluble, ion-exchange resins and humic substances exhibit physiocochemical properties as if they exist in a separate gel phase when suspended in an aqueous medium[1-4] for the following reasons: (1) the ligands on the ion exchange resins and humic material exist as ensembles and are not distributed uniformly throughout the aqueous medium, and (2) these polymers that chelate metals have negatively charged ligands which attract positively charged counterions such as Na^+, H^+, and M^{n+} (n-valent metal ion) to the polymer domain. Thus, a more rigorous description of the dissociation reaction ($HA = H^+ + A^-$; K_{HA}) and metal binding reaction ($M^{n+} + mA^- = MA_m^{n-m}$; β_{MA_m}) at thermodynamic equilibrium should be based on counterion and ionized ligand activities in the gel domain. Marinsky et al.[5] define the gel domain as the excluded volume of the resin or polymer matrix which includes the aqueous solution contained therein.

In the potentiometric titration of the macroscopic rigid Sephadex CM-25 gel and the flexible Sephadex CM-50 gel,[6,7] it was found that the curves of apparent pK_{HA} versus α (degree of ionization or neutralization) at different ionic strengths separate from each other; these curves did not converge to a

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common value at $\alpha = 0$. When the pH value of the gel domain [calculated from the solution pH by eq. (1) below] was used to define pK_{HA} in the Henderson–Hasselbalch equation, the curves of pK_{HA} versus α converged to a common value at $\alpha = 0$. This result implied that the polymer chain enclosed by the surface of the gel can be viewed as a suspension of polyelectrolytes in the gel-phase fluid.

An aqueous solution of the acidic polysaccharide, alginic acid, which forms a flexible microscopic colloid, was found to exhibit physiocochemical properties similar to those of macroscopic gels.[8,9] The data from base titration of the alginic acid, when plotted as the apparent pK_{HA} versus α at different ionic strengths, did not converge to a common value at $\alpha = 0$. This indicated that the electric field from the charged ligands on the polymer was not solely responsible for the deviation of the dissociation constant (and metal-binding constant) from the intrinsic values. Deviation likely occurred as a result of the existence of a separate polymer phase.

The reason that the model developed in Refs. 5 and 8 for macrosized ion-exchange resins has not been applied to microsized colloids has, in part, been due to the difficulty in determining the volume of the colloidal domain. Even if the colloidal domain were identifiable, its volume might vary with the degree of ionization and the extent of metal binding by the polymer, as well as with the ionic strength of the bulk solution.

In this paper, an iterative numerical procedure is described that predicts the volume of the polymer subphase of alginic acid within the colloidal or gel phase that is created when the alginic acid is suspended in water. The procedure utilizes potentiometric titration data and Donnan equilibrium theory to estimate polymer subphase volume at different α, ionic strength I, moles of metal bound, and concentration of alginic acid. The polymer subphase volume provides a more accurate estimation of the volume of that region in which counterions and metal ions interact with polymer ligands. Estimation of the polymer subphase volume should facilitate determination of intrinsic metal binding constants of flexible biopolymers.

THEORY

Phase-partition Model for Colloidal Polymers

It was proposed that a cross-linked, ion-exchange resin in an aqueous medium (Fig. 1) may be viewed as a suspension of charged polyelectrolyte molecules separated from the bulk solution by a semipermeable membrane.[5] The hypothetical membrane is permeable to simple electrolytes but not to polyelectrolyte molecules. The membrane delineates two separate domains: a bulk liquid phase and a gel phase which contains the polyelectrolyte and any liquid trapped therein.

If the boundary between the two phases acts as a semipermeable membrane, the distribution of counterions between the phases may therefore be described by Donnan equilibrium theory:

$$p\overline{\mathrm{H}} - p\mathrm{H} = p\overline{\mathrm{Na}} - p\mathrm{Na} \tag{1}$$

$$p\overline{\mathrm{M}} - p\mathrm{M} = n\left[p\overline{\mathrm{Na}} - p\mathrm{Na}\right] \tag{2}$$

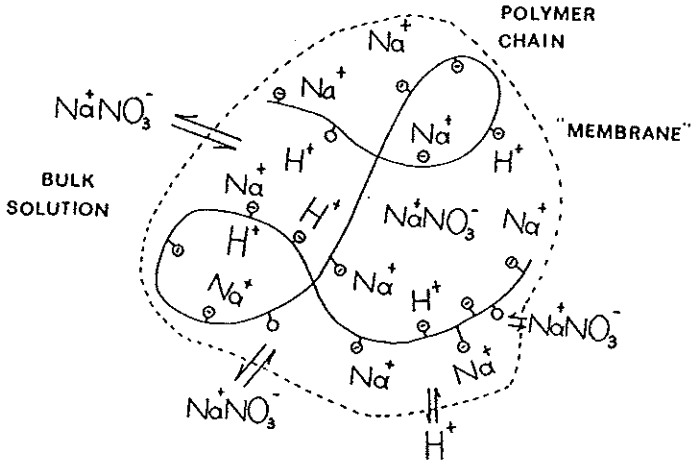

Fig. 1. The polymer chain separated from the bulk solution by a hypothetical membrane through which simple electrolytes can permeate. In salt-free solutions, $NaNO_3$ is not present. (○) The protonated, undissociated ligand; (⊖) the ionized ligand.

where a bar over a symbol refers to the gel phase (conceptually equivalent to the left compartment in Fig. 2) and symbols without bars are for the bulk liquid phase, and pX is the negative logarithm of the activity of X [where $X = H^+$, n-valent counterion M^{n+}, or Na^+ (introduced as an inert salt such as sodium nitrate to control the ionic strength of the polymer solution)].

As a counterion migrates across the hypothetical membrane into the gel phase and enters the electric field due to the proximity of the negatively charged ligands on the polymer, it becomes electrostatically bound (Fig. 2). The binding energy (between the counterion and the charged ligands) is much stronger than the thermal energy that allows the counterion to escape. Thus,

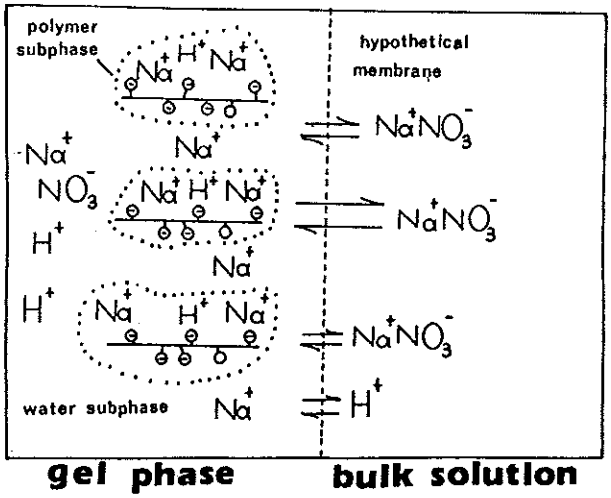

Fig. 2. The phase-partition model for the colloid of the polymer chain. The three polyelectrolyte molecules correspond to the polymer chain in Figure 1. The dotted line delineates polymer subphase volume V_p.

a small aqueous region surrounding the polymer chain in which a strong electrostatic attractive force exists should be included along with the polymer chain as a separate polymer subphase in the gel phase. One approach that may be useful for the determination of properties of the polymer subphase is the cell model of a polyelectrolyte solution.[10] According to this model, the condition of the remaining water trapped in the gel phase (i.e., the water subphase) determines the bulk properties of the gel phase (symbols with bars).

Within the polymer subphase, Na^+ is associated strongly with hydration water molecules and is bound only through electrostatic interactions with the charged polymer. A fraction of other counterions (such as H^+, Cu^{2+}, or Ca^{2+}) entering the polymer subphase, however, may lose their hydration water to form covalent-like bonds with ligands on the polymer chain. The polymer subphase can therefore be regarded as the reaction zone for protonation (or acid dissociation) and metal-binding reactions.

Since the counterions in the gel phase are under the influence of the same electrostatic field, activities of the electrostatically bound counterions in the polymer subphase can be related to those in the water subphase by Guoy–Chapman theory:

$$(\vec{\mathrm{Na}}^+) = (\overline{\mathrm{Na}}^+)\exp[-\epsilon\psi/kT] \tag{3}$$

$$(\vec{\mathrm{H}}^+) = (\overline{\mathrm{H}}^+)\exp[-\epsilon\psi/kT] \tag{4}$$

$$(\vec{\mathrm{M}}^{n+}) = (\overline{\mathrm{M}}^{n+})\exp[-n\epsilon\psi/kT] \tag{5}$$

where the arrow denotes the polymer subphase, the parentheses around the symbol of a counterion above and hereafter denote activity (concentration times activity coefficient), the brackets represent a group of mathematical terms, ϵ is the electric charge of a monovalent species, and ψ is the electrostatic potential on the polymer. The activities of the electrostatically bound counterions in the polymer subphase can be directly related to their activities in the bulk solution by combining eqs. (1) and (2) (between the bulk solution and the water subphase) and eqs. (3)–(5) (between the water subphase and polymer subphase):

$$p\vec{\mathrm{H}} - p\mathrm{H} = p\vec{\mathrm{Na}} - p\mathrm{Na} \tag{6}$$

$$p\vec{\mathrm{M}}^{n+} - p\mathrm{M}^{n+} = n\left[p\vec{\mathrm{Na}} - p\mathrm{Na}\right] \tag{7}$$

From eqs. (6) and (7), the activities of the electrostatically bound H^+ and M^{n+} in the polymer subphase can be obtained from their respective activities in the bulk solution provided that the activity of Na^+ in the polymer subphase can be determined. The Na^+ activity in the polymer subphase can be determined from the moles of electrostatically bound Na^+, the Na^+ activity coefficient, and volume of the polymer subphase. Estimation of the Na^+ activity in the polymer subphase thus provides a means to obtain the polymer subphase volume (conceptually equivalent to acid dissociation or metal binding reaction zone).

More detailed derivations of eqs. (1), (2), (6), and (7) are given in Refs. 6 and 7. In cross-linked gel systems, electroneutrality persists throughout the gel phase. In microscopic colloidal systems which behave much like the crosslinked gel systems yet appear to be homogeneous, neither the polymer subphase nor the water subphase in Figure 2 are electroneutral. However, electrochemical potentials, not chemical potentials, of ions are equal throughout the two phases of equilibrium. When comparing ions pairs in this way, the extra electrical force on each counterion cancels out to yield eqs. (6) and (7).

Physiocochemical Theory for Estimating Moles of Na^+ Immobilized by a Charged Polymer

The moles of electrostatically bound Na^+ can be estimated from a physiochemical theory of the solution of a charged polymer.[10] According to this theory, the practical osmotic coefficient $\phi_{p,Na}$ of Na^+ in a solution of charged polymer in the sodium form with no simple electrolytes added (the salt-free solution) can be calculated from the activity coefficient of the free Na^+, which escapes confinement by the electrostatic field of the polymer. In a salt-free solution, $\phi_{p,Na}$ is thus equivalent to the fraction of Na^+ which is unbound. If the number of moles of monovalent ionized ligands on a polymer molecule is A^- (equivalent to the moles of charged polyelectrolytes, on a monomeric basis in the left compartment of the model depicted in Fig. 2), the moles of electrostatically bound Na^+ may be defined as

$$\vec{N}a^+ = \left[1 - \phi_{p,Na}\right]A^- \tag{8}$$

In a salt-free solution containing a total of A^- moles of ionized ligands on all the polymer molecules, eq. (8) is also valid, since each polymer molecule forms an independent gel domain as depicted by the model in Fig. 2.

The degree of binding has been found to be practically independent of the ionic strength and is determined mainly by the charge density of the polymer.[11,12] Thus, eq. (8) may also apply to a polymer solution containing excess inert salt (such as $NaNO_3$). Likewise, the polymer concentration has little influence on the value of $\phi_{p,Na}$ as long as the polymer concentration remains low. A plot of $\phi_{p,Na}$ versus degree of ionization α for alginic acid is presented in Figure 3. The procedure of calculation, based on the model of rodlike polymer[13] and literature data[14] and our own experimental data for

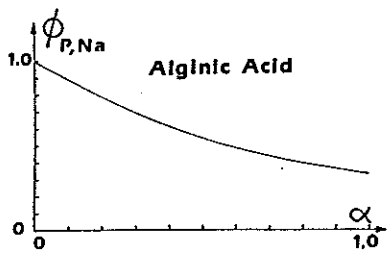

Fig. 3. The practical osmotic coefficient $\phi_{p,Na}$ versus degree of ionization α for alginic acid (sodium form).

$\phi_{p,\mathrm{Na}}$ at $\alpha = 1$, is presented elsewhere.[9,15] Basically, $\phi_{p,\mathrm{Na}}$ is determined by the charge density on the polymer chain, which can be calculated from the effective interligand distance and α.

Iterative Procedure for Calculating Polymer Subphase Volume in the Absence of Bivalent Metal Ions

The polymer subphase volume V_p at different α and I is obtained by an iterative procedure that tests different values of polymer subphase activity ($\vec{\mathrm{Na}}^+$) under different trial values of V_p until the Donnan equilibrium relationship for Na^+ [eq. (6)] is satisfied. This is achieved by first calculating the ionic strength $\vec{I}$ in the polymer subphase using the value of the electrostatically-bound Na^+ obtained in eq. (8), the moles A^- of ionized ligands determined by titration, and a trial value of V_p:

$$\vec{I} = [\vec{\mathrm{Na}}^+ + \mathrm{A}^-]/2V_p \tag{9}$$

The single-ion activity coefficient of Na^+ at ionic strength I (bulk solution) and $\vec{I}$ (polymer subphase) can be obtained from tabulated data[16] or from the mean activity coefficients of NaCl and KCl:[17]

$$[\gamma_{\mathrm{Na}^+}]_{\vec{I}} = [\gamma_{\pm\,\mathrm{NaCl}}]^2_{\vec{I}}/[\gamma_{\pm\,\mathrm{KCl}}]_{\vec{I}} \tag{10}$$

$$[\gamma_{\mathrm{Na}^+}]_I = [\gamma_{\pm\,\mathrm{NaCl}}]^2_I/[\gamma_{\pm\,\mathrm{KCl}}]_I \tag{11}$$

The rationale for using the above two equations is that the sizes of potassium and chloride ions are very close, and, therefore, their single-ion activity coefficients are about the same. Thus, the single-ion activity coefficient can be canceled from the numerators and the denominators of eqs. (10) and (11). However, at high ionic strengths the approach of either Biedermann or Pitzer provides a more reliable estimation of the single-ion activity coefficient of counter ions.[18,19]

If the initial guess of V_p is correct, the Na^+ activity in the polymer subphase will be related to the Na^+ activity in the bulk solution by

$$(\vec{\mathrm{Na}}^+) = (\mathrm{Na}^+)10^{\Delta pK} \tag{12}$$

where, according to eq. (6), $[\Delta pK = p\mathrm{H} - p\vec{\mathrm{H}} = p\mathrm{Na} - p\vec{\mathrm{Na}}]$. Equation (12) can also be expressed explicitly by

$$[\vec{\mathrm{Na}}^+][\gamma_{\mathrm{Na}^+}]_{\vec{I}}/V_p = [C_{\mathrm{Na}^+}][\gamma_{\mathrm{Na}^+}]_I 10^{\Delta pK} \tag{13}$$

where C_{Na^+} is the molar concentration of $NaNO_3$ added to the bulk solution (numerically equal to the ionic strength I of the bulk solution if no other salts are present in significant amounts). If the value of V_p is incorrect, an improved value can be obtained by rearranging eq. (13) to

$$V_p = [\vec{\mathrm{Na}}^+][\gamma_{\mathrm{Na}^+}]_{\vec{I}}/\{[C_{\mathrm{Na}^+}][\gamma_{\mathrm{Na}^+}]_I 10^{\Delta pK}\} \tag{14}$$

By iterating between eqs. (9) and (14), an acceptable V_p can be obtained when the values from two successive iterations converge.

The Partition Coefficient $10^{\Delta pK}$

The factor $10^{\Delta pK}$ which equals the ratio $(\vec{Na}^+)/(Na^+)$ in eq. (12) can be regarded as the partition coefficient for the distribution of monovalent species between the polymer subphase and the bulk solution. It can be shown that ΔpK also equals $pK_{HA}^{app} - pK_{HA}^{int}$, where K_{HA} is the acid dissociation constant of the polymer, and the subscripts app and int refer to apparent and intrinsic constants, respectively.

For the dissociation reaction $HA = H^+ + A^-$, K_{HA}^{app} based on the pH of the bulk solution can be described by

$$pK_{HA}^{app} = pH - \log\{\alpha/[1 - \alpha]\} \tag{15}$$

where

$$\alpha = A^-/A_t$$

$$= \left\{m_b + \left[V_s 10^{-pH}/\gamma_{H^+}\right]\right\}/m_{b,ep} \tag{16}$$

where m_b is the moles of NaOH added to reach any point in the titration of the polymer, V_s is the volume of the solution, and γ_{H^+} is the activity coefficient of H^+ (0.914, 0.830, 0.831, and 0.883 at ionic strengths of 0.01, 0.1, 0.3, and 0.5 M, respectively). In eq. (16) the moles of ionized ligands equal the moles of ligands neutralized by the added NaOH plus the moles of self-dissociated H^+; and the moles $m_{b,ep}$ of NaOH added to reach the endpoint of titration is taken as the moles A_t of total ionizable ligands.

Similarly, the intrinsic dissociation constant K_{HA}^{int} based on the pH of the polymer subphase can be described by

$$pK_{HA}^{int} = p\vec{H} - \{\alpha/[1 - \alpha]\} \tag{17}$$

Subtracting eq. (17) from eq. (15) yields

$$\Delta pK = pK_{HA}^{app} - pK_{HA}^{int} = pH - p\vec{H} \tag{18}$$

which also equals pNa $- p\vec{N}a$. Thus, the partition coefficient $10^{\Delta pK}$ for Na^+ at any α and I used in the iterative procedure can actually be estimated from the result of base titration of the polymer solution.

The value of pK_{HA}^{int} is identical to the pK_{HA} obtained from a polymer titration experiment that has been performed at a sufficiently high ionic strength to yield a constant value at different α. From a thermodynamic viewpoint, the deviation of K_{HA}^{app} (at some lower ionic strength) from K_{HA}^{int} is related to the change in free energy accompanying the ionization process. This free energy is equal to the work of moving a proton from an ionizable site in the polymer subphase across the hypothetical membrane into the bulk solution. In the ionization process, additional energy will be required if the

electrostatic field due to the ionized ligands is not effectively "blocked" or "neutralized" by electrolytes.

Effect of Trace Bivalent Metal Ions

The iterative procedure must be modified when a trace amount of n-valent metal ions such as Cu^{2+} is present in a solution which contains a polymer such as alginic acid with a strong metal chelating capacity. Under these circumstances, the majority of Cu^{2+} ions entering the polymer subphase is likely to be bound to sites on the ligand, with only a small fraction of the Cu^{2+} bound electrostatically. Cu^{2+} that interacts with the polymer occupies some of the deprotonated sites, so that the moles of unoccupied ionized ligands cannot be calculated from the pH change alone. Also, the Donnan equilibrium relationship for both electrostatically bound Na^+ and Cu^{2+} between polymer subphase and the bulk liquid phase must be satisfied simultaneously. By comparing V_p at any α between two cases (in the absence and presence of trace n-valent metal), it will be shown that trace n-valent metal ions may serve as excellent monitors for the change in the morphology of the polymer due to metal binding.

At any point in the titration, the moles of electrostatically bound Na^+ may be calculated from eq. (8), in which

$$A^- = \left\{m_b + \left[V_s 10^{-pH}/\gamma_{H^+}\right]\right\} - \vec{C}u_{sb} \tag{19}$$

where $\vec{C}u_{sb}$ is the moles of site-bound copper, which may be determined by subtracting the moles of free metal ion from the total moles of metal ion added.

The iterative procedure starts with an assumed value of V_p and an assumed value for the moles of electrostatically bound $\vec{C}u^{2+}$, which should be many times smaller than the total moles of $\vec{C}u_{sb}$ bound (to be validated by the iterative procedure). The ionic strength $\vec{I}$ in the polymer subphase is given by

$$\vec{I} = \left[4\vec{C}u^{2+} + \vec{N}a^+ + A^- + \vec{C}uA^+\right]/2V_p \tag{20}$$

where $\vec{C}uA^+$ is the moles of monodentate site-bound copper, which is assumed to be approximately equal to the moles of site-bound metal in a solution of dilute polymer containing trace bivalent metal. [The same assumption is used in eq. (19). In reality, the moles of monodentate complex and moles of bidentate complex have to be calculated from their respective binding stability constants, which are yet to be determined. However, since copper is present only in a trace amount, no significant error will be caused if the assumption about the type of copper complex is incorrect when evaluating I.] Then, the single-ion activity coefficients of Na^+ in the polymer subphase and the bulk solution can be obtained from the tabulated data or calculated by eqs. (10) and (11). Similarly, the single-ion activity coefficients Cu^{2+} can be obtained from the mean activity coefficients of $CuCl_2$ and KCl:

$$[\gamma_{Cu^{2+}}]_{\vec{I}} = [\gamma_{\pm CuCl_2}]^3_{\vec{I}}/[\gamma_{\pm KCl}]^2_{\vec{I}} \tag{21}$$

$$[\gamma_{Cu^{2+}}]_I = [\gamma_{\pm CuCl_2}]^3_I/[\gamma_{\pm KCl}]^2_I \tag{22}$$

The Donnan relations for the activity of Cu^{2+} is examined by using eq. (23)

$$(\vec{Cu}^{2+}) = (Cu^{2+})10^{2\Delta pK} \tag{23}$$

which can be rearranged to

$$[\vec{Cu}^{2+}] = V_p[C_{Cu^{2+}}][\gamma_{Cu^{2+}}]_I 10^{2\Delta pK}/\{[\gamma_{Cu^{2+}}]_{\vec{I}}\} \tag{24}$$

where $C_{Cu^{2+}}$ is the free cupric ion concentration in the bulk solution.

If eq. (23) does not hold, eq. (24) can be used to generate an improved value of $\vec{Cu}^{2+}$, and iteration is performed between eqs. (20) and (24) until values of $\vec{Cu}^{2+}$ from successive trials converge for the initial guess of V_p. Finally, the Donnan relation for Na^+ is examined by using eq. (13). If eq. (13) does not hold, an improved value of V_p can be generated using eq. (14), and the whole procedure is iterated until both V_p and $\vec{Cu}^{2+}$ from successive trials converge.

Potentiometric titration of the alginic acid solution in the presence as well as in the absence of trace Cu^{2+} can provide data necessary for testing the aforementioned iterative procedure.

EXPERIMENTAL

Biopolymer

Alginic acid (type III, A7003, Sigma Chemical Co., St. Louis, MO) extracted from kelp was used in the experiment as received. To examine the effects of polymer concentration and ionic strength, 0.0083 g or 0.0400 g of alginic acid was added to 10 mL of double-distilled water with ionic strength adjusted to 0.01, 0.1, 0.3, or 0.5 *M* $NaNO_3$.

Instrument

A *p*H/Ion meter (Accumet 825 MP, Fisher Scientific) controlled by a five-position switch box (Model 753, Fisher Scientific) was used to display *p*H (measured with a microglass electrode; AccupHast 13-639-280, Fisher Scientific) and free cupric ion concentration (measured with a cupric ion electrode; 94-29, Orion).

Base Titration of Alginic Acid in the Absence of Cupric Salt

The alginic acid solution was titrated with standard NaOH with a concentration identical to the ionic strength of the solution. The alginic acid solution was maintained at 25°C in a polypropylene vial in a water bath. The solution was blanketed with N_2 to prevent interference from CO_2 in the ambient air.

Titration of Alginic Acid with Cupric Sulfate: The First Kind of Titration

The alginic acid solution with ionic strength adjusted to 0.01 or 0.1 *M* ($NaNO_3$) was titrated with $CuSO_4$ until the total moles of $CuSO_4$ added reached about 5–7% of the total moles of ligands (determined from base

titration). The pH and free Cu^{2+} concentration were monitored alternatively by switching the electrode position on the switch box following each addition of the $CuSO_4$ solution.

Titration of Copper-containing Alginic Acid Solution with Standard Base: The Second Kind of Titration

Each alginic acid sample at the completion of the first kind of titration was continually titrated with standard NaOH until the pH reached 6.5. (At higher pH, the free Cu^{2+} concentration does not reflect the level of unbound Cu^{2+} because of the formation of $Cu(OH)_2$.) Under these conditions, pH increased while free Cu^{2+} decreased, because the moles of ionized ligands available for binding copper increased with added NaOH.

RESULTS

The curve of pH versus moles of NaOH added to each alginic acid sample in the absence of $CuSO_4$ had only one inflection point. The alginic acid used in this work contained 4.32×10^{-3} moles of titratable ligands per gram of dry material as calculated from the moles of NaOH needed to neutralize the sample. The values of pK_{HA}^{app} and α at each point of titration (Fig. 4) were calculated with eqs. (15) and (16). The plot of pK_{HA}^{app} versus α at $I = 0.5\ M$ fits a horizontal line which yields pK_{HA}^{int} value of 2.96 for alginic acid used in this work. (This result shows that a salt concentration level of 0.5 M is enough to eliminate the effect of a Donnan potential). The plots for different I do not converge to the intrinsic value at $\alpha = 0$, indicating that the alginic acid molecules form colloids and therefore should be viewed as a separate phase in the solution. From Figure 4 it can be observed that the ΔpK increased with increasing α and decreasing I.

The volume of the polymer subphase in the absence of copper is calculated by the iterative procedure eqs. (9)–(14) and presented in Figure 5 (0.083 g alginic acid/dL) and Figure 6 (0.400 g/dL). In Figures 5 and 6 the value of V_p

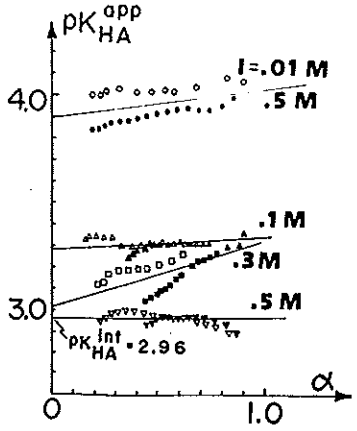

Fig. 4. Plots of pK_{HA}^{app} of alginic acid versus α at different ionic strengths (in $NaNO_3$) and alginic acid concentrations (solid symbols: 0.083 g/dL solution; open symbols: 0.400 g/dL solution).

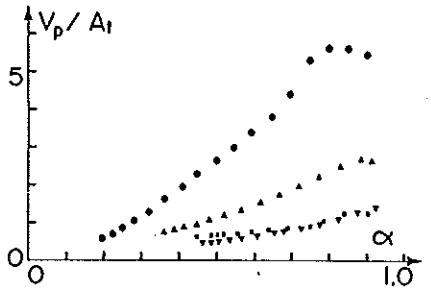

Fig. 5. The unit polymer-subphase volume V_p/A_t versus α for alginic acid solution of 0.083 g/dL in concentration without trace cupric salt added. The legends are the same as in Figure 4.

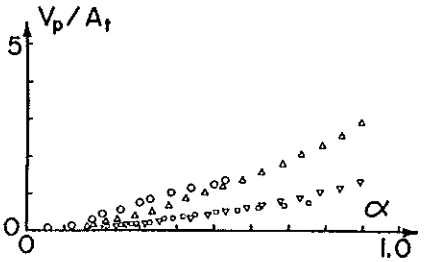

Fig. 6. The unit polymer-subphase volume V_p/A_t versus α for alginic acid solution at concentration 0.400 g/dL, without trace cupric salt added.

is normalized with respect to moles of total ionizable ligands A_t in each sample. The data demonstrate that the normalized polymer subphase volume V_p/A_t and its sensitivity to α increase with decreasing I. By comparing Figures 5 and 6 it is apparent that at each of the higher ionic strengths (0.1, 0.3, and 0.5 M $NaNO_3$), the curves for the two different alginic acid concentrations correspond closely with one another. However, at the lower ionic strength (0.01 M $NaNO_3$), the normalized polymer subphase volume for the higher alginic acid concentration is significantly lower than that for the lower alginic acid concentration.

The polymer subphase volume in the presence of trace amounts of copper, using data of the first kind and the second kind of titration, is presented in Figures 7 (alginic acid concentration 0.083 g/dL) and 8 (0.400 g/dL). Comparing curves of the same ionic strength in Figures 5 and 7 (and also in Figs. 6

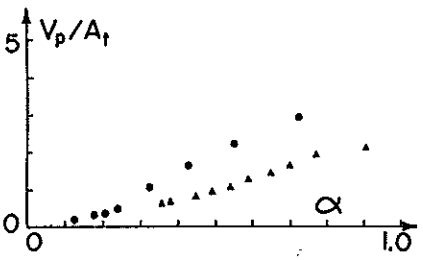

Fig. 7. The unit polymer-subphase volume V_p/A_t versus α for alginic acid solution, 0.083 g/dL, with trace cupric sulfate (5–7% of total ionizable ligands).

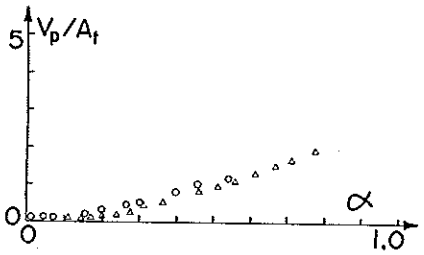

Fig. 8. The unit polymer-subphase volume V_p/A_t versus α for alginic acid solution, 0.400 g/dL, with trace cupric sulfate (5–7% of total ionizable ligands).

and 8), it is observed that the presence of trace copper causes a reduction in the normalized polymer-subphase volume. The number of moles of electrostatically bound cupric ions is less than 1% of site-bound cupric ions as determined by the iterative procedure.

DISCUSSION

The partition coefficient for the activity of a counterion between the polymer subphase and the bulk solution, $10^{n\Delta pK}$ ($n = 1$ for Na^+ and H^+; $n = 2$ for Cu^{2+}), is substantially greater than 1.0, especially at low ionic strengths and high degrees of ionization as suggested by Figure 4. Thus, the activity of a counterion in the polymer subphase, where binding reactions actually occur, can be many times higher than the activity in the bulk solution.

According to Figure 5, the greatest normalized polymer-subphase volume achieved with an alginic acid solution of 0.083 g/dL was 5.8 ml/mmol ionizable ligand when $\alpha = 0.8$ and $I = 0.01$ M. The corresponding V_p may therefore be calculated as 5.8 ml/mmol-ligand × 4.32 mmol-ligand/g × 0.0083 g = 0.21 ml, or 2.1% of the solution volume. Similarly, from Figure 6, the greatest normalized polymer subphase volume achieved with an alginic acid solution of 0.400 g/dL is 3.2 ml/mmol, yielding a V_p of 0.55 ml which is 5.5% of the solution volume. Therefore, the volume of polymer subphase at every point of titration only accounts for a small fraction of the solution volume. In other words, the density of ligands, associated or ionized, based on this polymer subphase volume should be many times larger than the ligand density based on the solution volume.

Based on the above discussion, the intrinsic metal binding constants of a polymer should be defined according to the counterion activity and the ligand density in the polymer subphase instead of bulk solution.[15]

Application of the iterative procedure to solutions of alginic acid demonstrated that the normalized polymer subphase volume varies according to the degree of polymer ionization, the amount of metal bound, ionic strength, and polymer concentration. Comparing the top curves in Figures 5 and 6, the alginic acid molecule is apparently more coiled (as indicated by the lower normalized polymer subphase volume) at the higher polymer concentration when the ionic strength is 0.01 M. Comparing the curves in Figures 5 and 7 as well as those Figures 6 and 8, the reduction in normalized polymer subphase

volume in the presence of copper is likely due to intramolecular and intermolecular cross-linking of the polymer chains by cupric ion.

Estimation of the volume of the polymer subphase made possible by the iterative procedure described here may serve as a useful index of polymer morphology. It is reasonable to assume that the volume of the polymer subphase increases with increasing repulsion among the charged ligands (and as a result, more expanded, less coiled colloids are formed). It should be possible to use rheological measurements to determine how the polymer morphology (which can be deduced from the shear stress measured at different shear rates) varies with the volume of polymer subphase calculated in this work. This approach appears to be well-suited to studying morphological changes in colloids that occur when the conditions of the bulk solution change.

Unlike other approaches such as the condensation theory,[20] the procedure described here is based purely on the potentiometric titration data and empirically derived osmotic coefficient; it does not require a complex mathematical model and a detailed knowledge of the structure and morphology of the polymer. The iterative approach could prove advantageous when characterizing complex biopolymers and their degradation products, whose structures are not well understood.

The possibility of invasion of neutral salt such as $NaNO_3$ in the polymer subphase was not taken into account in the iterative procedure illustrated above. It is reasonable to ignore neutral salt invasion when the electrostatic field is strong enough to repel negatively charged chloride ion (and the accompanying sodium ion from the solution). However, at low degrees of ionization and/or high ionic strengths of the solution, a significant amount of $NaNO_3$ could enter the polymer subphase. In this case, we can let $\overrightarrow{NaNO_3}$ be the moles of sodium nitrate entering the polymer subphase. Equation (9) should then be modified to

$$\vec{I} = \left[\vec{Na}^+ + A^- + 2\overrightarrow{NaNO_3}\right]/2V_p \tag{25}$$

where $\overrightarrow{NaNO_3}$ can be estimated from Donnan relation

$$\left\{\left[(1 - \phi_{p,Na})A^- + \overrightarrow{NaNO_3}\right]/V_p\right\}\left\{\overrightarrow{NaNO_3}/V_p\right\}$$

$$= \left[C_{NaNO_3}(\gamma_{\pm NaNO_3})\right]_I^2/\left[\gamma_{\pm NaNO_3}\right]_{\vec{I}}^2 \tag{26}$$

The two terms on the left-hand side of eq. (26) are the concentrations of sodium and nitrate ions, respectively, in the polymer subphase. In addition to V_p, the iterative procedure now involves a second variable, the moles $\overrightarrow{NaNO_3}$ entering the polymer subphase for testing the convergence of the iterative procedure. A partial listing of the results of calculation is given in Table I. It is shown that the extent of neutral salt invasion, as compared with the moles of electrostatically bound Na^+, is significant at higher ionic strengths of 0.5 *M* and 0.3 *M* over the whole range of degree of ionization. When the ionic strength is 0.1 *M*, the extent of salt invasion is only significant at low degrees of ionization. Very little neutral salt enters the polymer subphase at the low ionic strength of 0.01 *M*. In all cases, the values of V_p calculated are, however,

TABLE I
Effect of Neutral Salt Invasion on the Value of V_p

Ionic strength (M)	Degree of ionization	Sodium bound electrostatically (mmol)[b]	$NaNO_3$ entering polymer subphase (mmol)[b]	V_p/A_t[a] (ml/mmol)	
				Salt invasion considered	Not considered
0.5	0.29	8.3×10^{-3}	6.2×10^{-3}	0.112	0.108
	0.68	4.5×10^{-2}	3.7×10^{-2}	0.772	0.767
0.3	0.28	8.0×10^{-3}	4.5×10^{-3}	0.141	0.139
	0.68	4.5×10^{-2}	3.1×10^{-2}	1.04	1.05
0.1	0.29	8.3×10^{-3}	1.7×10^{-3}	0.218	0.218
	0.70	5.1×10^{-2}	1.3×10^{-2}	1.74	1.76
0.01	0.27	7.3×10^{-3}	1.0×10^{-4}	0.481	0.481
	0.70	4.9×10^{-2}	6.1×10^{-4}	3.51	3.51

[a] Volume of polymer subphase per millimole of titratable ligand when the concentration of copper-free alginic acid solution is 0.4 g/dL.
[b] Millimoles per 0.04 g alginic acid.

affected very slightly when the neutral salt invasion is considered.

CONCLUSIONS

1. The alginic acid colloids should be viewed as a separate phase in the aqueous solution.
2. The small aqueous region surrounding the charged polymer chain where a strong electrostatic attractive force for counterions exists should be regarded as a polymer subphase within the colloidal phase. Actual acid dissociation and metal binding reactions take place in this polymer subphase.
3. The iterative procedure based on Donnan equilibrium theory and potentiometric titration data provides a means to estimate the polymer-subphase volume at different ionic strengths, degrees of ionization, extents of metal binding, and polymer concentrations.
4. The polymer-subphase volume not only defines the reaction zone for acid dissociation and metal binding reactions but also serves as an index for the morphology of the polymer colloids in the aqueous solution.

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