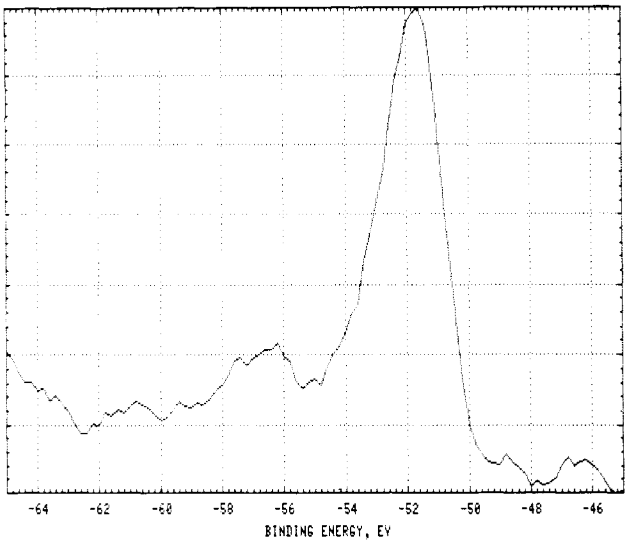

Figure 4. Li(I) XPS region after depth profiling of BTDA-ODA-LiCl film after 30 min.

decomposition were occurring during X-ray photoelectron spectroscopic examination. It is reasonable, therefore, to conclude that lithium oxide may be generated during thermal imidization via Li^+ reaction with the released water, followed by hydrolysis and dehydration. Similar lithium XPS observations have been made on films doped with Li_2PdCl_4. The peak at ~55.0 eV, which appears only at high lithium concentrations, arises when, no doubt, insufficient water of imidization is released to solvate all the lithium ion.

The nature of lithium on the film surface compared to the bulk is of interest. In other words, is the "lithium oxide-like" material dispersed throughout the film rather than on the film surface? First of all, the atmosphere side and glass side of each film show a comparable number of electron counts for lithium (Table II). This observation is contrary to our palladium findings in that the atmosphere side of the film contains appreciably more palladium than the glass side of the film. Information regarding the lithium content and chemical state inside the lithium-doped film has been obtained from angular and depth profile studies. At a 90° X-ray incidence angle, the spectrum of highly doped BTDA-ODA-LiCl is as per Figure 2b. On shifting to an incidence angle of 11°, the secondary peak is diminished significantly relative to the main peak. This suggests that "LiCl" is found only in the matrix of the polymer, whereas, "Li_2O" occupies the surface as well as the bulk. On depth profiling normally doped BTDA-ODA-LiCl for 30 min (argon ion sputtering), the shoulder at 55.0 eV, which originally could not be discerned, has grown to a distinct peak (Figures 3 and 4). The ratio of "LiCl" to "Li_2O" through the film appears to be a constant since spectra after 60- and 90-min sputtering are similar. One concludes, therefore, that "LiCl" is present in small quantities and that most has been converted into "Li_2O" during thermal imidization.

The primary question left unanswered is that of why some films conduct and others do not, when they apparently are identical in all aspects except monomer pair combinations. That there is some electrical conduction is realized due to the differences of volume resistivities measured. The final answer probably lies in an understanding of the mode of conduction in these lithium-doped films. It is reasonable to conclude that the quantity of Li(I) and consequently, its manner of distribution in the bulk of the polyimide films play pivotal roles in addressing this question.

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Polyelectrolyte Properties of Sodium Hyaluronate. 1.[†] Salt Exclusion in Sodium Chloride Solution

Robert L. Cleland

Department of Chemistry, Dartmouth College, Hanover, New Hampshire 03755.
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ABSTRACT: Experimental data from dialysis equilibrium on exclusion of NaCl from solutions of sodium hyaluronate are examined in terms of the Poisson–Boltzmann treatment of the uniformly charged rigid-rod model for polyions in excess salt. Values of the exclusion coefficient are fitted reasonably well by this model, although uncertainty in the data permits an estimate of the rod radius a only in the approximate range $a = 3.5$ Å (from the polymer molal volume) to $a = 7.5$ Å (from the estimated distance of closest approach of polyion and counterion).

The interpretation of data from the potentiometric titration of solutions of hyaluronic acid in the following paper[1] involved a consideration of polyion–small-ion interactions in polyelectrolyte solutions. The study of this problem led to a reexamination of existing data from dialysis equilibrium on salt exclusion[2-4] in solutions of sodium hyaluronate with NaCl. In this work the consequences of the treatment of salt exclusion by the Pois-

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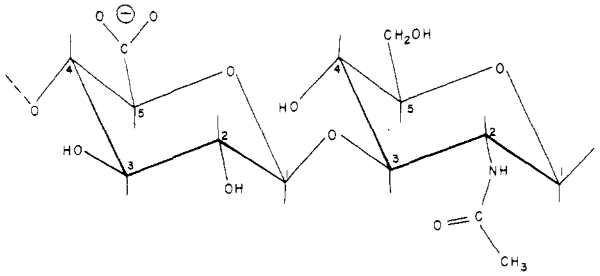

Figure 1. Disaccharide repeating unit of hyaluronate ion.

son–Boltzmann theory applied to the uniformly charged rigid-cylinder model of the polyion[5] are examined in the context of the thermodynamics of multicomponent solutions.[6,7]

The three-component system to be considered is defined in terms of the following choice of components: 1, the solvent, here H_2O; 2, the polymer (hyaluronate) ions with their accompanying counterions (Na^+); 3, the added electrolyte, here NaCl.

At osmotic pressure (or dialysis) equilibrium the solvent compartment consists of components 1 and 3 at concentrations C_i^* on the molar (mol L^{-1}) scale or m_i^* on the molal (moles of i per kilogram of component 1) scale. The equilibrium solution contains, in addition, component 2 and has concentrations C_i (or m_i), where C_2 (or m_2) is in terms of moles of monomer. The latter is taken as the chain repeating unit, of molecular weight M_2 (401), which is the disaccharide (see Figure 1) consisting of one residue of D-glucuronic acid (neutralized to carry one negative charge on a carboxylate ion with an accompanying counterion) β-glycosidically linked to carbon 3 of an N-acetylglucosamine residue, which is similarly linked to carbon 4 of the D-glucuronic acid of the next unit. The number of monomers in a polyion (degree of polymerization) is called N_2. With this definition C_2 is also the equivalent concentration. The absolute temperature T is taken constant and the pressures are written p^* and $p = p^* + \pi$, where π is the osmotic pressure of solution against solvent.

Salt Exclusion

The equilibrium distribution of the diffusible salt component 3 may be expressed by the expansion[5]

$$C_3/C_3^* = 1 + A_1(C_2/C_3^*) + A_2(C_2/C_3^*)^2 + ... \quad (1)$$

where the A_i are virial coefficients for the interactions of components 2 and 3. For a three-component system A_1 may be written following Casassa and Eisenberg[6] (see their eq 3.16) at the limit $C_2 = 0$ (represented by superscript zero)

$$-A_1 = (a_{32}^0/a_{33}^0)(1 - m_3^*\bar{V}_3^0/V_m^0) + \bar{V}_3^0/(V_m^0 a_{33}^0) + \bar{V}_2^0 m_3^*/V_m^0 \quad (2)$$

where

$$a_{32} = (\partial \ln a_3/\partial m_2)_{T,p,m_3} = (\partial \ln a_2/\partial m_3)_{T,p,m_2} = a_{23} \quad (3)$$

$$a_{33} = (\partial \ln a_3/\partial m_3)_{T,p,m_2} = (2/m_3)(1 + f_{33}) \quad (4)$$

where a_2 and a_3 are component activities, $\bar{V}_2$ and $\bar{V}_3$ are component partial molal volumes, and V_m^0 is the volume of solution containing 1 kg of component 1. The last equality of eq 4 makes use of the usual definition of the activity of a salt with singly charged ions, so that $f_{33} \equiv (\partial \ln \gamma_\pm/\partial \ln m_3)_{T,p,m_2}$, where $\gamma_\pm$ is the mean activity coefficient on the molal concentration scale. The limiting value a_{33}^0 is $2/m_3^*$, and the quantity $m_3^*\bar{V}_3^0$ is only about 2% of V_m^0 for a 1 m solution of NaCl. By anticipation of the experimental result $-A_1 = a_{32}^0/a_{33}^0 \simeq 0.3$ at small m_3^*, it is seen that the small terms containing $\bar{V}_3^0$ in eq 2 partly compensate and can be dropped at all m_3^* of interest to give, to a good approximation,

$$-A_1 = \frac{m_3^*(\partial \mu_2^0/\partial m_3^*)_{T,p}}{RT[2(1 + f_{33}^0)_{T,p}]} + \bar{V}_2^0 m_3^*/V_m^0 \quad (5)$$

In eq 5, $RT \ln a_2 \equiv \mu_2 - \mu_2^0$, where R is the molar gas constant and μ_2 and μ_2^0 are the molar chemical potential of component 2 and its value in the standard state at infinite dilution in solvent (m_3^*), respectively. The first term on the right-hand side of eq 5, to be called $-A_1^{el}$ for the electrical part of $-A_1$, may be interpreted in terms of a theoretical expression for μ_2^0 as a function of m_3^*. While the second term of eq 5 occurred thermodynamically only because eq 1 was expressed in volume concentrations C_i rather than molalities,[6,7] it will be referred to here as $-A_1^{ex}$ because it has the form of an excluded volume term. In the context of the rigid circular cylinder model discussed below $\bar{V}_2^0$ may be interpreted as the molar volume of the monomer regarded as a cylindrical element. Additional excluded volume is introduced if the cylinder radius a is taken as a distance of closest approach of, for example, polyion and small ion, when $\bar{V}_2^0$ is replaced in $-A_1^{ex}$ by $\bar{V}_2'$, the mutual excluded volume, given by

$$\bar{V}_2' = \pi a^2 N_A L/N_2 \quad (6)$$

where L is the length of the polyion cylinder and N_A is Avogadro's number. A similar expression can be obtained from the treatment of exclusion of uncharged solutes by hyaluronate chains regarded as fibers. Laurent[9] applied Ogston's expression[10] for the probability P of placing a spherical particle of radius r_s in a system of noninterfering fibers of radius r_r, $P = \exp(-\bar{V}_2'C_2/1000)$ with $a = r_r + r_s$. When P is taken as C_3/C_3^*, expansion of the exponential and comparison with eq 1 gives $-A_1^{ex}$ with $\bar{V}_2'$, since $C_3^*/1000 = m_3^*/V_m^0$.

The usual approach to interpretation of the thermodynamic behavior of polyelectrolyte solutions involves an application of the Poisson–Boltzmann equation originally proposed by Debye and Hückel. A fairly successful model of the polyion for this purpose consists of a long circular cylinder with a uniform continuous (smeared) distribution of surface charge. Numerical integration of the Poisson–Boltzmann equation has been carried out for such a model by several authors[8,11-13] and the errors in its use have been examined by Fixman.[14] A formulation which is particularly convenient to use was given by Stigter,[8] who calculated the free energy μ^{el} per mole of counterions for charging the cylinder of length L and radius a at infinite dilution (m_3^*) using the electrostatic potential obtained from such a numerical integration. The result (see eq 23 of ref 8) is

$$\mu^{el}/RT = \xi K_0(\kappa a)/\delta \kappa a K_1(\kappa a) \quad (7)$$

where $K_0(\kappa a)$ and $K_1(\kappa a)$ are modified Bessel functions of the second kind evaluated at κa, with κ^{-1}, the Debye length, defined as usual by

$$\kappa^2 = \lambda \sum_i C_i \quad (8)$$

where $\lambda = 4\pi N_A e^2/10^3 DkT$, D is the bulk solvent dielectric constant, k is Boltzmann's constant, e is the electronic charge, and the sum in eq 8 is taken over the mobile small ions in the solution. The charge density parameter ξ is defined by

$$\xi \equiv e^2 Q/DkTL \quad (9)$$

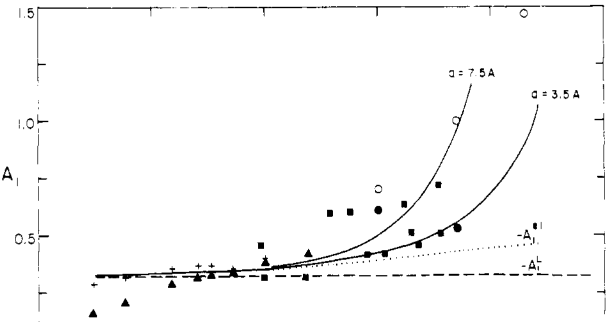

Figure 2. Salt exclusion coefficient $-A_1$ obtained from dialysis equilibrium data for sodium hyaluronate in NaCl solution by measurement of ^{22}Na concentration[4] (▲) and refractive index increments (■, ref 2; ○, ref 3), the latter being corrected to (●) as described in the text. The filled points are compared with theoretical results from the line charge theory[16] ($-A_1^L$) and the uniformly charged rigid-rod model[8] of radii a = 3.5 Å (corresponding to the partial molal volume of the polymer) and a = 7.5 Å (corresponding to an estimate of distance of closest approach of polyion and counterion). The dotted line $-A_1^{el}$ represents the first term in eq 11 (for a = 7.5 Å) and is added to the excluded volume terms to obtain the solid curves as given by eq 11. Crosses are values of $(C_3^*/C_2) \ln (C_3^*/C_3)$ calculated from data of ref 4 (see text).

where Q is the number of charges (in electron units) in length L. The quantity δ was tabulated by Stigter[8] as a correction to the corresponding Debye–Hückel linearization approximation to eq 7, for which $\delta = 1$. The standard-state chemical potential may be written[7]

$$\mu_2^0/RT = \mu_2^{00}/RT + \ln m_3^* + \mu^{el}/RT \qquad (10)$$

where μ_2^{00} is the standard-state potential excluding the polyion–small-ion interaction and mixing terms (at m_3^*) explicitly appearing in eq 10.

Evaluation of the derivative in the numerator of eq 5 with the use of eq 7 and 10 gives

$$-A_1 = -A_1^{el} - A_1^{ex} = \frac{1 + (\xi/2\tau)[(K_0^2/K_1^2) - 1]}{2(1 + f_{33}^0)} + \frac{\bar{V}_2'C_3^*}{1000} \qquad (11)$$

where τ is a numerical correction, which may be computed[15] from the tabulated results of Stigter.[8] The line charge theory result[16]

$$-A_1^L = (1 - \xi/2)/2 \qquad (\xi < 1) \qquad (12)$$

is recovered from eq 11 as m_3^* (and κ) approaches zero, provided that τ approaches unity, as calculations from the tabulations of Stigter indicate for the appropriate value of ξ. The latter was taken equal to 0.70, appropriate at 25 °C for a length of 10.2 Å for the disaccharide repeating unit.[17] In fact, τ does not exceed about 1.06 at any value of κa and approaches unity for both large and small κa.

Comparison with Experimental Data

Preston et al.[4] investigated dialysis equilibrium for a deproteinized sample of sodium hyaluronate extracted from bovine synovial fluid by equilibrating with NaCl solutions containing ^{22}Na, whose isotopic concentration was used as an assay for Na$^+$ concentration. The values of $-A_1$ (their Γ) = $(C_3^* - C_3)/C_2$ (for $A_2 = 0$) are plotted as the triangles in Figure 2 against $\log C_3^*$. Other data[2,3] from dialysis equilibrium were originally reported in terms of the increment dn/dc_2 of the refractive index n with weight concentration c_2 (g cm^{-3}) for both dialyzed and undialyzed samples. Values of $-A_1$ (again for $A_2 = 0$) may be estimated from such data by the method of Cleland[3] (whose δ is the present A_1), which is equivalent to the result of Casassa and Eisenberg[6] (their eq 4.68) to the good approximation of neglecting terms of the order of volume fractions of solutes, as in eq 5. The experimental values of Cleland (open circles) were not corrected for the change with C_3 of $(\partial n/\partial c_2)_{T,p,C_3}$ observed by Preston et al.[2] Application of their correction procedure leads to the $-A_1$ values shown as filled circles at 0.1 and 0.5 M NaCl. At 2 M NaCl overcorrection evidently occurs, since the corrected value of $-A_1$ (not shown) becomes negative, which seems unlikely; in any event, the experimental correction was determined[2] only up to C_3 = 0.35 M and extrapolation to 2 M solutions is not warranted empirically.

Also plotted in Figure 2 are the line charge result of eq 12 and the theoretical curve for $-A_1^{el}$ calculated from a slightly modified version of eq 11. The modification involved addition of a term $\ln \gamma_2$ to μ_2^0/RT as given by eq 10, where γ_2 is an activity coefficient resembling those of the Debye–Hückel treatment of singly charged ions. This term, which does not appear in the Poisson–Boltzmann treatment of the rigid cylinder, has been included to allow for the observed effect of ionic strength on the apparent ionization constants of hyaluronic acid at zero degree of ionization.[1] This modification to eq 10 requires addition of a quantity $f_{23}^0 \equiv (\partial \ln \gamma_2/\partial \ln m_3^*)_{T,p}{}^0$ resembling numerically f_{33}^0 in the numerator of the first term of eq 11. In calculating the curve $-A_1^{el}$ the approximations $\tau = 1$ and $f_{23}^0 = f_{33}^0 = 0$ were used with small error, since the various small corrections tend to compensate each other. The curve shown was calculated for a = 7.5 Å; doubling or halving a merely shifts the calculated curve along the $\log C_3^*$ axis by $\log 2^{1/2} = 0.15$ unit to the left or right, respectively (since κ is reciprocal to a at fixed κa), which has little effect on its magnitude. The result is thus not very sensitive to the choice of the cylinder radius.

The solid lines for the indicated values of a represent both terms in eq 11. The smaller value of a corresponds to $\bar{V}_2' = \bar{V}_2^0$, as in eq 5. The partial molal volume $\bar{V}_2^0 = \bar{v}_2^0 M_2$ was assumed to be independent of C_3^* for this purpose and was taken to be 224 cm^3 mol^{-1}, based on the experimental value[18] for the partial specific volume $\bar{v}_2^0$ of 0.55$_7$ cm^3 g^{-1}. This value of $\bar{V}_2^0$ substituted into eq 6 gives a = 3.5 Å, very similar to that estimated[19] from the experimental crosssectional radius of gyration from small-angle X-ray scattering. The value a = 7.5 Å is a reasonable estimate of the distance of closest approach of the polyion and a Na$^+$ counterion.[1] The excluded volume clearly leads to large increase of $-A_1$ at larger C_3^*. This experimental effect has been reported previously for DNA[20] and polyphosphates[21] and interpreted in terms of the similar theoretical treatment of $-A_1$ of Gross and Strauss.[12]

Discussion

The expression for $-A_1$ given by eq 12 is a limiting law result and can therefore not be expected to be valid to C_3^* above about 0.01 M. The data of Figure 2 are probably fitted within the rather large experimental uncertainty at C_3^* values within the range of its validity by eq 12, as well as by the nearly equivalent result (eq 11) from numerical integration. Above about 0.05 M salt the latter clearly provides a better fit. While the data lack sufficient precision to permit a choice of radius a for the cylinder model or to distinguish between the alternative forms for $-A_1^{el}$, the absolute values of $-A_1$ are in reasonable agreement with the theoretical models used. More precise experimental data, preferably obtained with the use of an isotope in the salt component, are clearly desirable.

At low salt concentrations, especially below 0.001 M, values of $-A_1$ calculated with the assumption $A_2 = 0$ fall below the limiting law value. While experimental errors are probably largest on a fractional basis in this concentration region, the effect is consistent. Since the points in question were obtained at $X > 1$, and the largest deviations occur at largest X, where $X \equiv C_2/C_3$, the question arises whether higher terms in eq 1, which is a power series in XC_3/C_3^*, should not be allowed for in calculating A_1. It is of possible interest in this connection that values of $(C_3^*/C_2) \ln (C_3^*/C_3)$, plotted as crosses in Figure 2, are rather close to the limiting theoretical value of $-A_1$. In fact, Scatchard et al.[22] used a similar function to represent salt distribution data for serum albumin in NaCl solution. There seems to be no theoretical basis for such use, nor, as Casassa and Eisenberg point out,[6] does the logarithmic function provide a better fit to the data than the linear first term $(C_3^* - C_3)/C_2$.

To examine whether the higher order terms in eq 1 might justify such a representation, the suggested equality is expanded in powers of $(C_3^* - C_3)/C_2$

$$-A_1C_2/C_3^* = -\ln (C_3/C_3^*) = \ln \left(1 - \frac{C_3^* - C_3}{C_3^*}\right) = \frac{C_3^* - C_3}{C_3^*} + \frac{1}{2}\left(\frac{C_3^* - C_3}{C_3^*}\right)^2 + \ldots = \left(\frac{C_3^* - C_3}{C_3^*}\right) + A_2\left(\frac{C_2}{C_3^*}\right)^2 + \ldots \qquad (13)$$

where the last equality results from eq 1. Solving for $[(C_3^* - C_3)/C_3^*]^2$ from eq 1, substitution into eq 13, and comparison of coefficients of $(C_2/C_3^*)^2$ give $A_2 = A_1^2/2$ as the requirement for the correctness of eq 13 to two terms.

Stigter[7] has given the thermodynamic relations between the virial coefficients for salt distribution and those for the osmotic pressure Π. The usual expansion for Π in powers of the weight concentration c_2 is

$$\Pi/RT = (N_2M_2)^{-1}c_2 + B_2^{(c)}c_2^2 + \ldots \qquad (14)$$

where $B_2^{(c)}$ is the familiar second virial coefficient for these concentration units. To the approximation that the volume fraction of component 3 and other small terms may be neglected relative to unity and that activity coefficient terms are neglected, the relation[7] for A_2 may be written in the present notation

$$2A_2 = -10^{-3}B_2^{(c)}C_3^*M_2^2(\partial \log B_2^{(c)}/\partial \log m_3^*)_{T,\mu_1} \qquad (15)$$

The derivative in eq 15 may be evaluated in terms of the experimental data for $B_2^{(c)}$ for sodium hyaluronate in NaCl solution plotted in Figure 3. The straight line fitted to that data is represented by

$$B_2^{(c)} = 1.4 \times 10^{-3}(m_3^*)^{-0.68} \qquad (16)$$

Substitution of this result into eq 15 leads to

$$A_2 = 0.075(C_3^*)^{0.32} \qquad (17)$$

Estimated values of A_2 from eq 17 and A_1 from the curve for $a = 3.5$ Å in Figure 2 give values of A_2/A_1^2 from 0.18 at $C_3^* = 0.2$ M to 0.07 at $C_3^* = 0.001$ M, all of which are much smaller than 0.5. The estimated A_2 values are therefore too small to account for the observed discrepancies in this way; correction for the effect of the estimated A_2 term alone on $-A_1$ would not increase A_1 at any C_3^* by more than the height of the plotted triangles in Figure 2. The reason for the discrepancies between experiment and theory at low C_3^* must therefore be sought elsewhere. It would be of interest in this regard to have a series of experimental values of apparent A_1 as a function of C_2 at fixed C_3^* to provide an experimental determination of the virial coefficients in eq 1.

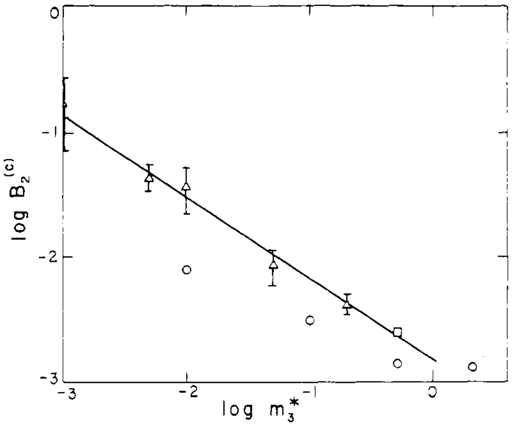

Figure 3. Data for the osmotic second virial coefficient $B_2^{(c)}$ for sodium hyaluronate as a function of the molality m_3^* of NaCl. Experimental points represent data from light scattering[3] (○), osmotic pressure[2] (□), and sedimentation equilibrium (△). The solid line is a least-squares fit to the points with omission of those from light scattering.

The conclusion is that, with the possible exception of data at very low salt concentrations, available data on salt exclusion by hyaluronate in NaCl solutions are fitted within experimental accuracy by the cylindrical rod model with a radius in the approximate range 3–8 Å. More precise data would presumably permit a more precise estimate of the radius.

Acknowledgment. Since it is scarcely discernible from the following list of references, I acknowledge in this way the inestimable influence on the scientific content of this and similar works in the field of polyelectrolytes of the late George Scatchard, who was also a valued early personal mentor and guide into the byways of solution thermodynamics.

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$$\tau = \frac{\xi[(K_0^2/K_1^2) - 1]}{2[(\beta\epsilon)^{-1} - 1]}$$

where β and ϵ are the quantities tabulated in that reference and K_0 and K_1 are to be evaluated at κa. Substitution of τ into eq 11 gives a more compact result

$$-A_1 = 2\beta\epsilon(1 + f_{33}^0) + V_2'C_3^*/1000$$

which was reported previously, without the factor $1 + f_{33}^0$ by: Schellman, J. A.; Stigter, D. *Biopolymers* **1977**, *16*, 1415.

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Polyelectrolyte Properties of Sodium Hyaluronate. 2. Potentiometric Titration of Hyaluronic Acid

Robert L. Cleland,* John L. Wang,[†] and David M. Detweiler

Department of Chemistry, Dartmouth College, Hanover, New Hampshire 03755.

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ABSTRACT: Titrations of 0.01 *m* glucuronic acid (GA) with NaOH were carried out in cells employing glass electrodes with a saturated calomel electrode (cell A) or a silver chloride electrode (cell B). The dissociation constant at zero ionic strength was given for either cell by $\mathrm{p}K = 3.23$ (±0.02), consistent with previous determinations. Corrections for the liquid-junction potential (cell A) led to the expected behavior of apparent pK with concentration of added $NaNO_3$. Similar titrations of 0.0085 *m* hyaluronic acid (HA) from bovine vitreous humor gave essentially linear plots of apparent pK against degree of ionization α over the range $\alpha = 0.3$–0.8 in the presence of added salt. Least-squares fits to these plots provided slopes which were fitted better as a function of concentration of added salt by the uniformly charged cylinder model than the infinite line charge model of a polyion. The cylinder radius required to obtain a good fit with the structural charge density is about 10 Å, however, which is larger than the structural radius (4–5 Å) of the charge sites. The discrepancy may be due in part to effects of charge discreteness and low dielectric constant of the polyion. The intrinsic dissociation constant for the polymer was estimated to be $\mathrm{p}K = 2.9$ (±0.1), where the large error estimate reflects uncertainties in extrapolation to $\alpha = 0$. The difference between polymer (HA) and monomer (GA) pK was attributed to effects of substitution at carbon 4 of the monomer. Although data for electrophoretic mobility at zero polymer concentration are limited for hyaluronate, agreement at one ionic strength (0.1 M) of the surface (ζ) potential calculated from this method and from potentiometric titration suggests that these techniques measure the same potential.

Hyaluronic acid, which occurs in its ionized form in many connective tissues and fluids, is a linear polysaccharide whose repeating disaccharide unit (see Figure 1, paper 1[1]) consists of D-glucuronic acid (β-linked at carbons 1 and 4) and *N*-acetyl-D-glucosamine (β-linked at carbons 1 and 3).[2] At complete ionization of the COOH groups on the glucuronic acid residues, charges occur regularly on every second glucoside and the average charge spacing approximates the length of the disaccharide unit, about 1 nm.

The thermodynamic properties of polyelectrolyte solutions have been extensively studied theoretically; in this work we investigate treatments which model the polyion as a smeared-out charge on an infinite line[3] or rigid cylinder[4,5] or as a flexible polymer chain with discrete charges.[6] In all of these treatments the chain may be characterized by a dimensionless charge density parameter ξ

$$\xi \equiv e^2Q/DkTL \qquad (1)$$

where e is the electron charge, Q is the number of (electron) charges in length L, D is the bulk solvent dielectric constant, k is the Boltzmann constant, and T is the absolute temperature. For monovalent counterions the value $\xi = 1$ has been sometimes regarded (see ref 3 and citations therein) as a critical value, above which counterion condensation occurs in some fashion near the polyion and below which the latter may be regarded as fully ionized. The value of ξ for the hyaluronate polyion has been taken previously[1] to be 0.70, which is below the critical value. The polymer can, therefore, be tentatively assumed to be fully ionized in the Debye–Hückel sense.

Various experimental features of hyaluronate behavior agree rather well with this assumption in terms of the line charge theory.[3] These include data for the enthalpy of mixing of NaCl and hyaluronate[7] and for activity coefficients in hyaluronate solutions.[8,9] The assumption of complete ionization at $\xi < 1$ has been questioned in a recent discussion of activity coefficients in solutions of polyacrylate copolymers of low charge density,[10] a point which will be dealt with in our discussion.

In the present work the potentiometric titration behavior of hyaluronic acid is examined experimentally and the results are investigated in terms of the available theoretical models as applied to this experimental technique. The most extensive previous investigation by this method was that of Laurent.[11]

Experimental Section

Materials. A crude sample of bovine vitreous humor hyaluronate (K^+ form, Nutritional Biochemical Corp.) weighing 6.14 g was dissolved in 500 cm^3 of deionized water containing (as in all solutions of polymer) 1 mg cm^{-3} of 5,7-dichloro-8-quinolinol (Eastman Kodak Co.) as preservative. The soluton, after dialysis for 48 h against deionized water, was treated with 5.0 g of kaolin (technical grade, washed and ignited, J. T. Baker Co.) for 30 min with stirring and 40 h unstirred, at room temperature. The suspension was clarified by centrifugation at ca. 10^4g for 15 min to give a supernatant containing about 0.08 mg cm^{-3} of protein contaminant. The supernatant (about 400 cm^3) was dialyzed against approximately 5 kg of stirred deionized water for 5 days

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[†] Present address: Department of Biochemistry, Michigan State University, East Lansing, MI 48824.

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