Electrophoretic Mobility of Wormlike Chains. 1. Experiment: Hyaluronate and Chondroitin 4-Sulfate

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ABSTRACT: As measured by the free-zone technique of Hjertén, the reduced electrophoretic mobility u' of tryptophan-labeled polymers decreased with increasing ionic strength I in Tris acetate buffers (pH ca. 8.2). For hyaluronate values of u' were proportional to the slope $\Delta pK/\Delta\alpha$ of plots of apparent pK against degree of ionization α (COOH groups) from potentiometric titration. The proportionality constant was about 50% larger than that predicted by the uniformly charged cylinder model having the radius (1 nm) required to fit potentiometric titration data. Labeling with tryptophan did not significantly affect the mobility. Hyaluronate polyions containing a small number (no more than 140) of disaccharide subunits gave values of u' 10–20% lower than a sample having several thousand such subunits. In buffers of constant ionic strength and varying pH u' increased with α for these polyions in satisfactory agreement with theoretical predictions of the wormlike cylinder model.

Introduction

The electrophoretic mobility of particles has long been regarded as a measure of the electrical potential at the particle surface. Potentiometric titration has also frequently been treated[1,2] in terms of this potential. The latter technique was used to investigate[3] the polyelectrolyte behavior of hyaluronic acid, the acid form of the important connective tissue polysaccharide hyaluronan. The surface potential obtained from the titration with use of the uniformly charged cylinder (UCC) model of the polyion gave approximate agreement with that derived from a single previous measurement[4] of the electrophoretic mobility. A continuing interest in the relationship between these two experimental techniques has now led to the present investigation. Results are reported of electrophoretic mobility measured by the free-zone technique pioneered by Hjertén.[5,6] These results show that the electrophoretic mobility is substantially larger than that predicted by the UCC model used to fit titration data of hyaluronic acid. The accompanying theoretical work[7] deals with this problem by treating electrophoresis in terms of the discrete-site wormlike chain model used previously in dealing with potentiometric titration.[8]

Experimental Section

Analytical Methods. Hexuronic acid analysis was carried out as described previously.[9] For small samples (0.1 mL maximum) microanalytical quantities of reagents used were one-fifth of the "economy proportions" of Bitter and Muir[10] and absorbance was measured in a semimicrocuvette.

The tryptophan content of the labeled samples was estimated by measurements of optical density at wavelengths in the near-ultraviolet between 260 and 400 nm. Absorption at 325 nm and longer wavelengths was assumed to be due entirely to light scattering, and the peak absorption at 280 nm was corrected by assumption of an inverse fourth-power dependence of scattering absorption on wavelength. The corrected absorption was converted to molar tryptophan concentration by use of the value ϵ_{280} = 5559 L mol^{-1} (0.1 M phosphate buffer, pH 7.1) from the literature.[11] The degree of substitution was estimated from this result combined with hexuronic acid analysis under the assumption that neither analysis was affected by the presence of the other component.

Reagents. L-Tryptophan (Sigma), dimethyl sulfoxide (Merck), acetaldehyde (Merck), and cyclohexyl isocyanide (Fluka) were of reagent grade. The Tris acetate buffers were prepared by half-neutralization of tris(hydroxymethyl)aminomethane (Sigma reagent) with acetic acid (Merck reagent). These buffers varied in ionic strength I (molar acetate concentration) from 0.002 (pH 8.2) to 0.05 (pH 8.3). Other buffers were prepared by mixing reagent grade components (Merck) to give the compositions and characteristics compiled in Table I. Hyaluronic acid samples prepared from rooster comb[9] were gifts from Pharmacia AB (Uppsala, Sweden).

Chondroitin 4-sulfate fraction A8 (CS-8) came from a previous fractionation carried out on DEAE-cellulose by a procedure similar to that used for hyaluronate,[12] except that higher NaCl concentrations were required for fraction elution. The molecular weight of 3.0×10^4 was calculated from the limiting viscosity number $[\eta] = 60$ cm^3 g^{-1} in 0.2 M NaCl by use of the proportionality $[\eta] = 0.0020M_r$ derived[13] from the experimental results of Wasteson.[14]

Degradation of hyaluronic acid of high molecular weight (similar to sample H-0) was performed as described previously[9] by hydrolysis in approximately 1 M HCl at 50 °C for different reaction times. The hydrolysates were neutralized with 5 M NaOH and dialyzed exhaustively against deionized water at about 5 °C for several days.

Solution viscosities η_{rel} of these samples relative to 0.2 M NaCl were measured as described previously[9] at a single polymer concentration. The values of the intrinsic viscosity (or limiting viscosity number) $[\eta]$ given in Table II were calculated from a form of the Kraemer equation[15]

$$\ln \eta_{rel} = [\eta]c - k''[\eta]^2c^2 \quad (1)$$

with k'' taken to be 0.125 for $[\eta]$ in cm^3 g^{-1}. Molecular weights M_r were calculated from values of $[\eta]$ as indicated in the footnote to Table II.

Labeling with Tryptophan. Polymeric acids were labeled by use of a four-component condensation reaction[16,17] involving the acid, a primary amine (tryptophan), a carbonyl compound (acetaldehyde), and an isonitrile (cyclohexylisonitrile) in mixtures of water and dimethyl sulfoxide of approximately equal volume. The aldehyde and isonitrile were used in roughly equimolar amounts, and the acid at about 50% greater molarity than the amine. Reagents were added in the order and amounts shown in Table III. After 4–5 h of reaction time at room temperature (ca. 20 °C), the labeled polymer was precipitated by addition of aqueous NaCl and gradual addition of methanol until precipitation was complete. The supernatant liquid was removed by suction of a water aspirator. The wet precipitate was washed twice by suspension in ca. 90% (v/v) methanol containing NaCl (0.05–0.2 M) followed by removal of supernatant liquid by suction or decantation. The resulting residue was washed repeatedly by resuspension in 90% (v/v) methanol followed by centrifugation at 5000 rpm (Sorvall GSA rotor) for 10 min and decantation. The sample was finally dissolved in ca. 10 mL of 0.2 M NaCl and

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Table I
Composition and Properties of Buffers

	buffers for pH variation at $I = 0.0100$			
pH	buffer anion	$[H^+]$	$[Na^+]$	$10^3\kappa_{sp}$,[a] S cm^{-1}
2.05	phosphate	0.0200	0.0000	3.62
2.51	phosphate	0.0102	0.0098	1.78
3.19	formate	0.0323	0.0093	1.17
3.69	formate	0.0102	0.0098	1.04
5.19	acetate	0.0033	0.0100	0.84

[a] Values of the measured specific conductivity κ_{sp} are reported as corrected to 25 °C.

Table II
Hyaluronate Samples

sample	hydrolysis time, min	$[\eta]$	$10^{-4}M_r$[a]
H-0			ca. 300
H-I	25	488	20.3
H-II	90	157	5.6
H-III	185	82	2.9

[a] Calculated from $[\eta]$ according to the method of Cleland and Wang[26] for $M_r > 10^5$ or Cleland[9] for $M_r < 10^5$.

Table III
Labeling of Polysaccharides with Tryptophan

	mmol of reagent				
starting material	disaccharide	tryptophan	acetaldehyde	isonitrile[a]	degree of substitution[b]
H-I	0.11	0.090	0.9	1.0	0.16
H-II[c]	0.13	0.084	3.5	4.0	0.12
H-II	0.13	0.086	0.18	0.20	0.01
H-0	0.13	0.091	3.5	4.0	0.08
CS-8	0.10	0.091	0.9	1.0	0.11

[a] The isonitrile reagent was cyclohexylisonitrile. [b] See text for method of estimation. [c] This experiment produced sample H-II-T.

dialyzed against 300 mL of 0.2 M NaCl for 48 h with one change of solvent at about 24 h. Tryptophan-labeled samples are designated by a final -T.

Electrophoretic Mobility. Electrophoretic velocity v was measured by a zonal technique with an apparatus described by Hjertén.[5,6] Two electrode vessels containing the solvent buffer were connected by a slowly rotating quartz capillary tube of 3 mm i.d. in which electrophoretic transport occurred. A small sample (typically about 10 μL) containing the polymer of interest was injected by microsyringe into the end of the tube to form a thin zone. Samples were prepared from stock polymer solutions by appropriate dilutions with stock buffer solutions. Multiple samples could be run by injecting at different initial positions along the tube, and typically two samples were run simultaneously: (1) two differing concentrations of the same polymer in experiments at varying ionic strength (set 1 below); (2) the two polymers at a single concentration each in experiments at varying pH (set 2). Temperature control was achieved by immersing the tube assembly in a thermostated cooling bath. The constant direct current i from a high-voltage source was measured by a precision milliammeter.

In this arrangement the zone moved as a well-defined sharp band, provided electroendosmosis (motion of the tube liquid as a whole) was suppressed, which can be achieved adequately by coating the inner tube wall with a thin layer of cross-linked methylcellulose. Detection of the zone position was based on the absorption of ultraviolet light by the sample, in the present case at about 280 nm. The tube assembly was moved through the filtered beam from a hydrogen lamp which passed through the tube and struck a photomultiplier tube. The current output of the latter was arranged to give a signal proportional to the ratio of transmission in two narrow bands, an adsorbing region centered on 280 nm and a reference region centered on 313 nm. The recorded output produced a peak when the UV-absorbing zone was traversed, and measurement on the recording for several scans at known time intervals provided an accurate determination of the zone velocity along the tube. Evidence of the absence of electroendosmois was provided by the shape of the peaks generated.

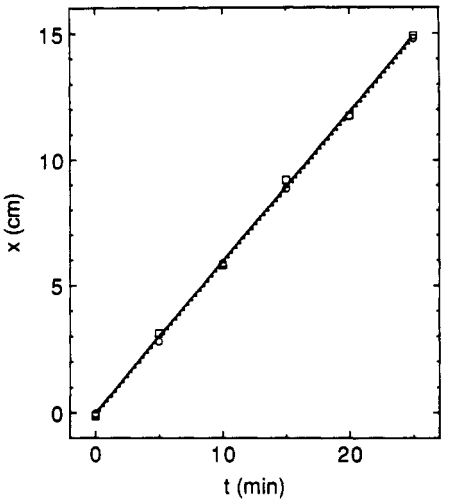

Figure 1. Position of maximum absorption in electrophoresis tube for velocity measurements of (□) tryptophan-labeled (H-II-T) and (○) unlabeled (H-II) hyaluronate. Samples (ca. 10 μL) of 2.5–2.6 mg/mL concentration were injected into a Tris acetate buffer ($I = 0.025$, pH 8.26). Experimental conditions: current, $i = 4.0$ mA; temperature, 19.0 °C. Electrophoretic velocities were determined from least-square fits to the plotted points.

In a few preliminary runs a number of fractions were taken with a microsyringe from the electrophoresis tube at the conclusion of the run. Each fraction corresponded approximately to 0.5 cm of tube length. A scale attached to the tube for this purpose[5] permitted determination of the position of such fractions relative to the zero of the zone detector. The fractions were weighed and analyzed microanalytically for hexuronic acid content.

The electrophoretic mobility $u = v/E$, where E is the magnitude of the electric field, was calculated in units of cm^2 V^{-1} s^{-1} from the equation $u = vq\kappa_{sp}/i$, where q is the cross-sectional area of the tube and κ_{sp} is the specific conductivity, which was measured separately on the solvent with a precision conductivity meter (Philips) calibrated with standard solutions of reagent grade KCl of known conductivity. To reduce dependence on solvent conditions, experimental mobilities are reported in this work as the reduced mobility[18] u' defined by

$$u' \equiv 6\pi\eta|u|/Dk_BT \qquad (2)$$

where η is the solvent viscosity, D is the bulk solvent dielectric constant (taken here to be 78.3 at 25 °C), k_B is Boltzmann's constant, and T is the absolute temperature.

Results

The question naturally arises whether the labeling procedure used affects the measured mobility. The following experiments were designed to compare the mobility of labeled and unlabeled hyaluronate. Unsubstituted sample HA-II and labeled sample HA-II-T, each injected at a concentration of 2.5–2.6 mg/mL in water into a buffer of Tris acetate ($I = 0.025$, pH 8.26), were run in separate experiments with the bath at 19 °C. The labeled sample gave the expected large absorption peak, while the unlabeled sample produced a small peak, presumably due to light scattering from the sample zone, which could be observed throughout the run. Peak widths at half-maximum absorption at a given migration time were similar for the two experiments. The measured positions of maximum absorbance are shown in Figure 1 as a function of time. The calculated zone velocity was 5.94 (±0.03) mm min^{-1} for the labeled and 5.96 (±0.09) mm min^{-1} for the unlabeled sample, where uncertainties represent the standard deviation of each slope in Figure 1.

Table IV
Reduced Mobility u' in Tris Acetate Buffers

sample	polymer concn, mg/mL	u' at buffer[a] ionic strength, I, of 0.05	0.01	0.005	0.002
H-0-T	0.32	1.80	2.60	3.00	3.80
	0.16	1.78	2.54	3.08	3.85
H-I-T	0.26	1.77	2.51	2.90	
	0.13	1.77	2.54	2.93	
H-II-T	0.69	1.66	2.24	2.65	
	0.35	1.65	2.13	2.59	
CS-8-T	0.37[b]	2.44	2.91	3.55	4.7
	0.18	2.46	2.90	3.56	4.8

[a] The buffers were Tris acetate [tris(hydroxymethyl)aminomethane half-neutralized with acetic acid] of pH 8.2–8.3. [b] The CS-8-T concentrations were 1.5 times larger than this value at $I = 0.05$.

In addition, fractions were removed at the completion of each experiment. Analysis gave hexuronic acid contents substantially proportional to the corresponding area under the optical peak trace of the labeled sample. For the labeled sample the position of maximum concentration estimated from these analyses agreed within 0.5% with that obtained as the midpoint of the area under the optical peak. The maximum hexuronic acid concentrations for the two samples also gave consistent results within 1–2% for the electrophoretic velocity. Comparison of the results of experiments with and without labeling thus show that the labeling does not affect the mobility within experimental error. The remainder of the experimental data were therefore obtained exclusively by optical detection.

The effect of injected polymer concentration on mobility was not investigated systematically. A few preliminary experiments in which concentrations up to 2.5 mg/mL were injected indicated that the mobility of either polymer at such concentrations differed by no more than about 10% from those extrapolated to zero concentration. To minimize all possible errors from concentration effects, however, the results reported below were obtained from experiments carried out at injected concentrations (typically 0.1–0.5 mg/mL) which gave small, but well-defined, absorption peaks. The data given in Table IV were all obtained from experimental runs in which two such small concentrations of the same polymer differing by a factor of 2 were run simultaneously. Differences in mobility in such cases were typically no more than 1 or 2%, without systematic increase or decrease attributable to concentration. Reported mobilities for zero polymer concentration in a given buffer solution were therefore reported as a weighted average of these two values. The weighting factors were, again, based on standard deviations of the slopes of position–time plots, which were typically about 1% and rarely more than 2% of the slopes themselves.

Two sets of experiments were performed with the labeled samples of Table III: set 1, variation of ionic strength I at complete ionization in Tris acetate buffers (pH 8.2–8.3), as reported in Table IV; set 2, variation of degree of ionization by use of buffers having different pH at approximately constant ionic strength, as reported in Table V.

Discussion

Effect of Ionic Strength. Values of u' from Table IV are plotted in Figure 2 against the common logarithm of 1:1 electrolyte concentration C_3^* (where * refers to the equilibrium dialysis solvent). Qualitatively, u' behaves in the same way with log C_3^* as the slope $m \equiv (\partial pK'/\partial\alpha)_I$ from potentiometric titration data for hyaluronic acid,[3]

Table V
Reduced Mobility in Buffers ($I = 0.01$) of Different pH

sample	u' at buffer[a] pH of 2.05	2.51	3.19	3.69	5.19
H-0-T[b]	0.45	0.70	1.53	1.91	2.62
CS-8-T[b]	2.46	2.47	2.86	3.01	3.46

[a] The buffer compositions are given in Table I. [b] Polymer concentrations were 0.32 mg/mL for H-0-T and 0.37 mg/mL for CS-8-T.

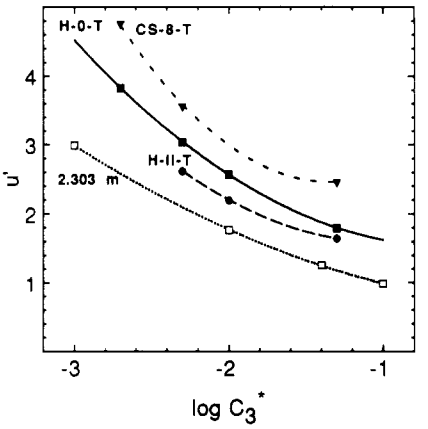

Figure 2. Reduced electrophoretic mobility u' as a function of concentration of C_3^* (molar acetate concentration) in half-neutralized Tris acetate buffers, pH 8.2–8.3. The samples shown are (■) H-0-T, (●) H-II-T, and (▼) CS-8-T. The open squares and the dotted line fit are values of $2.303m$, where $m \equiv (\Delta pK'/\Delta\alpha)_I$ from potentiometric titration of hyaluronic acid.[3] This function is plotted to show the predicted result, $u' = 2.303m$, of the uniformly charged cylinder model.

when pK' ($\equiv -\log K'$, where K' is the apparent acid dissociation constant) is plotted against α, the degree of ionization of the acidic groups. In fact, when the relatively small ion-atmosphere relaxation effects for this polymer are neglected, the UCC model of the polyion predicts[7] that $u' = 2.303m$. The dotted line in Figure 2 represents a quadratic least-squares fit to experimental values of $2.303m$ (open squares) for hyaluronic acid.[3] The values of u' (solid squares) for hyaluronate of high molecular weight all lie at about 50% above the predictions of the UCC model. The failure of the latter is well outside experimental error and large enough to justify an attempt at a more adequate theoretical explanation, which is the subject of the accompanying paper.[7] The higher values of u' for the chondroitin 4-sulfate sample are due to the higher linear charge density of this polymer, as discussed further below.

Effect of Degree of Ionization. The accepted chemical structures of hyaluronic acid and chondroitin 4-sulfate represent them as repeating disaccharides with alternating glucuronic acid and *N*-acetylhexosamine residues. The weakly acidic COOH groups in the glucuronic acid residue have a degree of ionization α which varies with solution acidity in the pH range below pH 5 or 6. In addition, chondroitin 4-sulfate contains a strongly acidic sulfate group, which is fully ionized under all buffering conditions used in this work. The object of the next set of experiments at varying pH reported in Table V was to investigate the effect on u' of varying α, the fraction of ionized COOH groups. Since the data are most easily interpreted in terms of the latter quantity, a procedure for estimation of α from pH is required.

(a) Hyaluronic Acid. In this case, for which potentiometric titration data are available, the calculation of α

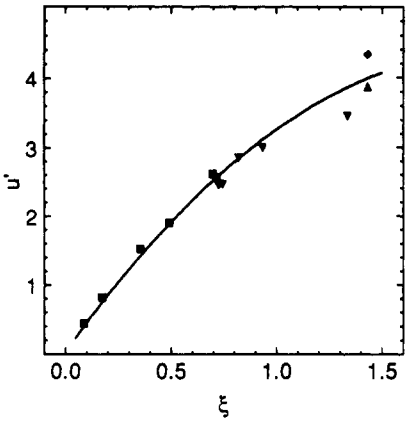

Figure 3. Reduced electrophoretic mobility u' for (■) hyaluronate (HA) sample H-0-T and (▼) chondroitin 4-sulfate (CS) sample CS-8-T from measurements at $I = 0.01$ in buffers of varying pH. Values of the charge density parameter ξ were estimated from buffer pH as described in the text. The solid line is the theoretical prediction by the method described elsewhere[7] for the unperturbed wormlike (radius $a = 0.55$ nm) with axial charge sites (corrected for the nonlinear Poisson–Boltzmann potential and for ion-atmosphere relaxation effects). For purposes of comparison points from the literature[26] are shown for polygalacturonic acid (Na^+ salt) (♦) and alginic acid (Na^+ salt) (▲).

may be readily carried out with use of the equation

$$\mathrm{pH} = 2.82 + 0.77\alpha + \log [\alpha/(1-\alpha)] \qquad (3)$$

derived from eq 9 and Table II (for $I = 0.01$) of the experimental titration work.[3] The assumption involved here is that the only parameter affecting the apparent value pK', aside from the small temperature dependence, is the ionic strength and not the chemical nature of the specific ions in the buffer.

To facilitate comparison of the two polyions, the values of α have been converted to the dimensionless charge density parameter ξ of the linear charge site model[19]

$$\xi \equiv e^2\alpha/Dk_BTd \qquad (4)$$

where e is the charge on the proton and d the length per unit charge of the fully ionized polyion. For hyaluronic acid with d taken to be 1.0 nm, and for $T = 298.15$ K, $\xi = 0.716\alpha$, and for chondroitin sulfate $\xi = 0.716(1 + \alpha)$ to allow for the ionized sulfate group.

Examination of the values of u' given in Table IV in conjunction with the data for hyaluronate samples in Table II reveals slight decreases in u' with decreasing degree of polymerization N ($\equiv M_r/M_0$, where M_0 is the molecular weight of a disaccharide subunit), particularly for sample H-II-T ($N \approx 140$). Such behavior is expected theoretically as a result of end effects, which diminish to give asymptotic values of u' at high N.[7] Values of u' for sample H-0-T ($N \approx 8 \times 10^3$) are therefore taken here to represent the latter and are plotted in Figure 3 as the squares.

(b) Chondroitin 4-Sulfate. In this case there appear to exist no published potentiometric titration data for $I = 0.01$. To estimate ξ values, we must therefore assume parameters for the analogue to eq 3. The method followed here has been to estimate the expected effect on pK', the apparent ionization constant for the glucuronic acid groups, due to the ionized sulfates, which are axially substituted at C_4 on the N-acetylhexosamine units (here galactosamine).

An estimate of the distance of the nearest-neighbor SO_4^- charge sites from an ionizing COOH group may be made from a slight modification of the conformational model used in a previous estimate of the electrostatic free energy of hyaluronic acid.[8] The modification consists of replacing the axially substituted hydrogen on C_4 in the hexosamine (B) unit of the latter polymer by SO_4^- with use of the geometry for the latter found for the sulfate group in potassium ethyl sulfate.[21] The charge site was taken to lie at the midpoint of the three external oxygen atoms. The conformational averaging followed the usual procedure,[8] including a Debye–Hückel (DH) term for the electrostatic energy, except that the steric effects of the sulfate group were not accounted for. The average site separation between SO_4^- and COO^- was estimated to be 0.85 (AB rotation) or 0.71 nm (BA rotation). The average DH electrostatic energy for the two nearest neighbors to a COO^- site at $I = 0.01$ is then $1.44kT$.

The electrostatic effects of the higher neighbors can be dealt with by use of the *linear* discrete-site model with a modification of Manning's procedure[22] for estimation of the electrostatic free energy. For the present case the ionized sulfates are located on the odd-numbered monosaccharide neighbors of a given COOH group. If we take the monosaccharide spacing to be represented by d, the electrostatic free energy G_{el} for the interaction between unlike groups on a chain of Z disaccharide units becomes, in the DH approximation

$$\frac{G_{el}}{ZkT} = \frac{2e^2}{DkTd}\sum_{\text{odd } t=1}^{\infty}\frac{x^t}{t} = \frac{e^2}{DkTd}\ln\left(\frac{1+x}{1-x}\right) \qquad (5)$$

where $x = \exp(-\kappa d)$. When d is taken to be 0.5 nm, the constant becomes $\xi_{CS} = 1.432$ at 25 °C, and $G_{el}/ZkT = 3.58$, of which the nearest-neighbor terms contribute 2.43. If the latter contribution is replaced by the value 1.44 from the conformation calculation and the contributions from the higher neighbors given by the linear model are retained, the estimate becomes $G_{el}/ZkT = 1.44 + 1.15 = 2.59$. The estimated effect on pK of these interactions would then be $\Delta\mathrm{p}K = 2.59/2.303 = 1.12$. A reasonable correction to this value for the full Poisson–Boltzmann equation for the potential would be that estimated[7] for the uniformly charged cylinder at $I = 0.01$ from the tabulations of Stigter,[23] which would correct $\Delta\mathrm{p}K$ to 1.04. Other possible corrections are ignored, since the linear discrete-site model appeared in the case of hyaluronic acid[8] to introduce compensating errors relative to the wormlike chain model, which made such refinements unnecessary.

The slope $m = 0.60$ might reasonably be expected for a chondroitin sulfate titration at $I = 0.01$ based on the model used previously[3] to predict correctly the experimental value at $I = 0.1$. The analogue to eq 2 becomes

$$\mathrm{pH} = 3.86 + 0.60\alpha + \log [\alpha/(1-\alpha)] \qquad (6)$$

While the prediction of eq 6 that pK' is linear in α is quite possibly incorrect in this titration region, the errors in estimated α values should not be significantly affected by this assumption. The experimental values of u' for chondroitin sulfate of Table IV are plotted in Figure 3 against values of ξ estimated by use of eq 6.

Comparison with Theory. The experimental variation of u' with ionic strength for hyaluronate agrees reasonably well with that expected theoretically for the wormlike cylinder of appropriate radius when corrections for nonlinearity of the Poisson–Boltzmann equation and for ion-atmosphere relaxation are taken into account.[7] Comparison of experiment with predictions of this model for variation of u' with charge density at fixed I are also of interest. The solid line in Figure 3 represents calculated results for the *unperturbed* wormlike chain with discrete axial charge sites, when corrections for the same effects

are made. Expansion of the polyion due to charge interactions can be ignored here, since calculated u' values change by less than 1% at $I = 0.01$ when this effect is included,[7] provided that possible expansion effects due to charge separations of less than a Kuhn length can be neglected. While the relaxation corrections were made here for a small-ion electrolyte approximating the conductance of KCl rather than those used in the experiments, the small errors resulting tend to be compensated by the use of an axial-site rather than a surface-site model.

Agreement of the calculated result of Figure 3 with experimental data for hyaluronate is quite satisfactory. The data for chondroitin sulfate are more scattered and vary somewhat less with ξ than predicted by the theory. It should be noted that the values of u' obtained by Tuffile and Ander[24] for the sodium salts of polygalacturonic and alginic acids, which have the same charge density, show reasonably good agreement with theoretical prediction.

A complication in any attempt to compare chondroitin 4-sulfate with theory results from the fact that the degree of polymerization of the latter lies in the region where end effects are almost certainly present. For the prelabeling degree of polymerization ($N \approx 60$) and $I = 0.01$, theoretical calculation (see Figure 3 of ref 7) suggests a correction of about 6% increase in u' to give the asymptotic value u_∞'. The correction procedure is rendered quantitatively uncertain by two factors: (1) the values of u' observed experimentally for hyaluronate sample H-II-T (prelabeling $N \approx 140$) at $I = 0.01$ are 15–20% smaller than for the sample (H-0-T) of high molecular weight and (2) there arises also the question of whether the labeling procedure itself introduces changes in the degree of polymerization due to chain scission. De Belder and Wik[25] examined the latter question by measurements of intrinsic viscosity and size exclusion gel chromatography before and after labeling a hyaluronate sample by the procedure used in this work and concluded that the molecular weight dropped from 1.1×10^6 by about 10%. Factor 1 supports the qualitative prediction that end effects are important. However, the correction required for H-II-T is larger than expected if degradation during labeling is small, and that required for labeled chondroitin 4-sulfate may therefore be significantly greater than that suggested above. While the quantitative nature of the end-effect corrections requires further investigation, the results for chondroitin 4-sulfate appear to be in rough agreement with theoretical expectation. It should also be pointed out in this respect that the bulky SO_4^- group may require assignment of a somewhat larger effective cylinder radius for this polyion.

Conclusions. The electrophoretic mobilities of hyaluronate and chondroitin 4-sulfate have been measured in a variety of buffer solutions. The experiments were designed to determine the effects on mobility of (a) ionic strength (at complete ionization) and (b) degree of ionization α at fixed ionic strength. The results confirm qualitative expectation that increasing ionic strength causes a decrease in mobility and that increasing α (or ξ) causes an increase. The UCC model which fits potentiometric titration data for hyaluronic acid underestimates the expected mobility. For ionized polysaccharides having values of ξ up to about 1.5, the cylindrical wormlike chain model provides a good account of experimental mobilities as a function of ξ and, as shown elsewhere,[7] as a function of ionic strength.

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References and Notes

(1) Hartley, G. S.; Roe, J. W. *Trans. Faraday Soc.* **1940**, *36*, 101.
(2) Katchalsky, A.; Shavit, N.; Eisenberg, H. *J. Polym. Sci.* **1954**, *13*, 69.
(3) Cleland, R. L.; Wang, J. L.; Detweiler, D. M. *Macromolecules* **1982**, *15*, 386.
(4) Niedermeier, W.; Gramling, E. S. *Carbohydr. Res.* **1968**, *8*, 317.
(5) Hjertén, S. *Free Zone Electrophoresis*; Almqvist and Wiksell: Uppsala, Sweden, 1967.
(6) Hjertén, S. *Methods Biochem. Anal.* **1970**, *18*, 55.
(7) Cleland, R. L. *Macromolecules* **1991**, following paper in this issue.
(8) Cleland, R. L. *Macromolecules* **1984**, *17*, 634.
(9) Cleland, R. L. *Biopolymers* **1984**, *23*, 647.
(10) Bitter, T.; Muir, H. M. *Anal. Biochem.* **1962**, *4*, 330.
(11) *Handbook of Biochemistry and Molecular Biology, Proteins*; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. I, p 186.
(12) Cleland, R. L.; Cleland, M. C.; Lipsky, J. J.; Lyn, V. E. *J. Am. Chem. Soc.* **1968**, *90*, 3141.
(13) Cleland, R. L. *Arch. Biochem. Biophys.* **1977**, *180*, 57.
(14) Wasteson, A. *Biochem. J.* **1971**, *122*, 477.
(15) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University: Ithaca, NY, 1953; p 310.
(16) Ugi, I.; Steinbrückner, C. *Chem. Ber.* **1961**, *94*, 2802.
(17) Gokel, G.; Lüdke, G.; Ugi, I. In *Isonitrile Chemistry*; Ugi, I., Ed.; Academic Press: New York, 1971; Chapter 8.
(18) Wiersema, P. H.; Loeb, A. L.; Overbeek, J. T. G. *J. Colloid Interface Sci.* **1966**, *22*, 78.
(19) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924.
(20) Stigter, D. *J. Phys. Chem.* **1979**, *83*, 1663.
(21) Truter, M. R. *Acta Crystallogr.* **1958**, *11*, 680.
(22) Manning, G. S. *Acc. Chem. Res.* **1979**, *12*, 443.
(23) Stigter, D. *J. Colloid Interface Sci.* **1975**, *53*, 296.
(24) Tuffile, F. M.; Ander, P. *Macromolecules* **1975**, *8*, 789.
(25) De Belder, A. N.; Wik, K. O. *Carbohydr. Res.* **1975**, *44*, 251.
(26) Cleland, R. L.; Wang, J. L. *Biopolymers* **1970**, *9*, 799.