

## Extent of Charge Screening in Aqueous Polysaccharide Solutions

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The absorption optical system of a Beckman XL-I ultracentrifuge has been used to monitor the Donnan distribution of ions in polysaccharide solutions dialyzed against sodium phosphate buffer (pH 6.8, *I* 0.08) supplemented with 0.2 mM chromate as an indicator ion. For dextran sulfate, heparin, and polygalacturonate, the effective net charges are shown to be only one-third of those deduced from the chemical structures—a reflection of charge screening (counterion condensation) in aqueous polyelectrolyte solutions. Whereas the extent of charge screening for the first two polysaccharides agrees well with theoretical prediction, the disparity in the corresponding comparison for polygalacturonate reflects partial esterification of carboxyl groups, whereupon the experimental parameter refers to the effective charge per hexose residue rather than the effective fractional charge of each carboxyl group.

### Introduction

In recent years, there has been a tendency to identify the charges on polysaccharides and polynucleotides with the values calculated from the chemical structure. However, the phenomenon of charge-screening (counterion condensation) has long been an established feature of polyelectrolyte theory.<sup>1–3</sup> To that end, the relatively small magnitudes of the effective net charges of nucleic acids<sup>4,5</sup> and polysaccharides<sup>6–8</sup> afford examples with direct relevance to biology. In the present investigation, we employ measurements of the Donnan distribution of small ions in dialysis<sup>9</sup> to reinforce earlier evidence of charge-screening effects in polysaccharide anions.<sup>6,7</sup> The study thereby draws attention to an aspect of the solution behavior of polyelectrolytes that is generally overlooked in biochemistry and molecular biology textbooks and which is therefore not appreciated by many researchers in biological chemistry.

In equilibrium dialysis of a solution of a polyanion (valence  $Z_P$  negative) with molar concentration  $C_P$  against a solution of uni-univalent electrolyte CA ( $C$  = cation,  $A$  = anion) with molar concentration  $C_{CA}$ , the requirement for equal chemical potentials of the salt in the polyanion ( $\alpha$ ) and diffusate ( $\beta$ ) phases dictates that

$$(\gamma_{\pm}^{\beta}/\gamma_{\pm}^{\alpha})C_C^{\beta} = C_C^{\alpha}[1 + (Z_P C_P^{\alpha}/C_C^{\alpha})]^{1/2} = (\gamma_{\pm}^{\alpha}/\gamma_{\pm}^{\beta})\left[C_C^{\alpha} + \frac{1}{2}Z_P C_P^{\alpha} + \dots\right] \quad (1a)$$

$$(\gamma_{\pm}^{\beta}/\gamma_{\pm}^{\alpha})C_A^{\beta} = C_A^{\alpha}[1 - (Z_P C_P^{\alpha}/C_A^{\alpha})]^{1/2} = (\gamma_{\pm}^{\alpha}/\gamma_{\pm}^{\beta})\left[C_A^{\alpha} - \frac{1}{2}Z_P C_P^{\alpha} + \dots\right] \quad (1b)$$

Comparison of the concentrations of either the cation or the anion in the two phases thus has potential for evaluating the polyanion valence, provided that estimates of the mean ion activity coefficient ( $\gamma_{\pm}$ ) are available for each phase.

From a biological viewpoint, eq 1 is not a very useful formulation of the Donnan distribution because of its inability to accommodate the more realistic situation in which the supporting electrolyte comprises several cations and anions, some of which are bivalent (e.g.,  $\text{HPO}_4^{2-}$ ,  $\text{Ca}^{2+}$ , etc.). To obtain more-suitable expressions, eqs 1a and 1b are therefore divided by  $C_C^{\beta}$  and  $C_A^{\beta}$ , respectively, to give

$$(C_C^{\alpha}/C_C^{\beta}) = (\gamma_{\pm}^{\beta}/\gamma_{\pm}^{\alpha}) - \left(\frac{1}{2}Z_P C_P^{\alpha}/C_C^{\beta}\right) + \dots \quad (2a)$$

$$(C_A^{\alpha}/C_A^{\beta}) = (\gamma_{\pm}^{\beta}/\gamma_{\pm}^{\alpha}) + \left(\frac{1}{2}Z_P C_P^{\alpha}/C_A^{\beta}\right) + \dots \quad (2b)$$

Upon noting that  $C_C^{\beta} = C_A^{\beta} = I^{\beta}$ , the molar ionic strength of the uni-univalent electrolyte in the diffusate phase, these two expressions can be approximated by the single expression

$$(C_i^{\alpha}/C_i^{\beta})^{1/Z_i} \approx 1 - Z_P C_P^{\alpha}/(2I^{\beta}) \quad (3)$$

in instances where the mean ion activity coefficient ratio is essentially unity. Furthermore, as realized by Svensson,<sup>10</sup> expression of the Donnan distribution of small ions in this manner has two advantages in that (i) eq 3 applies to each type of small ion in situations where the supporting

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electrolyte is not restricted to single cationic and anionic species and (ii) multivalence of a small ion is also accommodated.

In the current equilibrium dialysis study of charged polysaccharides, an indicator ion, L (chromate), is included in the supporting electrolyte medium (phosphate buffer, pH 6.8,  $I$  0.08) to allow assessment of the effective net charge of the polyanions via a modified form of eq 3 written for the indicator ion, namely,

$$(C_L^\alpha/C_L^\beta)^{1/Z_L} = 1 - fZ_p C_p / (2I^\beta) + \dots \quad (4)$$

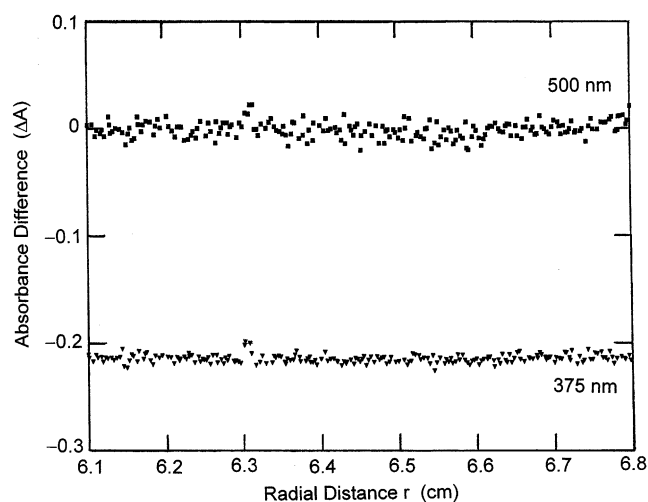
where  $Z_L$  is the chromate valence ( $-2$ ). The factor  $f$  is included to allow expression of the effective net charge as a fraction of the nominal valence,  $Z_p$ , that is indicated by the chemical structure of the saccharide repeat unit:  $f = Z_{\text{eff}}/Z_p$ . Unavailability of information on the magnitudes of the mean activity coefficients in this physiologically more-relevant environment means that the estimate of the extent of charge screening ( $f$ ) is an apparent value because of the necessity to assume a value of unity for the activity coefficient ratio in eq 2, upon which eqs 3 and 4 are based.

### Experimental Section

Sodium salts of dextran sulfate (D6001), heparin (H3393), and polygalacturonate (P3850) were used as supplied by Sigma Chemical Co, the charge characteristics of the dextran sulfate (prepared from dextran 500) being indistinguishable from those of a corresponding Pharmacia preparation with a specified sulfur content equivalent to the presence of two sulfonate residues per monosaccharide residue. These polysaccharides were dissolved directly in phosphate buffer (0.02 M  $\text{NaH}_2\text{PO}_4$ –0.02 M  $\text{Na}_2\text{HPO}_4$ ), pH 6.8,  $I$  0.08 M, supplemented with 0.2 mM  $\text{K}_2\text{CrO}_4$  to provide an indicator ion, whereupon the solutions (5 mL) were encased in 18/32 dialysis tubing. After extensive dialysis against the same chromate-supplemented buffer to establish the Donnan equilibrium distribution of small ions ( $4 \times 250$  mL, 48 h, 21 °C), the difference between chromate concentrations in the polysaccharide and diffusate solutions was monitored by means of the absorption optical system of a Beckman XL-I ultracentrifuge.<sup>11</sup> experiments were performed in triplicate. In that regard, the ultracentrifuge is merely being used as a double-beam spectrophotometer when a sufficiently low speed (3000 rpm) is used to ensure uniformity of solution composition throughout the cell. As in classical difference spectroscopy, diffusate was placed in the reference sector of the cell to allow direct measurement of the absorbance difference from a scan at 375 nm. A baseline for these measurements ( $\Delta A_{375} = 0$ ) was determined routinely from scans at 500 nm, a wavelength at which chromate does not absorb. The molar concentration of chromate in the dialyzed polysaccharide solution,  $C_L^\alpha$ , was then obtained from the corrected  $\Delta A_{375}$  measurement by means of the relationship

$$10^3 C_L^\alpha = (1.084 + \Delta A_{375})/5.42 \quad (5)$$

which was established by measuring the absorbance differences in calibration experiments with 0.2 mM chromate in



**Figure 1.** Ultracentrifuge scans (3000 rpm) at 375 and 500 nm (baseline) comparing the chromate concentrations in a dialyzed solution of dextran sulfate (10.1 mg/mL) with that in the diffusate, 0.08  $I$  phosphate buffer (pH 6.8) supplemented with 0.2 mM  $\text{K}_2\text{CrO}_4$ .

the reference sector and a range of chromate concentrations in the sample sector.<sup>11</sup>

Weight-concentrations of the dialyzed polysaccharide solutions  $c_p^\alpha$  (mg/mL) were obtained refractometrically by means of the expression<sup>12</sup>

$$c_p^\alpha = J/(17.5dn/dc) \quad (6)$$

where  $J$  is the number of Rayleigh interference fringes observed in a synthetic boundary run on the dialyzed solution of polysaccharide with specific refractive index  $dn/dc$  (mL/g). Values used for the various polysaccharides were the following: heparin, 0.132 mL/g;<sup>12</sup> dextran sulfate, 0.114 mL/g;<sup>13</sup> polygalacturonate, 0.155 mL/g.<sup>14</sup>

### Results and Discussion

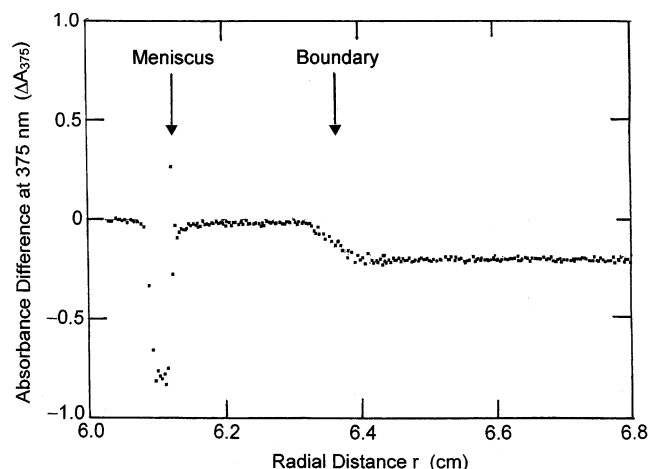
Scans at 375 and 500 nm obtained by subjecting a solution of dextran sulfate (10.1 mg/mL) dialyzed against the chromate-supplemented phosphate buffer (pH 6.9,  $I$  0.08 M) to centrifugation at 3000 rpm are presented in Figure 1. In keeping with the anionic nature of dextran sulfate, the negative magnitude of  $\Delta A_{375}$  ( $-0.215 \pm 0.002$ ) signifies that chromate is not a counterion.

Although the negative value of  $\Delta A_{375}$  is certainly consistent with the absence of chromate binding, that observation alone merely establishes a dominance of the Donnan effect over the increase in  $\Delta A_{375}$  that would stem from any binding of the indicator ion. Indeed, the choice of an XL-I ultracentrifuge as the double-beam spectrophotometer was predicated on our earlier demonstration with bovine serum albumin that the Donnan contribution to  $\Delta A_{375}$  could be distinguished from that reflecting chromate binding by increasing the rotor speed to 60 000 rpm to generate a protein-free region with a free chromate concentration equal to that in the dialyzed macroion solution.<sup>11</sup> For the present system, however, that means of distinguishing between the two effects did not materialize because the movement of the dextran sulfate boundary ( $s_{20} \approx 1.8$  S) was sufficiently slow to permit reestablishment of the Donnan equilibrium distribution of small ions across the

**Table 1.** Net Charges of Polysaccharide Anions in 0.08 *I* Phosphate, pH 6.8

polysaccharide	$c_P^a$ (mg/mL)	$M_P^a$	$C_P^a$ (M)	$Z_P$	$C_L^a/C_L^b$	fractional charge $f$	
						exptl <sup>c</sup>	theor <sup>d</sup>
dextran sulfate	10.1	366	0.0276	-2	0.800 ( $\pm 0.010$ )	0.34 ( $\pm 0.02$ )	0.33
heparin	9.0	665	0.0135	-4	0.815 ( $\pm 0.005$ )	0.32 ( $\pm 0.01$ )	0.33
polygalacturonate	13.6	198	0.0687	-1	0.765 ( $\pm 0.015$ )	0.33 ( $\pm 0.02$ )	0.60

<sup>a</sup> Molecular weight of the repeat unit (sodium salt). <sup>b</sup> Ratio of chromate concentrations in the solution and diffusate phases ( $C_L^b = 0.200$  mM). <sup>c</sup> Calculated from eq 4. <sup>d</sup> Calculated from eqs 10–12.



**Figure 2.** Ultracentrifuge scan at 375 nm obtained for the dialyzed dextran sulfate solution (Figure 1) after increasing the rotor speed to 60 000 rpm to determine the chromate concentration in the polysaccharide-free region of the sedimentation velocity pattern. Vertical arrows denote the positions of the air–liquid meniscus and the dextran sulfate boundary.

migrating boundary (Figure 2). This situation is signified by the identity of chromate concentrations in the diffusate and polysaccharide-free region of the scan ( $\Delta A_{375} = 0$ ): the observed decrease in  $\Delta A_{375}$  coincides with the position of the sedimenting boundary of dextran sulfate. No additional information has thus been afforded by the sedimentation velocity experiment on the dialyzed polysaccharide solution.

Although this finding that  $\Delta A_{375} = 0$  is at variance with the earlier result for serum albumin,<sup>10</sup> which signified an essential lack of Donnan reequilibration across the faster-sedimenting protein boundary ( $s_{20} \approx 4.2$  S), the observation does have a counterpart in a sedimentation velocity study of heparin.<sup>15</sup> Indeed, it provides visible proof of the assertion therein that the Donnan redistribution of small ions does occur across the migrating heparin boundary ( $s_{20} \approx 2$  S) and that the effective net charge can therefore be calculated from the electrolyte concentration in the polysaccharide-free region of the sedimentation velocity pattern for undialyzed heparin in 0.15 M NaCl. Adoption of the comparable procedure (sedimentation velocity without prior dialysis) here would have eliminated the need for prior dialysis but would not have provided a solution to the dilemma about the existence of any chromate binding.

It is therefore necessary to proceed on the basis that the negative value of  $\Delta A_{375}$  in Figure 1 reflects solely the consequences of the Donnan redistribution of small ions. Incorporation of this  $\Delta A_{375}$  of  $-0.215 (\pm 0.002)$  into eq 5 leads to an estimate of  $0.160 (\pm 0.002)$  mM for  $C_L^a$ , the chromate concentration within the polysaccharide solution that is in dialysis equilibrium with 0.200 mM chromate in

the diffusate ( $C_L^b$ ). These values now need to be rationalized in terms of eq 4. For dextran sulfate, the repeat unit is a glucose residue bearing two sulfonates, whereupon the molecular mass of the repeat unit ( $M_P$ ) is 366 Da. Consequently,  $C_P^a = c_P/M_P = 0.0276$  M in the present experiment with  $c_P^a = 10.1$  mg/mL. Substitution of the relevant parameters into eq 4 yields an estimate of  $0.34 (\pm 0.02)$  for the charge-screening parameter  $f$ . These findings are summarized in Table 1, which also includes the corresponding results for heparin (a disaccharidic repeat unit with four negatively charged groups) and polygalacturonate (nominally a single carboxylate group per hexose residue).

For all three polysaccharides, the value of  $f$  signifies that only a third of the  $\text{Na}^+$  counterions associated with the macroions are reflected in the Donnan distribution of small ions; the remainder are seemingly involved in counterion condensation with charged groups of the polyanion. In other words, the effective net charge is only one-third of that deduced from the chemical structure of the polysaccharide repeat unit. Admittedly, this conclusion is dependent upon the presumed absence of chromate binding, but that assumption should be a reasonable approximation for polysaccharide anions. Furthermore, the effect of any chromate binding would be to elevate the experimentally determined value of  $f$ , which would then underestimate the actual extent of charge-screening/counterion condensation.

Another potential source of error in the above analysis is its reliance upon a Donnan expression (eq 4) in which the ratio of mean ion activity coefficients in the two phases has been taken as unity. However, the present estimate of 0.34 for the effective fractional charge on each sulfonate group of dextran sulfate in 0.08 *I* phosphate (pH 6.8) is supported by analysis of equilibrium dialysis data in a more rigorous study of a comparable Pharmacia preparation ( $Z_P = -2$ ) with  $\text{Na}^+$  and  $\text{Cl}^-$  as the only small ions.<sup>7</sup>

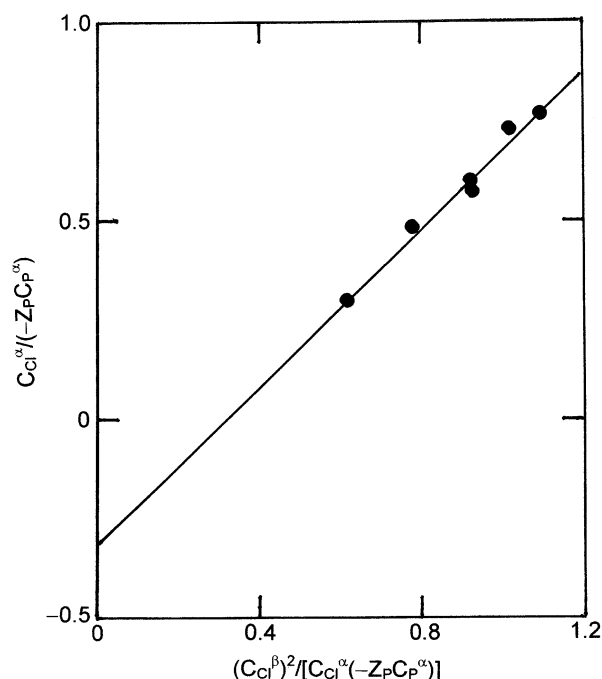
For a polyanion ( $Z_P$  negative) in the presence of a single uni-univalent electrolyte such as NaCl, the Donnan requirement is more rigorously written<sup>1,2</sup> as

$$(C_{\text{Cl}}^b)^2 = C_{\text{Cl}}^a(C_{\text{Cl}}^a - fZ_P C_P^a)(\gamma_{\pm}^a/\gamma_{\pm}^b)^2 \quad (7)$$

This equation is readily rearranged to the form<sup>7</sup>

$$\frac{C_{\text{Cl}}^a}{(-Z_P C_P^a)} = \frac{(\gamma_{\pm}^b/\gamma_{\pm}^a)^2 (C_{\text{Cl}}^b)^2}{C_{\text{Cl}}^a(-Z_P C_P^a)} - f \quad (8)$$

where  $(-Z_P C_P^a)$  is the concentration of counterion ( $\text{Na}^+$ ) required to neutralize the charges on the polysaccharide ( $C_{\text{Na}}^a = C_{\text{Cl}}^a - Z_P C_P^a$ ). It is reasonable to presume that the effective charge fraction ( $f$ ) should be constant at constant ionic strength, whereupon a linear dependence of  $C_{\text{Cl}}^a/(-$



**Figure 3.** Evaluation of the effective fractional charge ( $f$ ) from equilibrium dialysis studies of dextran sulfate in the presence of 0.1 M NaCl, the results being plotted according to eq 8. (Data taken from Table 4 of Preston and co-workers.<sup>7</sup>)

$Z_P C_P^{\alpha}$ ) on  $(C_{Cl}^{\beta})^2/[C_{Cl}^{\alpha}(-Z_P C_P^{\alpha})]$  is predicted in situations where the activity coefficient ratio is essentially constant.<sup>7</sup> Radiochemical quantification of sodium and chloride concentrations was used to ascertain  $C_{Na}^{\alpha}$  and  $C_{Cl}^{\alpha}$ , whereupon  $(-Z_P C_P^{\alpha})$  was determined as  $(C_{Na}^{\alpha} - C_{Cl}^{\alpha})$ .

Results from Table 4 of Preston and co-workers<sup>7</sup> for dialyzed dextran sulfate solutions with  $C_{Cl}^{\beta} = 0.096\text{--}0.100$  M certainly exhibit the predicted linear dependence (Figure 3). Furthermore, the magnitude of  $0.32 (\pm 0.06)$  for  $f$  that is deduced from the ordinate intercept essentially matches the current value for dextran sulfate (Table 1) at a slightly lower ionic strength ( $I = 0.08$  M, cf., 0.10 M). It is also of interest to note that the ratio of mean activity coefficients (deduced from the slope) is effectively unity  $[(\gamma_{\pm}^{\beta}/\gamma_{\pm}^{\alpha})^2 = 0.99 (\pm 0.07)]$ , an assumption made for chromate ion in the current application of eq 4 to obtain the effective fractional charge of each sulfonate group. Admittedly, the present estimates of  $f$  and  $(\gamma_{\pm}^{\beta}/\gamma_{\pm}^{\alpha})^2$  differ from the respective values of 0.21 and 0.88 that were obtained by comparable analysis of the whole data set presented in Table 4 of Preston and co-workers.<sup>7</sup> However, those values were influenced by the inclusion of results from experiments with a range of diffusate concentrations ( $0.014 \text{ M} \leq C_{Cl}^{\beta} \leq 0.100 \text{ M}$ ) and were therefore subject to the validity of more-questionable assumptions that the magnitudes of  $f$  and the activity coefficient ratio are independent of ionic strength.

The important point to emerge from this reappraisal of the earlier equilibrium dialysis data on the Donnan distribution of ions in dextran sulfate solutions<sup>7</sup> is its confirmation of the extent of charge-screening deduced from the present study with chromate as an indicator ion. By vindicating enforced assumptions about the absence of chromate binding and a value of unity for the mean ion activity coefficient ratio in the current study of dextran sulfate in 0.08  $I$

phosphate (pH 6.8), the results presented in Figure 3 also engender confidence in the estimates of the extent of charge-screening deduced from present studies of the two other polysaccharide anions, heparin and polygalacturonate (Table 3). In that regard, the estimated  $f$  of 0.32 for heparin also receives support from the value of  $0.25 (\pm 0.06)$  that is inferred (product of valence and equivalent weight divided by the corrected molecular weight) from Tables 1 and 2 of Braswell,<sup>6</sup> in which the effective net charge was deduced from its effect on thermodynamic nonideality in sedimentation equilibrium studies.<sup>16</sup> It remains to compare the present findings with the values of effective fractional charge ( $f$ ) that are predicted for the three polysaccharides by polyelectrolyte solution theory.<sup>2,3</sup>

A fundamental parameter in the calculation of net charge for a polyelectrolyte in an aqueous solution of uni-univalent electrolyte CN is the charge density parameter,  $\xi$ , defined<sup>2,3</sup> as

$$\xi = e^2/(\epsilon k_B T b) \quad (9)$$

where  $e$  is electronic charge,  $\epsilon$  the dielectric constant of the medium,  $k_B$  the Boltzmann constant,  $T$  the absolute temperature, and  $b$  the mean intercharge distance for the fully extended chain. For dextran sulfate, the intercharge spacing parameter ( $b$ ) is 0.26 nm<sup>17</sup>—a value shared by heparin, which also possesses two anionic groups per hexose residue: the corresponding parameter for polygalacturonate is 0.52 nm (one carboxyl group per hexose residue). Substitution of these intercharge spacings into eq 9 gives rise to respective charge densities ( $\xi$ ) of 2.78 and 1.39 for doubly and singly charged monosaccharide residues at 21 °C. On the grounds that  $\xi > 1$ , the effective fractional charge ( $f$ ) for each of the polysaccharides is given by the relationship

$$f = \phi/\xi \quad (10)$$

where  $1/\xi$  accounts for the decreased fractional charge reflecting mandatory anion condensation and where  $\phi$ , the osmotic coefficient, takes into account the further charge screening arising from Debye–Hückel considerations.<sup>2,3</sup> This parameter is in turn calculated from  $\xi$  via the expressions (see eqs 18, 23, 34, 35, and 40 of Manning<sup>2</sup>)

$$\phi = [X/(2\xi) + 2]/(X + 2) \quad (11)$$

$$X = (Z_P C_P^{\alpha}/\xi)/[C_{CN}^{\beta} - Z_P C_P^{\alpha}/(4\xi)] \quad (12)$$

The final column of Table 1 presents the values of  $f$  that have been calculated from eqs 10–12 on the basis that  $C_{CN}^{\beta}$  can again be replaced by the ionic strength of the diffusate ( $I^{\beta}$ ) for these three polysaccharides in phosphate buffer (see eq 4). There is excellent agreement between theoretical and experimental estimates of the effective fractional charge for dextran sulfate and heparin, but the predicted value of 0.60 for polygalacturonate greatly exceeds the experimental value (0.33). In view of the agreement between theory and experiment observed for the other two polysaccharides, the disparity can reasonably be attributed to partial esterification of the polygalacturonate ( $-\text{COOCH}_3$  rather than  $-\text{COO}^-$  for some residues). Despite its label, the polygalacturonate



was merely a purified pectin preparation (from orange juice), which may also contain neutral sugar residues such as rhamnose. Under those circumstances,  $f$  becomes the effective charge per hexose residue rather than the fractional charge on each carboxylate, whereupon comparison with theoretical prediction is precluded by the inability to assign magnitudes to  $b$ , and hence  $\xi$ , without knowledge of the degree of esterification and the neutral sugar content.

### Concluding Remarks

The purpose of this investigation has been to refocus attention on the need for experimental determination of the effective net charge on polysaccharidic macroions. For two-thirds of the charged groups on dextran sulfate and heparin in 0.08 *I* phosphate buffer (pH 6.8), the interactions with counterion are sufficiently strong to preclude participation of those counterions in the Donnan distribution of small ions during dialysis. Because such "binding" or condensation<sup>1,2</sup> of counterions to the carboxylate/sulfonate groups eliminates their consideration as sources of net charge, the effective valence is only one-third of that deduced from the chemical structure of dextran sulfate and heparin. For polygalacturonate, the effective charge per hexose residue is also one-third of that based on the structural formula; however, in this case, some of the carboxylate residues bear no charge because of esterification rather than counterion condensation. Although polysaccharides may still qualify for consideration as highly charged macroions, the magnitude of the effective charge density is diminished markedly by the various effects of charge-screening. For proteoglycans, the smaller but significant extent of this effect<sup>7,8</sup> presumably reflects, at least in part, the confinement of counterion condensation to the carbohydrate portions of these protein-polysaccharide complexes.

As well as drawing attention to the need for account to be taken of the diminished net charge on polysaccharide macroions, this study has also illustrated a relatively simple procedure for its determination. Despite the undesirability of the enforced assumption in eq 4 that the mean activity coefficient ratio for the indicator ion system is unity, the present findings suggest that this course of action is likely to represent a reasonable approximation. Indeed, a more-serious objection to the procedure has been the necessity to assume no binding of the indicator ion—a potential phenomenon that would have been quantified by the sedimentation velocity experiment if the rate of Donnan reequilibration across the macroion boundary had been slower.<sup>4,18</sup> In that regard, the substitution of a more-rapid separation procedure, such as ultrafiltration, may well provide an unequivocal estimate of  $C_L^\alpha$ .<sup>19,20</sup> Therefore, there seems to be scope for

optimism that equilibrium dialysis in the presence of an indicator ion (preferably a noncounterion) has the potential to provide a ready means of determining the effective net charge ( $fZ_p$ ) of polysaccharides (and polynucleotides presumably) in buffered electrolyte media that resemble more closely the physiological environment.

Finally, this investigation has served to highlight the ease with which fundamental findings can become lost in the burgeoning scientific literature. Although the existence of charge-screening in macroions may be a revelation to many current biochemical researchers, the concept has long been recognized as being central to our understanding of the charge-related characteristics of nucleic acids<sup>4,5</sup> and synthetic polyelectrolytes, such as polyacrylate<sup>21</sup> and polymethacrylate,<sup>1,17,22–24</sup> as well as polysaccharides.<sup>6–8,17,25</sup> May this communication help to reinforce this important aspect of the solution behavior of polyelectrolytes that is frequently overlooked in biochemistry and molecular biology textbooks.

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