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The theoretical distributions and diffusivities of small ions in chondroitin sulphate and hyaluronate

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The electrostatic interactions between polyionic glycosaminoglycans and small mobile ions are investigated using the Poisson–Boltzmann equation and a rod-in-cell model of the polyelectrolyte. Calculations are made for the range of polyelectrolyte concentrations and buffer compositions for which measurements of ion distributions and diffusivities are reported in a companion paper (Maroudas et al., Biophys. Chem. 32 (1988) 257). We conclude that the distribution of mobile ions is largely determined by the 'far-field' potential and is adequately described by the Poisson–Boltzmann theory and also by more approximate theories such as ideal Donnan or 'condensation' theory. The measured variations in cation diffusivities, particularly the increase in diffusivity with increasing matrix concentration at low ionic strengths, are predicted qualitatively using an approximate diffusion theory together with the calculated potential fields. However, the same theory applied to anion diffusion gives qualitatively wrong results.

1. Introduction

In a companion paper [1] we drew attention to the fact that studies on the interactions of small inorganic ions with glycosaminoglycans in well-defined model systems had been carried out only at polyion concentrations much lower than those occurring in the dense connective tissues and we attempted to close this gap in the experimental literature. Comparison of the experimental data obtained at low matrix concentrations either with the Donnan theory of ionic equilibrium or with the predictions of more detailed polyelectrolyte theory has led to the conclusion that the ion–polyion interactions are principally electrostatic [2,3], although there is some evidence of minor, more specific interactions [4]. Our experimental data described in ref. 1 suggest that at higher matrix concentrations electrostatic interactions between matrix elements become important and may help to screen mobile ion interactions. The principal aim of this paper is to investigate whether this behaviour can also be described within the framework of polyelectrolyte theory.

Much of the previous discussion of polyelectrolytes has centred around Manning's condensation theory [5,6] which in some sense can be shown to be an approximation to Poisson–Boltzmann theory [7–10]. Although the theory has been formulated in terms of general mixtures [11], most of the results which have been obtained are for the limiting case of infinite dilution and do not provide an appropriate formalism for the consideration of effects of changing matrix concentration. In the present work we have adopted the full non-linear Poisson–Boltzmann theory. The Poisson–Boltzmann formulation is also an approximation because of its neglect of direct polyion–polyion interactions but its theoretical foundations

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have been clarified [10] and the rod-in-cell model proposed by Katchalsky [12] is both straightforward and consistent with a number of widely used models of polymer matrices [13–15].

We have obtained numerical solutions for the equilibrium distributions of the mobile ions over a wide range of polyion concentrations and added salt concentrations and for various mixtures of monovalent and divalent counterions. These solutions display all of the trends observed in the experimental measurements of partition coefficients. However, the theoretical solutions depend quite sensitively on parameters such as charge spacing and polyion diameter and we find that uncertainties in these parameters preclude a detailed quantitative comparison between theory and experiment.

Several authors have also observed that the prediction of colligative properties is, in any case, only a weak test of a polyelectrolyte theory [12] and theoretical analysis of data on ion diffusivity should provide a more stringent test. There are, however, two problems: firstly, the effects of steric interactions are combined with electrical ones and, secondly, only a fairly simplistic theory of ion diffusion in an electrical potential gradient is available [16]. Interest in the movement of ions through charged polymer matrices first arose in relation to ion exchange membranes and one of the earliest models of transport ignored electrical interactions completely and considered the polymer merely as a physical obstacle [17]. This theory has been applied to the diffusion of ions in cartilage with some success [18]. A later, more elaborate statistical analysis of the 'obstruction' effect has also proved quite successful in describing the diffusion of a range of neutral solutes in biological polymers [19].

In the present work, we apply both of these models to our experimental data [1] and find that both provide a reasonable description of the diffusion of anions over the whole range of experimental conditions and of cations at high matrix concentration and high ionic strengths. Under 'physiological conditions', therefore, the obstruction effect alone is sufficient to account for the observed reduction in diffusivity even for divalent cations. At lower concentrations of salt and polyion, however, electrostatic effects become important. Although the theory of ion diffusion in a spatially varying electrostatic field [20–22] predates condensation theory by a number of years, most comparisons with experimental data appear to have combined both theories and made the additional assumption that the condensed ions have the same mobility as the polyelectrolyte molecules (essentially zero) [23]. The comparison has been made only over a restricted range of conditions but discrepancies have been noted both for biopolymers [3] and synthetic polymers [24–28]. In the final section of this paper we combine the Lifson–Jackson–Coriell–Manning expression for ion diffusivity with the Poisson–Boltzmann model of the electrostatic interaction. We show that the most striking experimental observation, that at low ionic strength diffusivity first falls and then rises as the matrix concentration increases for monovalent cations and even more dramatically for divalent cations, is predicted quite accurately by the theoretical model. The theory in its present form fails, however, by predicting similar behaviour for anions, which is not observed experimentally.

2. Theory

2.1. The Poisson–Boltzmann equation

We shall assume that the ionic interactions are described by the Poisson–Boltzmann equation and adopt the rod-in-cell model of the polyelectrolyte matrix as proposed by Katchalsky [12]. The first assumption rests upon the neglect of polyion–polyion and ion–ion interactions and its validity at higher concentrations must be questioned [10]. The second assumption is a gross over-simplification of the geometry of the matrix molecules but it may provide a reasonable description of the local configuration of the polyion even at relatively large matrix concentrations. For polyelectrolyte solutions without added salts, Mandel [15] has used scaling arguments to suggest that the rod-in-cell model is justified except in very dilute solutions where end effects are important and in very concentrated solutions where the obvious de-

fect that cylinders are not space filling becomes important. In polyelectrolytes with added salts, we would expect their screening effects of the mobile ions to extend the range of concentrations over which the cylindrical model is valid.

Consider a solution of polyions and small mobile ions in a solvent with a uniform dielectric constant. In the region 'outside' the polyion, the electrostatic interactions can be described by the Poisson equation

$$\nabla^2\psi = -\rho/\epsilon \tag{1}$$

where ψ is the potential, ρ is the local charge density and ϵ is the dielectric constant (SI units are used throughout). If the mobile ions are in local equilibrium, their distribution is given by the Boltzmann relationship

$$n_i = n_i^0 \exp\frac{-z_i e\psi}{kT} \tag{2}$$

where n_i is the local number density of the ith ion, n_i^0 is the number density at the reference point (or region) where the potential is taken to be zero, z_i is the valency of the ith ion, e is the charge on the electron, k is Boltzmann's constant and T is the absolute temperature. The local charge density is the sum taken over all of the mobile ions including both counterions and added electrolytes

$$\rho = e\sum_i z_i n_i \tag{3}$$

Eqs. 1 and 3 combine to give the Poisson–Boltzmann equation which we write in terms of the nondimensional potential, $\phi = -e\psi/kT$,

$$\nabla^2\phi = \frac{e^2}{\epsilon kT}\sum_i z_i n_i^0 e^{z_i\phi} \tag{4}$$

The coefficient in eq. 4 has the dimensions of length and can be written more conveniently in terms of the Bjerrum length $4\pi l_B = e^2/\epsilon kT$ which depends only upon the solvent and the temperature, and for water at 4°C is about 0.7 nm.

The specific (yet fairly general) problem we consider is that of a polyanion in a solution containing additional mono- (subscript 1) and di- (subscript 2) valent cations all with a common monovalent anion (subscript 3). For this case, eq. 4 is

$$\nabla^2\phi = 4\pi l_B\left(n_1^0 e^{\phi} + 2n_2^0 e^{2\phi} - n_3^0 e^{-\phi}\right) \tag{5}$$

In the rod-in-cell model, the polyion, of radius a, is located along the axis of a cylinder of radius R. These cylinders are assumed to be space filling so that

$$\pi R^2 b n_0 = 1 \tag{6}$$

where n_0 is the equivalent number density of the polyion and b is the average distance between charges on the polyion chain. The radius R is most conveniently determined from the volume fraction of the polyion, which for lack of better information we calculate from the specific volume, v

$$c_0 v = (a/R)^2 \tag{7}$$

where c_0 is the concentration of the polyion expressed as weight/weight of solution and a is the effective radius of the polyion molecule.

One boundary condition for eq. 4 comes from the assumption of symmetry of the potential on the outer boundary of the cells

$$\frac{d\psi}{dr} = 0; \quad r = R \tag{8}$$

The other boundary condition comes from the requirement of overall neutrality within each cell, which can be written as a condition on the potential gradient at the inner boundary by using the divergence theorem

$$\frac{d\psi}{dr} = \frac{e}{2\pi\epsilon ba}; \quad r = a \tag{9}$$

Introducing the nondimensional radius, $x = r/a$, the governing equation and its boundary conditions are:

$$\frac{1}{x}\frac{d}{dx}\frac{(x\,d\phi)}{dx} = \alpha_1 e^{\phi} + 2\alpha_2 e^{2\phi} - \alpha_3 e^{-\phi} \tag{10}$$

$$\frac{d\phi}{dx} = \frac{-2l_B}{b}; \quad x = 1$$

$$\frac{d\phi}{dx} = 0; \quad x = R/a$$

where $\alpha_i = 4\pi l_B a^2 n_i^0$ are measures of the number density of the ith ion at the reference point. It is convenient in analysing this equation to introduce a new spatial variable $y = \ln x$. In terms of y

$$\frac{d^2\phi}{dy^2} = e^{2y}\left(\alpha_1 e^{\phi} + 2\alpha_2 e^{2\phi} - \alpha_3 e^{-\phi}\right) \qquad (11)$$

$$\frac{d\phi}{dy} = -2\xi; \quad y = 0$$

$$\frac{d\phi}{dy} = 0; \quad y = Y$$

where $\xi = l_B/b$ is the measure of linear charge density on the polyion used by most previous workers, and $Y = \ln(R/a)$.

The solution of this equation depends upon the ionic strengths through the α_i, the linear charge density on the polyion through the inner boundary condition and the concentration of the polyion through its effect on the location of the outer boundary condition. The solution also depends upon the experimental conditions in that they determine the appropriate choice of the reference point where $\phi = 0$. Much of the previous work on polyelectrolytes has considered isolated solutions for which a reasonable choice for the reference point is the point of symmetry, $r = R$. In this case, the reference concentrations, n_i^0, are not known a priori but must be chosen so that the integral of the concentrations over the whole volume correspond to the amounts actually added. In the equilibrium dialysis experiments which we are modelling, the polyelectrolyte solution is separated from another solution by a membrane permeable to the mobile ions but not the polyions. In this case, it is more appropriate to choose the reference point as the region in the outer solution very far from the membrane, and use the equilibrium condition of uniform chemical potential for each of the ions to determine the reference potential inside the polyelectrolyte [29]. If the volume of the outer solution (the bath) is very large compared to the volume of the polyelectrolyte solution (the dialysis sac) then the reference concentrations, n_i^0, can be set a priori by the composition of the bathing solution. It may be remarked that for the familiar case of no added salt the choice of reference concentration is equivalent to a gauge transformation on the potential and therefore no ambiguity arises; this simplicity is lost in the general case.

Eq. 11 has analytical solutions for two special cases. For the case of no added salt, $\alpha_1 = \alpha_2 = 0$, the solution has been derived and discussed extensively by Katchalsky and his co-workers [12]. The other solution is the Debye–Huckel approximation where, for small ϕ, the exponentials are approximated by the first two terms of their power series. The resulting equation is linear and the solution depends only upon the Debye length

$$\lambda = 1/\sqrt{4\pi l_B \sum z_i^2 n_i^0} \qquad (12)$$

This approximation is frequently employed and is assumed, for example, in condensation theory. The domain of validity of this linear approximation, discussed at length by Lampert and Crandall [30], is too restrictive for our present purpose and we resort instead to numerical methods.

2.2. *The Donnan theory of ion distributions*

The Donnan theory is widely used, at least in the biological literature, to predict ionic partition coefficients and it is therefore of interest to compare its predictions with those of the more detailed electrostatic theory. This theory [31] presumes a uniform potential within the polyelectrolyte solution and determines the distribution of diffusible ions from the simultaneous requirements of electroneutrality and the uniformity of chemical potential of each diffusible species across the semipermeable membrane. For the case of mono- and di-valent added salts with a common anion considered here, electroneutrality within the polyelectrolyte solution requires

$$n_1 + 2n_2 - n_3 = n_0 \qquad (13)$$

For the ideal case, the activity of each diffusible salt depends only upon the concentrations of its ions, so the uniformity of chemical activity requires

$$n_1 n_3 = n_1^0 n_3^0 \qquad (14)$$

$$n_2 n_3^2 = n_2^0 n_3^{0\,2}$$

In an infinite bath the external concentrations, n_i^0, are specified and the solution to these equations in terms of the ideal Donnan partition coefficients, $K_i = n_i^0/n_i$ is

$$K_1 = 1/K_3; \quad K_2 = 1/K_3^2 \tag{15}$$

where K_3 is the solution to the cubic equation.

$$K_3^3 + XK_3^2 - \frac{n_1^0}{n_3^0}K_3 - \frac{2n_2^0}{n_3^0} = 0 \tag{16}$$

and $X = n_0/n_3^0$ is the molality ratio of polyion to added salt which is commonly taken as a fundamental parameter in polyelectrolyte theory.

2.3. Ion diffusivity

2.3.1. Steric interactions

Mackie and Meares [17] considered the effect of steric hinderance on the diffusion of solutes through ion exchange membranes and concluded that

$$\frac{D}{D^0} = \frac{1 - c_0 v}{1 + c_0 v} \tag{17}$$

where v is the specific volume of the membrane polymer and D^0 is the free diffusivity. Ogston et al. [19] considered diffusion through a random array of rod-like obstructions and concluded that

$$\frac{D}{D^0} = \exp\frac{-(a + r_s)}{r_s}\sqrt{c_0 v} \tag{18}$$

where a is the radius of the matrix molecule and r_s is the radius of the diffusing solute.

2.3.2. Electrostatic interactions

The effect of electrostatic interactions on the diffusivity of ions in a polyelectrolyte matrix has been considered by a number of authors [12,20–22]. Since the general theory of diffusion in a varying field is an unsolved problem of classical physics, all of these theories are, to some degree, approximations or limits deduced from special cases.

Jackson and his co-workers [20,21] have derived an expression for the effective diffusivity of an ion in a periodic field

$$\frac{D_i}{D_i^0} = \frac{1}{\langle \exp(z_i\phi)\rangle\langle \exp(-z_i\phi)\rangle} \tag{19}$$

where D_i^0 is the free diffusivity and $\langle\ \rangle$ indicates the spatial average. This expression is exact for one-dimensional diffusion and in three dimensions it can be shown, by variational arguments, to be a lower bound. It should be noted that the expression is an even function of ionic charge and therefore the diffusivities of anions and cations of the same valency should be reduced by a similar amount. Manning [22] has obtained an expression for ion diffusivity in a regular array of line charges using the Debye–Huckel linearisation

$$\frac{D_i^u}{D_i^0} = 1 - \frac{z_i^2}{3}\sum_{-\infty}^{\infty}\sum_{-\infty}^{\infty}\Phi_{mn}^2 \tag{20}$$

Assuming that the diffusivity of the condensed ions is zero, the coefficients are for monovalent ions [22]

$$\Phi_{mn} = \left[\pi(m^2 + n^2) + 1 + 2\xi/X\right]^{-1} \tag{21}$$

and for divalent ions [3]

$$\Phi_{mn} = \left[\pi(m^2 + n^2) + 1 + \xi(6/X - 1)\right]^{-1} \tag{22}$$

3. Results

3.1. Ion distributions

In the present work, eq. 11 has been solved numerically without further restrictions using a second order Runge-Kutta method. The potential field calculated for different matrix concentrations, ionic strengths and mixtures of monovalent and divalent counterions are shown in fig. 1. The parameters used in these calculations are given in table 1 and are chosen to reflect the properties of CS and HS as closely as possible within experimental uncertainties. The effect on the potential field of added monovalent salt at a constant, relatively large polyion concentration is shown in fig. 1a. At physiological ionic strength, $X < 1$, the counterions are relatively plentiful and effectively shield the polyions from interacting with each other. As a result, the potential is low. As the ionic strength in the bath is decreased, the polyions begin to interact with each other as their potential

fields begin to overlap. This occurs at $X \approx 1$ for the conditions in fig. 1a. Once this polyion interaction dominates, a further decrease in ionic concentration results in an increase in the calculated potential with a corresponding increase in the partition coefficient, K_1. It should perhaps be noted that although the parameter X is frequently used as the independent variable in discussion of polyelectrolyte phenomena, its use is limited to the cases of low polyion concentration and a single added salt. The former restriction arises because changes in n_0 and n_3 are reciprocal only when the overlap of polyion fields is negligible. In the case of mixed added salts, however, the symmetry between ion and polyion charge is further destroyed and a more physically meaningful parameter is possibly the ratio of Debye length (dependent on ion concentration) to cell radius (dependent on polyion concentration).

The effect of changing polyion concentration whilst maintaining the total salt concentration constant is shown in fig. 1b for a low concentration of monovalent added salt (0.0015 M). The effect is qualitatively similar to that in fig. 1a in that as the polyion concentration decreases, the radius of the cell increases and the interaction between the polyions decreases. For each ionic composition there is a polyion concentration at

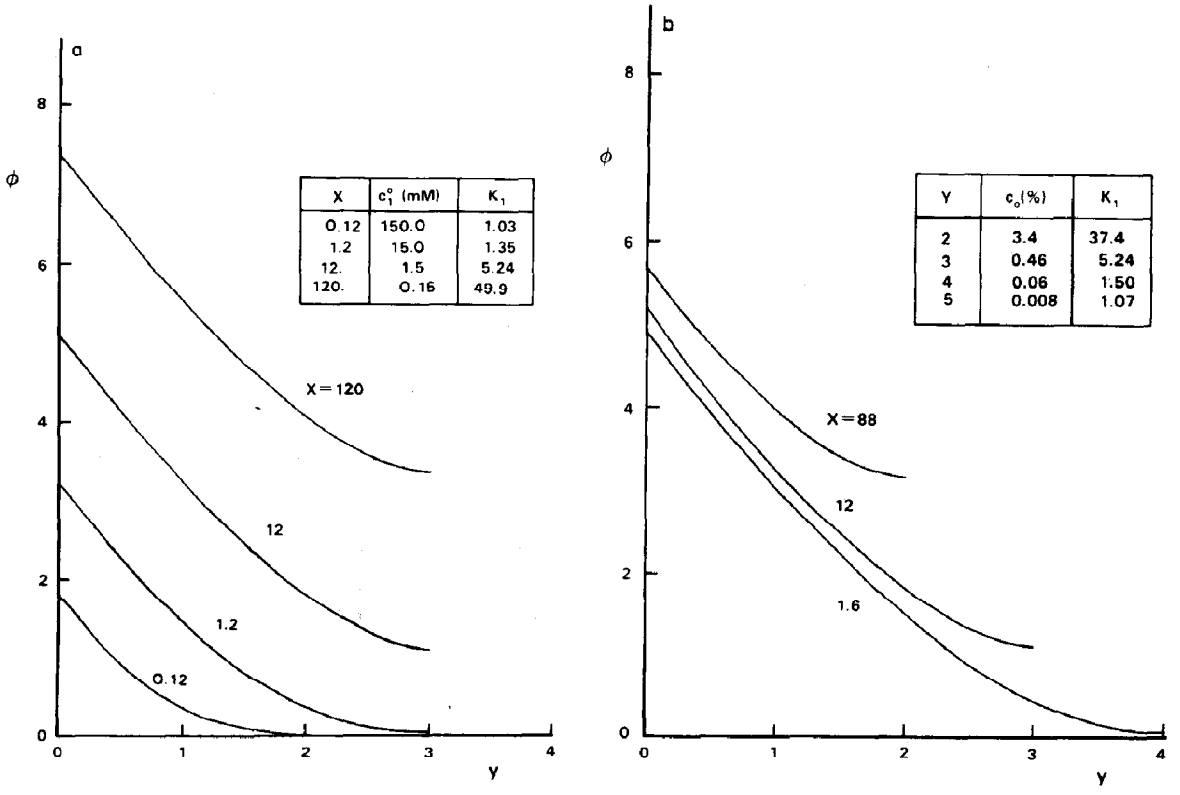

Fig. 1. The reduced potential ϕ as a function of $y = \ln(r/a)$ where a is the effective radius of the polyion calculated for different matrix concentrations and ionic strengths. The parameters used in the calculations as representative of CS and HA are given in table 1. (a) The effect of ionic strength at fixed CS concentration in a monovalent salt solution calculated for $Y = 3.0$, which is equivalent to $c_0 = 0.46\%$. (b) The effect of CS concentration at fixed ionic strength in a monovalent salt solution calculated for $c_1^0 = c_3^0 = 1.5$ mM, $c_2^0 = 0$. (c) The effect of divalent salt at fixed ionic strength and fixed CS concentration calculated for $c_3^0 = 1.5$ mM. (d) The effect of ionic strength at fixed HA concentration in a monovalent salt solution calculated for $Y = 3.0$, which is equivalent to $c_0 = 0.38\%$. The effect of polyion charge density can be seen by comparing these results with those in panel a calculated for CS, which has twice the linear charge density.

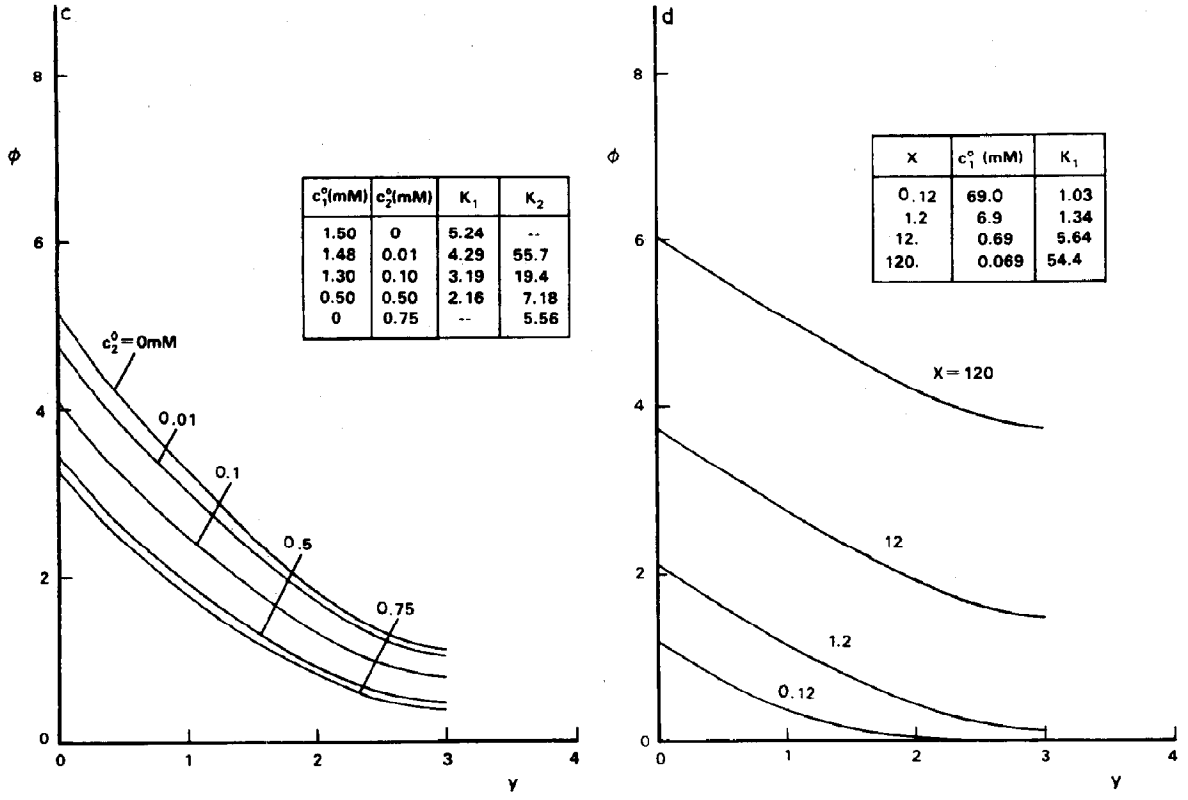

Fig. 1 (continued).

which this interaction effectively vanishes and a further decrease in concentration has little effect on the potential or the ion distributions. For the conditions in fig. 1b, this occurs at $Y \approx 4$ which, for CS, corresponds to a concentration of about 0.06% (w/w H_2O).

Fig. 1c shows the effect of adding divalent counterions whilst maintaining a fixed polyion concentration and a fixed total ionic strength in the external solution. For low total ionic strength the presence of even a small amount of divalent ions can alter the potential field significantly. Because of the stronger interaction between the divalent ions and the polyions, the divalent partition coefficient, K_2, is much larger than K_1 and it is much more sensitive to factors which affect the potential distribution. The change in the potential distribution as the monovalent counter ions are replaced by divalent ions at constant ionic strength reflects the greater effectiveness of the divalent ions in screening the polyion charge. At high ionic strength the divalent ions again provide better screening, but since there is little or no polyion

Table 1

Parameters used in the calculations [2,19,32,33]

GAG	Effective radius a (nm)	Inter-charge distance b (nm)	Linear charge density 2ξ	Specific volume v
CS				
Value used	0.5	0.7	2.0	0.54
Experimental range	0.35–0.9	0.5–0.8	1.8–2.9	–
HA				
Value used	0.5	1.4	1.0	0.65
Experimental range	0.35–1.1	1.2–1.6	0.8–1.1	–

interaction in any case, both partition coefficients are effectively one.

The effect of the linear charge density of the polyion can be seen by comparing fig. 1d, calculated for a lower charge density comparable to that of HA, with fig. 1a, calculated for the same conditions for a charge density comparable to that of CS. Decreasing the charge on the polyion makes the potential distribution flatter, but, at least for the conditions of these calculations, the predicted partition coefficients depend almost entirely upon X.

The effects of the potential field on the partition coefficients of mono- and di-valent cations, K_1 and K_2, are summarised in fig. 2 and the partition coefficients are compared with those predicted by the ideal Donnan theory. The Donnan distribution depends only on the parameter X but the partition coefficients predicted by the Poisson–Boltzmann theory depend independently on ionic strength, as discussed in relation to fig. 1a and d, and also matrix concentration and polyion radius. As shown in fig. 2a, the distribution coefficient for divalent cations is most sensitive to matrix concentration at large X (i.e. low ionic strength) when the fields of the polyion overlap, whilst for monovalent ions the sensitivity is greatest at large X. This difference reflects the non-linear dependence of ion distribution on valency. The ion distribution most closely agrees with the Donnan theory at high matrix concentrations where fluctuations of the potential about the mean are lower due to the overlap of the fields from neighbouring polyions (see, for example, fig. 3 in ref. 1).

It is evident in fig. 2b that the Poisson–Boltzmann predictions are extremely sensitive to polyion radius and the effect of only a small change in

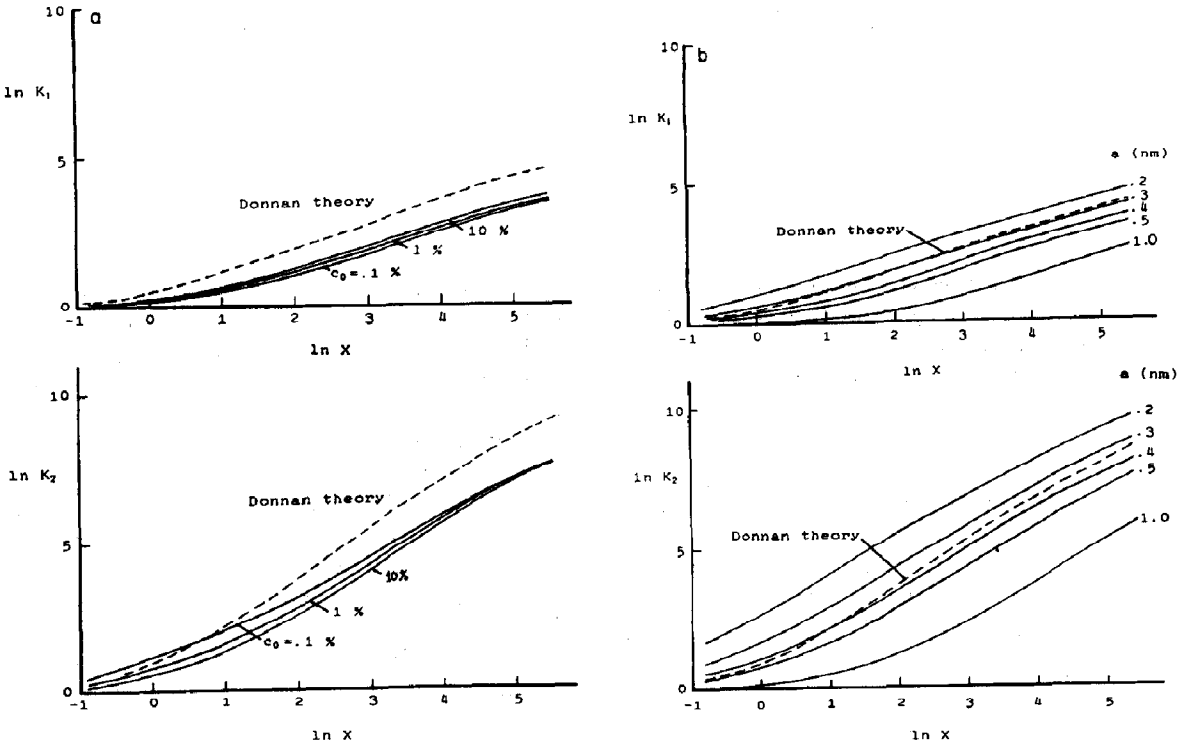

Fig. 2. Partition coefficients for monovalent, K_1, and divalent, K_2, cations as a function of X (the ratio of polyion molality, n_0, to the molality of added salt, n_3^0). (———) Poisson–Boltzmann theory calculated for $c_1^0/c_2^0 = 60$ which corresponds to experimental conditions in ref. 1; (- - - - -) ideal Donnan theory. (a) The effect of matrix concentration calculated for a polyion radius, $a = 0.5$ nm. (b) The effect of polyion radius calculated for a matrix concentration, $c_0 = 1\%$. Note the logarithmic scales.

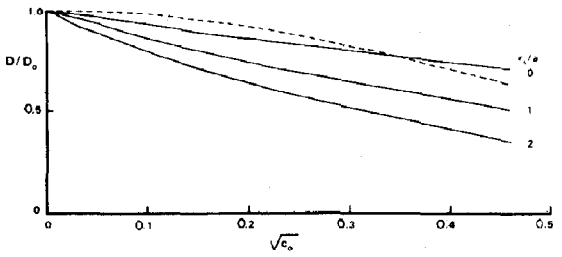

Fig. 3. The theoretical reduction of diffusivity in CS due to steric hindrance calculated for different ratios of solute radius to polyion radius, r_s/a. The parameters used in the calculations are given in table 1. (- - - - - -) eq. 17 [17]; (———) eq. 18 [19].

polyion radius is greater than deviations from the Donnan theory. As can be seen in fig. 2, if the polyion radius is taken as 0.5 nm (the value most frequently quoted in the literature) the partition coefficients for monovalent ions predicted by the theory invariably lie below those predicted by the Donnan theory. The same is true for divalent cations except at the lowest polyion concentrations. Since experimentally determined values of K in GAGs are close to those predicted by the Donnan theory, it seems that the effective radius of the GAGs should be closer to 0.3 nm.

3.2. Ion diffusion

The predictions of the two theories of the effect of purely steric hindrance on ion diffusion (eqs. 17 and 18) are illustrated graphically in fig. 3 for different solute radii. The predictions of the Ogston model are very sensitive to the radii of the diffusate and the polyion. However, both models provide a reasonable description of the behaviour of, for example, Na^+ at high ionic strength within the uncertainties of a, r_s and v, but there is a serious discrepancy with the data for Ca^{2+} at low ionic strength and low polyion concentration. It should be noted, though, that at higher matrix concentrations the electrostatic interaction appears to be of little consequence even at low ionic strength.

In order to quantitate the effect of electrostatic interactions, we have used eq. 19 together with numerical solutions of the Poisson–Boltzmann equation to calculate ion diffusivity at low ionic strength. It should be noted that the calculations differ in detail from those used to determine the partition coefficients, because under the conditions of the tracer diffusion experiment described by us [1] it is not possible to determine the n_i^0 a priori. They are determined by the volume averaged concentrations, $\langle n_i \rangle$, together with the assumption of $\nabla\phi = 0$ at $r = R$ and it is therefore necessary to use an iterative technique to solve for any particular experimental condition. The results of these calculations for monovalent and divalent ions is shown in fig. 4 for high and low ionic strengths. In these calculations, $\langle n_1 \rangle \gg \langle n_2 \rangle$; i.e. the divalent ion was present in relatively small amounts. The exact solution depends upon the ratio of the two ions, but the results, like those for the partition coefficient, are only weakly dependent upon the monovalent–divalent ion ratio compared to the effect of total ionic strength. It will be seen that at 'physiological' ionic strength the reduction in diffusivity is small but at low ionic strength there is a very large effect which, because of interactions between polyions, actually decreases at higher polyion concentration.

Fig. 5 shows the comparison between the predictions of eq. 19 and the experimental measurements of the diffusivity of Na^+ and Ca^{2+} in different concentrations of CS at low ionic strength. The agreement is extremely good except for Na^+ at the highest CS concentrations where the effects of steric hindrance by the CS chains, neglected in the derivation of eq. 19, become important. The predictions of condensation theory

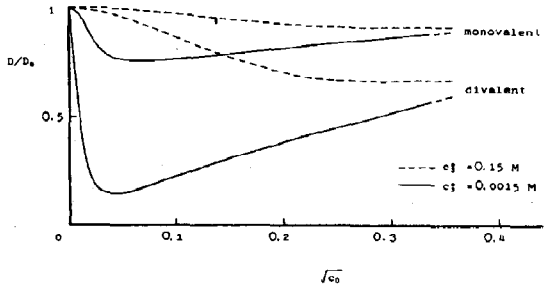

Fig. 4. The theoretical reduction of diffusivity in CS due to electrostatic interactions calculated from eq. 19 for monovalent and divalent cations. (- - - - - -) high ionic strength ($c_3^0 = 0.15$ M); (———) low ionic strength ($c_3^0 = 0.0015$ M).

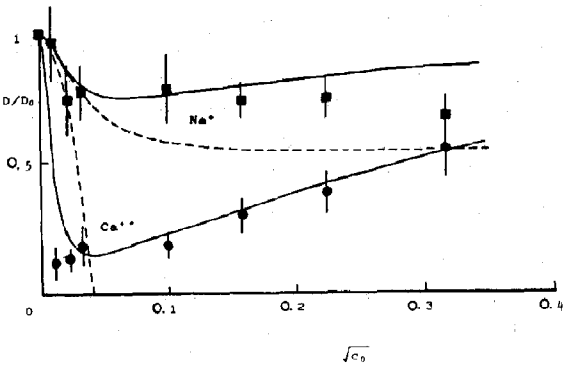

Fig. 5. The diffusivity of monovalent and divalent cations in CS at low ionic strength (1.5 mM). The symbols are experimental data from ref. 1 (fig. 2): (■) Na^+, (●) Ca^{2+}. The bars represent standard deviations. (———) Poisson–Boltzmann theory (from fig. 4); (------) condensation theory (eq. 20) assuming the condensed ions are immobilised.

calculated assuming that the diffusivity of the condensed ions is essentially zero are also included in fig. 5. As found by other workers [3], this theory provides a reasonable description of ion diffusivity at low polyion concentration where the assumptions leading to eq. 20 are expected to be valid. At higher concentrations, however, the limiting conditions are no longer valid and ion selectivity with increasing matrix concentrations should be taken into account [11].

One of the immediate implications of eq. 19 is that the presence of polyelectrolyte will have an identical effect on the diffusivities of both cations and anions of the same numerical charge. The uncertainties in the measurements of Cl^- diffusivity in ref. 1 were too large to test the prediction of symmetry between cation and anion diffusivity for monovalent ions. A discrepancy clearly emerges, however, for divalent ions, because in contrast to Ca^{2+}, SO_4^{2-} diffusivity is not reduced at low matrix concentrations. Eq. 19 is derived from a linearised analysis which exhibits a symmetry between positive and negative charges which does not exist in the nonlinear problem and so the theoretical explanation of these results may involve a full nonlinear analysis of the problem [16,34].

4. Discussion

Polyelectrolyte theory has not previously been tested experimentally at the high matrix concentrations and for the salt mixtures encountered in biological tissues. In the present paper we have attempted such a comparison, based on our experimental data [1] using the full Poisson–Boltzmann equation and a simple rod-in-cell model of matrix geometry.

We looked first at the equilibrium distribution of mobile ions. At low matrix concentrations the potential distributions given by the Poisson–Boltzmann equation close to the polyion are rather different from those of the linearised Debye–Huckel approximation. However, the volume averaged distribution of ions under these conditions is dominated by the contribution of the far field where the potential is approximately uniform and there is, therefore, little difference in the partition coefficients predicted by the Poisson–Boltzmann theory and condensation theory and even the more approximate Donnan theory gives an adequate description of the experimental data. At the higher matrix concentrations typical of connective tissues such as cartilage, the ratio of cell radius to polyion radius is smaller and the volume averages are less dominated by the far field and so larger differences between the predictions of the various formulations of polyelectrolyte theory might be expected. The qualitative agreement between the predictions of the Poisson–Boltzmann equation and the measured partition coefficients remained good. Unfortunately, however, the Poisson–Boltzmann theory contains a number of physical parameters which are known only approximately for the biological polymers we have used. In particular, we found that the theoretical results were very sensitive to the polyion radius (which does not appear in condensation theory) and that uncertainties in this parameter made quantitative comparisons difficult. At conditions of matrix concentrations and total ionic strengths typical of physiological conditions, the electrostatic effects of the polyion were effectively shielded by the mobile counterions and ideal Donnan theory was adequate to describe the ionic partition properties.

The prediction of colligative properties does

not constitute a sensitive test of polyelectrolyte theory, but diffusion which depends upon other factors including the gradient of the potential might be expected to provide a more sensitive test of a polyelectrolyte theory. Unfortunately, the underlying theory for the effect of a varying electrostatic potential on the diffusivity of an ion is only approximate and so the predictions of the theory are at best limits or bounds. In addition to the difficulty in characterising the interaction of the small ions with a single polyion there is the problem of describing the geometry of the real polymer network. We have employed a parallel array of rods. Recent work [34] suggests that regular parallel arrays give similar results, but random networks may behave differently. These uncertainties suggest to us that it is dangerous at present to interpret experimental diffusivity data as evidence either for or against a particular formulation of polyelectric theory. With this caveat, the excellent agreement between the predictions of eq. 20 and the data for Na^+ and Ca^{2+} at low matrix concentration to ionic strength shown in fig. 5 is unexpected. However, the lack of symmetry found experimentally in the behaviour of counter- and co-ions is clearly at variance with the simple theory (of diffusion rather than of polyelectrolytes, it should be emphasised) and as a minimal requirement, it appears to be necessary to elaborate eq. 19 to take account of the distribution of diffusing ions in the potential field.

Another conclusion of our study is that the effects of steric hindrance of the matrix on the diffusivity of ions must be taken into account at large matrix concentrations. In fact, with the exception of the diffusion of divalent cations at low ionic strengths, almost all of our diffusion results could be accounted for by the steric hindrance theory of Ogston et al. [19] which does not consider electrostatic interactions at all. The exception was, however, a dramatic one with the effective diffusivity of Ca^{2+} being decreased by an order of magnitude at low ionic strength and low matrix concentrations. This effect has been observed previously at low matrix concentrations [3,4] and is predicted by condensation theory. To our knowledge, the reduction of this decrease with increasing matrix concentration has not been reported previously. The success of the rod-in-cell model in predicting the large effect of the polyion at low concentrations as well as the decreasing of this effect with increasing matrix concentration gives us some confidence in the model.

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