The Osmotic Properties of Sulphoethyl-Sephadex

A MODEL FOR CARTILAGE

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1. Observations are reported on the variation of the swelling of sulphoethyl-Sephadex C-50 and C-25, and of the partition of NaCl between solution and gel, with the concentration of NaCl. 2. The results were interpreted in terms of Manning's (1969) treatment of the interactions between polyionic polymers and simple electrolytes and of Flory's (1953) treatment of the swelling of gels. 3. The results indicate net inner osmotic pressures as high as 3.6×10^5 Pa (140 mosmolar = 3.6×10^5 N/m²) in 0.15 M-NaCl. 4. It is suggested that cartilage may have net inner osmotic pressure of the order of 10^5 Pa.

The present paper describes the result of a study that we have made of the osmotic properties of SE-Sephadex* C-50 and C-25. We thought that this material might provide a model for cartilage, in view of the similarity between the polyanionic cross-linked dextran structure of SE-Sephadex and the cartilage system, where polyanionic glycosaminoglycans are immobilized within a collagen network. We hoped to obtain from the study quantitative estimates of the internal osmotic pressure of cartilage. Linn (1968) has suggested that this may be important in determining its mechanical properties.

We therefore measured first the effects of salt concentration and of dextran added to the outer solution on the swelling of the gel, and secondly the partition of Cl⁻ ion between outer solution and gel over a range of salt concentrations. From the partition measurements we obtained the activity coefficients of NaCl within the gel and compared these with the predictions of the theory of Manning (1969). We hoped to be able to use these and the swelling results to estimate the inner osmotic pressure of the gel over wide ranges of both degree of swelling and ionic strength. However, the relatively small effect of dextran on the swelling of the gel, compared with that of the concentration of electrolyte, made it possible to obtain estimates only in very restricted ranges of swelling at each ionic strength. Therefore we were only able to estimate the possible order of magnitude of the inner osmotic pressure of cartilage.

Terminology

We assume that there are two uniform phases in equilibrium, one composed of the particles of swollen gel, the other the solution that surrounds them. These are referred to as 'gel' and 'solution' or as 'inner' and

'outer'; the concentrations of a partitioned component are called 'inner' and 'outer' concentrations. The concentration (in the gel phase) of gel itself is called 'gel concentration', expressed as g/ml or g/g of water.

Experimental

Materials

SE-Sephadex C-50 and C-25 were from Pharmacia Fine Chemicals, Uppsala, Sweden, lots 2238 and 8761 respectively. They were washed in 1 M-NaCl, then thoroughly in water, then in ethanol and were dried in air at 105°C. Their sulphur contents, estimated by Dr. J. E. Fildes, were 2.36 and 2.18 mmol/g.

Dextran 500 was from Pharmacia, batch T 5406. Solutions were made and their concentrations measured as described by Ogston & Wells (1970). Titration of solutions (see below) showed a very low content of Cl⁻ ion.

NaCl, AgNO₃ and acetic acid were analytical grade. Solutions were made by weight. Water, twice distilled in an all-glass still, was used throughout.

Measurements of gel concentration

(i) Known weights of SE-Sephadex C-50 or C-25 were equilibrated with known weights of solutions of NaCl that contained known concentrations of Dextran 500. The relative amounts of SE-Sephadex and of solution were chosen so that, in a total volume of about 10 ml, about 1 ml of supernatant solution remained after equilibration. After 24 h with periodic mixing, the supernatant solution was analysed for Cl⁻ by titration with AgNO₃ (see below) and for dextran by polarimetry (Edmond *et al.*, 1968). The final dextran concentrations were low enough (less than 5 mg/ml) to have no significant effect on

* Abbreviation: SE-Sephadex, sulphoethyl-Sephadex.

the gel concentration. The apparent gel concentration was calculated on the assumption that no dextran enters the gel.

(ii) Relative changes of gel concentration with concentration of NaCl were measured from the changes of diameter of single spherical beads of SE-Sephadex C-50 by the method of Edmond *et al.* (1968).

Measurement of the effects of Dextran 500 on the gel concentration

Measurements were made, by the single-bead method of Edmond *et al.* (1968), of the effects on the gel concentration of various concentrations of Dextran 500 (up to 0.15g/ml) at several approximately constant concentrations of NaCl.

Partition measurements

The method closely resembled that of Ogston & Silpananta (1970) for the partition of neutral macromolecular solutes with neutral Sephadex. Equilibrium between SE-Sephadex and NaCl solution was for 24 h with occasional mixing, after which a sample of supernatant solution was withdrawn for Cl^- analysis. For calculation of the final inner concentration of Cl^- , the gel volumes were calculated from the experimental values of gel concentration at the final outer concentration of NaCl (Figs. 1 and 2). Inner and outer concentrations were expressed as molalities. The partial specific volume of the SE-Sephadex was taken to be 0.5 ml/g, that of dextran being 0.6 ml/g (Edmond *et al.*, 1968) and that of dextran sulphate of twice the sulphate content of the SE-Sephadex being 0.4 ml/g (J. D. Wells, unpublished work).

Determination of Cl^- ion

Supernatant solutions were used directly or after suitable dilution by weight. Samples (0.1–0.5 g containing 0.5–10 μequiv) were weighed into a 5 ml weighing bottle, sodium acetate buffer (1 ml, 0.2 M, pH approx. 4.7) was added and the solution was titrated with 0.05 M AgNO_3 , added in successive portions from an Agla micrometer syringe, with constant stirring by a magnetic stirrer. After each addition the e.m.f. between a Leeds and Northrup micro glass-electrode and a lightly chloridized silver wire, both dipping in the solution, was read (to 1 mV) with a Leeds and Northrup pH-meter. The steepest point of the e.m.f.-titre curve was taken as a first approximation to the end-point. From this titre and the solubility product of AgCl at the appropriate (room) temperature, the change of e.m.f. between the original solution and the equivalence point was calculated, and the final value of the titre was read from the curve at this point. The accuracy, checked

with known solutions of NaCl, was 1/300 or better. Dextran 500 did not interfere with the titration.

Results and Discussion

Gel concentration measurements

The dextran-dilution method should be reliable unless the dextran penetrates the gel significantly; if this happens, the gel concentration will be overestimated. Fig. 1 shows the results of measurements on SE-Sephadex C-50 by dextran dilution and by variation in the diameters of single beads (the latter were calculated on the assumption that the dextran-dilution value is correct in 0.2 M-NaCl); the fact that these results agree well over a wide range, and that the former tend to be lower in the region of greatest swelling of the gel, suggests that significant penetration does not occur. It is interesting that this should be so even at values of the gel concentration half that of Sephadex G-200 in water (cf. Edmond *et al.*, 1968). Since this must apply even more to SE-Sephadex C-25, only dextran-dilution measurements were made with this grade (Fig. 2).

The greater degrees of swelling of SE-Sephadex compared with those of corresponding grades of neutral Sephadex (from which, we understand, they are made) is to be expected because of the osmotic effects of the counter-ions in the inner phase only partly compensated by the Gibbs-Donnan distribution of neutral salt.

Fig. 1. Variation of gel concentration of SE-Sephadex C-50 with outer NaCl concentration (molal), measured by dextran dilution (●) and single bead diameters (○)

The relative bead-diameter values obtained from three separate beads were converted into absolute concentrations by assuming that $10^2 \times \text{gel concn.} = 5.5 \text{ g/ml}$ in 0.2 M-NaCl (shown by arrow).

Fig. 2. Variation of gel concentration of SE-Sephadex C-25 with outer NaCl concentration (molal)

Known weights of dry SE-Sephadex C-25 were added to known quantities of stock solutions containing Dextran 500, and the equilibrium gel volumes were calculated from the final dextran concentrations of the external solutions.

Partition of Cl^- ion

The mean molal activity coefficient, γ'_2 , of Na^+ and Cl^- ions in the gel at equilibrium is defined by the equation:

$$(\gamma'_2)^2 m'_2 (m'_2 + z_3 u'_3) = (\gamma_2)^2 (m_2^*)^2 \quad (1)$$

where m_2 is the outer molality of NaCl, γ_2 is its mean ionic activity coefficient (from Robinson & Stokes, 1955), m'_2 is the inner molality of Cl^- ion, z_3 is the stoichiometric value of anionic groups/g of SE-Sephadex and u'_3 is the weight of SE-Sephadex/g of water in the inner volume. The theoretical method of Manning (1969) for calculating the mean ionic activity coefficient of a 1:1 valent electrolyte in the presence of polyions contains the simplifying assumption that the 1:1 valent electrolyte itself behaves ideally. Consequently, where this condition is not satisfied (as in our experiments, particularly at higher concentrations of NaCl), this method predicts only a part of the actual activity coefficient. To enable comparison to be made we assume the additivity of excess free energies and write:

$$\gamma'_1 = \gamma_2^{\text{PM}} \cdot \gamma_2^{\text{MM}} \quad (2)$$

where γ_2^{PM} is the quantity calculated by Manning (1969) and γ_2^{MM} is the mean activity coefficient of NaCl at the same molality of Cl^- ion (Robinson & Stokes, 1955) in the absence of polyelectrolyte. We can then obtain experimental values of γ_2^{PM} for

comparison with those predicted by Manning's (1969) treatment, by use of eqns. (1) and (2). Similar treatment of measurements made on solutions of un-cross-linked polyelectrolyte of similar charge density (J. D. Wells, unpublished work) has given results agreeing well with Manning's (1969) theory over a wide range of conditions.

The results shown in Tables 1 and 2 (column 3) agree satisfactorily with values calculated by Manning's (1969) theory at higher salt concentrations (column 5), but there are increasing deviations at lower salt concentrations. Such deviations could have arisen from the presence within the gel either of bubbles (which are sometimes visible within the beads) or of regions that have partly or wholly escaped sulphation. Either would be inaccessible to dextran, and would therefore be included in the estimated gel volume: they would, however, contain NaCl at, or approaching, the concentration of the external solution. Values of γ_2^{PM} calculated on the assumption that the effective volumes of gel are lower than the measured volumes (by 10% for SE-Sephadex C-50 and by 4% for SE-Sephadex C-25) agree reasonably well with the theoretical values throughout the range (Tables 1 and 2, column 4).

Osmotic pressures

We can regard the equilibrium swelling of a gel as a balance between its net osmotic pressure and its 'elastic' resistance to swelling (Flory, 1953) as expressed by an equation of the type:

$$\frac{\pi_{\text{diff.}}}{RT} = \frac{\pi'}{RT} - \frac{\pi''}{RT} = \text{Pf} \quad (3)$$

(Edmond *et al.*, 1968) where π' and π'' are the total osmotic pressures of the inner and outer phases respectively, P is a constant depending on the structure of the gel and f is a function of its inner concentration. In the context of the possible osmotic swelling of cartilage, we are interested in the value of $\pi_{\text{diff.}}/RT$. For SE-Sephadex this could be estimated in several ways.

(i) Since the cross-linked structure of the original neutral Sephadex should not be seriously affected by the insertion of ethanesulphonic acid groups, one could assume the value of P to be the same in SE-Sephadex as in the corresponding neutral Sephadex, with adjustment for the change of mean residue weight. This would suggest a value of 2.8×10^{-5} mol/ml for P in the case of SE-Sephadex C-50. However, this value was established from measurements on Sephadex G-50 at gel concentrations of 0.2 g/ml and above (Edmond *et al.*, 1968), whereas the range for SE-Sephadex C-50 is 0.09–0.017 g/ml.

(ii) From the gel swelling and Cl^- partition measurements we know the equilibrium compositions of both gel and solution phases over a wide range

Table 1. Mean inner activity coefficients (γ_2^{PM}) of NaCl in SE-Sephadex C-50, calculated from the outer (m_2'') and inner (m_2') concentrations of Cl^- ion

Column 3 gives the directly calculated experimental values, column 4 the corrected values (see the text) and column 5 the values calculated by the theory of Manning (1969).

$10^3 \times \text{Concn. of } \text{Cl}^-$		γ_2^{PM}		
External (m_2'' , molal)	Internal (m_2' , molal)	Experimental	Corrected	Theoretical
1.978	0.345	0.477	0.677	0.685
1.982	0.365	0.489	0.636	0.685
7.923	2.405	0.666	0.733	0.740
8.109	2.474	0.670	0.737	0.741
28.73	16.12	0.733	0.735	0.768
29.58	16.93	0.732	0.741	0.772
114.3	86.8	0.849	0.851	0.858
116.4	89.2	0.847	0.850	0.860
233.0	188.3	0.914	0.915	0.900
239.5	197.0	0.906	0.907	0.900
1054	982	0.965	0.965	0.959
1068	986	0.973	0.973	0.960

Table 2. Mean inner activity coefficients (γ_2^{PM}) of NaCl in SE-Sephadex C-25, calculated from the outer (m_2'') and inner (m_2') concentrations of Cl^- ion

Column 3 gives the directly calculated experimental values, column 4 the corrected values (see the text) and column 5 the values calculated by the theory of Manning (1969).

$10^3 \times \text{Concn. of } \text{Cl}^-$		γ_2^{PM}		
External (m_2'' , molal)	Internal (m_2' , molal)	Experimental	Corrected	Theoretical
2.694	0.1469	0.362	0.674	0.707
2.733	0.1548	0.358	0.633	0.707
11.22	0.939	0.564	0.734	0.710
35.85	7.51	0.603	0.644	0.715
121.5	52.88	0.698	0.706	0.755
128.9	61.39	0.687	0.693	0.762
343.7	217.7	0.811	0.811	0.823
1042	851	0.881	0.881	0.905
1081	875	0.894	0.894	0.907

of concentrations. We can calculate π'/RT directly by use of the known osmotic coefficients of NaCl (Robinson & Stokes, 1955). The inner osmotic coefficient can be obtained from Manning's (1969) treatment by an expression analogous to eqn. (2) for osmotic coefficients; thence we can calculate π''/RT , and finally $\pi_{\text{diff.}}/RT$ and P by eqn. (3). The result would, however, be subject to errors, particularly at the highest and lowest concentrations of salt. At the highest concentrations $\pi_{\text{diff.}}/RT$ is a relatively small difference between two relatively large quantities; at the lowest concentrations the possible errors

Table 3. Values of $\pi_{\text{diff.}}/RT$ and P (see the text, eqn. 3) calculated for SE-Sephadex C-50 by method (ii) (see the text) from gel swelling and Cl^- partition results

$10^3 \times \text{External}$ concn. of Cl^- (molal)	$10^6 \times \pi_{\text{diff.}}/RT$ (mol/ml)	$10^5 \times P$ (mol/ml)
114.3	14.5	5.5
116.4	15.6	5.9
28.7	19.4	8.0
29.6	19.5	8.0

Table 4. Values of P (see the text, eqn. 3) estimated for SE-Sephadex C-50 by method (iii) (see the text) from measurements of diameters of single beads with Dextran 500 in the external solution

No. of experiments	External concn. of NaCl (molal) (Range)	External concn. of dextran (g/ml) (Range)	Gel concn. (g/ml) (Range)	Estimates of $10^5 \times P$ (mol/ml)	
				(Range)	(Mean)
5	0.010–0.012	0–0.145	0.024–0.028	3.1–9.7	7.4
4	0.051–0.060	0–0.145	0.037–0.047	6.2–6.5	6.4
5	0.26–0.31	0–0.146	0.059–0.093	–0.6–2.5	1.3
4	1.06–1.28	0–0.149	0.079–0.150	–0.3–1.3	0.7

in estimating the effective gel volume, discussed above, become serious. However, neither of these errors should be very serious at intermediate concentrations. Accordingly estimates of P have been made in this range (Table 3).

(iii) We also attempted to estimate P from the changes of gel concentration that result from increasing the outer osmotic pressures by addition of Dextran 500 to the outer solution. It was necessary, however, to allow for the changes of $\pi_{diff.}/RT$ that arise from the consequent changes of gel concentration and of ionic partition, again by using values of the inner activity and osmotic coefficients calculated from Manning's (1969) treatment. Even in the most favourable case (at low ionic strength) the latter effect accounted for at least two-thirds of the total change of net osmotic pressure and the calculated values of P are therefore not very accurate. The results, given in Table 4, suggest a variation of P with degree of swelling: this is in accordance with Flory's (1953) conclusion that gels should become less extensible at high degrees of swelling. Although these values are not highly accurate, they agree satisfactorily with those of method (ii) (Table 3) in the same range of salt concentrations, and are of the same order of magnitude as that of method (i).

In the case of SE-Sephadex C-25 we cannot apply method (iii) because of the very small changes of gel concentration caused by external osmotic pressures that can be produced by dextran. For the same reason, we have no estimates of the swelling parameters of neutral Sephadex G-25 which would allow the use of method (i). However, a rough estimate of P can be made for Sephadex G-25 from its gel concentration in water (0.34 g/ml), assuming that it has the same inner virial coefficients as Sephadex G-50. Allowing for the increase in mean residue weight the corresponding value of P for SE-Sephadex C-25 is 9×10^{-5} mol/ml. The measurements of chloride partition allow the application of method (ii) (Table 5).

In both cases the values of P estimated by methods

Table 5. Values of $\pi_{diff.}/RT$ and P (see the text, eqn. 3) calculated for SE-Sephadex C-25 by method (ii) (see the text) from gel swelling and Cl^- partition results

$10^3 \times$ External concn. of Cl^- (molal)	$10^6 \times \pi_{diff.}/RT$ (mol/ml)	$10^5 \times P$ (mol/ml)
0.764	130	30.6
0.391	150	36.3
0.376	150	36.0

(ii) or (iii) are higher than those estimated by method (i). Because the SE-Sephadex gels are much more highly swollen than the corresponding neutral grades, the higher values of P are to be preferred in estimating $\pi_{diff.}$. Taking values of P of 5×10^{-5} and 35×10^{-5} mol/ml for SE-Sephadex C-50 and C-25 respectively, we obtain net osmotic pressures in 0.15 molal outer NaCl of 0.4×10^5 and 3.6×10^5 Pa (14 and 140 mosmolar). Under these conditions the inner fixed charge concentrations are 0.12M and 0.46M. Since the polyanionic materials in cartilage have linear charge densities similar to that of SE-Sephadex and are present in equivalent concentrations of the order of 0.2M (Maroudas *et al.*, 1969), these values indicate that cartilage may have net inner osmotic pressures of the order of 10^5 Pa. They are substantially lower than might be expected on the basis of ideal Gibbs–Donnan behaviour, because of the lowered activity and osmotic coefficients due to the non-ideal interactions between the polyanionic structure and the counter ions, which can be viewed as a partial 'binding' (cf. Katchalsky *et al.*, 1966).

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