Donnan membrane equilibrium is not directly applicable to distributions of ions and water in gels or cells

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ABSTRACT Equilibration of ions and water with a charged gel does not follow the simple equations of the classical Gibbs-Donnan membrane equilibrium. Partition of ions between the gel and the external solution show specific effects, which require that activity coefficients are different in the two compartments. Highly hydrated ions, such as Na^+ and H^+ are accumulated into the gel water, whereas less highly hydrated ions, such as K^+ and NH_4^+ accumulate in the external water. This selectivity is the obverse of that found for gels containing low-density, expanded water. Water in a charged gel equilibrated with solutions of $MgCl_2$ was found to be more dense than bulk water at the same temperature. It is proposed that gels imbibe water to maximize the entropy of the system. Ions and water then equilibrate under those constraints. The chemical potential of water in the two compartments equalizes by an increase in density in the compartment of higher osmolality (the charged gel) and a decrease in density in the compartment of lower osmolality (the external solution). Electrolytes equilibrate so that macroscopic electroneutrality is conserved, and the chemical potential of an electrolyte is the same in each compartment. Because activity coefficients are different in the two compartments this results in asymmetric distributions of ions.

Because real gels usually contain both charged and hydrophobic regions of surface, populations of water molecules of different density coexist even in very small pores. This accounts for the common failure to detect this phenomenon experimentally.

INTRODUCTION

The Gibbs-Donnan membrane equilibrium is a thermodynamic treatment of the equilibrium between two aqueous compartments separated by a membrane, permeable to water and ions, but impermeable to a charged macromolecule, which is therefore confined to a single compartment. Donnan and Harris (1911) developed the theory and subsequently tested it experimentally. The essentials of the theory are that (*a*) macroscopic electroneutrality is conserved in each compartment, (*b*) the electrochemical potential of an ion is the same in each compartment, and (*c*) the chemical potential of water is the same throughout. Using equations describing these conditions he derived the familiar relationship that shows that if the mean activity coefficient of the electrolyte is the same in both compartments, the concentration of diffusible ions is higher in the compartment containing the charged macromolecule. Therefore, for water to equilibrate between the two compartments, a pressure equal to the difference in osmotic pressure of the two solutions must be applied to the compartment containing the macromolecule and its excess of ions. Procter (1914) and Procter and Wilson (1916) applied the same equations to charged gels. "The gelatin salt," they said, "like other salts, is highly ionized into the anion and a colloid cation, which either from polymerization or other causes peculiar to the colloid state, cannot diffuse." They concluded that the same equations applied: gels accumulate a Donnan excess of diffusible ions, and, in the absence of some restraining force, should imbibe water until they disperse. By analogy with the membrane systems, the restraining force has always been assumed to be a pressure exerted on the liquid inside the gel by elastic forces of the gel matrix. This would allow water and ions both to equilibrate, in spite of an excess concentration of ions in the gel water. Helfferich (1962) calculated pressures of hundreds of atmospheres, apparently exerted by a gel matrix upon its internal solution.

Gels are most remarkable, however, for their ability to imbibe and retain large volumes of water; if the gel material is cross-linked or the chains tangled, there must be an endpoint to gel swelling, but it is not obvious that in that resultant steady state the gel exerts a pressure on the internal solution. Wiggins and van Ryn (1990) have described a steady-state system with constant pressure and temperature throughout, but with a gradient in osmolality between two contiguous aqueous solutions. They showed that water in these two compartments must equilibrate by increasing its density in the solution of higher osmolality and decreasing its density in the solution of lower osmolality. In this communication we explore the possibility that in the steady state of a charged gel in an electrolyte solution, further influx of

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water is prevented, not by pressure, but because water equilibrates between the compartments of different osmolality by increasing its density inside the gel, and decreasing its density outside the gel.

When the density of water changes, the strength of water–water hydrogen bonds and the viscosity, reactivity, and solvent properties of the liquid all change; highly hydrated small cations go preferentially into the more dense of two contiguous populations of water molecules, whereas larger cations accumulate in less dense water (Wiggins and van Ryn, 1986; Wiggins and van Ryn, 1990). The change in selectivity occurs between Na^+ and K^+, which partition in opposite fashion. We have therefore looked for indirect evidence for changes in density of water in the pores of a charged gel, by measuring the pH at equilibrium, of an external solution, containing either Na^+ or K^+ salts.

MATERIALS AND METHODS

Materials

Biogel P-4 (Bio-Rad Laboratories, Richmond, CA) was washed exhaustively in HCl (0.1 M) and then water, and dried at 38°C in air. Residual water was estimated by weighing, drying at 110°C for 2 h, and reweighing. Weights were then corrected for this residual water. Drying at 110°C caused hydrolysis of end amide groups, increasing the charge on the gel and producing some NH_3. Agarose Isogel of zero electroendosmosis (EEO = 0) was a gift from Dr. John MacKay of the Auckland Hospital. Polyethylene glycol 20M (PEG 20M) was obtained from British Drug House Ltd. (Poole, England); dextran sulphate of average molecular weight 500,000 was obtained as the sodium salt from Sigma Chemical Company (St Louis, MO).

Methods

Osmolality was measured using a freezing point osmometer (Advanced Instruments Inc., Needham Heights, MA).

Densities were measured in 10-ml density bottles, calibrated with deaerated water. To allow for slight variations of room temperature from day to day (20–21°C), all final weighings of a single experiment were made on the same day. The temperature did not change significantly during this weighing. The density of the $MgCl_2$ solution was measured in five bottles; the density of the dry gel was estimated as previously described (Wiggins and van Ryn, 1990). This density was measured on one occasion, and assumed to be constant for subsequent weighings. Then up to 12 different weights of Biogel P-4 were weighed into the density bottles and left for a week to equilibrate, with gentle stirring. Finally, the bottles were all topped up with $MgCl_2$ solution and weighed. This method gave extremely reproducible results.

Viscosity was measured by timing movement of a miniscus past two calibrations in a 0.1-ml graduated pipette. Viscosity and density of dextran sulphate solutions were measured on the same day at the same temperature, which, again, varied only from 20–21°C, and not during a series of measurements. Times were reproducible to <0.5 s, in 50 s. Each solution was timed at least twice. Dynamic viscosity (η) was calculated from the relationship

$$T = k\eta/d,$$

where T was the time in seconds, d the density in grams per milliliter^{-1}, and η the viscosity in centipoise. The constant k was determined by calibrating the pipette with glycerol/water solutions. This extremely simple method was adequate for these experiments, but will be refined for future use.

The agarose gel was prepared and equilibrated as previously described (Wiggins and van Ryn, 1990). At the end of incubation its water content was determined by weighing wet, drying at 110°C, and reweighing. Equilibrations with Biogel P-4 were left for a week with daily rotamixing. The pH of the original solution (which had been adjusted to the required pH before putting it on the gel) and the pH of the supernatant were measured at the same time.

RESULTS

Biogel P-4 is a polyamide gel with an exclusion limit of 4,000. Hydrolysis of end amide groups produces free carboxyls so that the gels can be lightly or quite highly charged. Two batches of P-4 had quite different levels of charge. One (#307192-00) was much more charged than the other (#321374).

Fig. 1 illustrates the different selectivities of the two gels. Solutions contained either 10 mM KP_i, pH 7, plus increasing concentrations of KCl, or 10 mM NaP_i, pH 7, plus increasing concentrations of NaCl, where P_i is a convenient abbreviation for the mixture of $H_2PO_4^-$ and HPO_4^{2-} characteristic of the particular pH. When the gel was lightly charged, addition of KCl increased the pH of the supernatant, whereas increasing concentrations of NaCl decreased it. When the gel was highly charged, addition of NaCl increased the pH, whereas addition of KCl decreased it at first and then increased it slightly. These are surprising findings for what would normally be treated as an ion exchange experiment. This experiment

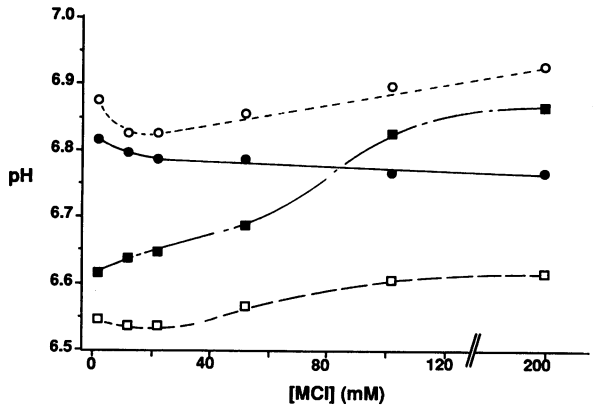

FIGURE 1 The effect of increasing concentrations of NaCl or KCl on the pH of a solution external to Biogel P-4. NaCl added to 10 mM NaP_i, pH 7: ■, #307192-00; ●, #321374; KCl added to 10 mM KP_i, pH 7; □, #307192-00; ○, #321374. 0.4 gel was equilibrated with 2.8 ml (#307192-00) or 2.4 ml (#321374) of solution. Each point is the mean of duplicates with standard deviation smaller than the symbol.

was reproducible in these two gels (each point is a mean of duplicates, with the standard deviation smaller than the size of the symbol). Other batches of Biogel P-4 gave different patterns of selectivity. Biogels with larger pores (P-10 to P-100) showed no selectivity at all.

Fig. 2 shows another example of the opposite selectivities of these two gels. Ethanol decreased the external pH when the lightly charged P-4 was buffered with KP_i, but had little effect when the buffer was NaP_i. When the gel was highly charged, ethanol increased the external pH when the buffer was NaP_i, but had little effect in KP_i.

Fig. 3 shows that other alcohols also decreased external pH when the gel was lightly charged and the buffer KP_i. The effect increased with the number of carbon atoms.

Fig. 4 illustrates the reason for abandoning buffered solutions and physiological pH and using changes in pH diagnostically in acid solutions. Lightly charged P-4 was equilibrated with buffer solutions which were initially all 10 mM and at pH 7. The final pH, and the time taken to reach it, differed from buffer to buffer, in many cases obscuring the magnitude of its change. Unbuffered neutral solutions fluctuate in pH too much to be reliable, but unbuffered acid solutions have a stable pH. Therefore, in the following series of experiments we used charged gels at low pH, and manipulated the solvent properties of their water by opposing the internal Donnan excess of ions with an external impermeant polymeric solute.

Fig. 5 shows specific effects of small cations on the pH of an unbuffered solution external to the lightly charged gel, in the presence of increasing concentrations of PEG 20M, which is too large to enter the pores of the gel. Fig. 6 shows the result of using dextran sulphate of average molecular weight as the impermeant solute. When the gel was a charged biogel, the pH of the external solution rose with increasing concentration of dextran sulphate, but when the gel was a neutral agarose gel contained in a dialysis membrane, the pH fell steadily.

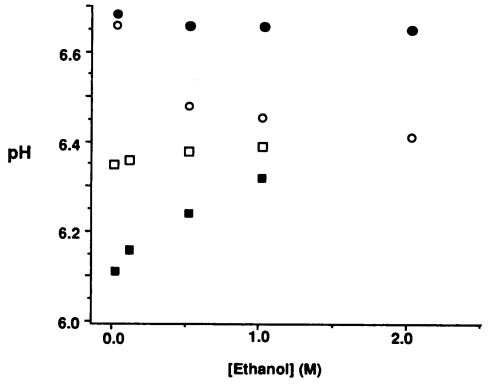

FIGURE 2 The effects of increasing concentrations of ethanol in either 10 mM NaP_i or 10 mM KP_i, pH 7, on the pH of a solution external to Biogel 4. NaP_i; ■, #307192-00; ●, #321374; KP_i; □, #307192-00; ○, #321374. Conditions were the same as in Fig. 1.

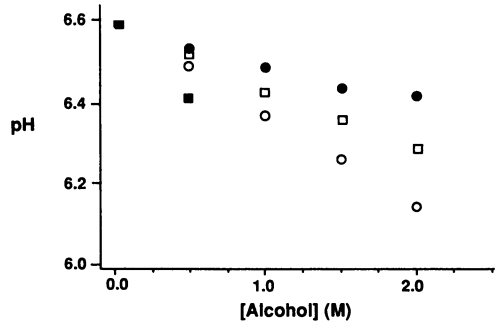

FIGURE 3 The effects of increasing concentrations of alcohols on the pH of a solution of 10 mM KP_i, pH 7, equilibrated with Biogel P-4 #321374, ●, methanol; □, ethanol; ○, propanol; ■, butanol.

Density of water in charged gels

Graded weights of the highly charged gel (P-4 307192-00) were equilibrated with solutions of $MgCl_2$, which should be most effective in generating dense water by accumulating into the gel. The average density of solution on the gel was then calculated; the results are shown in Fig. 7.

At three different initial concentrations of $MgCl_2$, the average density of solution on the gel increased with increasing gel weight. As the weight of gel in the 10-ml density bottle increased, a relatively greater proportion

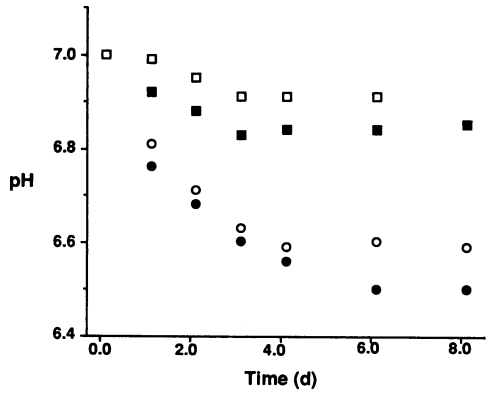

FIGURE 4 The change in pH of buffer solutions equilibrated with Biogel P-4 #321374. The solutions were all initially 10 mM and pH 7. □, Tris; ■, histidine; ○, KP_i; ●, NaP_i.

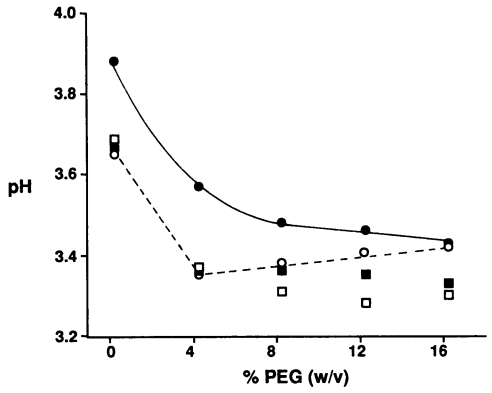

FIGURE 5 Changes in pH of unbuffered solutions (made pH 3 with HCl, and containing increasing concentrations of PEG 20M) equilibrated with Biogel P-4 #307192:00 ●, no added salt; □, 5 mM NaCl; ■, 5 mM KCl; ○, 5 mM NH_4Cl.

of the solution was inside the pores of the gel. The increase in average density, therefore, indicates that the internal water was more dense than the external water.

Changes in viscosity of dextran sulphate solutions

Viscosity is another property of water which is sensitive to its density. Fig. 8 shows the effects of added $MgCl_2$ on

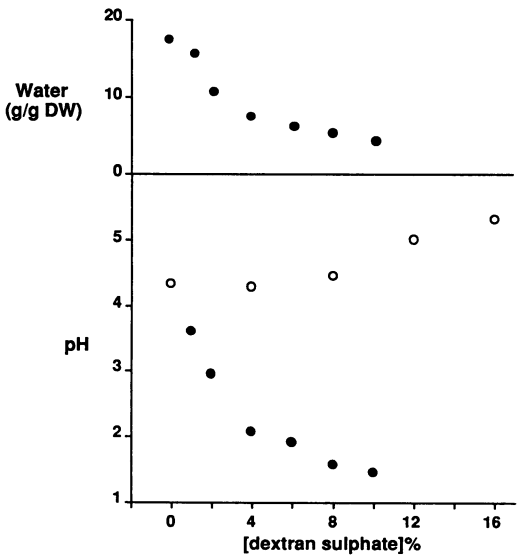

FIGURE 6 The effect of increasing concentrations of dextran sulphate on the pH of a solution, initially pH 3, equilibrated with a gel. ○, Biogel P-4 #307192-00; ●, agarose isogel (EEO = 0) in a dialysis bag; Top ●, the water content of the agarose gel, in gram/gram dry weight.

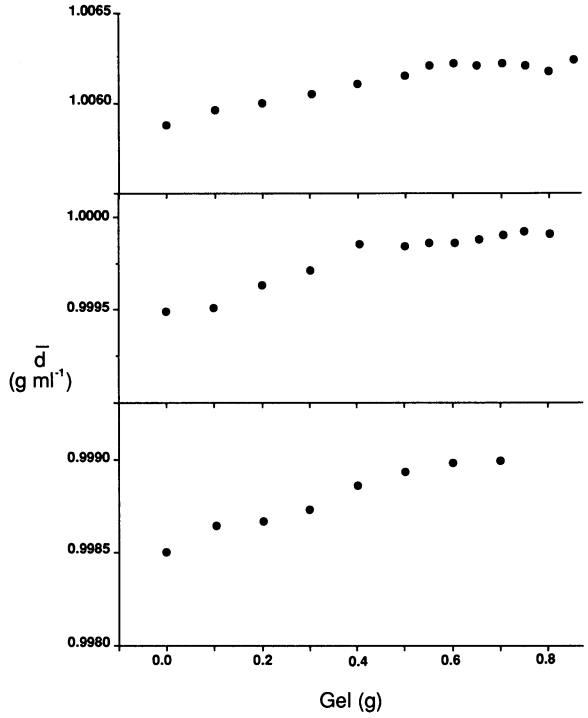

FIGURE 7 The average density of solutions of $MgCl_2$ equilibrated in density bottles with increasing weights of Biogel P-4 #307192-00. Top, 100 mM; middle, 20 mM; bottom, 5 mM.

the viscosity, density, and osmolality of 10% dextran sulphate. The viscosity decreased with increasing concentrations of $MgCl_2$; its value at 200 mM $MgCl_2$ was nearly halved. The increase in density of solutions with added salt was not significantly different from the increase when $MgCl_2$ was added to water. There was, however, a significant difference between the osmolality observed, and that calculated for simple addition of $MgCl_2$, with the appropriate osmotic coefficient, to the osmolality of 10% dextran sulphate; i.e., the observed osmolality was higher than that of the added components.

DISCUSSION

If the distribution of ions between the two batches of Biogel P-4 and their external solutions obeyed the classical equations of the Donnan Membrane Equilibrium, there would be no specificity in the effects of added Na^+ and K^+ salts on the measured pH. Specific effects of univalent cations are generally attributed to their selective binding to negatively charged sites on the gel. This, however, cannot explain the opposite effects of Na^+ and K^+ phosphates on the external pH in Figs. 1 and

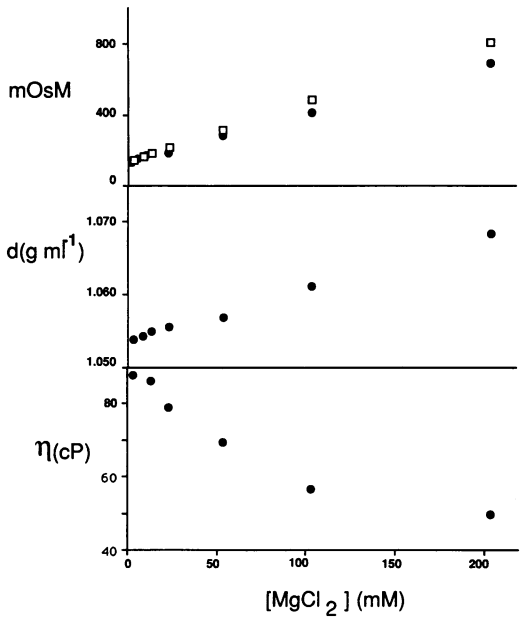

FIGURE 8 The osmolality, density, and viscosity of a solution of 10% dextran sulphate to which increasing concentrations of $MgCl_2$ were added. □, observed osmolality; ●, osmolality calculated assuming that the contributions of $MgCl_2$ and dextran sulphate were additive.

2. The two gels are identical in composition and pore size, and differ only in the number of carboxyl groups. If the specific effects of Na^+ and K^+ were due to binding to the surfaces of the gels, they would be qualitatively the same, and differ only in degree. The clear qualitative differences shown in Figs. 1 and 2 can therefore be attributed to differences in the properties of water inside the gel pores, differences which are to be expected on thermodynamic grounds.

The surfaces lining the pores consist of N-H and C = 0 groups, both of which make weak hydrogen bonds with water, cross-linking $-CH_2$ chains, which do not hydrogen bond at all, and the ionized carboxyls. Water molecules adjacent to the hydrophobic patches of surface or bonding weakly to the amide groups are in a state of higher energy than those water molecules further away from the surfaces, making strong hydrogen bonds with one another. Because hydrogen-bonding is cooperative, this state of high energy is propagated through several layers of water molecules. At constant pressure, temperature, and activity, this zone of water can equilibrate with bulk water only by decreasing its density. Water immediately adjacent to the carboxyl groups, on the other hand, has a lower activity than water further away from the surface, because of the strong electrostatic force which retains counterions in solution near the fixed charges. This water, therefore, must increase its chemical potential by increasing its density. Therefore, there are mixed poulations of water molecules coexisting even in these very small pores, with low-density water predominating in the lightly charged gel, and high-density water predominating in the highly charged gel.

Thus, in Fig. 1, as NaCl was added to the highly charged gel, it accumulated into the dense patches of water surrounding the carboxyl groups, increasing the local concentration of solutes still more, and decreasing the local activity of water. That water, therefore, increased still further in density, and became more strongly H^+-selective. H^+ diffused into the gel and the external pH increased. In the lightly charged gel, on the other hand, water inside the pores was predominantly of low density: NaCl was largely excluded from that water, which therefore increased in activity relative to the external water, and decreased its density still further. H^+ diffused out into the external solution. The results with KCl are less clear cut because KCl tends to normalize both kinds of water. It accumulates selectively into the lower-density populations of water, offsetting, to a degree, the excess concentration of ions round the carboxyl groups, and increasing the volume of low-density water at the expense of that of high density water. The activity of water near hydrophobic surfaces decreases so that it can equilibrate with bulk water with a smaller decrease in density than was necessary in the absence of KCl. Overall, therefore, water structure tends toward normal, and the two gels show similar patterns of selectivity.

This result illustrates the difficulty of designing experiments which will characterize these different water populations quantitatively. Only if pores contain water of one uniform density, and the external solution of water of another uniform density, can partition coefficients be measured. Real surfaces probably always have both weakly hydrogen bonding regions and charged regions, so that there is not a sharp interface at the mouth of a pore. Large-pored gels show little or no selectivity because most water is so far from a surface that it is continuous with the external solution and has the same solvent properties.

In Fig. 2, ethanol added to the highly charged gel accumulated selectively into the dense water surrounding the charged groups, increasing the local concentration of solutes and forcing local water to become more dense and more H^+ selective. NaP_i reinforced this effect, and H^+ diffused into the gel, increasing external pH. KP_i, by accumulating into the low-density water, decreased the volume of dense, H^+-selective water, and damped down the effect of ethanol. Ethanol added to the lightly

charged gel was largely excluded from the predominantly low-density water in the pores, generating an osmolality gradient which decreased still further the water density. NaP_i amplified this effect. In subsequent experiments (manuscript in preparation) we have found that the failure of pH, in this case, to give any indication that ethanol had changed the properties of water inside the pores, was due to the extremely low density state of the water, in which the carboxyls were all protonated. Urry et al. (1988) also showed a pK shift of a functional group due to hydrophobic effect. KP_i relieved this extremely low-density state by accumulating into the low-density water and decreasing its activity; the carboxyls ionized, and the low density state of the water was reflected in the fall in external pH with efflux of H^+. Fig. 3 shows the same decrease in pH with increasing concentrations of other alcohols in the presence of KP_i.

In Fig. 5 the density of water inside the pores of the gel was decreased by the presence of the impermeant solute PEG 20M. These are more complicated experiments to interpret in detail because there are multiple influences on water structure. But external pH fell, as expected, as the concentration of PEG 6000 increased, and again specific cation effects were apparent.

Dextran sulphate in its Na^+ form is stabilized by 1% Na_2HPO_4, so that the increasing concentration of dextran sulphate in Fig. 7 was accompanied by an increasing concentration of Na_2HPO_4 up to approximately 11 mM. As the dextran sulphate increased in concentration and tended to make water in the gel less dense, Na_2HPO_4 accumulated into the water surrounding the carboxyl groups and made that water more dense and H^+ selective. The net effect was a small increase in external pH. When, however, the gel was an effectively uncharged agarose gel, contained in a dialysis membrane, dextran sulphate scavenged H^+ most effectively. This process had two components. First, water inside the gel decreased progressively in amount and density, as external osmolality increased with increasing concentrations of dextran sulphate. H^+ increased in activity coefficient and left the gel. Secondly, however, dextran sulphate itself is a potent scavenger of hydrogen ions, because around each polyanion in solution is a high concentration of Na^+ ions, prevented from dispersing randomly through the solution by strong electrostatic interactions. Water containing these counterions is therefore dense and H^+ selective.

The existence of different populations of water molecules in a dextran sulphate solution was confirmed by the extreme changes in viscosity when $MgCl_2$ was added to a 10% wt/vol solution (Fig. 8). Dense water surrounding each polyanion would selectively accumulate $MgCl_2$, increasing the local osmolality, which would be partially diluted by influx of some water. The overall result would be an increase in the volume of dense water, an increase in its density, and therefore fluidity, and a marked decrease in the average viscosity of the solution. The excess osmolality above the added osmolalities of dextran sulphate and $MgCl_2$ probably indicates dissociation of Na^+ ions from the dextran sulphate polyanion into the more Na^+-selective water. On the other hand the density measurements were no different from the sum of the densities. Presumably the average value of the density was no different from that of normal water, and in that single measurement there is nothing to indicate that the mean is a mean of extremes.

Donnan membrane equilibrium and pressure

Evidence for the existence of water of different solvent properties in two gels which differed only in the magnitude of their charges partially invalidates the simple equations of the Donnan Membrane Equilibrium, which require that activity coefficients of univalent cations are the same and are equal to their values in simple solutions. The possibility remains that equilibration of water in these systems involves both a pressure exerted by the gel matrix and a change in water density. This, however, is unlikely to be a common phenomenon, because in the dextran sulphate solution, where pressure must be constant throughout, populations of water molecules in regions of differing osmolality equilibrated by changing their density. As such a solution concentrated and finally formed a gel, the mechanism would presumably be the same. If, as in the classic experiments of Donnan and Harris (1911), a polyelectrolyte solution is separated from a simple electrolyte solution, by a membrane permeable only to small ions and water, application of pressure to the polyelectrolyte solution, prevents influx of further solution from the simple electrolyte solution. It does not by itself make regions of water adjacent to polyions equilibrate with regions of water between polyions. Water must still change its density. Similarly, even if a gel matrix does exert a pressure on its internal solution, that solution contains populations of water molecules of different activity, experiencing an identical pressure. They can equilibrate with each other only by changing their densities. It seems, then, that changes in water density inside charged gels are necessary for equilibration of water, while differences in pressure may sometimes contribute to the overall steady state, but are not sufficient in themselves. When gel material is encased in a rigid capsule, the endpoint of its swelling must be partially determined by pressure.

Surfaces of biopolymers such as proteins, polynucleotides, and glycosamino glycans all consist of both hydrophobic patches and charged patches. Water adjacent to their surfaces must therefore be a mixture of high-density and low-density structures. It follows that whether cells are gels or solutions of proteins and polynucleotides, they must contain a mixture of water populations. As Clark (1987) demonstrated using skinned muscle fibres, the equations of the classical Donnan membrane equilibrium are not applicable to distributions of ions or water between cells and extracellular solution.

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