

The Osmotic Pressure of Macroions

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A theoretical study on the colloidal osmotic pressure and the Donnan-effect is presented. 1) An expression for osmotic pressure under ideal conditions is derived. 2) Deviations from the expression as regards colloidal osmotic pressure are explained by an "excluded volume" theory. 3) Deviations from the expression as regards the Donnan-effect are explained by a lowering of the effective charge of the macroion at salt concentrations greater than 0.1 M and in the case of hyaluronic acid by the formation of a network of hyaluronate molecules at salt concentrations lower than 0.1 M.

In this paper will be presented some theoretical considerations about the osmotic pressure of macroions, both in the case where the Donnan-effect is eliminated by high salt concentrations as where this is not so. The paper will fall in three parts: 1) The derivation of the osmotic pressure under ideal conditions; 2) The deviations from ideality when the Donnan-effect may be neglected; 3) The deviations from ideality as regards the Donnan-effect and the possibility of explaining this by accepting a lowering of the effective charge of the macroion when the salt concentration is increased. In all three cases the calculations will be illustrated with experimental data published earlier from this laboratory.

1. Let us denote the macroion R^- with the charge $-v$, and further:

Substance		H ₂ O	K ⁺	Cl ⁻	R ⁻
Symbol		M ₀	M ₊	M ₋	M _z
Mole fractions	outer liquid	$1 - 2y$	y	y	0
	inner liquid	$1 - x_+ - x_- - x_2$	x_+	x_-	x_2

The condition of electroneutrality yields:

$$x_+ = x_- + \nu x_2 \quad (0)$$

Assuming ideal conditions we have:

$$\mu_i = J_i + RT \ln z_i$$

where z_i is the mole fraction of M_i and J_i is independent of all the different mole fractions.

Denoting chemical potentials of the two liquids μ' and μ , respectively, and denoting the pressures by p^+ and p , respectively, we get:

$$\mu'_o = J_o^+ + RT \ln (1 - 2y)$$

$$\mu_o = J_o + RT \ln (1 - x_+ - x_- - x_2)$$

and from

$$u'_o = u_o:$$

$$1 - x_+ - x_- - x_2 = (1 - 2y)e^{-\alpha_o}$$

where

$$\alpha_o = (J_o - J_o^+)/RT = (p - p^+)V_o/RT = PV_o/RT$$

P is osmotic pressure and V_o the molar volume of pure water at the temperature in question. As P in the experiments is only a minute fraction of an atmosphere, it never exceeds 20 cm water, and is usually, much lower, we can substitute $1 - \alpha_o$ for $e^{-\alpha_o}$. Consequently we get:

$$x_+ + x_- + x_2 - 2y(1 - \alpha_o) = \alpha_o \quad (1)$$

Next we express that the potential μ_1 of KCl must be the same on both sides of the membrane. With $J_+ + J_- = J_1$ we get:

$$\mu'_1 = J_1^+ + 2RT \ln y$$

$$\mu_1 = J_1 + RT \ln(x_+ x_-)$$

proceeding in the same way as above, and substituting $e^{-\alpha_1}$ with $(1 - \alpha_1)$, where $\alpha_1 = P(V_+ + V_-)/RT = PV_1/RT$ we get:

$$x_+ x_- = (1 - \alpha_1)y^2 \quad (2)$$

Expressing (0) by the two equations:

$$\begin{aligned} x_+ &= Z + \frac{1}{2} \nu x_2 \\ x_- &= Z - \frac{1}{2} \nu x_2 \end{aligned}$$

we get from (1)

$$Z = \frac{1}{2} (\alpha_o - x_2) + y(1 - \alpha_o) \quad (1a)$$

and from (2)

$$Z^2 = y^2 (1 - \alpha_1) + \frac{1}{4} \nu^2 x_2^2 \quad (2a)$$

Squaring (1a) and accepting that α_0 is so much smaller than 1 that α_0^2 can be neglected compared to 1, we arrive at the equation:

$$0 = \frac{1}{4}(x_2^2)(\nu^2 - 1) + x_2[\frac{1}{2}\alpha_0 + y(1 - \alpha_0)] - y\alpha_0(1 - 2y + \alpha_0) - y^2\alpha_1 - \frac{1}{4}\alpha_0^2 \quad (3)$$

In the case where $y = 0$ this equation becomes:

$$x_2^2 (\nu^2 - 1) + 2x_2\alpha_0 - \alpha_0^2 = 0$$

which gives:

$$P = RTx_2 (\nu + 1)/V_0$$

If we now consider the case where $\alpha_0 \ll 1$ and $\alpha_0 \ll y$ eqn. (3) is reduced to:

$$\frac{1}{4} x_2^2 (\nu^2 - 1) + x_2 y - \alpha_0 (y - 2y^2) - y^2 \alpha_1 = 0 \quad (3a)$$

and introducing again that $\alpha_0 = PV_0/RT$ and $\alpha_1 = PV_1/RT$ we find for the osmotic pressure:

$$P = \frac{\frac{1}{4}x_2^2(\nu^2 - 1) + x_2y}{\frac{V_0}{RT}(y - 2y^2) + \frac{V_1}{RT}y^2}$$

or by rearrangement

$$P = \frac{RTx_2}{V_0(1 - 2y) + V_1y} \left[1 + \frac{x_2(\nu^2 - 1)}{4y} \right] \quad (4)$$

Eqn. (4) differs from a commonly accepted formula¹ by having the term $(\nu^2 - 1)$ instead of ν^2 . In the case of hyaluronic acid where the mean charge exceeds several hundred this is of course insignificant, but in the case of proteins near the isoelectric point the difference may be significant.

2. It is a well known fact that even when the salt concentration is so high that the figure in the brackets in (4) is 1, deviations from the theoretical expression are found in many cases. Jensen and Marcker² have demonstrated that dextran obeys the theoretical laws with good accuracy, but this is definitely not true when measurements are performed with hyaluronic acid, where Jensen and Marcker² find the law

$$c/p = A (1 - Bc)$$

to be the one which fits the experimental results best. (c is concentration of hyaluronate in g/l, P is osmotic pressure in cm water and A and B are constants, of which A is proportional to the molecular weight of the sample in question and B is the same for different samples.)

The problems concerned with the dependence of osmotic pressure on concentration have been studied theoretically by Güntelberg and Linderstrøm-Lang³ and Scatchard⁴ specially in the case where the high molecular substance is a protein. Here we shall present a simple explanation of the experimental results obtained earlier in this laboratory.

The assumption which is the basis of the following calculations is that the volume of the solvent should be corrected by subtraction of the "effective" volume of the dissolved molecules. In the case of dextran this volume could be expected to be relatively small at the concentrations used by Jensen and

Marcker, but in the case of hyaluronate where we have molecules with high negative charges the correction may be important either because of the repulsive forces of the charges or because of the ionic clouds which make it impossible for the molecules to come so close to each other, as when uncharged.

The following symbols will be used: c_g = weight concentration, P = osmotic pressure, N = number of molecules, v = total volume of solution, M = molecular weight of preparation used.

Let us have N molecules in a total volume of v . The "effective" volume of the molecules is then BNM , where B is a constant that includes the "effective" volume of the charged molecular unit. We then assume that the osmotic pressure is proportional to the "effective" concentration, *i. e.*:

$$P = A' \frac{N}{v - BNM}$$

substituting $N = c_g v / M$ and rearranging we get:

$$c_g / P = AM (1 - Bc_g) \quad (5)$$

which is identical with the expression found empirically by Jensen and Marcker².

It must be supposed that B varies with the salt concentration, pH and other factors influencing the charge of the molecule. In the case of ampholytes as, *e. g.*, proteins, it must further be assumed that B has its smallest value at the isoelectric point in good agreement with Christiansen, Jensen and Marcker⁵ who measured the osmotic pressure of bovine plasma albumin at different pH values and found a minimal osmotic pressure at pH 4.64.

Let us suppose that in this case the constant B could be written $B = D(1 + E \Delta\text{pH})$, where ΔpH is the difference between the actual pH and the isoelectric pH, and D and E are constants. Introducing this in eqn (5) we find at constant concentration

$$K_1 P - L P |\Delta\text{pH}| - 1 = 0$$

where K_1 and L are new constants. This means that p plotted *versus* pH should be a hyperbole, in good agreement with Fig. 2 in Christiansen, Jensen and Marcker⁵. This could be re-written as:

$$1/P = K - L |\Delta\text{pH}|$$

and $1/P$ plotted *versus* pH should yield a straight line. This is shown in Fig. 1, using the points from the right half of Fig. 2 in Christiansen, Jensen and Marcker. (Note in this figure the point at about pH 3.6 which makes it reasonable to believe that the curve should have shown a higher degree of symmetry.)

3. We have now accounted for the osmotic pressure in the case where the Donnan-effect has been eliminated by a suitably high concentration of salt. However measurements performed by Robinson and Jensen⁶ show that the salt concentration necessary to eliminate the Donnan effect is much smaller than calculated from (4). A fraction of hyaluronate with a molecular weight of 500 000 will have ν about 1 000. Inserting this in (4) gives that a concentra-

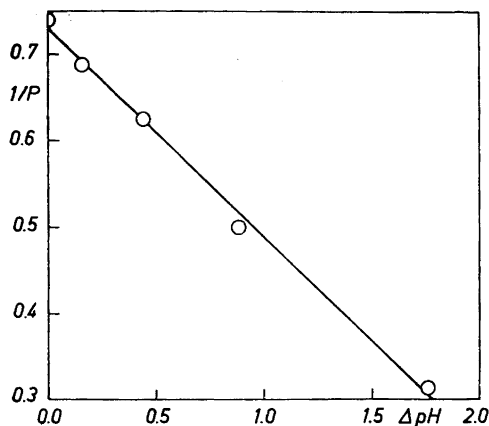


Fig. 1. A graph of $1/P$ cm water⁻¹ plotted against ΔpH for the experiment recorded in Fig. 2 in Christiansen, Jensen and Marcker⁶.

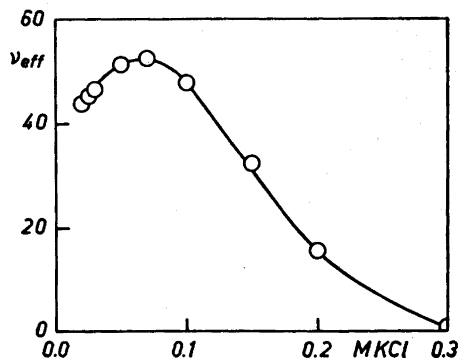


Fig. 2. A graph of ν_{eff} calculated from (7) using figures from Robinson and Jensen⁶ plotted against concentration of KCl. Molecular weight of sample is about 120 000, $\nu_{theor.} \approx 240$.

tion of hyaluronate at 10 g/l demands about 100 M KCl to reduce the Donnan effect to 5 % of the measured osmotic pressure. In this case, however, Robinson and Jensen find that there is no change in osmotic pressure when salt concentration varies from 2 M to 1 M and only when salt concentration is lower than 1 M is an increase in osmotic pressure observed. It seems reasonable to explain this by assuming an effective charge of the hyaluronate lower than the theoretical charge, due to the presence of potassium ions. To see whether this assumption is justified we shall use the figures recorded by Robinson and Jensen⁶ to calculate the effective charges necessary to make eqn (4) fit with the experimental results.

We change (4) to

$$P = P_{min} \left[1 + \frac{x_2(\nu^2 - 1)}{4y} \right] \quad (6)$$

where P_{min} is the osmotic pressure measured at salt concentrations so high that the addition of more salt would cause no change. By calculating the concentration of hyaluronate, x_2 we use the apparent molecular weight at the concentration used by Robinson and Jensen (10 g/l) and do not try to extrapolate to infinite dilution, as this procedure should automatically correct for volume effect and possibly other deviations from the van't Hoff theory. For the same reason we simply use P_{min} as the factor, and do not try to calculate this factor. Solving (6) in respect of ν gives us

$$\nu_{eff} = \sqrt{\frac{4y}{x_2} \left(\frac{P}{P_{min}} - 1 \right) + 1} \quad (7)$$

If we now calculate ν_{eff} we do not find as expected that ν_{eff} increases with decreasing salt concentration, with the theoretical value as the upper limit, but we find as demonstrated in Fig. 2 that this is only the case when the salt concentration is higher than about 0.1 M, and that below this concentration ν_{eff} decreases with decreasing salt concentration.

It should be noticed that a change in the behaviour of hyaluronic acid at salt concentrations about 0.1–0.2 M is observed in other properties of hyaluronic acid^{6,7}. This may be explained by the assumption that hyaluronic acid at low salt concentrations forms some sort of a network and only at higher salt concentrations is it dissolved as single molecules. This too can explain the results found here, as this would cause a decrease in x_2 , which would make the values of ν_{eff} calculated from (6) higher than those recorded in Fig. 2.

Acknowledgements. We are very much indebted to Professor, Dr. J. A. Christiansen for his interest in this work and for his many valuable suggestions.

The authors' thanks are due to Dr. C. E. Jensen who has kindly allowed us to use his experimental results in our calculations and has given us valuable information about the behaviour of hyaluronic acid, as well as about the immense literature on the subject.

Financial support from *NOVO Terapeutisk Laboratorium* and *Købmand i Odense Johann og Hanne Weimann, født Seedorf's foundation* is acknowledged by Kjeld Marcker.

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Received August 4, 1959.