Physical Chemistry of Protein Solutions. I. Derivation of the Equations for the Osmotic Pressure[1]

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Introduction

The osmotic pressure of a system containing a membrane permeable to the solvent and to some of the solutes, but impermeable to some solute ions, and the distribution of the diffusible ions across the membranes were determined for ideal solutions by Donnan many years ago.[2] Little progress has been made toward determining the relations between these quantities and the activity coefficients in non-ideal solutions. More recently Donnan[3] has even questioned whether the ratio of the osmotic pressure to the molal concentration of the non-diffusible ions approaches RT as a limit as this concentraton approaches zero but the concentration of diffusible ions remains finite.

It is possible to show that the conditions for the approach to this limit are essentially the same as the conditions for the approach to the same limit of the ratio of the "total osmotic pressure" to the total molal concentration as the total concentration approaches zero. This limit would not necessarily be approached if there were a second non-diffusible solute maintained at the same concentration on each side of the membrane, and the simplicity depends upon the fact that the solutes which can diffuse are at the same potential, or activity, on the two sides and not necessarily at the same concentration.[4] We shall also show how the changes in the logarithm of the activity coefficient of all the solutes with changes in the concentration of the non-diffusible solute may be calculated from the osmotic pressure and the equilibrium distributions of the diffusible solutes, and shall develop these functions as Taylor series.

General Equations

By osmotic pressure we shall mean the difference in pressure between two solutions at equilibrium across a semipermeable membrane when the concentration of non-diffusible solutes on one side of the membrane is zero, and the pressure on the side containing the non-diffusible solute is the atmospheric pressure. This pressure is sometime

called the "oncotic pressure" and sometimes the "colloid osmotic pressure" to distinguish it from "the total osmotic pressure," which is defined as the difference in pressure at equilibrium with pure solvent on one side of a membrane impermeable to all the solutes.

It is convenient to express the concentrations relative to that of one of the diffusible components present in large quantities, though not necessarily of the component in largest quantity. We call it component 1 and designate it with the subscript 1, and express other concentrations as moles per kilogram of component 1. Moles per mole of component 1 would do equally well. Component 2 is the non-diffusible component which may be treated as a single component even when it is not homogeneous for we can determine only the average values of its molecular weight, valence and effects on the various potentials.[5] We indicate other diffusible components by higher odd numerals or by capital letters, and diffusible species, including ions, by lower case letters. It is convenient both for experiments and for calculations to keep the pressure constant on the side containing the non-diffusible component. The constant pressure on the side containing non-diffusible solute is p. The pressure on the side of variable pressure is p', so that $p - p'$ is the osmotic pressure P. The superscript ^{0} indicates the limit as P or m_2 approaches zero, and the superscript $^\infty$ indicates the standard state of atmospheric pressure and each m equal to zero. The superscript $'$ will be used when distinction is necessary to indicate the solution without non-diffusible component.

At equilibrium the chemical potential of each diffusible component is the same on the side of constant pressure as on the side of varying pressure, and the change in chemical potential with concentration of non-diffusible component is also the same on the two sides. At constant temperature the independent variables may be chosen as the pressure and the molalities of the components. We will use derivatives with round ∂'s to indicate that each of these variables not specifically mentioned is kept constant, and derivatives with square d's to represent the total change at equilibrium. Then for any diffusible component K, if J is also any diffusible component

$$\frac{\partial \mu_K}{\partial m_2} + \Sigma_J \frac{dm_J}{dm_2} \frac{\partial \mu_K}{\partial m_J} = \frac{dp'}{dm_2} \frac{\partial \mu_K}{\partial p'} + \Sigma_J \frac{dm_J'}{dm_2} \frac{\partial \mu_K}{\partial m_J} \quad (1)$$

multiplying by m_K and summing for all the diffusible components gives

(1) Some of these equations were presented at the Buffalo meeting of the American Chemical Society, September 10, 1943, and are given by E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Company, New York, 1943. This paper is so closely related to the studies on plasma proteins of the Department of Physical Chemistry of the Harvard Medical School that it is given the number V in the series, "Preparation and Properties of Serum and Plasma Proteins" of that laboratory.

(2) F. G. Donnan, *Z. Elektrochem.*, 17, 572 (1911).

(3) F. G. Donnan, *Trans. Faraday Soc.*, 31, 80 (1935).

(4) A similar relation has already been treated for the effect of gas at constant pressure on freezing point depressions (G. Scatchard, P. T. Jones and S. S. Prentiss, THIS JOURNAL, 54, 2676 (1932)).

(5) We reserve the higher even numerals for non-diffusible components in case the composition is known from independent measurements.

$$\Sigma_K m_K \frac{\partial \mu_K}{\partial m_2} + \Sigma_J \frac{dm_J}{dm_2} \Sigma_K m_K \frac{\partial \mu_K}{\partial m_J} =$$
$$\frac{dp'}{dm_2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial p'} + \Sigma_J \frac{dm_J'}{dm_2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial m_J'} \quad (2)$$

By the Gibbs–Duhem equation

$$\Sigma_K m_K \frac{\partial \mu_K}{\partial m_2} = - m_2 \frac{\partial \mu_2}{\partial m_2}$$

So

$$m_2 \frac{\partial \mu_2}{\partial m_2} - \Sigma_J \frac{dm_J}{dm_2} \Sigma_K m_K \frac{\partial \mu_K}{\partial m_J} =$$
$$- \frac{dp'}{dm_2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial p'} - \Sigma_J \frac{dm_J'}{dm_2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial m_J'} \quad (3)$$

$$m_2 \frac{\partial \mu_2}{\partial m_2} + m_2 \Sigma_J \frac{dm_J}{dm_2} \frac{\partial \mu_2}{\partial m_J} =$$
$$- \frac{dp'}{dm_2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial p'} - \Sigma_J \frac{dm_J'}{dm_2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial m_J'} \quad (4)$$

Further differentiation of equation 4 yields

$$\frac{\partial \mu_2}{\partial m_2} + m_2 \frac{\partial^2 \mu_2}{\partial m_2^2} + 2m_2 \Sigma_J \frac{dm_J}{dm_2} \frac{\partial^2 \mu_2}{\partial m_2 \partial m_J} + \Sigma_J \frac{dm_J}{dm_2} \frac{\partial \mu_2}{\partial m_J} +$$
$$m_2 \Sigma_J \frac{d^2 m_J}{dm_2^2} \frac{\partial \mu_2}{\partial m_J} + m_2 \Sigma_{JK} \frac{dm_J}{dm_2} \frac{dm_K}{dm_2} \frac{\partial^2 \mu_2}{\partial m_J \partial m_K} =$$
$$- \frac{d^2 p'}{dm_2^2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial p'} - \frac{dp'}{dm_2} \Sigma_K \frac{dm_K}{dm_2} \frac{\partial \mu_K'}{\partial p'} -$$
$$\left(\frac{dp'}{dm_2}\right)^2 \Sigma_K m_K \frac{\partial^2 \mu_K'}{\partial p'^2} - 2 \frac{dp'}{dm_2} \Sigma_J \frac{dm_J'}{dm_2} \Sigma_K m_K \frac{\partial^2 \mu_K'}{\partial p' \partial m_J'} -$$
$$\Sigma_J \frac{d^2 m_J'}{dm_2^2} \Sigma_K m_K \frac{\partial \mu_K'}{\partial m_J'} - \Sigma_J \frac{dm_J'}{dm_2} \Sigma_K \frac{dm_K}{dm_2} \frac{\partial \mu_K'}{\partial m_J'} -$$
$$\Sigma_{IJ} \frac{dm_I'}{dm_2} \frac{dm_J'}{dm_2} \Sigma_K m_K \frac{\partial^2 \mu_K'}{\partial m_I' \partial m_J'} \quad (5)$$

If we define the non-diffusible component so that one mole of component 2 contains one mole of non-diffusible species, we may define the potentials with no further loss of generality by the equations

$$\frac{\mu_2}{RT} = \ln m_2 + \Sigma_i \nu_{2i} \ln m_i + \beta_2 + \mu_2^{00}/RT \quad (6)$$
$$= \ln m_2 + \Sigma_i \nu_{2i} \ln (\Sigma_J \nu_{Ji} m_J + \nu_{2i} m_2) + \beta_2 + \mu_2^{00}/RT$$

$$\frac{\mu_K}{RT} = \Sigma_i \nu_{Ki} \ln m_i + \beta_K + \frac{\mu_K^{00}}{RT} \quad (7)$$
$$= \Sigma_i \nu_{Ki} \ln(\Sigma_J \nu_{Ji} m_J + \nu_{2i} m_2) + \beta_K + \mu_K^{00}/RT$$

in which ν_{2i} is the number of moles of species i in one mole of component 2, and ν_{Ki} is the number of moles of species i in one mole of component K, μ^{00} is the value of μ in the standard state at the same temperature and pressure, and β is the excess chemical potential divided by RT, so that

$$\beta_2 = \ln \gamma_2 \quad (8)$$
$$\beta_K = \Sigma_i \nu_{Ki} \ln \gamma_K \quad (9)$$

if γ is the activity coefficient, or the mean activity coefficient of the ions of an ionized component. Then

$$\frac{\partial \mu_2}{\partial m_2} \Big/ RT = \frac{1}{m_2} + \Sigma_i \frac{\nu_{2i}^2}{m_i} + \beta_{22} \quad (10)$$

$$\frac{\partial \mu_2}{\partial m_K} \Big/ RT = \frac{\partial \mu_K}{\partial m_2} \Big/ RT = \Sigma_i \frac{\nu_{2i}\nu_{Ki}}{m_i} + \beta_{2K} \quad (11)$$

$$\frac{\partial \mu_K}{\partial m_J} \Big/ RT = \frac{\partial \mu_J}{\partial m_K} \Big/ RT = \Sigma_i \frac{\nu_{Ji}\nu_{Ki}}{m_i} + \beta_{JK} \quad (12)$$

$$\frac{\partial^2 \mu_2}{\partial m_2^2} \Big/ RT = - \frac{1}{m_2^2} - \Sigma_i \frac{\nu_{2i}^3}{m_i^2} + \beta_{222} \quad (13)$$

etc., if

$$\beta_{22} = \frac{\partial \beta_2}{\partial m_2}, \quad \beta_{2K} = \frac{\partial \beta_2}{\partial m_K} = \frac{\partial \beta_K}{\partial m_2}, \text{ etc.}$$

These correspond to the relation

$$- m_1 \frac{\partial \mu_1}{\partial m_2} \Big/ RT = 1 + \Sigma_i \nu_{2i} + \beta_{22} m_2 + \Sigma_K \beta_{2K} m_K \quad (14)$$

We also use the relations

$$- dp'/dm_2 = dP/dm_2$$
$$\partial \mu_K'/\partial p' = \overline{V}_K \text{ and } \Sigma_K m_K \overline{V}_K' = V_m' \quad (15)$$

in which $\overline{V}_K$ is the partial molal volume of component K, so that V_m' is the volume of the solution on the side of variable pressure which contains one kilogram of component 1.

The Limiting Osmotic Pressure Law

At the limit of zero concentration of component 2, the two solutions are identical so that each $m_K = m_K' = m_K^0$ and $V_m' = V_m^0$. Then equation 3 becomes

$$m_2 \frac{\partial \mu_2}{\partial m_2} = \frac{dP}{dm_2} V_m^0 + \Sigma_J \left(\frac{dm_J}{dm_2} - \frac{dm_J'}{dm_2}\right) \Sigma_K m_K' \frac{\partial \mu_K'}{\partial m_J'} \quad (16)$$

or

$$1 + m_2 \Sigma_i \frac{\nu_{2i}^2}{m_i} + \beta_{22} m_2 = \frac{dP}{dm_2} \frac{V_m^0}{RT} + \frac{1}{RT} \Sigma_J \left(\frac{dm_J}{dm_2} - \frac{dm_J'}{dm_2}\right) \Sigma_K m_K' \frac{\partial \mu_K'}{\partial m_J'} \quad (17)$$

Van't Hoff's law of osmotic pressure may be written

$$1 = \frac{dP V_m^0}{dm_2 RT} \quad (18)$$

The necessary conditions for equation 17 to reduce to equation 18 are that

$$m_2 \Sigma_i \frac{\nu_{2i}}{m_i}, \quad \beta_{22} m_2 \text{ and } \left(\frac{dm_J}{dm_2} - \frac{dm_J'}{dm_2}\right) \Sigma_K m_K' \frac{\partial \mu_K'}{\partial m_J'}$$

vanish as m_2 approaches zero.

The condition that the first shall vanish is that the non-diffusible component shall contain no diffusible species which is not contained in one of the diffusible components. This condition offers no real difficulty except when the non-diffusible species is electrically charged and none of the diffusible components is ionized. In this case there is usually a more serious difficulty caused by a reaction which is called membrane hydrolysis in aqueous solutions. The water reacts with the non-diffusible component to form an acid and a base, one of which is diffusible. Similar reactions may occur in non-aqueous systems.

The condition that the second term shall vanish is the same as the condition that Raoult's law shall hold in a solution without other solute, as in equation 13 with each m_K except m_1 equal to zero. The Debye theory tells us that at very low ionic strengths $\beta_{22} m_2$ is proportional to the square root of m_2 if component 2 contributes to the ionic strength, but that it is proportional to m_2 for non-electrolytes or for electrolytes at larger values of

the ionic strength. If the ion concentration is high enough to prevent difficulties due to membrane hydrolysis, β_2 or any β_K may be expanded in a Taylor series in m_2.

Since $\Sigma_K m'_K \frac{\partial \mu'_K}{\partial m'_J}$ is zero by the Gibbs–Duhem relation, a sufficient condition for the third term to vanish is that each $\left(\frac{dm_J}{dm_2} - \frac{dm'_J}{dm_2}\right)$ be finite. It is possible to keep each m_J (or each m'_J) constant and to choose component 1 so that each dm'_J/dm_2 (or each dm_J/dm_2) is less than m^0_J/m_1. Equation 18 for any mixed solvent has, therefore, the same validity as the other laws of dilute solutions.

The Distribution of Solute

Substituting equations 11, 12 and 18 in equation 1 at the limit as m_2 goes to zero yields

$$\Sigma_i \frac{\nu_{2i}\nu_{Ki}}{m_i{}^0} + \beta^0_{2K} + \frac{\overline{V}^0_K}{V^0_m} =$$
$$- \Sigma_J \left(\frac{dm_J}{dm_2} - \frac{dm'_J}{dm_2}\right)^0\left(\Sigma_i \frac{\nu_{Ji}\nu_{Ki}}{m^0_i} + \beta^0_{JK}\right) =$$
$$- \Sigma_J \left(\frac{d \ln m_J/m'_J}{dm_2}\right)^0\left(\Sigma_i \frac{\nu_{Ji}\nu_{Ki}m^0_J}{m^0_i} + \beta^0_{JK}m^0_J\right) \quad (19)$$

Each $\overline{V}^0_K$ and each β_{JK} may be determined from independent measurements in the absence of component 2 and the membrane. The values for many solutions of single salts are known as well as a few values for mixed solutions. $\overline{V}^0_K$ and V^0_m may easily be determined for any mixture, and a method has been given[6] for determining the approximate value of β^0_{JK} from β^0_{JJ} and β^0_{KK}. The remaining variables are β^0_{2K} and $(d \cdot \ln (m_J/m'_J)/dm_2)$ for each diffusible component. If the distributions are measured, β^0_{2K} can be determined from a single equation. If the β's are known and the distribution is to be determined, it is necessary to use simultaneously the $K - 1$ equations for all the diffusible components except component 1. The K'th equation which was eliminated in the summation may be considered to correspond to the first component, for which dm_1 and dm'_1 are obviously zero.

If there are only two diffusible components, 1 and K, equation (19) reduces to

$$\Sigma_i \frac{\nu_{2i}\nu_{Ki}}{m_i{}^0} + \beta^0_{2K} + \frac{\overline{V}^0_K}{V^0_m} =$$
$$- \left(\frac{d \ln (m_K/m'_K)}{dm_2}\right)(\nu_K + \beta^0_{KK}m^0_K) \quad (20)$$

in which $\nu_K = \Sigma_i \nu_{Ki}$.

Experiments indicate that the distribution of a diffusible solute may be represented by the simple expression

$$- \ln m_K/m'_K = b_{2K}w_2 = b_{2K}\overline{W}_2m_2 \quad (21)$$

in which w_2 is the concentration of component 2 in grams per kilogram of component 1, and $\overline{W}_2$ is its number average molecular weight. Application of Taylor's theorem yields, with equation 20

(6) G Scatchard. *Chem Rev.*, **19**, 309 (1936).

$$b_{2K}\overline{W}_2 = -\left(\frac{d \ln \frac{m_K}{m'_K}}{dm_2}\right)_0 = \frac{\Sigma_i \frac{\nu_{2i}\nu_{Ki}}{m_i{}^0} + \beta^0_{2K} + \overline{V}^0_K/V^0_m}{\nu_K + \beta^0_{KK}m^0_K} \quad (22)$$

For small molecules, or salts with small ions, $\overline{V}^0_K/V^0_m$ is less than the experimental error. For aqueous serum albumin and sodium chloride, β^0_{2K} ranges from 40 to 80 while $\overline{V}^0_K/V^0_m$ is less than 0.02.

There is a great convenience in defining component 2 so that the total number of moles of this component is the same as the number of moles of non-diffusible species, while the total charge on the component must be zero. We therefore define the number of moles of the diffusible species i in one mole of the non-diffusible component 2, ν_{2i} as

$$\nu_{2i} = - z_2z_im_i{}^0/\Sigma_j z_j{}^2m_j{}^0 \quad (23)$$

in which z_i is the valence of the species i. Then the total number of moles in one mole of the non-diffusible component, $\Sigma\nu_2$, is

$$\Sigma\nu_2 = 1 - \frac{z_2\Sigma_i z_im_i{}^0}{\Sigma_j z_j{}^2m_j{}^0} = 1 \quad (24)$$

The second equality follows because the solution is electrically neutral, so $\Sigma_i z_im_i{}^0 = 0$. The component is electrically neutral since

$$z_2 - \frac{z_2\Sigma_i z_i{}^2m_i{}^0}{\Sigma_j z_j{}^2m_j{}^0} = z_2 - z_2 = 0 \quad (25)$$

Then equation 22 becomes

$$b_{2K}\overline{W}_2 = \frac{\beta^0_{2K} + \overline{V}^0_K/V^0_m}{\nu_K + \beta_{KK}m^0_K} \quad (26)$$

which for small diffusible ions reduces to

$$\beta^0_{2K} = b_{2K}\overline{W}_2 (\nu_K + \beta_{KK} m^0_K) \quad (27)$$

Osmotic Pressure at Moderate Concentrations

In the limit of m_2 approaches zero, equation 5 becomes

$$\frac{z_2^2}{\Sigma_i m_i z_i{}^2} + \beta_{22} + \Sigma_J \frac{dm_J}{dm_2}\left(\Sigma_i \frac{\nu_{2i}\nu_{Ji}}{m_i} + B_{2J}\right) =$$
$$\frac{d^2P}{dm_2^2} V^0_m/RT + \Sigma_K \frac{\overline{V}^0_K}{V^0_m}\frac{dm_K}{dm_2} - \frac{RT}{V^{02}_m}\frac{\partial V^0_m}{\partial p'} -$$
$$\Sigma_J \frac{dm'_J}{dm_2}\Sigma_K \frac{dm_K}{dm_2}\left(\Sigma_i \frac{\nu_{Ji}\nu_{Ki}}{m_i} + \beta_{JK}\right) +$$
$$\Sigma_J \frac{dm'_J}{dm_2}\Sigma_K \frac{dm'_K}{dm_2}\left(\Sigma_i \frac{\nu_{Ji}\nu_{Ki}}{m_i} + \beta_{JK}\right) \quad (28)$$

$$\frac{z_2^2}{\Sigma_i m_i z_i{}^2} + \beta_{22} -$$
$$\Sigma_J \left(\frac{dm_J}{dm_2} - \frac{dm'_J}{dm_2}\right)\Sigma_K\left(\frac{dm_K}{dm_2} - \frac{dm'}{dm_2}\right)\left(\Sigma_i \frac{\nu_{Ji}\nu_{Ki}}{m_i} + \beta_{JK}\right) =$$
$$\frac{d^2P}{dm_2^2} V^0_m/RT + 2 \Sigma_K \frac{\overline{V}^0_K}{V^0_m}\frac{dm_K}{dm_2} - \frac{RT}{V^{02}_m}\frac{d V^0_m}{dp'} \quad (29)$$

Experimental observation shows that the osmotic pressure of protein solutions may often be represented up to rather high concentrations by the equation

$$P = Aw_2 (1 + Bw_2) = Aw_2 + ABw_2^2 =$$
$$A\overline{W}_2m_2 + AB\overline{W}_2^2m_2^2 \quad (30)$$

For other protein solutions the expression

$$P = Aw_2 + Bw_2 P = \frac{Aw_2}{1 - Bw_2} = Aw_2 + ABw_2^2 +$$

$$\frac{AB^2 w_2^3}{1 - Bw_2} = A\overline{W}_2 m_2 + AB\overline{W}_2^2 m_2^2 + \frac{AB^2 \overline{W}_2^3 m_2^3}{1 - B\overline{W}_2 m_2} \quad (31)$$

Application of Taylor's theorem yields, with equation 18

$$A\overline{W}_2 = \left(\frac{dP}{dm_2}\right)_0 = \frac{RT}{V_{m_1}^0} \quad (32)$$

and with equation 29

$$2B\overline{W}_2 = \frac{V_m^0}{RT}\frac{d^2P}{dm_2^2} = \frac{z_2^2}{\Sigma_i m_i z_i^2} + \beta_{22} -$$

$$\Sigma_J \left(\frac{dm_J}{dm_2} - \frac{dm_J'}{dm_2}\right)\Sigma_K\left(\frac{dm_K}{dm_2} - \frac{dm_K'}{dm_2}\right)\left(\Sigma_i \frac{\nu_{Ji}\nu_{Ki}}{m_i^0} + \beta_K\right) -$$

$$2\Sigma_K \frac{\overline{V}_K^0}{V_m^0}\frac{dm_K}{dm_2} + \frac{A\overline{W}_2}{V_m^0}\frac{dV_m^0}{dp'} \quad (33)$$

The last two terms are usually negligibly small in solutions of proteins and moderately small diffusible solutes or ions. For two diffusible components, 1 and K, this then becomes

$$2B\overline{W}_2 = \frac{z_2^2}{\Sigma_i m_i z_i^2} + \beta_{22} - \left(\frac{dm_K}{dm_2} - \frac{dm_K'}{dm_2}\right)^2\left(\frac{\nu_K}{m_K} + \beta_{KK}\right) \quad (34)$$

$$= \frac{z_2^2}{\Sigma_i m_i z_i^2} + \beta_{22} - \frac{\beta_{2K}^0{}^2 m_K^0}{\nu_K + \beta_{KK}^0 m_K^0} \quad (35)$$

Buffer solutions offer difficulties of interpretation, which arise from the fact that it is not possible to tell the valence of the protein component, or the concentrations of the buffer components, when there is a possibility of a reaction

$$P + H^+B \rightleftharpoons H^+P + B$$
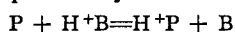
in which P and B are the basic forms of the protein and of the buffer, and H⁺P and H⁺B are the acid forms, each with a net charge one unit more positive than the corresponding base. These difficulties do not interfere with the determination of the molecular weight, and there are many approximate methods for determining the extent of the neutralization reaction. However, the uncertainties in any method appear to be of the same order as the other quantities we wish to determine.

Mixed Non-diffusible Components

If there are two or more non-diffusible components, it is of interest to discuss their behavior in terms of the mole fractions, such as $x_M = m_N/\Sigma_M m_N$, and of the weight fractions, such as $y_M = w_N/\Sigma_N w_N$. z_2, β_2 and β_{2K}, are linear functions of either fraction, while $\overline{W}_2$ is linear in the mole fraction and $1/\overline{W}_2$ is linear in the weight fraction. Although z_2^2 is a quadratic function of either fraction, it may be calculated from the valence of the various components. On the other hand, β_{22} is a quadratic for which each cross product must be determined in a binary mixture. The resulting coefficients in equations 21, 32 and 34, omitting the volume term in the latter, are

$$A_2 = (RT/V_{m_1}^0)/\Sigma_N x_N \overline{W}_N = (RT/V_{m_1}^0) \Sigma y_N/\overline{W}_N \quad (36)$$

$$b_{2K} = (\Sigma_N x_N \overline{W}_N b_{NK})/(\Sigma_M x_M \overline{W}_M) \quad (37)$$

$$2B_2 = \frac{1}{(\Sigma_N x_N \overline{W}_N)}\left[\frac{\Sigma_{MN} z_M z_N x_M x_N}{\Sigma_i z_i^2 m_i^0} + \Sigma_{MN}\beta_{MN}x_M x_N - \left(\frac{dm_K}{dm_2} - \frac{dm_K'}{dm_2}\right)^2\left(\frac{\nu_K}{m_K} + \beta_{KK}\right)\right] =$$

$$\frac{1}{(\Sigma_N y_N/\overline{W}_N)}\left[\frac{\Sigma_{MN}(z_M/\overline{W}_M)(z_N/\overline{W}_N)y_M y_N}{\Sigma_i z_i^2 m_i^0} + \Sigma_{MN}(\beta_{MN}/\overline{W}_M \overline{W}_N)y_M y_N - \left(\frac{dm_K}{dw_2} - \frac{dm_K'}{dw_2}\right)^2\left(\frac{\nu_K}{m_K} + \beta_{KK}\right)\right] \quad (38)$$

Both A_2 and b_{2K} are linear functions of the y's and can therefore be determined by measurements on solutions of the single components. A_2B_2 is a quadratic function, of which the Donnan term can be determined from measurements on solutions of the single components but the non-ideal term requires one measurement on each binary mixture, preferably at about equal weight fractions. The generalization to the case of several non-diffusible components and several diffusible components is obvious but complicated.

Membrane Potentials

The membrane potential cannot be obtained from any treatment which chooses neutral molecules as the components. If the ions are chosen as the components, there will be one more component than if neutral molecules are chosen. To compensate there will be a new equation of condition, automatically satisfied if neutral molecules are chosen, restricting the compositions to those which make each phase electrically neutral so that the quantity of one ion is fixed if the quantities of all the other ions are known. The equations for the chemical potentials contain a new variable which we will call ϵ. We will give the subscript π to the non-diffusible ion. Then

$$\mu_\pi/RT = \ln m_2 + \beta_\pi + \mu_\pi^{00}/RT + \epsilon z_2$$

$$\mu_k/RT = \ln m_k + \beta_k + \mu_k^{00}/RT + \epsilon z_k$$

These μ's are what Guggenheim calls "electro-chemical potentials," and are the only potentials of the ions we need consider.

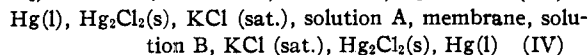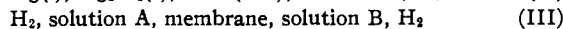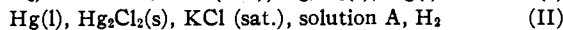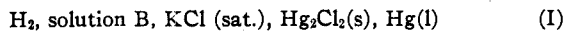The quantity ϵ is related to the membrane potential, E_m, by the equation

$$E_m = RT(\epsilon - \epsilon')/F$$

in which F is the faraday. If E_m and the distribution of each diffusible ion is measured and if each β_{ij} for diffusible ions is already known, ϵ and each β_{2i} is determined. Then the measurement of the osmotic pressure determines $\beta_{\pi\pi}$.

The assumptions necessary to interpret an experimental measurement as E_m are the same as those necessary to interpret a similar measurement as determining a single ion activity. If the hydrogen electrode with saturated potassium chloride bridge and calomel electrode measures the activity of the hydrogen ion, it follows that a silver–silver chloride electrode with saturated potassium chloride bridge and calomel electrode measures the activity of the chloride ion, and also that two

saturated potassium chloride bridges with calomel electrodes, one on each side of the membrane, measure the membrane potential.

It is simpler to measure this potential by a pH determination in each of the two solutions if the pH is stable in each, or to use an electrode reversible to some ion present in moderately high concentration. If we have the four cells

H_2, solution B, KCl (sat.), Hg_2Cl_2(s), Hg(l)   (I)

Hg(l), Hg_2Cl_2(s), KCl (sat.), solution A, H_2 (II)

H_2, solution A, membrane, solution B, H_2 (III)

Hg(l), Hg_2Cl_2(s), KCl (sat.), solution A, membrane, solution B, KCl (sat.), Hg_2Cl_2(s), Hg(l)   (IV)

passing a current through I, II and III in series produces the same change in state as passing the same current through IV. So the sum of the electromotive forces of I, II, and III must equal that of IV. However, the equilibrium electromotive force of III is zero, so the sum for I and II must equal the electromotive force of IV.

From equations 6 and 7 it follows that

$$\beta_2 = \beta_\pi + \Sigma_i \nu_{2i} \beta_i$$

$$\beta_K = \Sigma_i \nu_{Ki} \beta_i$$

$$\beta_{22} = \beta_{\pi\pi} + 2\Sigma_i \nu_{2i} \beta_{\pi i} + \Sigma_{ij} \nu_{2i} \nu_{2j} \beta_{ij}$$

$$\beta_{2K} = \Sigma_i \nu_{Ki} \beta_{\pi i} + \Sigma_{ij} \nu_{2i} \nu_{Kj} \beta_{ij}$$

$$B_{KJ} = \Sigma_{ij} \nu_{Ki} \nu_{Jj} \beta_{ij}$$

The derivatives of the activity coefficients of the neutral molecule components are easily calculated from those of the ions. The inverse calculation requires the knowledge of one β_i, which may be obtained from measurements with a saturated potassium chloride bridge. β_{kj} cannot be uniquely determined because it is impossible to add the single ions independently. So we obtain only such results as $(\nu_i \beta_{ki} + \nu_j \beta_{kj})$ with ν_i and ν_j so chosen that $(\nu_i z_i + \nu_j z_j)$ is zero. Perhaps our most important conclusion regarding membrane potentials is that the determination of a membrane potential is equivalent to two determinations of the activity of a single ion.

Other Definitions of Non-diffusible Components

For some purposes it may be desirable to make a choice of the non-diffusible component other than the one we have chosen. If the new component is electrically neutral, it will differ from ours by a certain number of moles of one or more diffusible components for each mole of non-diffusible component. We will designate the new set of functions with stars. The concentration of the non-diffusible component will be the same in the two sets, but its potential will be different. For the diffusible components, on the other hand, the potentials will be the same in the two sets, although the concentration will usually be different except when the concentration of component 2 is zero.

The most usual case will probably be the defi-

nition of component 2 as the salt with a single ion whose valence has the opposite sign to that of z_2. From the composition of component 2, it will always be possible to calculate dm_j^*/dm_2^*, β_2^*, β_{22}^* and each β_{2J}^* from the corresponding functions in the unstarred set. For example, in the case in which the protein solution is diluted with sodium chloride, which we will call component 3

$$\frac{dm_3^*}{dm_2} = \frac{dm_3}{dm_2} - \frac{|z_2|}{2}$$

$$\beta_2^* = \beta_2 + \frac{|z_2|}{2} \beta_3$$

$$\beta_{22}^* = \beta_{22} + |z_2| \beta_{23} + \frac{z_2^2}{4} \beta_{33}$$

$$\beta_{23}^* = \beta_{23} + \frac{|z_2|}{2} \beta_{33}$$

if $|z_2|$ is the absolute value of z_2. For any other diffusible species k

$$\frac{dm_k^*}{dm_2} = \frac{dm_k}{dm_2}$$

$$\beta_{2k}^* = \beta_{2k} + \frac{|z_2|}{2} \beta_{3K}$$

It would be extremely useful to have expressions for the potential of a nondiffusible component with valence other than z_2, for example of the isoionic form with valence zero. The expressions are about the same formally as the foregoing, but they involve the distribution of an acid or a base and are very much more difficult to correlate with experimental measurements.

Summary

Equations are derived relating the osmotic pressure and the distribution of diffusible solutes across a semi-permeable membrane to the variations of the activities, or potentials, of the components with changing composition of the solution, particularly with changing concentration of the non-diffusible solute.

It is shown that the conditions necessary for van't Hoff's law of osmotic pressure to hold with a semi-permeable membrane are essentially the same as those for the other generally accepted laws of dilute solutions.

To simplify the relations, the composition is defined so that the total number of moles of the non-diffusible component is the same as the number of moles of the non-diffusible species, but the component is electrically neutral even when the species is charged. The transformation to other definitions is shown.

The equations for the membrane potential and other relations involving single ion activities are given, and their validity is discussed.

The equations are extended to mixtures of non-diffusible components whose concentrations may be independently varied.