[CONTRIBUTION FROM THE LOW TEMPERATURE RESEARCH STATION]

THE THERMODYNAMIC ACTIVITIES OF THE PROTEINS

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I. Introduction

In solutions of finite concentrations the osmotic pressures of protein salts may be very different from the values predicted by the ideal solution laws.[2] This paper is concerned with the derivation of formulas correlating the thermodynamic activities of protein salts with the measurements of osmotic pressure and the practical application of these formulas to a salt of hemoglobin.

The empirical formula for a salt like the sodium hemoglobinate, $HbNa_n$, includes a number, n, which is not an integer, because it represents the average number of sodium atoms combined with a protein molecule, in a system in which the number of atoms combined with individual protein molecules may have a wide range of variation. The average value of n is a variable ranging from 0 to 80 in accordance with variations in the activities of sodium and hydroxyl ions.

In certain respects there is a close resemblance between protein salts and typical strong electrolytes, but the theoretical and experimental treatment of the activities of protein salts is modified by the condition that n is neither an integer nor a constant independent of the concentrations of diffusible electrolytes.

II. Thermodynamical Relationship between the Osmotic Pressures and the Average Values of the Potentials and Activities of Protein Salts

The activity or effective concentration of a protein salt is here defined by Formula 1.

$$\mu_{ps} = RT \ln a_{ps} + K_1 \qquad (1)$$

K_1 = a constant, defined by the convention that the activity equals the concentration in very dilute solutions. μ_{ps} = the "average value" of potential of the protein salt per gram mole, at the temperature T and the pressure P. a_{ps} = the "activity" of the protein salt, defined by this formula, and is the antilogarithm of the "average value" of $\ln a_{ps}$ in the assemblage of salts represented by an empirical formula of the type $HbNa_n$.

It is well known that the potential or activity of a single substance can be determined by the application of Gibbs' equations[3] (Lewis and Randall[4])

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[2] G. S. Adair, *Proc. Roy. Soc. London*, **120A**, 573 (1928).

[3] J. W. Gibbs, *Trans. Conn. Acad.*, **3**, 108 (1876); "Scientific Papers," New York, **1906**, p. 88.

[4] G. N. Lewis and M. Randall, "Thermodynamics and the Free Energy of Chemical Substances," McGraw-Hill Book Co., Inc., New York, **1923**.

and under certain conditions, discussed below, it is possible to determine the average value of the potential, μ_{ps}, by the application of Equation 2, a restatement of Gibbs' equation numbered 97.

$$V\mathrm{d}P = \eta\mathrm{d}T + m_{ps}\mathrm{d}\mu_{ps} + m_1\mathrm{d}\mu_1 + m_2\mathrm{d}\mu_2 \ldots + m_n\mathrm{d}\mu_n \qquad (2)$$

V = the volume of the solution; P = the hydrostatic pressure. η = the entropy of the system; T = the absolute temperature; m_{ps} = mass of the protein salt in gram moles in the volume V; m_1 = the mass of water in gram moles; m_2, m_n, denote the masses of other crystalloids; μ_1 = the potential of water; and μ_2, μ_n = the potentials of other crystalloids.

The conditions that the protein system must be stable and that the protein itself must be equivalent to one component can be realized in solutions of hemoglobin at 0°.[2] The protein system, however, differs from an aqueous solution of a typical strong electrolyte in that the presence of a third component such as sodium hydroxide is a condition for the existence of a salt like sodium hemoglobinate. In this work the number of diffusible components has been increased to four by the addition of phosphate mixtures, in order to maintain the protein salt at well-defined hydrogen-ion concentrations.

The practical application of Gibbs' formula is simplified if some of the variables are kept constant, so that their differentials can be omitted, as stated in Formulas 3 and 4 below.

$$m_{ps}\mathrm{d}\mu_{ps} = -m_1\mathrm{d}\mu_1 - m_2\mathrm{d}\mu_2 \ldots\ldots - m_n\mathrm{d}\mu_n \qquad (3)$$

μ_{ps} symbolizes the potential of the protein in systems where T, P and m_1, m_2, m_n are constants and m_{ps} is varied.

In the protein systems considered in this work the degree of accuracy obtained in measurements of osmotic pressure is much greater than the accuracy attainable in the measurements of the potentials of the diffusible substances, and therefore the calculations of μ_{ps} have been made by Formula 5 (derived from Formula 4 below) rather than the well-known and theoretically more accurate Formula 3.

The function $(\mu_{ps})_\mu$ defined by Formula 4 differs from μ_{ps} because it refers to conditions where T, μ_1, μ_2, μ_n are constant and the hydrostatic pressure P is a variable.

$$m_{ps}\mathrm{d}(\mu_{ps})_\mu = V\mathrm{d}P = V\mathrm{d}p \qquad (4)$$

In the experiments described below the potentials of the crystalloids have been kept constant by the equilibration of the protein solutions in collodion membranes with a standard solution of crystalloids in which the temperature, the hydrostatic pressure P'' and the composition have been kept constant. Gibbs' equation for membrane equilibrium (No. 77) shows that μ_1, μ_2, μ_n are then constant in the protein solutions, as they are constant in the standard solution or "outer fluid."

p = the observed osmotic pressure is here defined as the pressure difference $P - P''$, measured with a membrane permeable by all the crystal-

loids but impermeable by protein, under conditions where the protein concentration is varied, T and μ_1, μ_2, μ_n are constants and P'' is equal to 1 atmosphere.[5]

Since the function $(\mu_{ps})_\mu$ refers to a special set of conditions, the results calculated by Formula 4 require correction before they can be compared with the observations on solutions of crystalloids published by previous workers.

In general this correction is complicated by the fact that the "solvent" is not a pure substance but a mixture with three or more components. In the protein systems considered below m_{ps} is relatively small and it appears that a correction which is accurate enough for the purpose of this work can be obtained by assuming that $d(\mu_{ps})_\mu$ is equal to $(\partial\mu_{ps}/\partial P)dp + d\mu_{ps}$.

It is known that the effect of hydrostatic pressure on the potential of a component is represented by an equation of the form $(\partial\mu_{ps}/\partial P) = \bar{v}_{ps}$, where $\bar{v}_{ps}$ is the partial specific volume of the protein salt per gram mole, and it follows that μ_{ps} at a constant hydrostatic pressure is determined by Formula 5.

$$m_{ps}d\mu_{ps} = Vdp - m_{ps}\bar{v}_{ps}dp \qquad (5)$$

The determination of the conditions under which the simple Formula 5 is equivalent to Formula 3 is facilitated by the consideration of the "corrected" osmotic pressure $(P - P'')\ P$ where the protein solution is at a constant pressure, P, and the "outer fluid" is at a pressure P'' less than one atmosphere.

It will be assumed that under these conditions the protein solution can be composed of two mixtures, each of which is of constant composition. The composition of the first mixture, designated the "solvent," is the same as the composition of the fluid outside the membrane. The second mixture, designated the "protein salt," includes the pure protein and a certain proportion of the crystalloids that are present in excess inside the membrane.

If these two mixtures of constant composition are treated as the components of the system, a formula for μ_{ps} in terms of μ_x', the potential of the "solvent," can be obtained by the application of Equation 3 as stated in Formula 6. The theory of membrane equilibrium shows that $\mu_x' = \mu_x''$, the potential of the "solvent" in the outer fluid, and by Formula 2 $\mu_x'' = v_x''dP''$, where v_x'' is equal to the volume of one mole of the mixture in the outer fluid at the pressure P''. The corrected osmotic pressure can then be correlated with $d\mu_x'$ and $d\mu_{ps}$ by Formula 6.

$$m_{ps}d\mu_{ps} = -m_x'd\mu_x' = +m_x'[v_x''d(P - P'')_P] \qquad (6)$$

[5] p is very much smaller than the osmotic pressure measured by a membrane permeable by water only (sympolized π in Formula 11). The relationship between π and the activity of a colloid in an aqueous solution has been worked out by Linderström-Lang, *Compt. rend. Lab. Carlsberg*, **16** (1926).

m'_x = gram moles of "solvent" in the solution containing m_{ps} gram moles of protein.

Experimental evidence given below indicates that the observed and the corrected osmotic pressures are in close agreement, and it follows that Formulas 5 and 6 are equivalent to the simplified Formula 7.

$$v_s dp = (V' - \bar{v}_{ps})dp = d\mu_{ps} = RT \, d \ln a_{ps} \qquad (7)$$

v_s = liters of solvent per mole of protein. $V' = V/m_{ps}$ = liters of solution per mole of protein.

A formula for the potentials and activities of protein ions (No. 8) can be obtained by the application of Formula 2 to a system in which the individual ions are treated as components. In the case of the sodium proteinate, $HbNa_n$, it is simpler to replace $d \ln a_{ps}$ in Formula 7 by the equivalent expression $d \ln a_p + n \, d \ln a_{Na}$, derived from Formula 12. Since $n = -n_p$, where n_p is the average valence of the protein ions, Formula 13 shows that $n \, d \ln a_{Na} = (n_p F/RT)dE$, where E = the membrane potential.

In the derivation of Formula 8 below, a small correction for the effects of pressure on the activities of the diffusible ions has been omitted

$$v_s dp = d\mu_{ps} = d\mu_p + n_p F dE = RT \, d \ln a_p + n_p F dE \qquad (8)$$

μ_p = average value of the potential of the protein ions; a_p = (logarithmic) mean value of the activity of the protein ions; n_p = mean valence of protein ions, defined as the equivalent cencentration divided by the molar concentration.

III. Definitions of the Standard State of a Protein Salt and the Functions a_{ps} and a_p

Relative values of the potentials and activities can be calculated by integrating Equations 7 and 8, as stated in Formulas 9 and 10, on the assumption that the composition of the protein salt is constant over the range of osmotic pressure from p° to p'. Experimental evidence concerning the degree of accuracy of this assumption is referred to below.

$$\int_{p^\circ}^{p'} v_s dp = \mu'_{ps} - \mu^\circ_{ps} = RT \ln a'_{ps} - RT \ln a^\circ_{ps} \qquad (9)$$

$$\int_{p^\circ}^{p'} v_s dp = \mu'_p - n_p FE' - \mu^\circ_p - n_p FE^\circ \qquad (10)$$

There are two conventional definitions of the "standard state" of the protein salt that must be considered in fixing the absolute values of the activities by Formulas 9 and 10.

The "Hypothetical Standard State" is a very dilute solution of the protein salt in the absence of diffusible crystalloids. The theoretical osmotic pressure of such a salt is given by Formula 11.

$$\pi = (1 + n)C_p RT \qquad (11)$$

C_p is the molar concentration and n the number of sodium atoms in the salt $HbNa_n$. With this definition of the standard state, the activity of the protein salt is comparable with the activity of typical strong electrolytes and the following formula may be applied.

$$(a_{ps})_w = (a_p)_w a_{Na}^n \tag{12}$$

$(a_{ps})_w$ and $(a_p)_w$ denote the activities of the protein salt and the protein ion referred to the hypothetical standard state. These activities cannot be determined by direct methods because the protein salt cannot exist in the theoretical standard state. Indirect methods of estimating these activities and a function f_0 referred to below will be discussed in a later paper. a_{Na}, the activity of sodium ions, can be calculated from measurements of the membrane potential by Formula[6] 13 below, as described in a previous paper.[2]

$$\ln a_i' = \ln a_i'' - n_i FE/RT \tag{13}$$

a_i' = the activity of any diffusible ion (of valence n_i) in the protein solution. a_i'' = the activity of this ion in the solution of crystalloids in diffusion equilibrium with the protein solution.

In this case a_i'' = a constant and it follows that $d \ln a_{Na} = -(n_i F/RT)dE$.

The "Experimental Standard State" is a very dilute solution of the protein in diffusion equilibrium with the standard solution of crystalloids. The osmotic pressure, p, approaches the value $C_p RT$ rather than $(1 + n)C_p RT$ under these conditions, as shown by the observations in Table I. The symbols a_{ps} and a_p denote activities calculated on assumption that a_{ps} is equal to the protein concentration in the experimental standard state. The application of Formula 10 shows that a_{ps} and a_p are related by Formula 14

$$a_{ps} = a_p e^n p^u \tag{14}$$

$u = EF/RT$, where E is the membrane potential; $e = 2.718$; a_p = the logarithmic mean value of the activity of the protein ions referred to the experimental standard state. In calculations of a_p at 0°, from the data given below, Formula 14 can be replaced by 14a.

$$\log a_{ps} = \log a_p + n_p FE/2.303RT = \log a_p + n_p E/0.0542 \tag{14a}$$

The relationships between a_p and a_{ps} and the activities referred to the hypothetical standard state are given below. (1) $d \ln a_p$ is equal to $d \ln (a_p)_w$, and therefore $(a_p)_w$ is equal to $f_0 a_p$, here f_0 is an undetermined integration constant. (2) The application of Formula 13 shows that $a_{Na} = a_{Na}'' e^{-u}$, and it follows that $(a_{ps})_w = f_0 (a_{Na}'')^n a_{ps}$.

The experimental investigations recorded below refer to the functions a_{ps} and a_p. The determination of f_0 and $(a_{ps})_w$ with the assistance of solubility measurements may be considered, but the thermodynamic treatment of solubility measurements must be postponed, because the

[6] F. G. Donnan and J. H. Allmand, *J. Chem. Soc.*, **105**, 1941 (1914).

composition of the crystalline phase is not a constant but a function of the concentrations of diffusible electrolytes.[7]

IV. Experimental Investigations

Five groups of measurements are required for the application of the thermodynamic formulas for the activities of protein salts and protein ions.

1. A series of measurements of osmotic pressures and volumes under conditions where the potentials of the crystalloids are nearly constant, supplemented by measurements of the temperature, the pressure and the composition of the solution of crystalloids in diffusion equilibrium with the protein solution.

2. A series of measurements of the effects of the temperature, pressure and the concentrations of the crystalloids on the osmotic pressure at a constant protein concentration. These observations are required to reduce the observed pressures under slightly different conditions to those corresponding to the theoretical standard state in which the potentials of the crystalloids are absolutely constant.

3. Measurements of the magnitude of the error introduced by the assumption that the average composition of the protein salt is independent of the protein concentration under conditions where the potentials of the crystalloids are constant. In the system considered in this work the error due to this assumption appears to be relatively small.

4. Measurements of the partial specific volumes of "solvent" and solute are required in calculations of activities at a constant hydrostatic pressure.

5. Measurements of membrane potentials and the valences of protein ions are required in calculations of the activity of the protein ion.

The subsidiary investigations 2–5 are not quite complete and the numerical values of the activities given in this paper are provisional.

1. Measurements of Osmotic Pressure.—A critical study of the direct method of measuring the osmotic pressure of hemoglobin with collodion membranes permeable by water and salts but impermeable by the protein has been given in a previous paper.[8]

This method has been applied for the purpose of determining the relationship between osmotic pressure and protein concentration under conditions where the potentials of the crystalloids are practically constant.

Collodion sacs containing about 15 cc. of hemoglobin solution have been equilibrated with relatively large volumes of the "Standard Solution" of phosphates, specified in Table I. The results of 32 measurements of the osmotic pressure of hemoglobin in equilibrium with this standard solution have been published in the tables and curves given in a previous

[7] S. P. L. Sörensen, *Compt. rend. Lab. Carlsberg*, **12**, 188 (1917). The solubility of hemoglobin in different mixtures of electrolytes has been measured by E. J. Cohn and A. M. Prentiss, *J. Gen. Physiol.*, **8**, 619 (1927).

[8] G. S. Adair, *Proc. Roy. Soc. London*, **108A**, 627 (1925).

paper.[2] Calculations of the "osmotic coefficients" for six of these observations are recorded in Table I. In dilute solutions the observed osmotic pressures approach the theoretical values given by van't Hoff's law stated in the form

$$p = C_p RT \qquad (15)$$

$C_p = C_{Pr}$, in grams of dry protein, divided by 68,000, where 68,000 ± 2,000 is the molecular weight of sheep's hemoglobin expressed in grams of protein dried at 103°.[9]

TABLE I

OSMOTIC PRESSURES OF A SALT OF HEMOGLOBIN DESIGNATED[a] $Hb(Na + K)_{8.5}$

Conditions: the potentials of the crystalloids have been kept constant by the equilibration of the protein solutions with a standard phosphate mixture containing 0.1 mole of KCl, 0.0613 mole of Na_2HPO_4 and 0.00533 mole of KH_2PO_4 per liter of solution. The temperature of the standard solution is 0° and the hydrostatic pressure is 760 mm. p = observed osmotic pressure in mm. of mercury at 0°. $RT = 22.41 \times 760 = 17{,}033$ at 0°.

Hb per liter of soln., g. C_{Pr}	Hb per liter of soln., mole C_p	Osmotic press., obs., p	Osmotic press., theoretical RTC_p	Osmotic coeff., p/RTC_p
22	0.00032	6.15	5.5	1.11
80	.00118	28.2	20.0	1.42
200	.00294	110.9	50.1	2.21
240	.00353	155.0	60.1	2.58
290	.00426	264.6	72.6	3.64
344	.00506	382.8	86.1	4.44

[a] The formula $Hb(Na + K)_{8.5}$ is provisional. A discussion of the complete formula for the protein salt as it exists in the solution will be given at a later date. The number of Na + K atoms combined with hemoglobin in blood is approximately the same as the number in the salt considered in this paper.

2. Corrections for Deviations from the Standard State.—The actual conditions in different experiments recorded in Table I did not correspond exactly with the theoretical standard state, namely, a solution of reduced hemoglobin in equilibrium with a solution containing 0.1 mole of KCl, 0.0613 mole of Na_2HPO_4 and 0.00533 mole of KH_2PO_4 at 0° and 760 mm.

The effects of different variables at a protein concentration (about 190 g. per liter) which gives a pressure of 100 mm. in the standard state have been summarized below.

(i) An increase in temperature from 0 to 1.0° raises the osmotic pressure from 100 mm. to 100.36 mm.

(ii) An increase in the barometric pressure from 760 to 775 increases the osmotic pressure from 100.0 to about 100.02 mm.

(iii) An increase of 10% in the total concentration of crystalloids diminishes the osmotic pressure from 100.0 mm. to 99.6 mm.

(iv) An increase in the hydrogen-ion concentration due to carbon dioxide which

[9] G. S. Adair, *Proc. Camb. Phil. Soc. Biol.*, **1**, 75 (1924); *Proc. Roy. Soc. London*, **109A**, 292 (1925); T. Svedberg and R. Fåhraeus, THIS JOURNAL, **48**, 430 (1926); T. Svedberg and J. B. Nichols, *ibid.*, **49**, 2920 (1927).

corresponds with a change in P_H from 7.80 to 7.60 diminishes the pressure from 100.0 mm. to 99 mm.

(v) The oxygenation of the hemoglobin and the formation of met-hemoglobin cause relatively little change in the observed osmotic pressure.

The variations considered above are greater than any that have been observed in the experiments included in Table I.[2] It seems probable that the observed pressures are within about 2% of the corrected pressures for the theoretical standard state.

3. The Effects of Variations in the Average Composition of the Protein Salts.—In practice it is impossible to alter the total concentration of a protein without altering some of the conditions which determine the proportions of individual protein salts and the average compositions of the mixture.

If the concentration of a protein salt is increased at a constant hydrogen-ion concentration, there is an alteration in the interionic attractions which tends to increase n, the average number of atoms of sodium in a salt like sodium hemoglobinate.[10]

If the concentration of the sodium hemoglobinate is increased under conditions where the potentials of the crystalloids are constant, there is an increase in a_H, the activity of hydrogen ions, (Table II) or a diminution in P_H ($= -\log a_H$) which tends to diminish the value of n by approximately 10 units for a diminution of 1 unit in the P_H. This estimate is based on the observations of Van Slyke and other workers, referred to in a previous paper.[2]

The range of variation in P_H from 7.80 to 7.77 (shown in Table II) diminishes n from about 8.5 to 8.2, and it seems unlikely that this change is exactly balanced by the effects of interionic forces.

It has been stated that Formulas 9 and 10 are not valid if n is not a constant, and therefore it is necessary to consider the error due to a variation in n. In the previous section it has been pointed out that a variation in the hydrogen-ion concentration from P_H 7.8–7.6 alters the osmotic pressure p by about 1%, and therefore it appears that the variation in n caused by a much smaller change in hydrogen-ion concentration cannot have any great effect on the pressure p or the function a_{ps}, which is defined in terms of osmotic pressures. The absolute value of n does not enter into the formula for a_{ps}. In the case of the function $(a_{ps})_w$, which is equal to $(a_p)_w a_{Na}^n$, the absolute value of n and the effects of slight variations in n are of much greater significance.

4. The Partial Specific Volumes of the Solvent and the Protein.—v_x'' = the volume of 1 mole of the standard solution (Table I). $\bar{v}_x$ = the "partial specific volume" of 1 mole of this mixture in the protein solution. $\bar{v}_{ps}$ = the partial specific volume of 1 mole of the hemoglobin.

Preliminary investigations show that the ratio $\bar{v}_x/v_x''$ varies from 0.9998

[10] S. P. L. Sörensen, K. Linderström-Lang and E. Lund, *J. Gen. Physiol.*, **8**, 543 (1927).

at low protein concentrations to 0.999 at higher concentrations. The application of the formula[11] $(\partial p/\partial P) = (1 - \bar{v}_x/v''_x)$ shows that the observed osmotic pressure, p, is not appreciably different from the theoretical pressure $(P - P'')_P$ in Formula 6.

The measurement of $\bar{v}_{ps}$ is more difficult because the protein cannot be dried without irreversible changes. As a first approximation it appears that $\bar{v}_{ps}$ is about 65 liters.[2]

5. The Membrane Potentials of the Protein Solutions.—A table of measurements of membrane potentials with saturated calomel electrodes has been given in a previous paper.[2] Calculations based on six of these observations recorded in Table II show that the simple empirical formula No. 16, agrees with the observed results within the limits of experimental error

$$E = -0.24\, m_p \qquad (16)$$

m_p = moles of protein per liter of solvent = $C_p/(1 - 65\, C_p)$.

TABLE II

MEMBRANE POTENTIALS AT DIFFERENT HEMOGLOBIN CONCENTRATIONS

Conditions: hemoglobin solutions in equilibrium with Standard Solution in Table I. KCl + Na_2HPO_4 + KH_2PO_4 at 0° (P_H = 7.8).

Hb per liter of soln., moles, C_p	Hb per liter of "solvent," moles, m_p	Membrane potential, (obs.), mv.	Membrane potential (calcd.), mv.	Corr. P_H of soln.
0.00032	0.00033	−0.07	−0.08	7.799
.00147	.0016	− .50	− .38	7.791
.00294	.0035	− .87	− .87	7.784
.00353	.0046	−1.05	−1.10	7.781
.00426	.0059	−1.45	−1.42	7.773
.00506	.0075	−1.98	−1.80	7.764

6. The Mean Valence of Protein Ions.—The mean valence of the protein ions is equal to the "equivalent concentration" of the protein divided by its molar concentration. This equivalent concentration must be equal to the difference between the equivalent concentrations of diffusible anions and cations that exist in the free state, uncombined with protein.

The concentrations of the free ions in the solution of strong electrolytes outside the membrane can be determined (Table I) and a provisional estimate of their concentrations in the protein solution can be obtained by applying Donnan's formula (13) to the membrane potentials recorded in Table II. It appears that $n_p = -5.9/f_e$, where f_e is a constant which is equal to unity if the concentrations of the ions are equal to their activities. If $f_e = 0.7$, then $n_p = -8.5$. The figure -8.5 has been adopted in the provisional calculations recorded in Table III. A revision of these calculations will be published when n_p has been determined by more accurate methods.

[11] A. W. Porter, *Proc. Roy. Soc. London*, **79A**, 519 (1907).

V. The Activity Coefficients of a Salt of Hemoglobin and its Ions

The activities of protein salts referred to the experimental standard state are different from the activities referred to a hypothetical solution of the protein salt in water, and therefore Formulas 17 and 18, which define the coefficients f_{ps} and f_p, are different from the conventional definitions of the activity coefficients of strong electrolytes.

$$RT \ln f_{ps} m_p = RT \ln a_{ps} = \int v_s dp + \text{constant} \qquad (17)$$

m_p = gram moles of protein salt per liter of solvent and $f_{ps} = a_{ps}/m_p$ is a coefficient which approached unity at low protein concentrations, as shown in Table III. The integration constant is determined by this condition and by the condition that p approaches RTm_p when m_p is very small.

The chief difference between f_{ps} and the activity of a typical strong electrolyte is due to the application of this formula rather than Formula No. 11, $\pi = (1 + n)C_pRT$. The function f_{ps} is not useful in comparisons of the properties of protein salts and crystalloids, but it is of importance as a step in the calculations of f_p, the activity coefficient of the protein ion, and in the treatment of the equilibrium of protein salts in gravitational fields of force, a subject which will be discussed in a subsequent communication.

The activity coefficient f_p defined by the formula $f_p = a_p/m_p$ can be calculated by Formula 14a, or Formula 18 given below.

$$m_p f_{ps} = m_p f_p e^{n_p w} \qquad (18)$$

Since $(a_p)_w$ is equal to $f_0 a_p$, where f_0 is an indetermined constant, the product $f_0 f_p$ is comparable with the activity coefficient of an ion defined by the formulas of Lewis and Randall, although the resemblance is not quite exact, because $f_0 f_p$ is an "average value" and n_p is an average valence.

The results recorded in Table III show that the function f_p increases as the protein concentration increases. An increase in the partial osmotic pressure of the protein ions under these conditions has been referred to in a previous paper.[2]

A discussion of the kinetic significance of the rise in the activity coefficient is not permissible in a purely thermodynamical treatment of osmotic pressures and activities but there are one or two points which are of interest in connection with the application of the interionic attraction theory of Debye and Hückel to protein ions.

The absolute values of f_p increase as the protein concentration increases, a variation in the opposite direction to that predicted by the theory. If, however, the increase in f_p is compared with the increase in f_{pi}, the activity coefficient of the isoelectric protein, it appears that the "relative activity coefficient" (f_p/f_{pi})[12] diminishes from 1.0 to 0.8 as the protein concentra-

[12] The ratio (f_p/f_{pi}) has been referred to in a preliminary note on the activity of protein ions, Adair, *Trans. Faraday Soc.*, **23**, 536 (1927).

tion increases from 0.0 to 0.004, and in this respect the protein salt resembles a typical strong electrolyte.

TABLE III

THE ACTIVITY COEFFICIENTS OF A SALT OF HEMOGLOBIN AND ITS IONS

Conditions: temperature, 0°; pressure, 760 mm.; composition of "solvent," 0.10 mole of KCl, 0.0613 mole of Na_2HPO_4, 0.00533 mole of KH_2PO_4 per liter. Composition of protein salt, Hb $(Na + K)_{3.5}$. Valence of protein ion, $n_p = -8.5$. The membrane potential factor (Formula 14) is calculated from data given in Table II. The activity coefficients of the sodium and potassium ions are approximately equal to 0.7.

Hb per liter of solvent, mole, m_p	Act. of protein salt,[a] a_{ps}	Membrane potential factor, $e^{n_p u}$	Act. coeff. of salt, f_{ps}	Act. coeff. of ion, f_p
0.0002	0.0002	1.02	1.05	1.03
.0010	.0014	1.09	1.40	1.3
.0020	.0040	1.20	2.0	1.7
.0030	.0090	1.30	3.0	2.3
.0040	.0200	1.40	5.0	3.6

[a] The determination of a_{ps} by the evaluation of the integral in Formula 17 may be facilitated by application of the empirical formulas discussed in a previous paper.[2] In dilute solutions the formula $p(v_s - b) = RT$ can be applied and by integration $RT \ln a_{ps} = \int v_s dp = RT \ln p + bp$. Over the range of pressures from 12 to 120 mm., a formula with two empirical constants can be applied, $p(v_s - 107.5) = 1.09\ RT$.

Conclusions

Under certain conditions the salt of a protein such as a sodium hemoglobinate is equivalent to one component, although the variations in the average composition of the salt caused by alterations in the activities of diffusible acids and bases prove that the sodium hemoglobinate is a mixture of a great number of individual protein salts.

The conditions for the application of Gibbs' equations to determine the "average values" of the potentials and activities of protein salts have been considered and the following formula has been obtained.

$$RT \ln a_{ps} = RT \ln a_p e^{n_p u} = \int_{p^\circ}^{p} v_s dp + \text{constant}$$

$\ln a_{ps}$ and $\ln a_p$ denote the logarithmic mean values of the activities of the protein salt and protein ion; n_p = the average valence of the protein ion; $u = EF/RT$, where E = the membrane potential; v_s = the volume of "solvent"—(a mixture) per gram mole of protein; p = the osmotic pressure measured with a collodion membrane under conditions where the potentials of the diffusible salts are kept constant by the equilibration of the protein with a standard solution of crystalloids at a constant pressure and temperature.

The integration constant is determined by the convention that a_{ps} is equal to the protein concentration in a very dilute solution in equilibrium with the standard solution of crystalloids. The relationship between a_{ps} and the activities of typical strong electrolytes has been formulated.

The activity of a salt of hemoglobin has been investigated by experiment in order to determine the degree of accuracy in the assumptions that have been made in the thermodynamical treatment of a salt of variable composition in a system of many components.

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[CONTRIBUTION FROM THE WOLCOTT GIBBS MEMORIAL LABORATORY, HARVARD UNIVERSITY]

SPECIFIC HEATS OF SODIUM AND POTASSIUM HYDROXIDE SOLUTIONS[1]

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In a previous publication[2] from this Laboratory it was pointed out that a serious discrepancy existed between the value then found for the specific heat of $NaOH \cdot 25H_2O$ and earlier values. Since this datum was to be made the basis for the specific heats of other solutions, heats of dilution, heats of neutralization, etc., the new and higher value demanded confirmation.[3]

Hence part of the work was repeated with even greater care, especially studying possible sources of error and inaugurating a few slight modifications and improvements in procedure. The outcome was in general essentially in accord with the work of Richards and Gucker, as will be shown.[4]

Procedure with Modifications

The twin calorimeter system used previously was rebuilt in essentially the same form as described on pp. 1878–1881 of ref. 2. Standardization of new thermocouples showed a sensitivity of 0.000340° per mm. of scale reading for the larger thermocouple and 0.000527° per mm. for the smaller ones. Slight changes in the apparatus made it necessary to redetermine the water equivalent. Later it was found advisable to use a smaller amount of liquid in each can to avoid errors from splashing or spilling in assembling and handling the apparatus.

The chief causes of minor variations were found in maintenance of adiabatic conditions at all times and in trouble from unequal evaporation from the calorimeter cans, one containing water and the other solutions. The former difficulty was in part eliminated by trying out and adjusting

[1] On account of the death of Professor Richards on April 2, 1928, the work here described was completed and the manuscript prepared by the junior author.

[2] Richards and Gucker, THIS JOURNAL, **47**, 1876 (1925).

[3] Earlier records present a bewildering variety of specific heat values for sodium hydroxide solutions, particularly for the more concentrated ones. Richards and Rowe, [THIS JOURNAL, **43**, 770 (1921)] took 0.855 for $NaOH \cdot 10H_2O$—choosing from among old values ranging all the way from 0.84 to 0.89. It will be shown that this choice, although perhaps the best possible at the time, was much too low in value.

[4] Ref. 2 and the following paper in this series.