

# THEORETICAL STUDIES UPON DIPOLAR IONS<sup>1</sup>

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## I

The influence of electrolytes on the behavior of the amino acids and proteins is perhaps one of the most important topics in the physical chemistry of these substances. It has long been known that there is a strong thermodynamic interaction between electrolytes and the amino acids and proteins. In recent years the accurate measurements of Cohn and his coworkers (1, 2, 3, 4, 5) have provided a large body of thermodynamic data relating to this question.

With the accumulation of evidence in favor of the dipolar ion or zwitter-ion structure of the amino acids and proteins in the isoelectric condition in solvents of high dielectric constant, it has become apparent that thermodynamic interaction between electrolytes and these substances can, in large measure, be attributed to the strong electrostatic intermolecular forces between dipolar ions and real ions in solution. A dipolar ion, while bearing no net charge, is characterized by electric multipole moments of large magnitude. For example, the glycine dipolar ion,  $\text{NH}_3^+\text{CH}_2\text{COO}^-$ , possesses a dipole moment of about 15 Debye units, about ten times that of an ordinary polar molecule. Thus a dipolar ion is in a sense a superpolar molecule, surrounded by an intense electrostatic field.

An extension of the Debye-Hückel theory describing the electrostatic interaction of dipolar ions and real ions in solution was developed by Scatchard and the writer (10) and later elaborated in some detail by the writer (8) for dipolar ions of spherical shape. It is the purpose of the present article to review the possible applications of the theory and to extend it to dipolar ions of elongated shape. In this extension of the theory we shall confine ourselves to limiting laws approached at high

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dilution, making use of a method similar to that developed by Fuoss (6) for the study of dipole-dipole interaction, rather than the somewhat more complicated method based upon the Debye-Hückel theory.

The theory may be employed in two ways. By means of it the influence of electrolytes upon the chemical potential of a dipolar ion of known electrical structure may be predicted. On the other hand, it may be used to determine the structure, for example, the distance between the charged groups and the dipole moments, of simple dipolar ions from data relating to the influence of electrolytes on their chemical potentials. For the latter purpose we shall make extensive use of the measurements of Cohn and his coworkers.

In this article we shall limit the discussion to the aliphatic amino acids and their peptides, since the proteins present certain ambiguities of interpretation with which we cannot concern ourselves here. Thus, so many parameters are required to specify the charge distribution of protein dipolar ions that they cannot be uniquely determined from the influence of electrolyte upon chemical potential. On the other hand, too little is known at present about the details of protein structure to permit a probable assignment of configuration to the dipolar ionic charges from which to predict the interaction with electrolytes. Nevertheless, as Cohn has shown, certain progress can be made along the latter lines, and observed electrolyte effects can, in the case of egg albumin and hemoglobin (5), be reproduced by hypothetical though not unique assignments of charge configuration.

## II

We shall be interested in the properties of a solution containing a dipolar ion component  $i$  at a molar concentration,  $c_i$ , and  $\nu$  ionic components at molar concentrations,  $c_1 \dots c_\nu$ , in a solvent of dielectric constant  $D$ . The pertinent thermodynamic properties of the solution may be derived from the chemical potentials of the several components. We shall be particularly concerned with the chemical potential of the dipolar ion species, which may be written in conventional form,

$$\begin{aligned}\mu_i &= RT \log \gamma_i c_i + \mu_i^0(T, p) \\ \mu_i^0 &= \lim_{\substack{c_1 \dots c_\nu \\ =0}} [\mu_i - RT \log c_i]\end{aligned}\tag{1}$$

where  $\gamma_i$ , the activity coefficient, is defined by this equation.

We shall make the simplifying assumption that the deviation of the solution from ideal behavior is due to electrostatic intermolecular forces

alone. If the solvent is idealized as a structureless dielectric continuum, the logarithm of the activity coefficient,  $\gamma_i$ , may be written as follows (8),

$$\log \gamma_i = \sum_{k=1}^{\nu+1} B_{ik} c_k$$

$$B_{ik} = \frac{N\beta}{1000} \int_{\omega}^{\nu} \int_0^1 V_{ik} e^{-\beta W_{ik}(\lambda)} d\lambda dv \quad (2)$$

$$\beta = 1/kT$$

where the sum extends over all  $\nu + 1$  solute components.  $V_{ik}$  is the electrostatic work required to bring the pair of molecules or ions,  $i$  and  $k$ , from infinite separation to the given configuration in the pure solvent, and  $W_{ik}(\lambda)$  is the average work (potential of average force) expended in the same process in the actual solution, all charges,  $e_1 \cdots e_s$ , of molecule  $i$  having a fraction,  $\lambda$ , of their full values. The integration extends over all values of the relative coordinates of the pair of molecules in the volume,  $\nu$ , of the solution and outside a region,  $\omega$ , of molecular dimensions determined by the size and shape of the two molecules, into which intermolecular repulsion at short range prevents penetration.<sup>2</sup>

According to the Debye-Hückel theory the potentials of average force,  $W_{ik}$ , necessary for the evaluation of the coefficients,  $B_{ik}$ , satisfy the following relation,

$$W_{ik}(\lambda) = z_k e \psi_i(\lambda, \mathbf{r}_k) \quad (3)$$

where  $\psi_i$  is the average electrostatic potential at the point  $\mathbf{r}_k$  from an origin in molecule  $i$  and  $z_k e$  is the charge of ion  $k$ . The potential,  $\psi_i$ , satisfies the Poisson-Boltzmann equation of the Debye-Hückel theory, which in its linear approximation is

$$\nabla^2 \psi_i - \kappa^2 \psi_i = 0$$

$$\kappa^2 = \frac{4\pi N e^2}{1000 D k T} \sum_{k=1}^{\nu} c_k z_k^2 \quad (4)$$

<sup>2</sup> Equation 2, while somewhat more convenient for our purposes, is essentially equivalent to the more usual expression, based on the Guntelberg-Müller charging process

$$kT \log \gamma_i = \sum_{l=1}^s e_l \int_0^1 \{\psi_i(\lambda e_1, \dots, \lambda e_s) - \psi_i^0(\lambda e_1, \dots, \lambda e_s)\} d\lambda$$

where  $\psi_i$  is the average electrostatic potential at the point of location of charge,  $e_l$ , of molecule  $i$  in the actual solution and  $\psi_i^0$  is its value at infinite dilution, all charges,  $e_1 \dots e_s$ , having a fraction,  $\lambda$ , of their full values.

outside the region of non-penetration, while in  $\omega$  Laplace's equation is satisfied.

$$\nabla^2\psi_i = 0 \quad (5)$$

Solution of equations 4 and 5, subject to the boundary conditions of electrostatics on the surface of  $\omega$ , continuity of the potential and the normal component of the dielectric displacement, yields  $\psi_i$ , which may be used in equation 2 or 2a for the calculation of the activity coefficient. This method has been employed by the writer to study the influence of electrolytes on the activity of spherical dipolar ions (8).

In the present article we propose to use a somewhat simpler method, which yields only a limiting law. Only linear terms in the expansion of the logarithm of the activity coefficient in the concentrations of the various solute species appear, thus allowing the treatment of non-spherical dipolar ions and permitting the inclusion of "salting-out" forces between an ion and a dipolar ion. The latter arise from a repulsion between the ionic charge and an image distribution in the cavity of low dielectric constant created by the dipolar ion in the solvent. It is evident that

$$\lim_{c_1, \dots, c_{r+1}=0} W_{ik}(\lambda) = \lambda V_{ik} \quad (6)$$

If we are content with the linear terms in a power series in the ionic concentrations, we may, therefore, write equation 2 in the following form,

$$\begin{aligned} \log \gamma_i &= \sum_{k=1}^r B_{ik}^0 c_k \\ B_{ik}^0 &= \frac{N}{1000} \int_{\omega}^{\infty} (1 - e^{-\beta v_{ik}}) dv \\ B_{ik}^0 &= \lim_{c_1, \dots, c_{r+1}=0} B_{ik} \end{aligned} \quad (7)$$

For simplicity we suppose that the term in the sum, equation 2, arising from the mutual interaction of dipolar ions is negligible and thus limit ourselves to solutions in which the concentration of dipolar ions is itself small. If desired, the  $B_{ii}^0$  for mutual dipolar interaction may be obtained from Fuoss' calculations for dipole molecules (6). The development (equation 7) is only possible when the integrals defining the  $B_{ik}$  exist. Although they diverge when the component  $i$  is a true ion, they exist when it is a dipolar ion, bearing no net charge, and the expansion is suitable for our purposes. In solvents of relatively high dielectric constant at ordinary temperatures, a satisfactory approximation to the  $B_{ik}$  may be

obtained by expansion of  $1 - e^{-\beta V_{ik}}$  with retention of the first two terms alone.

$$B_{ik}^0 = \frac{N}{1000} \int_{\omega} [\beta V_{ik} - \frac{\beta^2}{2} V_{ik}^2] dv \quad (8)$$

For a dipolar ion characterized by a set of charges,  $e_1 \cdots e_s$ , distributed in a cavity,  $\omega_0$ , of dielectric constant  $D_0$ , the electrical work,  $V_{ik}$ , required to bring a true ion of charge,  $z_k e$ , from infinity to a point  $\mathbf{r}_k$  from an origin fixed in the dipolar ion  $i$  is given by

$$V_{ik} = \frac{1}{2} \left\{ z_k e \psi_e^0(\mathbf{r}_k) + \sum_{l=1}^s e_l \psi_i^0(\mathbf{r}_l) \right\} \quad (9)$$

where  $\psi_i^0(\mathbf{r}_l)$  is the electrostatic potential in the interior of  $\omega_0$  at the location of the charge,  $e_l$ , and  $\psi_e^0(\mathbf{r}_k)$  is the potential at the point  $\mathbf{r}_k$  exterior to  $\omega_0$ , when the ion and dipolar ion are fixed in the given configuration in the pure solvent. Self-energy terms in equation 9 are to be omitted,  $V_{ik}$  vanishing when the ions are at infinite separation. The potentials  $\psi_i^0$  and  $\psi_e^0$  satisfy Laplace's equation,  $\nabla^2 \psi = 0$ , both interior and exterior to the surface of  $\omega_0$ , and fulfill the usual boundary conditions everywhere on this surface,

$$\begin{aligned} \psi_i^0 &= \psi_e^0 \\ D_0 \mathbf{n} \cdot \nabla \psi_i^0 &= D \mathbf{n} \cdot \nabla \psi_e^0 \end{aligned} \quad (10)$$

where  $\mathbf{n}$  is a unit vector normal to the surface. In addition, the potentials must have the singularities characteristic of the real charge distributions of the two ions. The details of the determination of the electrostatic potential for dipolar ions of several shapes are given in the appendix.

We first consider a spherical dipolar ion of radius  $b$ , the charge distribution of which may be characterized by a point dipole of moment,  $\mu$ , located at its center. The cavity,  $\omega_0$ , is thus a sphere of radius  $b$ , and the excluded region,  $\omega$ , in the integral (equation 8) is a sphere of radius  $a$ , the sum of the radii of the dipolar ion and the real ion, all real ions in the solution being spheres of the same radius. The potential energy,  $V_{ik}$ , has the following form,

$$V_{ik} = \frac{3z_k e \mu \cos \vartheta}{(2D + D_0)r^2} + \frac{z_k^2 e^2 b^3}{2\pi^4} \frac{D - D_0}{D} \sum_{n=0}^{\infty} \frac{n+1}{(n+2)D + (n+1)D_0} \left(\frac{b}{r}\right)^{2n} \quad (11)$$

where  $r$  is the distance between the centers of the ion and dipolar ion, and  $\vartheta$  is the angle between the vectors,  $\mathbf{r}$  and  $\mathbf{u}$ , the dipole moment. The second member of equation 11 represents a repulsion between the real

ion and an image distribution in the cavity,  $\omega_0$ , created by the dipolar ion in the solvent. A similar term, usually of much smaller magnitude, arising from the interaction of the dipolar ion and an image distribution in the ionic cavity has been suppressed.

Substitution of equation 11 in equations 7 and 8 and the neglect of small terms in  $D_0/D$  in the summation of the resulting series yields the following limiting law for the activity coefficient of the dipolar ion component of the solution,

$$\begin{aligned} \log_{10} \gamma_i &= -B_i \Gamma \\ \Gamma &= (1/2) \sum_{k=1}^{\nu} c_k z_k^2 \\ B_i &= \frac{2\pi N e^2}{2303 D k T} \left\{ \frac{3}{2} \frac{\mu^2}{D a k T} - \frac{b^3}{a} \alpha(\rho) \right\} \end{aligned} \quad (12)$$

where  $\Gamma$  is the ionic strength of the solution,  $\rho$  is the ratio  $b/a$ , and  $\alpha(\rho)$  is a function tabulated in table 1. Insertion of numerical values for the

TABLE 1  
 $\alpha(\rho) = (\frac{1}{3}\rho^4) \{ (\rho^3 - 2) \log(1 + \rho) - (\rho^3 + 2) \log(1 - \rho) - 2\rho^2 \}$

$\rho$	$\alpha(\rho)$
0.0	1.00
0.2	1.01
0.4	1.08
0.6	1.21
0.8	1.54
0.9	1.96

universal constants and the introduction of the dielectric constant of water,  $D_w$ , yields at 25°C.

$$\begin{aligned} B_i &= B_i^{(0)} - B_i^{(1)} \\ B_i^{(0)} &= 5.48 \times 10^{-3} (D_w/D)^2 \mu^2/a = 0.125 (D_w/D)^2 R^2/a \\ B_i^{(1)} &= 4.66 \times 10^{-3} (D_w/D) (v_i/a) \alpha(\rho) \end{aligned} \quad (13)$$

where  $R$  is the effective dipole separation,  $\mu/e$ , and  $v_i$  is equal to  $4\pi N b^3/3$ , the value at infinite dilution of the partial molal volume of the dipolar ion component under the idealization of the solvent as structureless continuum. The units employed in equation 13 are the Debye unit for dipole moment and the Ångström unit for the lengths  $R$  and  $a$ . The term  $B_i^{(0)}$  gives rise to a decrease in the chemical potential of the dipolar ion by electrolyte ("salting-in"), while the term  $B_i^{(1)}$  gives rise to an increase ("salting-out"). The latter effect was not included in the writer's previous

treatment (8), based on the Debye-Hückel theory. If the term  $B_i^{(0)}$  is omitted in equation 12, a limiting law in exact agreement with the writer's earlier one is reached.

We next consider a dipolar ion of prolate ellipsoidal shape, characterized by a charge distribution consisting of two charges,  $+e$  and  $-e$ , located at the foci. It is convenient to employ confocal elliptical coordinates in the treatment of this model. Thus a point situated at distances  $r_1$  and  $r_2$  from the respective foci of the ellipsoid and in a plane inclined at an angle,  $\varphi$ , to the reference plane containing the major axis is specified by the coordinates  $\lambda$ ,  $\eta$ , and  $\varphi$ .

$$\begin{aligned}\lambda &= (r_1 + r_2)/R \\ \eta &= (r_1 - r_2)/R\end{aligned}\tag{14}$$

where  $R$  is the interfocal distance. The cavity,  $\omega_0$ , is an ellipsoid of eccentricity,  $\epsilon$ , equal to  $1/\lambda_0$ , and the region  $\omega$ , into which ions cannot penetrate is, for simplicity, assumed identical with  $\omega_0$ , although this is strictly true only for vanishing ion size. The potential energy,  $V_{ik}$ , of the dipolar ion and a real ion of charge  $z_k e$  is closely approximated by the following expression,

$$V_{ik} = \frac{4z_k e^2}{DR} \frac{\eta}{\lambda^2 - \eta^2} \lambda_0^{-1} \left[ \lambda_0 - \frac{(\lambda_0^2 - 1)}{2} \log \frac{\lambda_0 + 1}{\lambda_0 - 1} \right]\tag{15}$$

Equation 15 is exact for the two limiting cases, zero and unit eccentricity, and only slightly inaccurate, owing to the approximate summation of an infinite series, for intermediate eccentricities. The term arising from the interaction of the ion with its image distribution in the dipolar ion cavity, included for the sphere, has been omitted in equation 15, since it introduces undue complication, which we postpone for later, in the evaluation of the integral (equation 8). Introduction of the coordinates  $\lambda$  and  $\eta$  as variables of integration in the latter equation leads to the following expression

$$B_{ik}^0 = - \frac{\pi N \beta^2 R^3}{1000} \int_{\lambda_0}^{\infty} \int_{-1}^{+1} (\lambda^2 - \eta^2) V_{ik}^2 d\eta d\lambda\tag{16}$$

the term linear in  $V_{ik}$  vanishing since it is an odd function of  $\eta$ . Equation 16, together with equations 7 and 15, yields for the logarithm of the activity coefficient of the dipolar ion,

$$\begin{aligned}\log_{10} \gamma_i &= - B_i \Gamma \\ B_i &= \frac{2\pi N e^4 g(\lambda_0)}{2303(DkT)^2} R \\ g(\lambda_0) &= \lambda_0^{-2} \left[ \lambda_0 - \frac{(\lambda_0^2 - 1)}{2} \log \frac{\lambda_0 + 1}{\lambda_0 - 1} \right]^{-1}\end{aligned}\tag{17}$$

Insertion of numerical values for the constants gives, at 25°C.,

$$B_i = 0.167(D_w/D)^2 g(\lambda_0) R \quad (18)$$

A tabulation of  $g$  as a function of the eccentricity of the ellipsoidal cavity is given in table 2. We remark that for constant eccentricity  $B_i$  is proportional to the first power of the distance,  $R$ , between the charges of the dipolar ion. For elongated ellipsoids in the neighborhood of unit eccentricity, the function  $g$  is approximately unity. For ellipsoids nearly spherical,  $g$  may be expanded in a power series in  $R/a$ , where  $a$  is the shortest distance of either focus to the surface, equal to the radius of the sphere at zero eccentricity. The initial term of the series,  $3R/4a$ , when substituted in equation 18 yields an expression identical with  $B_i^{(0)}$  (equation 13) for a dipole at the center of a sphere. No counterpart of  $B_i^{(1)}$  is obtained, since we have neglected the "salting-out" influence of image

TABLE 2  
*g as a function of the eccentricity of the ellipsoidal cavity*

$\epsilon$	$g(\epsilon)$	$\epsilon(1 - \epsilon^2)^{-1/2}g(\epsilon)$
0.00	0.00	0.00
0.20	0.30	0.061
0.33	0.49	0.17
0.50	0.71	0.39
0.60	0.83	0.58
0.70	0.94	0.82
0.80	1.01	1.14
1.00	1.00	

forces in the present case. While we shall not consider the problem in detail in the present article, it seems reasonable to suppose that the magnitude of  $B_i$  for an ellipsoid can be roughly estimated by an analog of the spherical formula, equation 13, in which  $v_i/l$  appears instead of  $v_i/a$ ,  $l$  being a length intermediate between the semi-major and semi-minor axes of the ellipsoid.

We shall also discuss a second model in which the dipolar ion is ellipsoidal in shape but characterized by a charge distribution consisting of a point dipole of moment,  $\mu$ , situated at one of the foci and parallel to the major axis. The calculations proceed in the same manner as for the first ellipsoidal model except that the potential,  $V_{ik}$ , is of the form,

$$V_{ik} = \frac{4\pi z_k e}{DR^2} \frac{\lambda\eta}{(\lambda + \eta)^3} \lambda_0^{-1} \left[ \lambda_0 - \frac{(\lambda_0^2 - 1)}{2} \log \frac{\lambda_0 + 1}{\lambda_0 - 1} \right]^{-1} \quad (19)$$



The logarithm of the activity coefficient of the dipolar ion is finally calculated to be

$$\begin{aligned} \log_{10} \gamma_i &= -B_i T \\ B_i &= \frac{3\pi N e^2 u(\lambda_0) \mu^2}{2303 (DkT)^2 R} \\ u(\lambda_0) &= \frac{8}{9} \frac{1 + 3(\lambda_0^2 - 1)^{-1} + 4(\lambda_0^2 - 1)^{-2}}{\lambda_0(\lambda_0^2 - 1) \left[ \lambda_0 - \frac{(\lambda_0^2 - 1)}{2} \log \frac{\lambda_0 + 1}{\lambda_0 - 1} \right]} \end{aligned} \quad (20)$$

With numerical values for the constants at 25°C.,  $B_i$  is given by the following equation,

$$B_i = 5.48 \times 10^{-3} (D_w/D)^2 u(\lambda_0) \mu^2 / R \quad (21)$$

in which the Debye and the Ångström are the units employed. The function  $u(\lambda_0)$  is listed for several eccentricities in table 3. In the limit

TABLE 3  
Values of the function  $u(\lambda_0)$

$\epsilon$	$u(\epsilon)$	$\epsilon(1 - \epsilon^2)^{-1/2} [u(\epsilon)]^{-1}$
0.00	0.00	0.50
0.20	0.47	0.43
0.33	1.03	0.33
0.50	2.92	0.19
0.60	6.31	0.11
0.70	16.5	0.053
0.80	81.5	0.014

of zero eccentricity of the ellipsoid,  $u(\epsilon)/R$  reduces to  $1/a$ , where  $a$  is the radius of the sphere, and equations 20 and 21 reduce to 12 and 13, except for the "salting-out" term.

### III

We are now ready for a brief review of the interpretation of the thermodynamic interaction of dipolar ions and electrolytes by means of the equations put forward in the preceding section. For this purpose we turn to the data of Cohn and his coworkers (1, 2, 3), relating to the influence of electrolytes on the solubility of the aliphatic amino acids and the peptides of glycine. If  $s$  and  $s_0$  are the solubilities of a dipolar ion in a given solvent in the presence and in the absence of electrolyte, the conditions of heterogeneous equilibrium require that the ratio  $s_0/s$  be equal to  $\gamma$ , the activity

coefficient in the presence of electrolyte, the solution being assumed ideal when the dipolar ion component is present alone. We may therefore write a limiting solubility law in the following form,

$$\log_{10}(s/s_0) = B\Gamma \quad (22)$$

where  $\Gamma$  is the ionic strength and  $B$  is given by one of equations 12, 17, or 20, according to the assumed structure of the dipolar ion. The data of Cohn confirm the form of the limiting law, in which the initial term is linear in the ionic strength, and the coefficient  $B$  may be obtained from the limiting slopes of his solubility curves. In order to correlate measurements in solvents of different dielectric constant, it is convenient to introduce a coefficient  $B_0$  equal to  $(D/D_w)^2 B$ , related to the solubility ratio as follows,

$$B_0 = \lim_{\Gamma \rightarrow 0} \left[ \frac{d(D_w/D) \log_{10}(s/s_0)}{d(D/D_w)\Gamma} \right] \quad (23)$$

From equations 12, 17, and 20 we remark that the theory predicts that  $B_0$  should be independent of the dielectric constant of the solvent if "salting-out" forces due to image repulsion are of small magnitude. This independence is approximately confirmed by Cohn's measurements (1) of the solubilities of the amino acids in alcohol-water mixtures, from which we may conclude that the "salting-out" forces are of secondary importance, though not negligible.

We shall first discuss the solubility data for the simplest amino acid, glycine, on the basis of the spherical dipolar ion model. The dipole moment,  $\mu$ , may be computed from the solubility coefficient,  $B_0$ , by equation 13.

$$\begin{aligned} \mu &= \sqrt{183B_0^{(0)}a} \\ B_0^{(0)} &= (D/D_w)^2 B^{(0)} \end{aligned} \quad (24)$$

From the partial molal volume of glycine at infinite dilution in water and the ionic radii of Pauling and Huggins (9), Cohn (1) estimates  $a$  to be 3.90 for glycine and lithium chloride. From the solubility of glycine in the presence of lithium chloride in alcohol-water solvents of decreasing dielectric constant, he determines  $B_0^{(0)}$  to be 0.32. Substitution of these values in equation 24 yields a value, 15 Debye units, for the glycine dipole moment. This value is in agreement with the dipole moment, calculated on the basis of structural considerations, for the glycine dipolar ion in which the terminal  $\text{NH}_3^+$  and  $\text{COO}^-$  carry residual charges  $+e$  and  $-e$ , respectively. It is also of interest to consider the "salting-out" coefficient  $B_0^{(1)}$ . Its relative importance becomes greater with increasing

dielectric constant of the solvent. Although in water the high solubility of glycine prevents the use of equation 22 without a term for the mutual dipolar ion interaction, the coefficient  $B_0$  may be obtained from electromotive force measurements of Joseph (7) and freezing point measurements of Scatchard and Prentiss (11). For glycine and sodium chloride in water at 25°C.,  $B_0$  has the value 0.24. The difference between this value and  $B_0^{(0)}$ , roughly 0.08, gives the "salting-out" coefficient,  $B_0^{(1)}$ , on the basis of the present theory. For glycine and sodium chloride Cohn calculates  $a$  to be 4.05 and  $\rho$  is 0.7. By linear interpolation in table 1, we obtain for  $\alpha(\rho)$  a value 1.37. With these values and Cohn's estimate, 57 cc., for the limiting partial molal volume of glycine, corrected for solvent electrostriction, we calculate from equation 13 the value 0.08 for  $B_0^{(0)}$ . Thus in water the "salting-out" contribution arising from image forces amounts to about 25 per cent of the "salting-in" contribution to the solubility coefficient,  $B_0^{(0)}$ , arising from the interaction of the ions of the electrolyte and the true charges of the dipolar ion.

We shall now discuss glycine and its peptides on the basis of the first ellipsoidal model, in which two charges of opposite sign are situated at the foci. The calculations will be somewhat more approximate than those for glycine on the spherical model, since the ion size of the electrolyte is neglected and the "salting-out" influence of image forces is not included. The distance  $R$  between the foci of an ellipsoid of eccentricity  $\epsilon$  and volume  $v/N$  may be expressed in the following manner

$$R = \epsilon p (1 - \epsilon^2)^{-\frac{1}{3}} \quad (25)$$

where the length  $p$  is equal to  $(6v/N\pi)^{\frac{1}{3}}$  and is to be expressed in Ångström units. With numerical values for the constants, we have

$$p = 1.47 v^{\frac{1}{3}} \quad (26)$$

where the molal volume  $v$  is in cubic centimeters. For a sphere  $p$  is the diameter. From equations 18, 25, and 26 we may write

$$\begin{aligned} B_0 &= 0.167 \epsilon p (1 - \epsilon^2)^{-\frac{1}{3}} g(\epsilon) \\ R &= \epsilon p (1 - \epsilon^2)^{\frac{1}{3}} \\ p &= 1.47 v^{\frac{1}{3}} \end{aligned} \quad (27)$$

From the first of these equations and the experimental value of  $B_0$  the eccentricity of the molecular ellipsoid may be obtained by linear interpolation in table 2. From the eccentricity and the molal volume, the

charge separation of the dipolar ion may then be computed with the second of the equations. Calculations for glycine, diglycine, triglycine, and  $\beta$ -alanine are summarized in table 4. They are based on Cohn's experimental values of  $B_0$  and on his estimates of molal volumes corrected for solvent electrostriction (1). The distance, 2.8 Å., for glycine, corresponds to a dipole moment of 13, which differs only slightly from the value 15 obtained on the basis of the spherical model. A significant part of the difference between the two values is due to the neglect of electrolyte ion size in the ellipsoidal calculation. It therefore appears that we cannot conclude much as to the shape of the glycine dipolar ion from the influence of salts on its activity. However, owing to this very insensitivity to shape, we can place considerable confidence in the value of the dipole moment computed from the salt effect. The distances 4.7 and 6.4 computed for diglycine and triglycine are considerably below the values, 6.7 and 10.2, estimated by Cohn (1) for an extended chain configuration. Thus our calculations suggest that the extended chain configuration is

TABLE 4  
*Values of  $B_0$ ,  $v$ , and  $R$  for four dipolar ions*

	$B_0$	$v$	$R$
Glycine.....	0.32	57	2.8
Diglycine.....	0.58	93	4.7
Triglycine.....	0.80	130	6.4
$\beta$ -Alanine.....	0.43	73	3.6

not the preferred one, but that, owing to internal rotation, the average separation of the charges in these dipolar ions lies intermediate between the extended chain value and the free rotation value. Under these circumstances the computed distances have only formal significance as average distances unless a single preferred configuration should happen to dominate all others in probability. To take internal rotation properly into account, we should compute a  $B_0$  for each internal configuration and then average over all configurations with an appropriate distribution function for comparison with the experimental value of  $B_0$ . At present it is not possible to do this; moreover, the ellipsoidal model would be an extremely rough approximation for "crumpled" configurations.

Finally we shall discuss a series of the aliphatic alpha-amino acids on the basis of the second ellipsoidal model in which a point dipole is located at one focus. We represent glycine by a sphere with a point dipole at its center, 2.8 Å. from the surface of the molecule, and the homologs of glycine by ellipsoids with point dipoles at a focus located at the same

distance, 2.8 Å., as in glycine, from the surface of the molecule measured along the major axis. The eccentricity of the ellipsoid is then determined by the relation

$$(1 - \epsilon)(1 - \epsilon^2)^{-\frac{1}{3}} = 2l/p \quad (28)$$

where  $p$  is defined by equation 26 in terms of the molecular volume, and  $l$ , the distance of a focus from the surface, is 2.8 Å. in the subsequent calculations. Equation 28 may be roughly solved by linear interpolation

TABLE 5  
*l/p as a function of the eccentricity of the ellipsoidal cavity*

$2(l/p)$	$\epsilon$	$2(l/p)$	$\epsilon$
1.00	0.00	0.46	0.60
0.81	0.20	0.38	0.70
0.70	0.33	0.28	0.80
0.55	0.50		

TABLE 6  
*Calculated values of the dipole moment*

ACID	$B_0$	$p$	DIPOLE MOMENT
Glycine.....	0.32	5.6	13
$\alpha$ -Alanine.....	0.31	6.1	13
$\alpha$ -Aminobutyric acid.....	0.31	6.6	13
Leucine.....	0.30	7.3	13

in table 5. From equations 21 and 25 the following relation between the dipole moment of the dipolar ion is obtained

$$\mu = \sqrt{183B_0\epsilon p(1 - \epsilon^2)^{-\frac{1}{3}}[u(\epsilon)]^{-1}} \quad (29)$$

Calculations based upon equation 29 and Cohn's values of  $B_0$  and molecular volumes for glycine,  $\alpha$ -alanine,  $\alpha$ -aminobutyric acid, and leucine are listed in table 6. The discrepancy between the dipole moment values 13 and 15, both based upon the spherical model, is merely due to the neglect of electrolyte ion size in the present calculation, a procedure which we see does not lead to great error. It is interesting that the calculated dipole moments of glycine and of the other aliphatic alpha-amino acids turn out to have identical values. This is essentially what would be expected on the basis of structural considerations, although small differences could possibly arise from induction effects in the different aliphatic chains.

The problem of internal rotation does not enter explicitly into the determination of the dipole moments of the alpha-amino acids as it did in the case of the peptides of glycine. However, the average configuration of the aliphatic chain to which the glycine residue is attached will determine how closely the actual molecule conforms to the ellipsoidal shape, which is at best an approximation.

## REFERENCES

- (1) COHN, E. J.: *Naturwissenschaften* **20**, 663 (1932); *Ann. Rev. Biochem.* **4**, 93 (1935); *Chem. Rev.* **19**, 241 (1936).
- (2) COHN, E. J., McMEEKIN, T. L., EDSALL, J. T., AND BLANCHARD, M. H.: *J. Am. Chem. Soc.* **56**, 784 (1934); *J. Biol. Chem.* **100**, Proc. XXVIII (1933).
- (3) COHN, E. J., McMEEKIN, T. L., GREENSTEIN, J. P., AND WEARE, J. H.: *J. Am. Chem. Soc.* **58**, 2365 (1936).
- (4) McMEEKIN, T. L., COHN, E. J., AND BLANCHARD, M. H.: *J. Am. Chem. Soc.* **59**, 2717 (1937).
- (5) FERRY, R. M., COHN, E. J., AND NEWMAN, E. S.: *J. Am. Chem. Soc.* **60**, 1480 (1938).
- (6) FUOSS, R.: *J. Am. Chem. Soc.* **56**, 1027 (1934); **58**, 982 (1936).
- (7) JOSEPH, N. R.: *J. Biol. Chem.* **111**, 479, 489 (1935).
- (8) KIRKWOOD, J. G.: *J. Chem. Phys.* **2**, 351 (1934); *Chem. Rev.* **19**, 275 (1936).
- (9) PAULING, L., AND HUGGINS, M. L.: *Z. Krist. Miner. Petrog.* **A87**, 205 (1934).
- (10) SCATCHARD, G., AND KIRKWOOD, J. G.: *Physik. Z.* **33**, 297 (1932).
- (11) SCATCHARD, G., AND PRENTISS, S. S.: *J. Am. Chem. Soc.* **56**, 1486, 2314 (1934)

## APPENDIX

We shall present here some of the mathematical details in the calculation of  $V_{ik}$ , the electrostatic work required to bring a dipolar ion  $i$  and a real ion  $k$  from infinite separation in the pure solvent to a given relative configuration. The real ion is represented by a point charge  $z_k e$  and the dipolar ion by a charge distribution  $e_1 \cdots e_s$  located in a cavity  $\omega_0$ , of dielectric constant  $D_0$ , in the solvent of dielectric constant  $D$ . The potential  $V_{ik}$  is then given by

$$V_{ik} = W - W_0 \quad (30)$$

where  $W$  is the work of charging the system in the given configuration and  $W_0$  the work of charging when the two ions are infinitely separated.  $W$  is to be calculated by means of the formula

$$W = \frac{1}{2} \left\{ z_k e \psi_e(\mathbf{r}_k) + \sum_{i=1}^s e_i \psi_i(\mathbf{r}_i) \right\} \quad (31)$$

where  $\psi_i(\mathbf{r}_i)$  is the electrostatic potential in the interior of  $\omega_0$  at the point  $\mathbf{r}_i$  of location of charge  $e_i$  and  $\psi_e(\mathbf{r}_k)$  is the potential exterior to  $\omega_0$  at the

point of location  $\mathbf{r}_k$  of the real ion. The potentials  $\psi_i$  and  $\psi_e$  both satisfy Laplace's equation,

$$\begin{aligned}\nabla^2\psi_i &= 0 \\ \nabla^2\psi_e &= 0\end{aligned}\tag{32}$$

as well as the boundary conditions

$$\begin{aligned}\psi_i(\mathbf{r}) &= \psi_e(\mathbf{r}) \\ D_0\mathbf{n}\cdot\nabla\psi_i(\mathbf{r}) &= D\mathbf{n}\cdot\nabla\psi_e(\mathbf{r})\end{aligned}\tag{33}$$

on the surface of the cavity  $\omega_0$ .

### *Sphere*

When the cavity  $\omega_0$  is a sphere of radius  $b$ , it is convenient to employ polar coordinates  $(r, \vartheta, \varphi)$  with origin at the center of the sphere. Potentials  $\psi_i$  and  $\psi_e$  satisfying Laplace's equation and possessing the appropriate singularities are

$$\begin{aligned}\psi_i &= \sum_{l=1}^s \frac{e_l}{D_0|\mathbf{r}-\mathbf{r}_l|} + \sum_{n=0}^{\infty} \sum_{m=-n}^{+n} B_{nm} r^n P_n^m(\cos \vartheta) e^{im\varphi} \\ \psi_e &= \frac{z_k e}{D|\mathbf{r}-\mathbf{r}_k|} + \sum_{n=0}^{\infty} \sum_{m=-n}^{+n} \frac{A_{nm}}{r^{n+1}} P_n^m(\cos \vartheta) e^{im\varphi}\end{aligned}\tag{34}$$

where the  $P_n^m(\cos \vartheta)$  are the associated Legendre functions of the first kind. On the boundary of the sphere  $b$ , we have

$$\begin{aligned}\psi_e(b, \vartheta, \varphi) &= \psi_i(b, \vartheta, \varphi) \\ D\left(\frac{\partial\psi_e}{\partial r}\right)_{r=b} &= D_0\left(\frac{\partial\psi_i}{\partial r}\right)_{r=b}\end{aligned}\tag{35}$$

for all values of  $\vartheta$  and  $\varphi$  in the intervals 0 to  $\pi$  and 0 and  $2\pi$ . On the surface of the sphere we may employ the following harmonic expansions

$$\begin{aligned}\frac{z_k e}{D|\mathbf{r}-\mathbf{r}_k|} &= \sum_{n=0}^{\infty} \sum_{m=-n}^{+n} F_{nm} r^n P_n^m(\cos \vartheta) e^{im\varphi} \\ F_{nm} &= \frac{z_k e}{Dr_k^{n+1}} \frac{(n-|m|)!}{(n+|m|)!} P_n^m(\cos \vartheta_k) e^{-im\varphi_k} \\ \sum_{l=1}^s \frac{e_l}{D_0|\mathbf{r}-\mathbf{r}_l|} &= \sum_{n=0}^{\infty} \sum_{m=-n}^{+n} \frac{G_{nm}}{r^{n+1}} P_n^m(\cos \vartheta) e^{im\varphi} \\ G_{nm} &= \frac{1}{D_0} \frac{(n-|m|)!}{(n+|m|)!} \sum_{l=1}^s e_l r_l^n P_n^m(\cos \vartheta_l) e^{-im\varphi_l}\end{aligned}\tag{36}$$

By substitution of equations 34 and 36 in equation 35 and use of the orthogonality of the functions

$$P_n^m(\cos \vartheta) e^{im\varphi}$$

on the surface of the sphere, we obtain the following set of linear equations for the coefficients  $A_{nm}$  and  $B_{nm}$

$$\begin{aligned} A_{nm} + b^{2n+1} F_{nm} &= G_{nm} + b^{2n+1} B_{nm} \\ (n+1)A_{nm} - nb^{2n+1} F_{nm} &= \sigma[(n+1)G_{nm} - nb^{2n+1} B_{nm}] \\ \sigma &= D_0/D \end{aligned} \quad (37)$$

Solution yields

$$\begin{aligned} B_{nm} &= \frac{2n+1}{n+1+n\sigma} F_{nm} + \frac{(n+1)(\sigma-1)}{n+1+n\sigma} \frac{G_{nm}}{b^{2n+1}} \\ A_{nm} &= \frac{n(1-\sigma)b^{2n+1}}{n+1+n\sigma} F_{nm} + \frac{(2n+1)\sigma}{n+1+n\sigma} G_{nm} \end{aligned} \quad (38)$$

Use of equations 34 and 38 in equations 30 and 31 and application of the addition theorem of spherical harmonics yield, when the dipolar ion has no net charge,

$$\begin{aligned} V_{ik} &= \frac{z_k e}{Dr_k} \sum_{n=1}^{\infty} \sum_{l=1}^n \frac{(2n+1)e_l}{n+1+n\sigma} \left(\frac{r_l}{r_k}\right)^n P_n(\cos \vartheta_{kl}) \\ &\quad + (1-\sigma) \frac{z_k e^2 b}{2Dr_k^2} \sum_{n=1}^{\infty} \frac{n}{n+1+n\sigma} \left(\frac{b}{r_k}\right)^{2n} \end{aligned} \quad (39)$$

where  $r_k$  is the distance of the real ion  $z_k e$  from the center of the sphere, and  $\vartheta_{kl}$  is the angle between the vectors  $\mathbf{r}_k$  and  $\mathbf{r}_l$  from the center of the sphere terminating in the real ionic charge  $z_k e$  and the charge  $e_l$  of the dipolar ion. When the dipolar ion contains a point dipole at the center, the first sum in equation 39 degenerates into

$$\frac{z_k e \mu \cos \vartheta_k}{(2D + D_0)r_k^2} \quad (40)$$

and equation 11 results at once from equation 39, with a slight change in the summation index in the second sum. In this case the dipolar ion may be regarded as possessing two charges  $+e$  and  $-e$  situated at equal distances  $r_0$  from the center, with  $\vartheta_{k2} = \pi - \vartheta_{k1}$ . If we pass to the limit  $r_0 = 0$  with

$$\mu = 2 \lim_{r_0 \rightarrow 0} (er_0)$$

all terms except for  $n = 1$  vanish in first sum of equation 39, the first term reducing to equation 40 if  $\vartheta_{k1}$  is designated simply by  $\vartheta_k$ .



Before leaving the spherical case, it is perhaps desirable to give the general expression for  $B_i^{(0)}$  when the charge distribution of the dipolar ion is arbitrary.  $B_i^{(1)}$  is still given by equation 13.

$$B_i^{(0)} = \frac{4\pi N e^2}{2303(DkT)^2} \sum_{n=1}^{\infty} \frac{(2n+1)}{(2n-1)(n+1+n\sigma)} \frac{M_n}{a^{2n-1}} \quad (41)$$

$$M_n = \sum_{\substack{l, l' \\ -1}}^s e_l e_{l'} r_l^n r_{l'}^n P_n(\cos \vartheta_{l'l})$$

where  $\vartheta_{l'l}$  is the angle between the vectors of length  $r_{l'}$  and  $r_l$ , joining the charges  $e_{l'}$  and  $e_l$  to the center of the sphere. For a molecule of the type of cystine, a model, consisting of two point dipoles of moment  $\mu$  perpendicular to a common diameter and each situated at a distance  $l$  from the center, is useful. In this case equation 41 reduces to

$$B_i^{(0)} = \frac{8\pi N e^2}{2303(DkT)^2} \frac{\mu^2(1+\cos\varphi)}{a} \sum_{n=0}^{\infty} \frac{2n+3}{(2n+1)[n+2+(n+1)\sigma]} \left(\frac{l}{a}\right)^{2n} \quad (42)$$

where  $\varphi$  is the angle between the two dipole moments.

### Ellipsoid

When the cavity  $\omega_0$  is ellipsoidal in form, we may conveniently employ confocal elliptical coordinates,  $\lambda, \eta, \varphi$ , where  $\lambda = (r_1 + r_2)/R$ , and  $\eta = (r_1 - r_2)/R$ ,  $r_1$  and  $r_2$  being the distances of the point from the respective foci and  $R$  the interfocal distance. The angle  $\varphi$  measures the inclination of the plane of  $r_1$  and  $r_2$  to a chosen reference plane containing the major axis. The cavity  $\omega_0$  is then specified by a value  $\lambda_0$  equal to the reciprocal of the eccentricity of its elliptical section. We suppose the charges  $e_1 \dots e_s$  of the dipolar ion to lie on the major axis of the ellipsoid. We shall further neglect the image distribution induced in the cavity  $\omega_0$  by the real ionic charge. Potentials satisfying Laplace's equation and having the proper singularities are the following

$$\psi_s = \sum_{n=0}^{\infty} A_n P_n(\eta) Q_n(\lambda) \quad (43)$$

$$\psi_i = \sum_{l=1}^{\infty} \frac{e_l}{D_0 |\mathbf{r} - \mathbf{r}_l|} + \sum_{n=0}^{\infty} B_n P_n(\eta) Q_n(\lambda)$$

where the  $P_n(\lambda)$  and  $Q_n(\lambda)$  are the Legendre functions of the first and second kinds, respectively.

The boundary conditions are

$$\left. \begin{aligned} \psi_s(\lambda_0, \eta) &= \psi_i(\lambda_0, \eta) \\ D \left( \frac{\partial \psi_s}{\partial \lambda} \right)_{\lambda=\lambda_0} &= D_0 \left( \frac{\partial \psi_i}{\partial \lambda} \right)_{\lambda=\lambda_0} \end{aligned} \right\} -1 \leq \eta \leq +1 \quad (44)$$

On the boundary of  $\omega_0$  the initial terms of the second of equations 43 may be developed in the Neumann expansion,

$$\sum_{i=1}^s \frac{e_i}{D_0 |\mathbf{r} - \mathbf{r}_i|} = \frac{2}{R} \sum_{n=0}^{\infty} (2n+1) G_n P_n(\eta) Q_n(\lambda_0) \quad (45)$$

$$G_n = \sum_{i=1}^s e_i P_n(\eta_i)$$

where  $(1, \eta_i)$  are elliptical coordinates of the dipolar ionic charge  $e_i$ . Application of the boundary conditions 44 to equations 43 and 45, use of the orthogonality of the functions  $P_n(\eta)$ , yields the set of linear equations,

$$A_n Q_n(\lambda_0) = B_n P_n(\lambda_0) + (2/R)(2n+1) G_n Q_n(\lambda_0) \quad (46)$$

$$A_n Q'_n(\lambda_0) = \sigma [B_n P'_n(\lambda_0) + (2/R)(2n+1) G_n Q'_n(\lambda_0)]$$

where  $Q'_n(\lambda_0)$  and  $P'_n(\lambda_0)$  are the first derivative of the indicated functions, and  $\sigma$  is the ratio  $D_0/D$ . Solution of the equations 46 and elimination of the derivative  $Q'_n(\lambda_0)$  by means of the formula

$$P_n Q'_n - P'_n Q_n = (1 - \lambda^2)^{-1}$$

yield

$$A_n = \frac{2\sigma}{R} \frac{(2n+1)G_n}{1 + (\lambda_0^2 - 1)(1 - \sigma)P'_n(\lambda_0)Q_n(\lambda_0)} \quad (47)$$

$$B_n = \frac{2(\sigma - 1)}{R} \frac{(2n+1)G_n}{1 - \sigma[P'_n(\lambda_0)Q_n(\lambda_0)]/[P_n(\lambda_0)Q'_n(\lambda_0)]}$$

We now calculate  $V_{ik}$  by means of the formula

$$V_{ik} = z_k e \psi_e(\lambda_k, \eta_k) \quad (48)$$

and obtain, neglecting  $\sigma$  in comparison with unity in the denominators on the right-hand side of equation 47,

$$V_{ik} = \frac{2z_k e}{DR} \sum_{n=1}^{\infty} \frac{(2n+1)G_n P_n(\eta_k) Q_n(\lambda_k)}{1 + (\lambda_0^2 - 1)P'_n(\lambda_0)Q_n(\lambda_0)} \quad (49)$$

The sum begins with  $n$  equal to unity since  $G_0$ , the total charge of the dipolar ion, vanishes. By a simple algebraic transformation equation 49 may be written as follows

$$V_{ik} = \frac{2z_k e}{DR [1 + (\lambda_0^2 - 1)Q_1(\lambda_0)]} \sum_{n=1}^{\infty} (2n+1) G_n P_n(\eta_k) Q_n(\lambda_k) + Y(\lambda_k, \eta_k)$$

$$Y(\lambda_k, \eta_k) = \frac{2(\lambda_0^2 - 1)z_k e}{DR} \sum_{n=2}^{\infty} \frac{(2n+1)G_n [Q_1(\lambda_0) - P'_n(\lambda_0)Q_n(\lambda_0)]}{[1 + (\lambda_0^2 - 1)P'_n(\lambda_0)Q_n(\lambda_0)][1 + (\lambda_0^2 - 1)Q_1(\lambda_0)]} P_n(\eta_k) Q_n(\lambda_k) \quad (50)$$

The term  $Y(\lambda_k, \eta_k)$  vanishes for the limiting eccentricities zero and unity of the ellipsoidal cavity  $\omega_0$  and can be neglected without great error for intermediate eccentricities. We therefore have, approximately,

$$V_{ik} = \frac{2z_k e}{DR[1 + (\lambda_0^2 - 1)Q_1(\lambda_0)]} \sum_{n=1}^{\infty} (2n + 1)G_n P_n(\eta_k) Q_n(\lambda_k) \quad (51)$$

When the charge distribution of the dipolar ion consists of two charges  $+e$  and  $-e$  situated at the foci  $(1, 1)$  and  $(1, -1)$ , respectively, we have

$$G_n = e[1 - (-1)^n] \quad (52)$$

and the series in equation 51 may be summed to give equation 15, when we note that  $1 + (\lambda_0^2 - 1)Q_1(\lambda_0)$  is equal to

$$\lambda_0 \left[ 1 - \frac{\lambda_0^2 - 1}{2} \log \frac{\lambda_0 + 1}{\lambda_0 - 1} \right]$$

On the other hand, when the charge distribution consists of a point dipole of moment  $\mu$  at the focus  $(1, -1)$ , the  $G_n$ 's have the form

$$G_n = \lim_{x \rightarrow 0} e \left[ P_n \left( -1 + \frac{2x}{R} \right) - (-1)^n \right] \quad (53)$$

$$\mu = \lim_{x \rightarrow 0} (ex)$$

and calculation yields

$$G_n = (-1)^n n(n + 1) \mu / R \quad (54)$$

Summation in equation 51 with the  $G_n$  of equation 54 yields equation 19.