Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. VIII. The Relation between the Activity Coefficients of Peptides and their Dipole Moments

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Whereas the activity coefficients of ions depend upon their valence, those of dipolar ions depend largely upon their dipole moments. This had been suggested on the basis of theoretical considerations,[1,2] but further development of the field had to await more detailed experimental studies.

For ions, the effect of electrostatic forces can be distinguished from that of non-electrical forces since the former varies as the square root of the concentration, whereas the latter varies as the concentration. This distinction cannot be so readily made for dipolar ions because the effect of electrostatic forces is linear with the concentration and does not vary as its square root. It follows that even in very dilute aqueous solutions of amino acids and proteins the forces are not necessarily purely electrostatic in nature.

Electrostatic forces due to dipolar ions increase not with the quantity κ of Debye's theory,[3] but with κ^2. κ^2 increases not only with increase in concentration but with decrease in temperature and in dielectric constant. Electrostatic forces thus become more important the lower the temperature and the lower the dielectric constant.

The study of the influence of neutral salts upon glycine in ethanol–water mixtures demonstrated that at sufficiently low dielectric constants the logarithm of the solubility ratio multiplied by the dielectric constant ratio $(D/D_0) \log N/N_0$ was a function of κ^2. So plotted measurements in 60, 80, 90 and 95% ethanol containing neutral salts essentially coincide suggesting that under these conditions the activity coefficients of the amino acids studied were largely ascribable to electrostatic forces.[2,4]

These preliminary experiments on amino acids led to the choice of conditions for the studies of peptides here reported. The solvent employed was 80% ethanol containing varying concentrations of sodium chloride. At this alcohol concen-

tration sodium chloride solutions up to 0.25 molar can be prepared, and the solubility of glycine in this most concentrated salt solution was 3.73 g. per liter, or approximately one-fourth the concentration of salt. In the absence of salt glycine is soluble only to the extent of 2.00 g. per liter in 80% ethanol. It is this solvent action associated with change in ionic strength with which we are here concerned, and not with the effect of change in medium which reduces the solubility of glycine in 80% ethanol one hundred-fold from its solubility in water of 216.6 g. per liter at 25°.

The peptides of glycine have been chosen for comparison rather than aliphatic amino acids of long dipole moment because of their far lower solubility in both water and alcohol–water mixtures. This is related to the far greater density of glycine peptide crystals.[5] As a result, although β-alanine and ϵ-aminocaproic acid are far more soluble than the corresponding α-amino acids (though they have closely the same densities in the solid state), the tightly packed glycine peptides are progressively less soluble in water the longer their molecules, despite their larger dipole moments. This is not true, as Fischer[6] pointed out, of certain peptides with paraffin side chains.

Lysylglutamic acid is extremely soluble in water. Its solubility in 80% ethanol is none the less very low, the very rapid falling off of solubility with dielectric constant depending upon its multipolar nature. In this its behavior resembles to a considerable extent proteins such as egg albumin, with which it is compared in a subsequent communication. The solubility of all of the peptides studied is so small in 80% ethanol that the composition of the solutions approaches closely those of the pure solvents.

I. Methods and Materials

Solubility has been determined by methods that have been described previously.[7,8] Fresh

(1) Scatchard and Kirkwood, *Physik. Z.*, **33**, 297 (1932).

(2) Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934).

(3) The term proportional to κ has been shown by Scatchard and Kirkwood[1] to vanish when the net charge is zero.

(4) Cohn, *Naturwissenschaften*, **20**, 663 (1932).

(5) McMeekin, Cohn and Weare, THIS JOURNAL, **57**, 626 (1935).

(6) Fischer, *Ber.*, **36**, 2982 (1903).

(7) Cohn, McMeekin, Edsall and Weare, THIS JOURNAL, **56**, 2270 (1934).

(8) McMeekin, Cohn and Weare, *ibid.*, **58**, 2173 (1936).

aliquots of solvent were repeatedly saturated with the solute. It was demonstrated that solubility was independent of the amount of saturating body and the time of equilibration. The temperature for these experiments was $25.00 \pm 0.05°$.

Glycine and its peptides were prepared and purified by the methods that have been described previously.[5,7] Lysylglutamic acid was prepared by the method of Bergmann, Zervas and Greenstein.[9]

The hydantoic acids of glycine and of diglycine were the same preparations as were studied in the previous paper.[8] The heptyl esters of the hydantoic acids were prepared by the method described by Fischer for the preparation of the ethyl esters of hydantoic acids.[10]

Hydantoic Acid Heptyl Ester.—The heptyl ester hydrochloride of glycine was dissolved in water and an equivalent of potassium cyanate was added. On standing, the heptyl ester of hydantoic acid crystallized out. After recrystallizing from benzene, it melted at 98–99°.

Anal. Calcd. for $C_{10}H_{20}O_3N_2$: N, 12.96. Found: N, 12.80.

α-Aminocaproic Hydantoic Heptyl Ester (α-Uramidocaproic Acid Heptyl Ester).—The substance melted at 70–71°.

Anal. Calcd. for $C_{14}H_{28}O_3N_2$: N, 10.29. Found: N, 10.10.

Glycylglycine Hydantoic Acid Heptyl Ester (Uramido Glycylglycine Heptyl Ester).—The substance melted at 123–125°.

Anal. Calcd. for $C_{12}H_{23}O_4N_3$: N, 15.39. Found: N, 15.12.

II. Composition of Solvents

The three-component solvents employed in this investigation are most conveniently characterized by first considering the ethanol–water mixture and then the changes in the properties of the ethanol–water mixture brought about by the neutral salt.

The densities of ethanol–water mixtures are reported in the "International Critical Tables." Over the range in the neighborhood of 80% ethanol, they are given by the relation

$$\rho_0' = 0.85510 - 0.1672 (N_2 - 0.5197)$$

where 0.5197 is the mole fraction of ethanol, 0.85510 the density of 80% ethanol and ρ_0' that of some other mixture of not very different mole fraction, N_2.

The addition of sodium chloride to an 80% ethanol–water mixture increases its density. The weight of ethanol in such mixtures is 628.05

(9) Bergmann, Zervas and Greenstein, *Ber.*, **65**, 1692 (1932).
(10) Fischer, *ibid.*, **35**, 1095 (1902).

g. per liter. In a subsequent paper in this series, we shall compare the results when the ratio of the weight of water to alcohol is retained constant with those here reported in which the weight of water is diminished as the concentration of salt increases. Solubility in solvents defined in either way may be computed from both sets of measurements, and yield the same result. In many of the experiments recorded in this paper, especially those with the peptides, it proved convenient to prepare the large volumes of solvent required by weighing the salt and ethanol into volumetric flasks, and then adding water to volume. All weights were corrected for air buoyancy.

Table I

Concn. of NaCl, C	Water g. per liter, g.	Density of solvent, ρ	$\dfrac{\rho - \rho_0'^a}{C}$	Apparent molal volume of NaCl.[a] Φ
0.00	227.04	0.85510		
.05	225.92	.85690	0.0408	20.7
.10	224.75	.85864	.0402	21.3
.15	223.52	.86033	.0398	21.8
.25	220.97	.86364	.0393	22.5

[a] The values of ρ_0' calculated from "International Critical Tables" for the weight per cent. of alcohol and water in the mixture were, respectively, 0.85486, 0.85462, 0.85436, 0.85382.

Since dielectric constant measurements cannot be made in the presence of electrolytes, it remains an open question whether solutions would be more nearly isodielectric where the volume occupied by the salt is considered to have the same properties as the water replaced, or whether the effect of the salt on the dielectric constant should be considered negligible.

III. Apparent Molal Volumes

When the apparent molal volumes of the sodium chloride in these solvents were at first calculated, we questioned the results since they were so much higher than those in water, defined by Gucker[11] by the equation

$$\Phi_{NaCl} = 16.28 + 2.22 \sqrt{C}$$

For this reason a 25-cc. pycnometer was constructed which was employed in most of the above measurements. The remaining density determinations were carried out with 10-cc. pycnometers. The more accurate results confirm the order of those calculated from our routine density determinations, and indicate a higher apparent molal volume in 80% ethanol than in water, although the reverse might have been ex-

(11) Gucker, *Chem. Rev.*, **13**, 111 (1933).

pected as a result of increased electrostriction of the solvent. These measurements as well as those to be reported subsequently may tentatively be defined by the equation

$$\Phi_{NaCl} = 19.5 + 6\sqrt{C}$$

Solubilities in these solvents of all of the peptides are smaller than those of glycine, and the densities of the solutions deviate appreciably therefore from those of the solvent only in the case of glycine and the hydantoic acids. The densities of these saturated solutions are given in Table II and apparent molal volumes in 80% ethanol calculated.

TABLE II

APPARENT MOLAL VOLUMES IN 80% ETHANOL

Concn. of NaCl in solvent	Density of solvent	Density of solution	Soly., g. per liter	Apparent molal vol., Φ
		Glycine		
0.05	0.85690	0.85801	2.34	46.0
.15	.86033	.86175	3.00	46.0
.25	.86464	.86537	3.73	46.6
		Hydantoic acid		
.05	.85690	.86392	15.42	74.9
.15	.86035	.86823	16.44	71.5
.25	.86364	.87145	17.09	74.4
		α-Aminocaproic hydantoic acid		
.05	.85690	.86148	15.52	143.4
.15	.86035	.86500	16.24	144.2
.25	.86364	.86831	16.83	145.8

IV. Solubility Measurements

The solubilities in the various solvents are reported in Table III, as well as the ratio of solubility, expressed as mole fraction, in systems containing salt to that in the salt-free ethanol–water mixtures. The results would be essentially the same if the solubility ratios were calculated as volume concentrations, S/S', since the very large medium effects considered in the previous communications[7,5,8] are retained as nearly constant as possible in this study in which we are concerned only with the influence of salts upon amino acids, peptides and their derivatives.

The logarithm of the solubility ratio is plotted as ordinate in Fig. 1 and the concentration of sodium chloride as abscissa. The curves appear to belong to the same family and reveal effects of two kinds. In the first place the longer the peptide the greater the solvent action of the neutral salt. In the second place the larger the peptide the more this solvent action diminishes with increasing salt concentration. The solvent action of sodium chloride upon the hydantoic acids

TABLE III

INFLUENCE OF NaCl UPON THE SOLUBILITY OF GLYCINE, PEPTIDES AND HYDANTOIC ACIDS IN 80% ETHANOL AT 25°

Concn. of NaCl, C	$(D_0/D)C$	Solubility — Moles per liter, S	Mole fraction, N	Logarithm of soly. ratio, log N/N'	(D/D_0) log (N/N')
		Hydantoic acid			
0.00		0.126	0.00485		
.05	0.110	.131	.00511	0.0227	0.0103
.15	.330	.139	.00538	.0451	.0205
.25	.550	.145	.00561	.0633	.0288
		Hydantoic acid of α-aminocaproic acid			
.00		.0867	.00337		
.05	.110	.0891	.00346	.0115	.0052
.15	.330	.0933	.00364	.0335	.0152
.25	.550	.0966	.00378	.0499	.0227
		Hydantoic acid of diglycine			
.00		.0220	.00084		
.05	.110	.0247	.00945	.0506	.0230
.15	.330	.0269	.00103	.0880	.0400
.25	.550	.0294	.00113	.128	.0584
		Glycine $\delta = 22.6$			
.00		.0267	.00102		
.05	.107	.0312	.00119	.0682	.0316
.10[a]	.213	.0361[a]	.00138	.123	.0576
.15	.321	.0400	.00153	.177	.0824
.25	.532	.0497	.00190	.272	.127
		Diglycine $\delta = 70.6$			
.00		.00374	.000143		
.05	.109	.00500	.000191	.126	.0579
.10	.217	.00650	.000248	.236	.109
.15	.325	.00780	.000298	.319	.147
.25	.539	.01060	.000406	.454	.211
		Triglycine $\delta = 113.3$			
.00		.000608	.0000231		
.05	.110	.000899	.0000343	.171	.0780
.10	.219	.00129	.0000492	.328	.150
.15	.328	.00180	.0000687	.473	.216
.25	.545	.00278	.000106	.661	.303
		Lysylglutamic acid $\delta = 345$			
.00		.0000778	.00000296		
.05	.110	.000137	.00000522	.247	.113
.10	.219	.000210	.00000801	.433	.197
.15	.329	.000288	.0000110	.570	.260
.25	.547	.000531	.0000203	.837	.383

[a] Calculated.

studied is far smaller than that upon these dipolar ions. It is greatest for the hydantoic acid of diglycine, which has two peptide linkages, and smallest for the hydantoic acid of α-aminocaproic acid which contains a larger number of nonpolar CH_2 groups.

Hydantoic acid contains one less CH_2 group than diglycine and the hydantoic acid of diglycine one less CH_2 group than triglycine. Additional CH_2 groups might be expected to decrease slightly

the solvent action of neutral salts. The far greater influence of sodium chloride upon the peptides may be ascribed, therefore, to their dipolar ion structure.
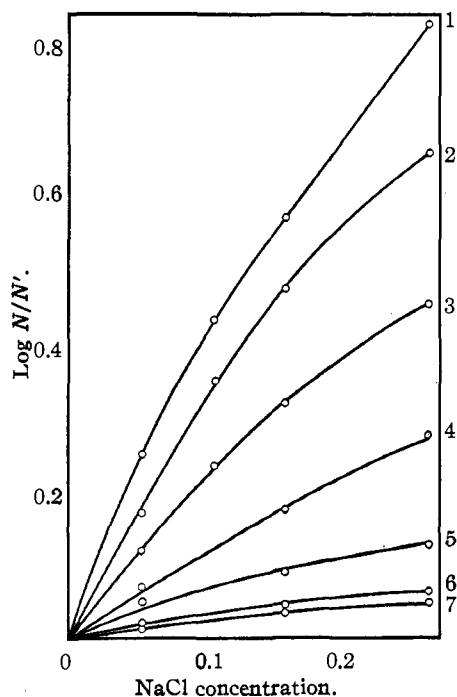

Fig. 1.—Solubility in 80% ethanol containing NaCl of 1, lysylglutamic acid; 2, triglycine; 3, diglycine; 4, glycine; 5, diglycyl hydantoic acid; 6, hydantoic acid; 7, α - aminocaproic hydantoic acid.

The logarithm of the activity coefficient varies as the reciprocal of the second power of the dielectric constant in Debye's theory. The dielectric constant of 80% ethanol is 35.72 and of water 78.54 at 25°.[12] The logarithm of the solubility ratio is therefore multiplied by D/D_0 in the last column of Table III. The concentration of salt, multiplied by D_0/D, given in the second column yields a quantity proportional to κ^2 in Debye's theory. The dielectric constants of the solvents are corrected in this calculation for the influence of glycine and the peptides. The values of δ tentatively adopted are those for water. The only amino acid sufficiently soluble to be studied thus far in 80% ethanol, α-aminobutyric acid, has a value of 24.0 in this solvent as compared with 23.5 in water.[13] Although the dielectric constant increments, δ, also given in Table III[14] are greater the longer the peptide, the solubility of

(12) Wyman, THIS JOURNAL, **53**, 3292 (1931).

(13) Wyman, ibid., **56**, 536 (1934).

(14) Wyman and McMeekin, ibid., **55**, 908 (1933).

all the peptides studied is so small in these solvents that the correction is appreciable only in the case of glycine. ' Correction for difference in volume between solvent and solution is negligible in 80% ethanol, though not in the lower ethanol-water mixtures subsequently to be reported. In order to facilitate comparison the hydantoic acids are also multiplied by the dielectric constant ratio of the solvent. Their own contributions to the dielectric constant of solutions is still in doubt.

Although it is certain that the longer peptides have the greater dipole moments, no satisfactory method is available for estimating the moments of dipolar ions. In a recent discussion of the problem two approximations were compared.[15] In the one, the molecules are considered extended in solution, as in the solid state, and the increment in the distance separating successive peptides taken as 3.5 Å. Structural considerations suggest that the dipole distance of glycine lies between 3 and 3.3 Å. The intermediate value of 3.17 Å. estimated by Kirkwood[2] from our solubility studies may be tentatively adopted. The ratio $[(D/D_0) \log N/N']/R'$, where R' represents the dipole distance estimated in this way, is calculated in Table IV. This ratio is plotted against $(D_0/D)C$ in Fig. 2. The agreement at low concentrations of salt indicates that *the logarithms of the activity coefficients of amino acids and peptides are, as a first approximation, proportional to their dipole moments.* At higher concentrations the influence of the larger molecules in diminishing their activity coefficients is reflected by the greater curvatures in Fig. 2.

TABLE IV

INFLUENCE OF THE MOMENTS OF DIPOLAR IONS ON THEIR ACTIVITY COEFFICIENT

Concn. of NaCl	Glycine	Diglycine	Triglycine	Lysylglutamic acid
		$[(D/D_0) \log N/N']/R$		
0.00	0.00	0.00	0.00	0.00
.05	.0100	.0105	.0111	.0092
.10	.0182	.0197	.0214	.0161
.15	.0260	.0267	.0308	.0212
.25	.0402	.0383	.0432	.0312
		$[(D/D_0) \log N/N']/R'$		
.00	.00	.00	.00	.00
.05	.0100	.0087	.0077	.0063
.10	.0182	.0163 '	.0147	.0111
.15	.0260	.0221	.0213	.0147
.25	.0402	.0316	.0298	.0216

(15) Cohn, "Annual Review of Biochemistry," Vol. IV, Stanford University, California, 1935, p. 93.

The dipole distances of lysylglutamic acid have been estimated from structural considerations by Greenstein, Wyman and Cohn.[16] The longest dipole is 14.23 Å. The shorter dipole, which has the same configuration as glycylglycine, has a length in the direction of the chain of 3.5 Å. The logarithm of the solubility ratio divided by the vector sum, 17.73 Å., also is plotted in Fig. 2.

These estimates of R' may be considered maximal. Presumably there is at least some bending around the free bonds in these molecules, resulting in smaller dipole moments than those estimated from structural considerations. As a lower limit we may tentatively adopt the square root rule proposed by Werner Kuhn[17] for very long hydrocarbon chains. Assuming again the dipole distance of glycine to be 3.17 Å. and that of the peptides to increase as the square root of their dielectric constant increments, values of R have been calculated for these peptides.[15] The ratio $[(D/D_0) \log N/N']/R$ is also given in Table IV.

The results in Fig. 2 and Table IV suggest that in dilute solution, as a first approximation, the logarithms of the activity coefficients of dipolar ions increase as the concentration of the salt, and the dipole distance, R or R', of the peptides. The limiting slopes $[(D/D_0) \log N/N']/(D_0/D)C$ are 0.30 to 0.32 for glycine, 0.57 to 0.59 for diglycine, 0.75 to 0.80 for triglycine and 1.12 to 1.2 for lysylglutamic acid. These slopes will be proportional to the first term of any equation for the change in free energy of dipolar ions with change in ionic strength. They are approximately one-tenth the dipole distances estimated as proportional to the square root of dielectric constant increments and taken as 3.17 for glycine and 12.25 for lysylglutamic acid.

Kirkwood has developed an equation for this effect for spherical molecules and applied it to glycine.[2] The first term for this equation is 0.125 R^2/a, where a is the sum of the radii of ion and dipolar ion. The peptides that have now been studied cannot be considered spherical. Rather they are cylindrical or ellipsoidal, depending on whether they are stretched or somewhat constricted in solution as a result of rotation around free bonds. Kirkwood has now calculated the effect for an elliptical model[18] and finds

(16) Greenstein, Wyman and Cohn, THIS JOURNAL, **57**, 637 (1935).
(17) Kuhn and Martin, Ber., **67**, 1526 (1934).
(18) Personal communication. These results will be recalculated by Kirkwood in terms of this equation in another place.

that under these circumstances the first term in the free energy equation is proportional to R, as indicated by these experiments.
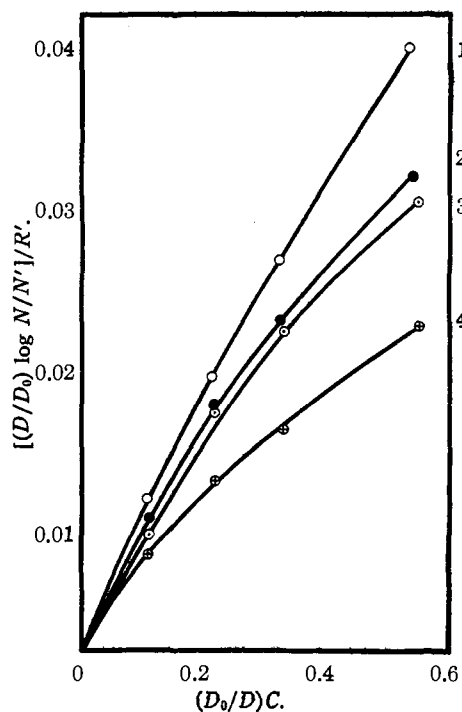

Fig. 2.—Solubility ratios of amino acids and peptides in 80% ethanol, containing sodium chloride, divided by their dipole distances as estimated by structural considerations: 1, glycine; 2, diglycine; 3, triglycine; 4, lysylglutamic acid.

The dipole moments of dipolar ions may be estimated by multiplying these distances by the charge on the electron. On this basis the moment of glycine is 15 × 10⁻¹⁸ e. s. u., and that of diglycine between 26 and 32 × 10⁻¹⁸ e. s. u. These moments are far greater than those due to the polar groups of these molecules. The moment of the ethyl ester of glycine, and those of other α-amino acids have been studied in benzene by Wyman and McMeekin[19] and estimated to be 2.1 × 10⁻¹⁸ e. s. u. That of the ethyl ester of diglycine they estimated to be 3.2 × 10⁻¹⁸ e. s. u.

Hydantoic acids terminate in NH_2 and $COOH$ groups. That is to say, their structure is that classically ascribed to amino acids before the studies of Adams and of Bjerrum. With the exception of the movement of a proton, hydantoic acid differs from diglycine by possessing one less CH_2 group. The ester of hydantoic acid and of hydantoic α-aminocaproic acid might therefore

(19) Wyman and McMeekin, THIS JOURNAL, **55**, 915 (1933).

be expected to have moments close to that of diglycine. Their heptyl esters have therefore been prepared and measured in benzene and found to have moments of 3.6 and 3.5 × 10⁻¹⁸ e. s. u., respectively.[20] The moments of these larger molecules in benzene fall off rapidly with concentration. They suggest a value of 1.4 or 1.5 × 10⁻¹⁸ e. s. u. for the peptide linkage, whereas the dipole moment of the amide group has been estimated to be 3.2[21] in the gaseous state and 3.6 to 3.8 in nonpolar solvents.[22,23] The solvent action of neutral salts upon the hydantoic acids studied also suggests higher moments than are indicated by the measurements in benzene.

The moments of the esters of amino acids, peptides and of their hydantoic acids are, however, small in comparison with the superimposed effects due to dipolar ionization, and it is for the most part the latter which are responsible for the very large activity coefficients of peptides in salt solutions in regions of low dielectric constant. In regions of high dielectric constants, where the solubilities of amino acids and peptides are rela-

tively high, the same contributions of Coulomb forces to activity coefficients presumably obtain, but are there supplemented by forces other than those due to the purely electrostatic interaction of ions and dipolar ions.

Summary

1. The interaction between ions and dipolar ions has been studied in regions of low dielectric constant in order to estimate the electrostatic forces involved.

2. Solutions of sodium chloride in 80% ethanol at 25° were employed as solvents, and glycine, diglycine, triglycine and the tetrapole lysylglutamic acid as solutes.

3. Hydantoic acids prepared from glycine and diglycine were studied in the same solvents in order to estimate what proportion of the effect was due to the dipolar ion structure of amino acids and peptides and what to the polar groups of these molecules.

4. The solvent action of neutral salts upon amino acids and peptides in dilute solution appears, as a first approximation, to be a function of their dipole moments.

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(20) We are indebted to Dr. Wyman for making these measurements.

(21) Zahn, Trans. Faraday Soc., 30, 804 (1934).

(22) Devoto, Gazz. chim. ital., 63, 491 (1933).

(23) Kumler and Porter, THIS JOURNAL, 56, 2549 (1934).

Studies in the Physical Chemistry of the Proteins. XIII. The Solvent Action of Sodium Chloride on Egg Albumin in 25% Ethanol at −5°

BY RONALD M. FERRY, EDWIN J. COHN AND ETHEL S. NEWMAN

Introduction.—The relation of the properties of amino acids and peptides to their chemical structure, and to the number and distribution of the electrostatic charges which they bear, has been considered in recent investigations.[1-3] Even when they have no net charge, these molecules, by virtue of their dissociated ammonium and carboxyl groups, have large electric moments and contribute markedly to the dielectric constants of their aqueous solutions, thereby diminishing electrostatic forces. The solubility of these dipolar ions diminishes, however, with diminution of the dielectric constant of the solvent, and under these conditions the properties of the solutions ap-

proach those of the pure solvents, and the effects of electrostatic forces are more readily observed.

It is of interest to extend these investigations to more complex dipolar and multipolar ions.[4] We have, accordingly, studied the solubility of isoelectric egg albumin in 25% ethanol–water mixtures at −5°. Egg albumin is a well-defined, easily purified, protein. Its molecules are roughly spherical with a radius of about 22 Å.[5] Early estimates revealed twenty-seven dissociable acids and the same number of dissociable basic groups,[6] whereas a recent study suggests a slightly higher number of dissociable groups.[7] Albumins are de-

(1) Cohn, Naturwissenschaften, 20, 663 (1932).

(2) Kirkwood, J. Chem. Phys., 2, 351 (1934).

(3) Cohn, McMeekin, Greenstein and Weare, THIS JOURNAL, 58, 2365 (1936).

(4) Such amino acids as cystine, such peptides as lysylglutamic acid, and all proteins are in reality multipoles. None the less they have an effective resultant dipole moment.

(5) Svedberg, Kolloid Z., 51, 10 (1930).

(6) Cohn, Physiol. Rev., 5, 349 (1925).

(7) Cannan, Biochem. J., 30, 227 (1936).