determined from X-ray data. The positive charge is assumed at the nitrogen atom and the negative charge is taken as being on the oxygen atom closest the nitrogen atom. Although X-ray crystal structure studies[12] indicate that the negative charge is probably located halfway between the two oxygens, the diagram here indicates the closest position of the negative charge to the nitrogen atom. The configuration of the —C—COO$^-$ group illustrated in the diagram corresponds to the distance of closest approach of the negative and positive charges while still retaining the normal bond angles and bond lengths. This configuration corresponds to a positive to negative charge distance of 2.48 Å. or a dipole moment of this length times the charge on the electron or 11.9×10^{-18} e. s. u.—still a very large dipole moment to account for small values of δ.

The possibility of bond angle distortion was raised by Edsall and Wyman.[11] However, the bond angle deformation necessary to account for such small dipole moments would be tantamount to breaking the chemical bonds.

Of course, dipole association could account for an apparent decrease in moment of the dipole. However, this would be expected to be dependent on the concentration of the dipoles giving a decrease in δ with increasing concentration. In these experiments no such effects were observed.

An explanation for the "anomalous" lowering of δ with lowering of ϵ_0 lies in the electrostatic behavior of dipoles in mixed solvents, a general theory of which is presented in the following paper.

Summary

1. The dielectric constants of solutions of glycine and of pyridine betaine in mixtures of water and dioxane were determined.

2. The dielectric increments both of glycine and of pyridine betaine showed a regular decrease with decreasing dielectric constant of the solvent mixture. This decrease is similar to that exhibited by glycine betaine and the benzbetaines.

3. The possibility of reversion of pyridine betaine to the un-ionized was examined. Calculations show that the charged groups could approach each other (and thus account for the experimental results) only if the valence bonds were tremendously distorted.

Cambridge, Mass. Received November 5, 1943

[12] G. Albrecht and R. B. Corey, This Journal, 61, 1087 (1939).

[Contribution from the Department of Chemistry, Cornell University]

The Dielectric Properties of Dipolar Ions in Mixed Solvents

By Gerald Oster[1]

Introduction

The similarity of physical properties of all the amino acids and betaines manifests itself in many ways. Their high melting points[2] as compared with their uncharged isomers, their relative insolubility in many organic solvents, and their dielectric behavior in various solvents,[3a,b] all indicate together with considerable supplementary evidence,[4] the presence of a common property, namely, the existence of these substances in the form of dipolar ions.

In this paper the dielectric properties of these dipolar ions (or zwitterions, as they are sometimes called) in solution will be considered since it is these properties which are so closely similar among the various amino acids and betaines. Glycine and different betaines when in mixed solvents exhibit similar behavior of the dielectric increment, the change in dielectric constant of the solution with concentration of solute, with changing dielectric constant of the solvent mixture. With all the amino acids and betaines studied (ortho, meta and para benzbetaines,[3a] glycine, pyridine betaine)[3b] a regular decrease in the dielectric constant of the solvent mixture (alcohol–water, dioxane–water, or benzene–alcohol mixtures) was observed. A decrease in the dielectric increment corresponds to an effective decrease in dipole moment, the quantitative relation being shown below. A detailed study of the structure of one of these substances[3b] indicates that rearrangement of the charged groups within the molecule to form an uncharged molecule is probably not possible. The argument presented in reference (3b) may be carried over to other betaines and amino acids. In fact, there is further evidence[5] that the benzbetaines and glycine betaine do not exist in the uncharged form in solvent mixtures of low dielectric constant in that the known uncharged isomers (esters of these dipolar ions, prepared by heating the dipolar solid to very high temperatures) have solubility properties vastly different from the dipolar substances.

A theory of the behavior of dipolar ions in mixed solvents will now be presented in which the existence (in any appreciable quantities) of the uncharged dipolar ion need not be assumed. The

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[2] E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Co., New York, N. Y., 1943; especially Chapter 8 by Edsall and Scatchard.

[3] (a) J. Wyman, Jr., *Chem. Rev.*, 19, 213 (1936); (b) D. Price, L. G. Joyner and G. Oster, This Journal, 66, 946 (1944).

[4] Reference 2, Chapter 2 by J. T. Edsall.

[5] J. T. Edsall and J. Wyman, Jr., This Journal, 57, 1964 (1935).

theory should be equally valid for all amino acids and betaines (aminophenol being a rare exception because of its unusual molecular structure). There is abundant evidence[2] that amino acids and betaines do exist almost completely in the dipolar form in water and mixtures of water and ethanol. However, there is no direct evidence that the uncharged form is present also. It will be shown that the presence of the uncharged form, a notion incompatible with many of the properties of these substances, need not be assumed in order to explain the dielectric behavior of betaines and amino acids.

It is known from electrostatic considerations[6] that ions in mixed solvents will cause a sorting of the components of the solvent mixture so that the component of the solvent having the higher dielectric constant will gather about the ions, this being an arrangement corresponding to minimum free energy. For the same reason we would expect sorting by dipolar ions in mixed solvents. The actual evaluation of the minimized free energy will not be attempted here since the problem is quite difficult even for ordinary ions; furthermore, the exact distribution of the solvent components around the dipolar ions need not be known except that we would expect the layer of solvent immediately surrounding the dipolar ions to be made up predominantly of that solvent mixture constituent having the greater dielectric constant. Thus, we would expect water in water–dioxane or water–alcohol mixtures to gather about the dipolar ions. Alcohol in alcohol–benzene mixtures would be expected to do likewise.

From electrostatic theory it will be shown that the net effect of surrounding the dipole with a shell of material of dielectric constant greater than that of the bulk of the liquid is to reduce the effective moment of the dipolar ion. This shielding effect becomes more prominent as the difference in dielectric constant of the shell surrounding the dipolar ion and the dielectric constant of the liquid in the bulk of the solution becomes greater. This, then, accounts for the decrease in dielectric increment of the dipolar ions as the dielectric constant of the solvent mixture is decreased.

Relation of Dielectric Increment to Dipole Moment.—The dielectric increment, δ, is defined by the relation that the dielectric constant of the solution of dipolar ions of concentration c is $\epsilon = \epsilon_0 + \delta c$ where ϵ_0 is the dielectric constant of the solvent. Wyman[2] found that δ is practically independent of concentration, c.

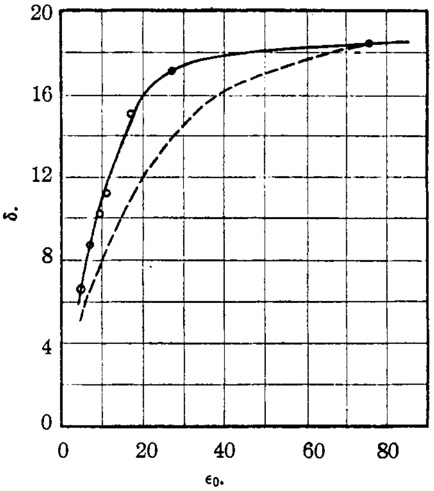

Fig. 1.—Experimental and theoretical dielectric increments of pyridine betaine in water–dioxane mixtures: ———, experimental; – – – –, theoretical.

A relation between the dielectric increment and the dipole moment of the solute may be derived from the general theory of dielectrics due to Kirkwood[7] which has been applied with some success to polar liquids by Oster and Kirkwood.[8] In the ternary system of solvents of mole fractions x_2 and x_3 with solute (the dipole ions) of concentration x_1 the dielectric constant of the solution, ϵ, is related to the mean molar volume, v, the mole fractions, and polarizations P_1, P_2 and P_3 of the constituents by the formula

$$\frac{(\epsilon - 1)\ (2\epsilon + 1)}{9} = \frac{x_1P_1 + x_2P_2 + x_3P_3}{v} \quad (1)$$

Combining equation (1) with the formula $\epsilon = \epsilon_0 + \delta c$, where ϵ_0 is the dielectric constant of the mixture of solvents, constituents 2 and 3, and c is the concentration of the dipolar ion (constituent 1) and after some algebraic manipulation and conversion of mole fractions to moles per liter one obtains the limiting polarization of the dipolar ions due to their permanent dipole moments

$$P_1 = \frac{4\pi N}{3}\frac{\mu\bar{\mu}}{3kT} = \frac{2000}{9}\delta + \frac{(\epsilon_0 - 1)(2\epsilon_0 - 1)}{9\epsilon_0}v - R \quad (2)$$

where $\mu\bar{\mu}$ is the scalar product of the dipole moment μ of the dipolar ion and dipole moment $\bar{\mu}$ of the dipolar ion and its shell of neighbors, beyond which the dielectric constant equals the macroscopically observed dielectric constant. v is the molal volume of the dipolar ion and R is its molar refractivity.

Equation (2) is valid for most substances when the dielectric constant of the solvent ϵ_0 is above ten. Between ten and five the first term on right side of equation (3) must be replaced by the approximation $\frac{2000}{9}\delta\left(1 + \frac{1}{2\epsilon_0^2}\right)$.

Inserting numerical values for the constants in equation (2) then for 25°

$$\delta = \frac{9}{2000}\left[20.6 \times 10^{36}\mu\bar{\mu} - \frac{(\epsilon_0 - 1)(2\epsilon_0 + 1)}{9\epsilon_0}v + R\right] \quad (3)$$

It is now necessary to calculate $\mu\bar{\mu}$ from an assumed model and so obtain values for δ which we can compare with experiment.

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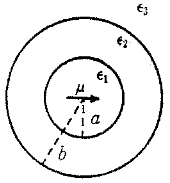
(6) P. Debye and J. McAuley, *Physik. Z.*, **26**, 22 (1925); G. Scatchard, *J. Chem. Phys.*, **9**, 34 (1941).

(7) J. G. Kirkwood, *ibid.*, **7**, 911 (1939).

(8) G. Oster and J. G. Kirkwood, *ibid.*, **11**, 175 (1943).

Consider a point dipole of moment μ at the center of a sphere (the dipolar ion) of radius a and dielectric constant ϵ_1. Surrounding the sphere is a shell of inner radius a, outer radius b, and dielectric constant ϵ_2. The sphere and its shell are imbedded in a medium of dielectric constant ϵ_3.



Since no free charges are present, the electrostatic potential must satisfy Laplace's equation, $\nabla^2\psi = 0$ everywhere. Across the boundaries the potential and the normal component of the displacement vector must be continuous, *i. e.*, at the boundaries the following conditions must be satisfied

$$\begin{aligned} \psi_1 &= \psi_2 \quad \epsilon_1 \frac{\partial \psi_1}{\partial r} = \epsilon_2 \frac{\partial \psi_2}{\partial r} \quad (r = a) \\ \psi_2 &= \psi_3 \quad \epsilon_2 \frac{\partial \psi_2}{\partial r} = \epsilon_3 \frac{\partial \psi_3}{\partial r} \quad (r = b) \end{aligned} \tag{4}$$

From the general solution of Laplace's equation in spherical coördinates for this spherically symmetric problem, namely

$$\psi = \sum_{n=0}^{\infty} \left(A_n r^n + \frac{B_n}{r^{n+1}}\right) P_n(\cos\theta) \tag{5}$$

the simplest solutions satisfying the conditions of the problem are taken. They are

$$\psi_1 = -Ar\cos\theta + (\mu/\epsilon_1 r^2)\cos\theta \tag{6a}$$

$$\psi_2 = -Br\cos\theta + (C/r^2)\cos\theta \tag{6b}$$

$$\psi_3 = (D/r^2)\cos\theta \tag{6c}$$

Applying the conditions (4) to equations (6) yields for the field inside the sphere (region 1)

$$A = \frac{2\mu}{\epsilon_1 a^3}\left[\frac{(\epsilon_2 - \epsilon_1)(\epsilon_2 + 2\epsilon_3) - (2\epsilon_1 + \epsilon_2)(\epsilon_2 - \epsilon_3)\left(\frac{a}{b}\right)^3}{(2\epsilon_2 + \epsilon_1)(2\epsilon_3 + \epsilon_2) - 2(\epsilon_2 - \epsilon_1)(\epsilon_2 - \epsilon_3)\left(\frac{a}{b}\right)^3}\right] \tag{7}$$

and for the field in the shell region (2)

$$B = \frac{6\mu}{b^3}\left[\frac{\epsilon_3 - \epsilon_2}{(2\epsilon_2 + \epsilon_1)(2\epsilon_3 + \epsilon_2) - 2(\epsilon_2 - \epsilon_1)(\epsilon_2 - \epsilon_3)\left(\frac{a}{b}\right)^3}\right] \tag{8}$$

Since $\bar{\mu}$ is the moment of the molecule and its shell of neighbors, then

$$\bar{\mu} = \mu + \frac{\epsilon_2 - 1}{4\pi}\frac{4\pi}{3}(b^3 - a^3)B \tag{9}$$

The second term on the right of the equation (9) comes from the electrostatic formula for the moment M of a region of volume v and dielectric constant ϵ in a field of intensity E, namely

$$M = \frac{\epsilon - 1}{4\pi} vE$$

In equation (9) μ does not equal the moment μ_0 of the molecule in free space, but rather

$$\mu = \mu_0 + \frac{\epsilon_1 - 1}{4\pi}\frac{4\pi}{3}a^3 A \tag{10}$$

Now μ and $\bar{\mu}$ act in the same direction so that $\mu\bar{\mu}$ is simply the product of equations (9) and (10). The equation for $\mu\bar{\mu}$ is considerably simplified if we take $\left(\frac{a}{b}\right)^3 << 1$. If, due to sorting, the outer radius of the shell is only three times that of its inner radius the quantity $(a/b)^3$ is sufficiently small so that the following equation holds to a good degree of approximation

$$\mu\bar{\mu} = \mu^2\left[1 - \frac{2(\epsilon_2 - 1)(\epsilon_2 - \epsilon_3)}{(2\epsilon_2 + \epsilon_1)(2\epsilon_3 + \epsilon_2)}\right] \tag{11a}$$

where

$$\mu = \frac{\mu_0}{1 - \frac{2(\epsilon_1 - 1)(\epsilon_2 - \epsilon_1)}{3\epsilon_1(2\epsilon_2 + \epsilon_1)}} \tag{11b}$$

Equation (11b) shows that μ is practically independent of ϵ_3. It will be noticed from equation (11a) that $\mu\bar{\mu}$ is decreased when the dielectric constant of the shell, ϵ_2, is greater than that of the outer medium, ϵ_3. $\mu\bar{\mu}$ equals μ^2 only when $\epsilon_2 = \epsilon_3$ as would be the case for dipolar ions in a single solvent, for, example, pure water. As the dielectric constant of the medium ϵ_3 (written ϵ_0 in equations (2) and (3)) is reduced, $\mu\bar{\mu}$ approaches zero (ϵ_2 is generally large compared with unity and $\epsilon_1 = n^2$, the square of the refractive index of the solute) and so by equation (3) δ should approach zero (the other two terms in equation (3) not containing δ are relatively small). The effect on ϵ_3 of the sorting out of the higher dielectric constant constituent of the solvent mixture to form a shell about the dipolar ion has been neglected. This is a small effect, however, since the concentrations of the dipolar ions in the systems studied are very small compared with the concentration of the high dielectric constant constituent of the solvent mixture, so that the dielectric constant of the solvent would hardly be expected to be altered appreciably by this sorting process.

The media 1, 2 and 3 have been treated as continuous right up to the surface of the adjacent media. It is known[7,8] that in order to account for the dielectric properties of pure polar liquids it is necessary to examine a region near the surface of a molecule of the liquid where the dielectric constant cannot be considered equal to that of the bulk of the liquid. It turns out, however, that the cruder continuum model proves to be sufficiently detailed so as to agree with the general features of the observed dielectric properties of dipolar ions in mixed solvents.

Application of the Theory

In order to test the theory, the dielectric increment of pyridine betaine in mixtures of water and dioxane have been calculated. This may be evaluated by combining equations (3) and (11a) to solve for δ. From the observed δ for pyridine betaine in pure water we calculate its dipole moment μ to be 16.9×10^{-18} e. s. u. In equation (11a) ϵ_1 is replaced by the square of the refractive index of pyridine betaine which is determined from a calculated molar refractivity R. The molar volume of pyridine betaine in water is $v = 99.6$ cc./mole.[6] The dielectric constant of water, ϵ_2, has the value 78.54.

The theoretical and experimental[3b] results are illustrated in the figure.

The deviations of the theoretical results from the observed suggest that the sorting of the water molecules by the dipolar ions is not complete and therefore the actual shielding effect is not as great as would be expected for a shell made up entirely of water molecules.

The writer wishes to thank Professor J. G. Kirkwood for his helpful suggestions.

Summary

A theory of the decrease in dielectric increment of dipolar ions with decrease in dielectric constant of the solvent mixture is presented. On the basis of electrostatic theory dipolar ions would be expected to sort out the solvent constituent of higher dielectric constant. The shell of material of higher dielectric constant thereby produced serves to act as an electrostatic shield thus decreasing the effective dipole moment (and therefore the dielectric increment) of the dipolar ion. The shielding effect becomes more prominent as the difference in dielectric of the shell surrounding the dipolar ion and that of the liquid in the bulk of the solution becomes greater.

This continuum treatment leads to results which reproduce the general features of the observed results.

CAMBRIDGE, MASS. RECEIVED NOVEMBER 5, 1943

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD UNIVERSITY AND RADCLIFFE COLLEGE]

Syntheses of Tyrosyltyrosyltyrosine and Tyrosyltyrosyltyrosyltyrosine

BY ARCHIE E. BARKDOLL[1] AND WILLIAM F. ROSS[2]

In relation to certain protein studies in progress in this Laboratory, there were desired polypeptides containing three, four and additional residues of the amino acid *l*-tyrosine[3] bound one to another with none but tyrosyl residues within the chain. For this reason the syntheses of tyrosyltyrosyltyrosine and tyrosyltyrosyltyrosyltyrosine have been carried out according to the procedures to be described in this paper.

The starting material was the compound O-acetyl-N-carbobenzoxytyrosyltyrosine ethyl ester, I, prepared according to the method of Bergmann, *et al.*,[4] which involves the coupling of O-acetyl-N-carbobenzoxytyrosyl chloride with the ethyl ester of tyrosine. This product may be applied in several ways toward the synthesis of the desired polypeptides. Its hydrogenation yields tyrosyltyrosine ethyl ester, II, to the free amino group of which additional tyrosine residues may be coupled through the action of the chloride or azide of a suitable tyrosine derivative.

The preparation of O-acetyl-N-carbobenzoxytyrosyl-O-acetyltyrosyl chloride, which after reaction with II would lead directly to a derived tetrapeptide, was first investigated. O-Acetyl-N-carbobenzoxytyrosyl-O-acetyltyrosine was obtained in good yield by the saponification and subsequent acetylation of I, but attempts to convert it satisfactorily into the chloride failed. The reaction of the free acid with phosphorus pentachloride yielded an oil, which in exploratory experiments with tyrosine ethyl ester gave no useful products. It appears probable that the failure of this approach was due to an attack on the peptide linkage by the phosphorus pentachloride. Similar results were obtained by Pacsu and Wilson,[5] who studied the action of phosphorus pentachloride and of thionyl chloride on carbobenzoxyglycylglycine.

The azide of N-carbobenzoxytyrosyltyrosine coupled with II, would also yield a derivative of the tetrapeptide. This line of approach was next investigated. N-Carbenzoxytyrosyltyrosine hydrazide was obtained readily by the action of hydrazine hydrate on I. The corresponding azide was prepared and, without isolation, added in ethyl acetate solution to a similar solution of the ester, II. The failure to isolate any definite product and the inaccessibility of the two reagents employed led finally to the simple, direct approach of adding single tyrosine residues stepwise to peptide esters of increasing length.

This procedure, although laborious and time-consuming, led finally to the desired products. For its consummation O-acetyl-N-carbobenzoxytyrosyl chloride was coupled with tyrosyltyrosine ethyl ester, to give O-acetyl-N-carbobenzoxytyrosyltyrosyltyrosine ethyl ester, III. The latter was converted by hydrolysis to the carbobenzoxytripeptide, IV, which was catalytically reduced to the desired tripeptide, V. Repeating this process, addition of another

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[3] *l*-Tyrosine was used throughout; consequently all of the compounds described in the text contain tyrosine of this configuration.

[4] Bergmann, Zervas, Salzmann and Schleich, *Z. physiol. Chem.*, **224**, 17 (1934).

[5] Pacsu and Wilson, *J. Org. Chem.*, **7**, 117 (1942).