THE INVESTIGATION OF PROTEINS BY DIELECTRIC MEASUREMENTS[1]

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The dielectric behavior of a solute of long relaxation time in a solvent of shorter relaxation time is discussed. Procedures adapted to the measurement of both the dielectric constant and the conductance of such systems are outlined, and the effect of electrode polarization upon the results is also discussed.

Such experiments yield data concerning (*1*) the dielectric increments at high and at low frequencies and (*2*) the behavior of the solution in the dispersion region. Interpretation of the increment results in terms of dipole moments and of the dispersion results in terms of the shape and size (and hence the hydration) of the molecules are discussed. Most of the dielectric studies upon protein solution reported in the literature are briefly reviewed.

The interactions of protein molecules with similar protein molecules, with other protein molecules, and with other types of molecules and ions are dependent upon the charge and dipole moment of the molecules or ions involved (7, 9). The dipole moments of protein molecules are often very large and play a most important part in the behavior of protein solutions, and in many cases these studies have been carried out in such a manner that information regarding the size and shape of the protein molecules can be obtained.

I. INTRODUCTION

The dielectric constant, ϵ, of a polar solution can be related to the number of dipoles oriented by the external applied field and the dipole moment, μ, of the individual dipoles. Orientation is hindered by the Brownian motion of the molecules and by frictional forces, these latter forces being proportional to the rate of orientation (measured by the frequency, ν, of the applied field) and a constant τ, designated as the "relaxation time," dependent on the viscosity of the solvent and the size and shape of the dipoles. Thus the degree of orientation at unit field strength will decrease in a frequency region where the hindering frictional forces and the orienting forces become of the same order of magnitude.

Figure 1 represents the dielectric behavior of a solution of protein molecules with relaxation times of the order of microseconds, dissolved in low-molecular-weight polar solvent molecules with relaxation times much smaller than this. The figure is divided into five regions, each of which exhibits a different dielectric behavior. In region A the orienting torque acting on both solute and solvent molecules is sufficient to overcome all frictional forces, and we find both being oriented and a constant and high value of ϵ, designated ϵ_0. In region B, however, the frictional forces on protein dipoles with the larger relaxation time

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can no longer be neglected, the orientation of these dipoles is no longer independent of the frequency, and we find a region of decreasing dielectric constant. In region C this frictional force has overcome completely the orienting force on the protein dipoles, and they then contribute very little to the dielectric constant of the solution, which we designate ϵ_∞. In regions D and E we have similar changes in the orientation of the solvent dipoles, but in the case of studies where the relaxation times of the solvent molecules are smaller than those of the protein dipoles by a factor of 100 or more, which is usually the case with protein solutions,[2] the dielectric behavior in this region does not complicate that observed in regions A, B, and C, and furnishes little or no information regarding the properties of the protein molecules.

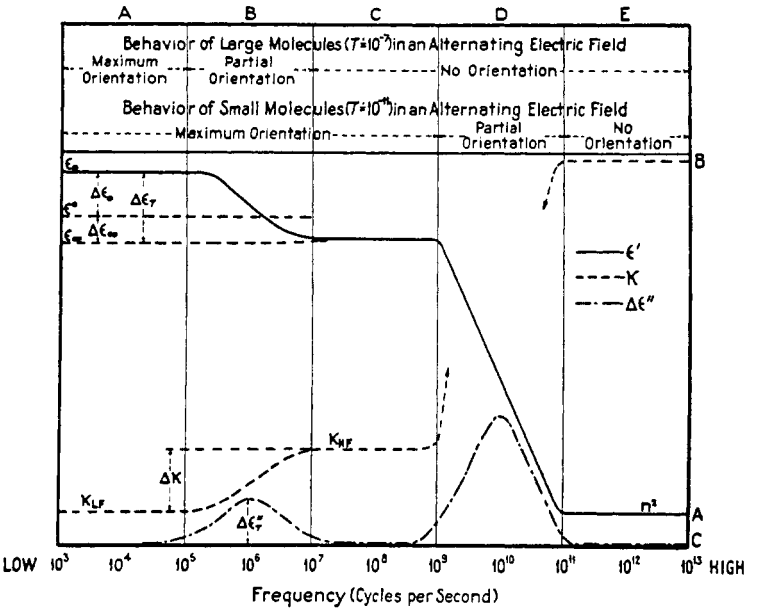

FIG. 1. Schematic diagram of anomalous dispersion of the dielectric constant (ϵ'), the specific conductivity (κ), and the dielectric absorption ($\Delta\epsilon''$) for two widely separated critical frequencies. (From Oncley, Ferry, and Shack: Ann. N. Y. Acad. Sci. **40**, 371 (1940)).

II. METHODS OF MEASUREMENT

The measurement of the dielectric properties of protein solutions over an extended frequency range can be carried out by any of several methods which have thus far been devised and employed. They are more or less classifiable as (*1*) bridge methods, (*2*) resonance methods, (*3*) force methods, (*4*) calorimetric methods, and (*5*) various other methods, involving comparisons of phase and magnitude of the voltage across an unknown and a standard.

(*1*) The bridge method involves the comparison of the resistance and capa-

[2] The relaxation time of water at room temperature is of the order of 10^{-11} sec., while that of the amino acids and simpler peptides is 10^{-10} sec.

citance of a cell containing the solution under investigation with some arrangement of standard resistances and capacitances. The main source of difficulty in measurements of this type with solutions of high conductance is in the selection of a suitable resistance standard. Apparatus used for the study of protein solutions has been described by Errera (18), by Daniels, Mathews, and Williams (11), by Oncley (32, 33), and by Ferry and Oncley (21). Apparatus suitable for such measurements has also been described by Cole and Curtis (10) and by Hemingway and McClendon (25). A modification of the bridge described previously (21, 32, 33) is now being used in our laboratory. The standard capacitance is a General Radio Type 722-D precision condenser, and the standard resistance is made up of a General Radio Type 669 compensated slide-wire resistor (1 ohm), of two General Radio Type 668 compensated decade-resistance units (10 and 100 ohms), and a series of 100-ohm resistance units such as are used in the General Radio Type 510 decade-resistance, which are shunted with trimming condensers adjusted to eliminate exactly the effective inductance of the entire resistance combination.[3] The ratio arms are General Radio Type 516-P4 units, of 1000 ohms each. The fourth arm of the bridge is made up of resistance units identical with those used for the standard, and of a series of variable air condensers. The unknown is connected in parallel with the standard arm of the bridge, and measured by substitution methods. By a suitable choice of detector and oscillator equipment, this bridge may be used over a wide frequency range.[4]

[3] A brief description of the standard resistance is necessary. Almost any type of resistor has a certain amount of residual capacitance or inductance. The type 668 and 669 units that we have employed have an almost constant residual inductance, and can be represented by a resistance R_a, variable from 0 to 111 ohms, and a series inductance L_a, of about 1.1 ± 0.05 microhenrys. The type 510 resistance, R_b, shunted with the trimming condenser, C_b, can as a first approximation (i.e., at low frequencies) be considered as a fixed resistance R_b and a fixed series inductance, $-C_bR_b^2$. When these units are connected in series, we have a total series resistance of $R_a + R_b$ and a total series inductance of $L_a - C_bR_b^2$. By a proper adjustment of C_b we can reduce this series inductance to zero at low frequencies. Experimentally we find this to be a perfectly feasible method for obtaining a variable resistor with very low residuals between 1000 and 5,000,000 cycles. The resistance R_b can be set equal to 50, 100, 200, or 300 ohms. Higher resistances cannot easily be used, since the capacitance of the switch and connections is so great that the value $L_a - C_bR_b^2$ becomes negative even without any shunting condenser.

[4] The equipment used in this laboratory consists of the following:

FREQUENCY RANGE	OSCILLATOR	AMPLIFIER	DETECTOR
50 to 8,000	Clough-Brengle Type 79-U audio frequency oscillator	General Radio Type 814A amplifier	General Radio Type 760A sound analyzer
10,000 to 100,000	General Radio Type 684A modulated oscillator	General Radio Type 814A amplifier	Sargent Model 11-UA all-wave receiver
100,000 to 5,000,000	General Radio Type 684A modulated oscillator	None	Sargent Model 11-UA all-wave receiver

(*2*) The resonance method is widely used, especially for the higher range of frequencies (above 10^6 cycles per second). It involves the measurement of the resonance frequency of a circuit which includes a cell containing the solution. It has been widely used by Drude (14) and others. Many modifications have been proposed, the most accurate of which is probably that of Wyman (48). Elliott and Williams (16) have recently described a method applied to protein solutions.

(*3*) The force method involves the measurement of the deflecting force exerted by an applied electric field upon a conducting ellipsoid suspended in the solution under investigation. It was originally described by Fürth (24) and has most recently been applied by Shutt and his coworkers (4, 15, 42). It is most effective at the low frequencies (10^2 to 10^4 cycles per second).

(*4*) The calorimetric method involves the determination of the expansion of the solution as a measure of the heating produced by an applied field of high frequency. It has been used for the study of various solutions by Malsch (29), Debye (13), Martin (30), Schmelzer (40), and others. Its application to protein solutions has been described by Shack (41). It is most effective at the higher frequencies, but is capable of use over most of the usual dispersion range for protein solutions of not too high conductance.

(*5*) Various other methods have been used for the measurement of the conductance and capacitance, or in some cases of the relaxation time directly, of a solution. Most of these methods depend upon the direct comparison of magnitude and phase of the voltage across an unknown and a standard capacitance, often by means of an oscillograph measurement. A recent publication of Wyman and Marcy (52) describes such an apparatus.

III. DIELECTRIC INCREMENT

The dielectric increment of a solution, $\Delta\epsilon$, is defined as the increase in dielectric constant of the solution, ϵ, over that of the solvent under similar conditions, ϵ^0; that is,

$$\Delta\epsilon = \epsilon - \epsilon^0 \tag{1}$$

In the case of most polar solutes in solvents of high dielectric constant, this increment is found to be at least approximately proportional to the concentration (50, 51). In the case of protein solutions, it is convenient to define a quantity $\Delta\epsilon/g$, designated the "increment per gram," where g is the concentration of the protein expressed in grams per liter. This increment per gram is related to the increment per mole, δ, by the equation $\Delta\epsilon/g = \delta/M$, where M is the molecular weight of the solute (51).

Low-frequency increment

The increment measured in the low-frequency region, indicated by A in figure 1, is called the low-frequency increment, and designated $\Delta\epsilon_0$. It is independent of frequency over a wide range of frequencies, if complications due to

polarization capacity are eliminated by proper methods of measurement. A linear relationship between the low-frequency increment $\Delta\epsilon_0$ and the concentration has been observed in dilute solutions of a considerable number of proteins. In many cases this linear relationship holds up to quite high concentrations, but in some cases, especially when $\Delta\epsilon_0/g$ is large, the increment per gram decreases somewhat with increasing concentration. In general we may write

$$\Delta\epsilon_0 = E_0 g - F_0 g^2 \tag{2}$$

where E_0 and F_0 represent parameters characteristic of the individual proteins. Terms in higher powers of g may sometime be required, but present indications are that the coefficients for these terms will be small, and negligible except in very concentrated solutions.

High-frequency increment

The increment measured in the high-frequency region, indicated by C in figure 1, is called the high-frequency increment, and designated $\Delta\epsilon_\infty$. The high-frequency increment has been measured for only a few proteins. If we assume that the dielectric constant at high frequencies, ϵ_∞, is due largely to the contribution of solvent molecules (assuming the volume occupied by the protein to have a high-frequency dielectric constant of unity),[5] then we obtain the equation

$$\Delta\epsilon_\infty/g = (\epsilon^0 - 1)v/1000 \tag{3}$$

where v is the volume of water (in cubic centimeters) displaced by 1 g. of anhydrous protein. We can set $v = \bar{v} + w/\rho_0$, where ρ_0 is the density of the solvent, $\bar{v}$ the partial specific volume of the solute (anhydrous), and w the number of grams of water which appear to be associated with each gram of anhydrous protein. If $w = 0$, and $\bar{v} = 0.75$, we obtain a value of $\Delta\epsilon_\infty/g = 0.059$ for water at 25°C. and 0.060 at 0°C. The observed values are of this order of magnitude, but slightly larger. If we assume reasonable values for w (34), the agreement is very good.

Total increment

The total increment is designated $\Delta\epsilon_t$, and is the sum of the low-frequency and high-frequency increments. In cases where proteins are dissolved in mixed solvents, there is sometimes difficulty in exactly defining the composition of the solvent, since one or more of the components may be more strongly absorbed by the protein molecules. In these cases it is not possible to compute accurately the low-frequency and high-frequency increments, but the total increment may still be accurately obtained.

[5] The value of unity for the high-frequency dielectric constant of the protein is too small, since it neglects entirely the optical polarization of the protein and the polarization of the hydrated water molecules. The value $(\epsilon^0 - 1)$ in equation 3 is, however, probably within 5 or 10 per cent of the proper value for solvents of high dielectric constant.

IV. ELECTRODE POLARIZATION

One of the most serious difficulties involved in measurements by any of these methods is the complication introduced by electrode polarization. This effect is most serious at low frequencies and high conductivities, but decreases in magnitude until it may properly be neglected at high frequencies or for solutions of very low conductivity. It is discussed in some detail by Jones and Christian (26), by Cole and Curtis (10), by Fricke and Curtis (22), by Oncley (32), by Ferry and Oncley (21), and by Dunning and Shutt (15) and must be considered as a possible source of error in all work of this type. Correction for this effect was made very successfully at frequencies higher than 25,000 cycles for salt solutions and protein solutions (32) by plotting capacities against $\nu^{-3/2}$ and determining B empirically as the slope of the resulting straight line. The dielectric increment $\Delta\epsilon$ is thus given by

$$\Delta\epsilon = (C_x - B\nu^{-3/2} - C_x^0)/Q \tag{4}$$

where C_x is the cell capacity at frequency ν with the protein or salt solution, C_x^0 the cell capacity with conductivity water, and Q the cell constant $(dC_x/d\epsilon')$. Below 25,000 cycles, however, the plot against $\nu^{-3/2}$ was often found to deviate from linearity, and it was no longer possible to correct for the polarization capacity effect by this simple procedure. To involve the least additional complication, a method of comparison between solutions of equal conductivities has been employed. If the polarization correction is represented by $B\nu^{-3/2} + f(\nu)$, where $f(\nu)$ takes care of the departure from linearity of the $\nu^{-3/2}$ plot, and if $f(\nu)$ should prove to be dependent only on the conductivity, but not on the nature of the solute, then the difference between the corrections for two solutions of different solutes, but with the same conductivity, should be proportional to $\nu^{-3/2}$. The parameter B will in general not be the same for the two solutions, but the difference, ΔB, can be determined from the plot of $(C_x - C_x')$ against $\nu^{-3/2}$, where C_x' is the capacity of the cell when filled with a potassium chloride solution of the same conductivity, and the values of $\Delta\epsilon$ obtained as

$$\Delta\epsilon = (C_x - C_x' - \Delta B\nu^{-3/2})/Q \tag{5}$$

This method has been tested by a series of measurements comparing solutions of potassium chloride, ammonium sulfate, glycine (with potassium chloride added to adjust the conductivity), and lactoglobulin in glycine, and proved to be satisfactory for frequencies above 10,000 cycles (21).

V. CALCULATION OF DIPOLE MOMENTS

The interpretation of dielectric increments obtained in polar solvents in terms of the dipole moments of the solute molecules has been discussed by numerous workers (23, 28, 31, 37, 43–45, 47, 50, 51). In the case of protein measurements, where both the high-frequency and the low-frequency increments can be obtained, we can eliminate some of the approximations which are often introduced. This is especially important when we are dealing with proteins with small values

of the low-frequency increment per gram. The relation between dipole moment and dielectric increment may be expressed by the equation

$$\mu^2 = [9000kT/4\pi Nb]\,[M(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)] \tag{6}$$

where N is Avogadro's number, k is Boltzmann's constant, T is the absolute temperature, and μ is the dipole moment of a single molecule in solution. This moment is not necessarily equal to the moment of a single molecule in the gas phase, but is in general somewhat greater (28).

The parameter b may be evaluated in several different ways:

(*1*) Direct application of the Debye theory to polar media (32) yields a value $b = (\epsilon_0 + 2)\,(\epsilon_\infty + 2)/3$, which may be approximated by the equation $b = (\epsilon^0 + 2)^2/3$ for solutions with sufficiently small increments.

(*2*) Studies by Wyman and others indicate that $b = (\epsilon - a)/p$ in pure liquids and $b = (\epsilon - \epsilon^0 - a)/p$ in binary mixtures, where p is the volume polarization and a another parameter. Wyman (50) suggests the values $b = 8.5$ and $a = -1$ as best representing the bulk of the available data.

(*3*) We may evaluate b by assuming values of $(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)$ and μ for the glycine molecule. Various measurements[6] indicate that μ is close to 15, and we will choose this value. The total increment per gram may be taken as 0.36.[7]

(*4*) Onsager (37) has computed b as a function of an internal refractive index, n, giving the result $b = (n^2 + 2)^2/2 = 4.5\,[1 + (n^2 - 1)/3]^2$.

(*5*) Kirkwood (28) has presented a method for computing b in terms of the hindered relative rotation of neighboring molecules. He gives the equation

$$b = 4.5\left[1 + \frac{N}{v}\int^{v^0} \cos\gamma e^{-W/kT}\,d\omega\,dv\right] = 4.5(1 + \beta)$$

where

$$\int^{v^0} e^{-W/kT}\,d\omega\,dv = 1$$

Here γ is the angle between the dipole moments of an arbitrary pair of molecules, and W is the potential between an arbitrary pair of molecules. The integration extends over all relative orientations ($d\omega$) and positions (dv) within a sphere of volume v_0 which includes the molecule and the first few shells of neighboring molecules.

For the calculation of dipole moments it is convenient to modify equation 6 to become:

$$\mu = \alpha\sqrt{M(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)} = \alpha\sqrt{M(\Delta\epsilon_t/g)}$$

[6] Kirkwood (27) estimates the moment as 15.0 Debye units, on the basis of studies of the solvent action of neutral salts on glycine at low dielectric constants by Cohn (5), and Scatchard and Prentiss (39) estimate the moment as 14.8 Debye units, on the basis of freezing-point measurements. A value near 15 is obtained by consideration of the distance separating the positive and negative charges in the glycine molecule.

[7] $(\Delta\epsilon_0/g)$ from Wyman and McMeekin (53) and $(\Delta\epsilon_\infty/g)$ calculated from equation 3, taking 0.582 for the partial specific volume of glycine (6).

Here a new parameter

$$\alpha = \sqrt{9000kT/(4\pi Nb)} = 0.403 \times 10^{-18}\sqrt{T/b}$$

has been introduced, and values of both b and this quantity at 0°, 20°, and 25°C. are tabulated in table 1. Values of n calculated from Onsager's equation and of β calculated from Kirkwood's equation are also given for the various values of b.

The assumption of $b = 5.8$, as obtained by method (*3*) using 15 for the dipole moment of glycine, appears to be the most reasonable choice we can make at the present time, and the values for α calculated from this b value are those used in the calculation of all protein dipole moments. It would seem unlikely that these values should vary more than perhaps ±15–25 per cent from the true ones.

VI. DISPERSION STUDIES

The study of the region B, figure 1, in which dielectric dispersion occurs is capable of giving us information dependent upon the size and shape of the

TABLE 1

Various evaluations of b and α for equation 7 and the corresponding values for the dipole moment of glycine

	b	$\alpha \times 10^{18}$			DIPOLE MOMENT OF GLYCINE	ONSAGER'S REFRACTIVE INDEX, n	KIRKWOOD'S PARAMETER, β
		0°C.	20°C.	25°C.			
Debye	2160	0.136	0.146	0.149	0.77	8.1	479
Wyman	8.5	2.28	2.36	2.38	12.3	1.457	0.89
Empirical	5.8	2.76	2.86	2.89	15.0	1.182	0.29
Onsager or Kirkwood*	4.5	3.13	3.24	3.28	17.0	1.000	0.0

* Minimum values for b.

molecules, and gives us one of the most powerful methods for the accurate measurement of these quantities that is at present available.

Dispersion of dielectric constant

The quantitative behavior of the dielectric constant in this dispersion region is somewhat complex. Debye (12) has treated the simplest case involving a single relaxation time, and has obtained the equations

$$\epsilon' = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/(1 + \nu^2/\nu_c^2) \tag{8}$$

Here ϵ' is the usual dielectric constant of the solution (often called the "real" dielectric constant), ϵ_0 and ϵ_∞ are the dielectric constants observed in regions A and C, respectively, ν is the frequency, usually expressed in cycles per second, or megacycles per second, and ν_c is the "critical frequency," defined as $1/(2\pi\tau)$, where τ is the relaxation time, which is the time required for $1/e$ of the molecules to become randomly distributed if they were completely oriented by a field, and then released at $t = 0$.

This simple theory, involving a single relaxation time and critical frequency, can be extended to include the case of several relaxation times, giving

$$\epsilon' = \epsilon_\infty + \Delta\epsilon_1/(1 + \nu^2/\nu_1^2) + \Delta\epsilon_2/(1 + \nu^2/\nu_2^2) + \cdots \quad (9)$$

where $\Delta\epsilon_1$, $\Delta\epsilon_2$, etc., represent the total dielectric increment associated with the critical frequencies ν_1, ν_2, etc. The critical frequencies are defined as before; that is, $\nu_i = 1/(2\pi\tau_i)$. An ellipsoidal molecule would in general have three relaxation times, but if we restrict ourselves to ellipsoids of revolution, then we have only two relaxation times, τ_a and τ_b, and hence two critical frequencies, ν_a and ν_b. Equations of Perrin (39) give τ_a and τ_b in terms of the ratio of axes of the ellipsoid, a/b, and the relaxation time τ_0 of a sphere of the same volume, and we can thus express ν_a and ν_b in terms of a/b and ν_0. If $\Delta\epsilon_t$ be taken as the

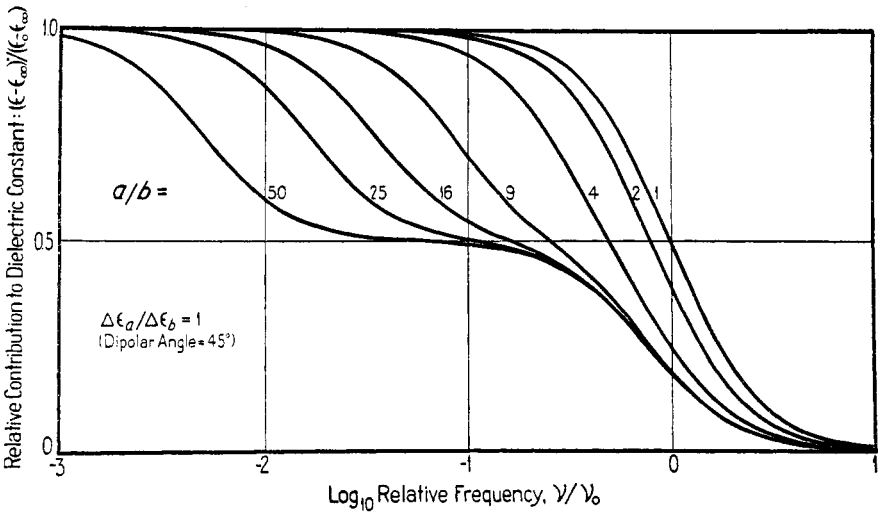

FIG. 2. Dielectric dispersion curves for elongated ellipsoids of revolution (according to Perrin). Constant dipole angle ($\theta = 45°$) and varying axial ratio (a/b from 1 to 50). (From Oncley: J. Phys. Chem. **44**, 1103 (1940)).

sum of $\Delta\epsilon_a$ and $\Delta\epsilon_b$, then equation 7 can be expressed in terms of a/b, ν_0, $\Delta\epsilon_t$, and $\Delta\epsilon_a/\Delta\epsilon_b$, and we can construct series of curves of $(\epsilon' - \epsilon_\infty)/\Delta\epsilon_t$ against ν/ν_0 for various values of asymmetry, a/b, and increment ratio, $\Delta\epsilon_a/\Delta\epsilon_b$. Such curves are illustrated in figures 2 and 3. By comparison of the experimental values obtained in region B (figure 1) with theoretical values calculated in this manner, we can evaluate a/b, $\Delta\epsilon_a/\Delta\epsilon_b$, and ν_0. The values of ν_0 (or τ_0) are directly related to the molecular volumes, V, of the particles:

$$\nu_0 = kT/(8\pi^2 ab^2\eta) = RT/(6\pi V\eta) = 1/(2\pi\tau_0) \quad (10)$$

or

$$V = RT/(6\pi\eta\nu_0) = RT\tau_0/(3\eta) \quad (11)$$

For water at 25°C., this becomes

$$V = 147{,}000/(\nu_0)_{25,w} = 9250(\tau_0)_{25,w} \times 10^8 \quad (11a)$$

where ν_0 is expressed in megacycles and τ_0 in seconds. The relaxation times are usually reduced to the value which would be obtained at 25°C. in water, using the equation

$$\tau_{25,w} = \tau T/(298 \cdot \eta/\eta_{25,w}) = T/(2\pi\nu_c \cdot 298 \cdot \eta/\eta_{25,w}) \quad (11b)$$

The increment ratio, $\Delta\epsilon_a/\Delta\epsilon_b$, may also be expressed as a "dipole angle," θ, defined as the angle between the geometric axis a of the ellipsoid and the electric moment vector. Since the electric moment μ will be the vector sum of the two moments μ_a and μ_b, and since the moments are proportional to the square roots of the increments, we have

$$\tan\theta = \mu_b/\mu_a = \sqrt{\Delta\epsilon_b/\Delta\epsilon_a} \quad (12)$$

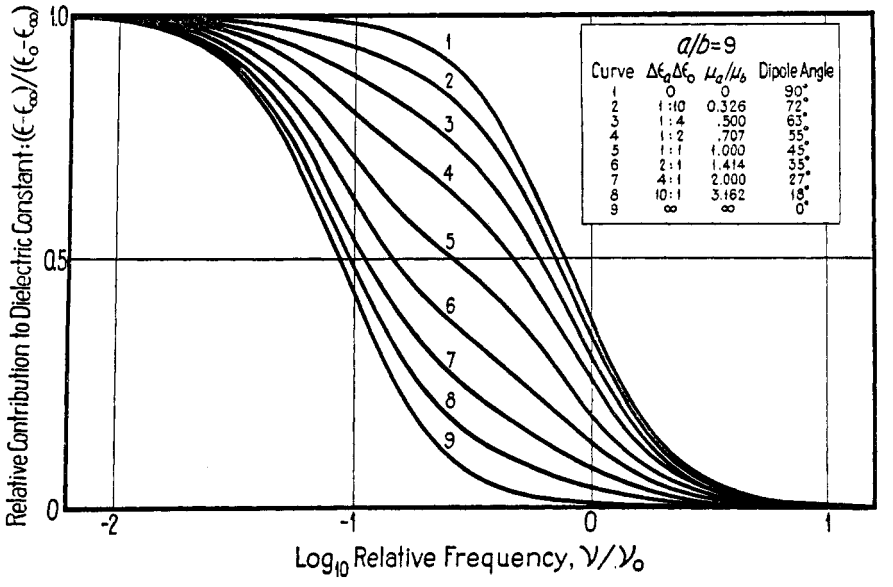

FIG. 3. Dielectric dispersion curves for elongated ellipsoids of revolution (according to Perrin). Constant axial ratio (a/b = 9) and varying dipole angle (θ from 0° to 90°). (From Oncley: J. Phys. Chem. **44**, 1103 (1940)).

Dispersion of conductance

Measurements of the specific conductance,[8] κ, of a protein solution can also be used to evaluate the total dielectric increment and relaxation times of mole-

[8] The specific conductance of a solution, κ, is determined from the measured parallel conductance, G, by the equation $\kappa = 0.0885G/Q = kG$, where k is the usual conductance cell constant and Q is the change in cell capacitance (in $\mu\mu$fds) caused by a unit change in the dielectric constant ($Q = dC/d\epsilon'$). Here G is expressed in μmhos and κ in μmhos per centimeter.

We may also express the dielectric losses in these solutions in terms of the imaginary part of the complex dielectric constant, or by the phase angle. The complex dielectric constant, ϵ, is composed of a real part, ϵ', usually simply called the dielectric constant, and an imaginary part, ϵ''. We have $\epsilon = \epsilon' - i\epsilon''$, where $i = \sqrt{-1}$. The phase angle, δ, is

cules. At low frequencies (region A, figure 1) this conductance will have the value κ_0, which increases in region B to the value κ_∞ in region C. We thus have the equations

$$\kappa = \kappa_\infty - (\kappa_\infty - \kappa_0)/(1 + \nu^2/\nu_c^2) = \kappa_0 + (\kappa_\infty - \kappa_0)(\nu/\nu_c)^2/(1 + \nu^2/\nu_c^2) \quad (13)$$

and

$$\begin{aligned}\kappa &= \kappa_\infty - \Delta\kappa_1/(1 + \nu^2/\nu_1^2) - \Delta\kappa_2/(1 + \nu^2/\nu_2^2) - \cdots \\ &= \kappa_0 + \Delta\kappa_1(\nu/\nu_1)^2/(1 + \nu^2/\nu_1^2) + \Delta\kappa_2(\nu/\nu_2)^2/(1 + \nu^2/\nu_2^2) \cdots \end{aligned} \quad (14)$$

TABLE 2

*Comparison of results obtained on several protein molecules studied by both bridge and calorimetric methods**

PROTEIN	METHOD	CRITICAL FREQUENCY ν_c	DIELECTRIC INCREMENT $\Delta\epsilon_i/g$	CONDUCTANCE INCREMENT $\Delta\kappa_i/g$
Egg albumin (8–9 per cent concentration), 1st relaxation	Bridge	0.88	0.092	(0.045)
	Calorimetric	0.80	(0.102)	0.046
	Best values	0.86	0.094	0.045
Egg albumin (8–9 per cent concentration), 2nd relaxation	Bridge	3.6	0.071	(0.142)
	Calorimetric	3.3	(0.062)	0.114
	Best values	3.4	0.063	0.119
Egg albumin (8–9 per cent concentration), total increments	Bridge		0.163	(0.187)
	Calorimetric		(0.165)	0.154
	Best values		0.157	0.164
Carboxyhemoglobin (horse)	Bridge	1.9	0.44	(0.46)
	Calorimetric	2.0	(0.40)	0.44
	Best values	1.9	0.42	0.44
Lactoglobulin (0.1 per cent in water)	Bridge	2.4	1.5	(2.0)
	Calorimetric	2.2	(1.4)	1.7
	Best values	2.4	1.5	2.0

* Taken in part from Oncley, Ferry, and Shack (36).

to correspond with equations 8 and 9 for the dielectric constant. Here $\Delta\kappa_i$ and ν_i represent the conductance increment and critical frequency of the i^{th} dispersion region. The relation

$$\Delta\kappa_i = \Delta\epsilon_i\nu_i/1.80 \quad (15)$$

defined by the equation $\tan\delta = \epsilon''/\epsilon'$. When dealing with solutions having D.C. conductance, κ_0, we must not include this part of the loss when computing the imaginary dielectric constant. The value as corrected for this D.C. conductance has been designated $\Delta\epsilon''$, and called the dielectric absorption (36, 41). It is related to the conductance by the equation $\Delta\epsilon'' = 1.80\ (\kappa - \kappa_0)/\nu$. Here the specific conductance κ (and κ_0) is expressed in μmhos per centimeter, and the frequency ν in megacycles per second.

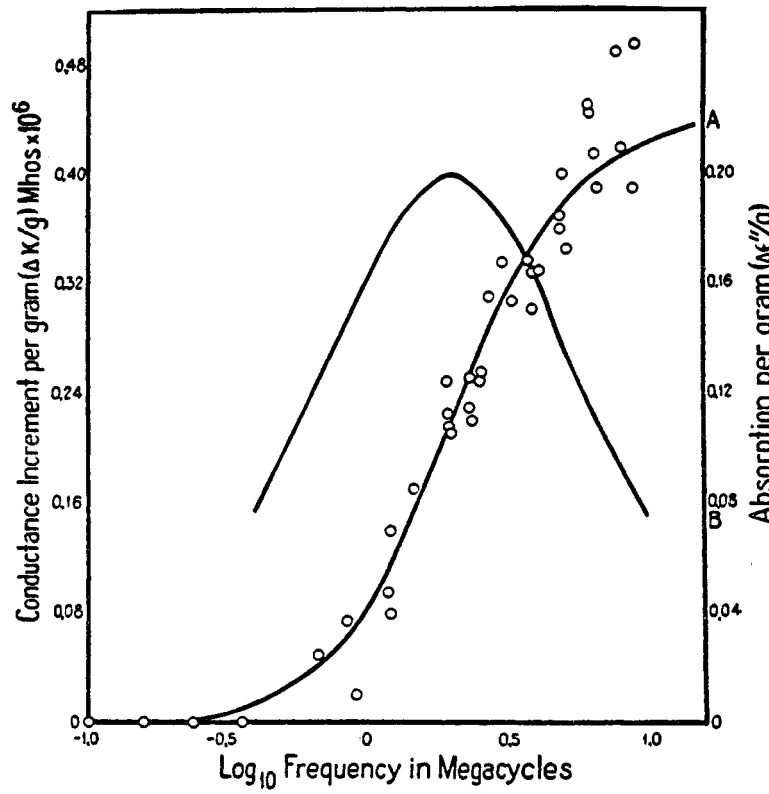

FIG. 4. Dielectric absorption of carboxyhemoglobin solutions: o, experimental measurements of $(\kappa - \kappa_0)/g$; curve A, $(\kappa - \kappa_0)/g$, and curve B, $\Delta\epsilon''/g$. (From Oncley, Ferry, and Shack: Ann. N. Y. Acad. Sci. **40**, 371 (1940)).

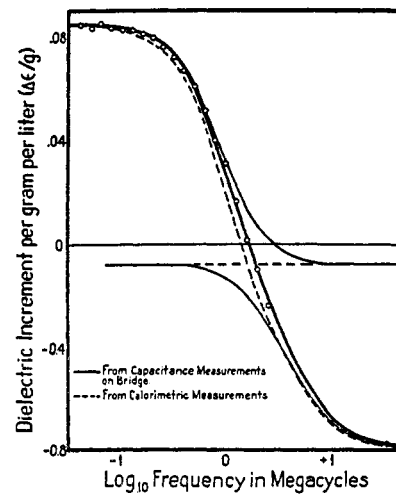

FIG. 5. Dispersion of the dielectric constant of egg albumin, showing resolution into two Debye curves: o, experimental measurements of $\Delta\epsilon/g$; heavy curve from bridge measurements, and broken curve from calorimetric measurements. (From Oncley, Ferry, and Shack: Ann. N. Y. Acad. Sci. **40**, 371 (1940)).

TABLE 3

Dielectric increments and dipole moments of various protein molecules

PROTEIN	SOLVENT	REFERENCE	TEMPERATURE °C.	E_0	F_0	$-E_\infty$	E_t	M	μ
Horse carboxyhemoglobin	Water	(18, 32, 41)	25	0.33	0	0.09	0.42	67,000	480
Pig carboxyhemoglobin	Water	(3)	20				0.3	(67,000)	410
Myoglobin	Water	(52)	25	0.15		(0.06)	0.21	17,000	170
Insulin	80% aqueous propylene glycol	(8)	25				0.38	40,000	360
Insulin	90% aqueous propylene glycol	(8)	25				0.29	40,000	310
Insulin	100% propylene glycol	(8)	25				0.26	40,000	300
Lactoglobulin	$M/2 + M/4$ glycine	(19, 21)	25	1.51	0.025	(0.07)	1.58	40,000	730
Lactoglobulin	$M/2 + M/4$ glycine	(21)	0	1.84	0.047	(0.08)	1.92	40,000	770
Egg albumin	Water	(35, 36, 41)	25	0.10	0	0.07	0.17	44,000	250
Horse serum albumin (carbohydrate-free)	Water	(20, 33)	25	0.17	0	0.07	0.24	70,000	380
Horse serum pseudoglobulin-γ	Water	(20, 33)	25	1.08	0.017	(0.06)	1.14	142,000	1100
Horse serum pseudoglobulin-γ	Water	(33)	0	1.26	0.023	(0.06)	1.32	142,000	1300
Edestin	2 M glycine	(33)	25	0.7		(0.1)	0.8	310,000	1400
Gliadin	56% aqueous ethanol	(2, 17)	25				0.10	42,000	190
Secalin	54% aqueous ethanol	(1)	25				1.0	24,000	440
Zein	72% aqueous ethanol	(16, 46, 49)	25				0.45	40,000	380

exists between the conductance increment, the critical frequency, and the dielectric increment of any dispersion region when conductances are expressed in μmhos per centimeter and critical frequencies in megacycles. Measurements

TABLE 4

Critical frequencies, relaxation times, and geometrical asymmetries of various protein molecules

PROTEIN	SOLVENT	REFERENCE	TEMPERATURE °C.	$\frac{\eta}{\eta_{25,w}}$	$\nu_{obsd.}$	$\frac{\Delta\epsilon_1}{\Delta\epsilon_2}$	$\tau_{25,w} \times 10^8$	$(r_0)_{25,w} \times 10^8$	a/b	$\Theta(^\circ)$
Horse carboxyhemoglobin	Water	(18, 32, 41)	25	1.00	1.9		8.4	(6.6)	(1.6)	
Pig carboxyhemoglobin	Water	(3)	20	1.12	1.1		13.0			
Myoglobin	Water	(52)	25	1.00	5.5		2.9			
Insulin	80% aqueous propylene glycol	(8)	25	17.0	0.59		1.6			
Insulin	90% aqueous propylene glycol	(8)	25	28.0	0.31		1.8			
Insulin	100% propylene glycol	(8)	25	48.0	0.19		1.8			
Lactoglobulin	*M*/4 glycine	(19, 21)	25	1.04	1.0; 3.0	0.25	15; 5.1	4.3	4	63
Lactoglobulin	*M*/2 glycine	(19, 21)	25	1.08	0.9; 2.6	0.25	16; 5.7	4.8	4	63
Lactoglobulin	*M*/2 glycine	(21)	0	2.12	0.44; 1.25	0.25	16; 5.5	4.6	4	63
Egg albumin	Water	(35, 36, 41)	25	1.00	0.86; 3.4	1.5	18; 4.7	3.7	5	40
Horse serum albumin (carbohydrate-free)	Water	(20, 33)	25	1.00	0.44; 2.1	1.0	36; 7.5	6.0	6	45
Horse serum pseudoglobulin-γ	Water	(20, 33)	25	1.00	0.064; 0.57	1.0	250; 28	22.0	9	45
Edestin	2 *M* glycine	(33)	25	1.33	0.050; 0.44	1.0	240; 27	21.0	9	45
Gliadin	56% aqueous ethanol	(2, 17)	25	2.58	0.23; 1.6	1.6	27; 3.8	3.1	8	38
Secalin	54% aqueous ethanol	(1)	25	2.59	0.21; 2.3	1.5	29; 2.7	2.1	10	40
Zein	72% aqueous ethanol	(16, 46, 49)	25	2.22	0.30; 1.7	2.0	24; 4.2	3.3	7	35
Zein		(16)	0.8	5.10		2.0	20; 3.5	2.8		

of this type on a solution yielding a single relaxation time are shown in figure 4. The results of a comparison of conductance increments, dielectric increments, and critical frequencies by independent bridge and calorimetric methods are recorded in table 2. The mean values in this table are weighted, more weight being given to the low-frequency bridge data and to the high-frequency calorimetric data. Values enclosed in parentheses are calculated from equation 15. Figure 5 also compares the data obtained by these two methods in the case of egg albumin.

VII. RESULTS

A survey of the dielectric constant literature for protein solutions reveals few data of significance before those of Wyman (49). Before this time most workers had made measurements at such high frequencies that the results ob-

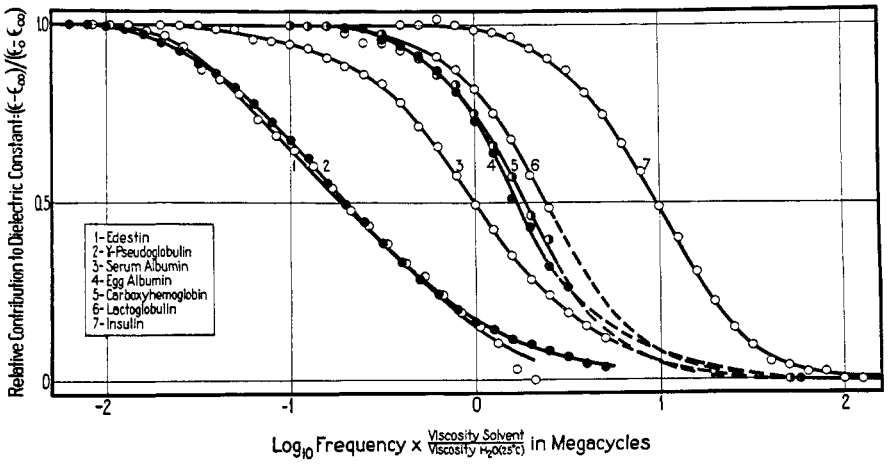

FIG. 6. Dielectric dispersion curves of various proteins. 1, edestin; 2, horse γ-pseudoglobulin; 3, horse serum albumin; 4, egg albumin; 5, horse carboxyhemoglobin; 6, lactoglobulin; 7, insulin.

tained fell in region C (figure 1), where the contribution of the protein molecules to the dielectric constant was very small, and even these measurements were very uncertain. The development of new techniques of measurement since 1928 have made observations in regions A and B much more reliable. The results which we will consider are confined almost entirely to these more recent investigations.

Dielectric increments

The dielectric increments and dipole moments of a number of protein molecules are recorded in table 3. The observed low-frequency increments vary from about 0.1 to over 1.8, a variation somewhat greater than has been observed in studies upon amino acids and peptides (33, 51). Dipole moments of from less than 200 to about 1400 Debye units have been computed from these results, using equation 7 with b equal to 5.8. These values of dipole moment seem very

large when compared with those of low-molecular-weight substances, but this is due largely to the high molecular weights of the proteins. A comparison of

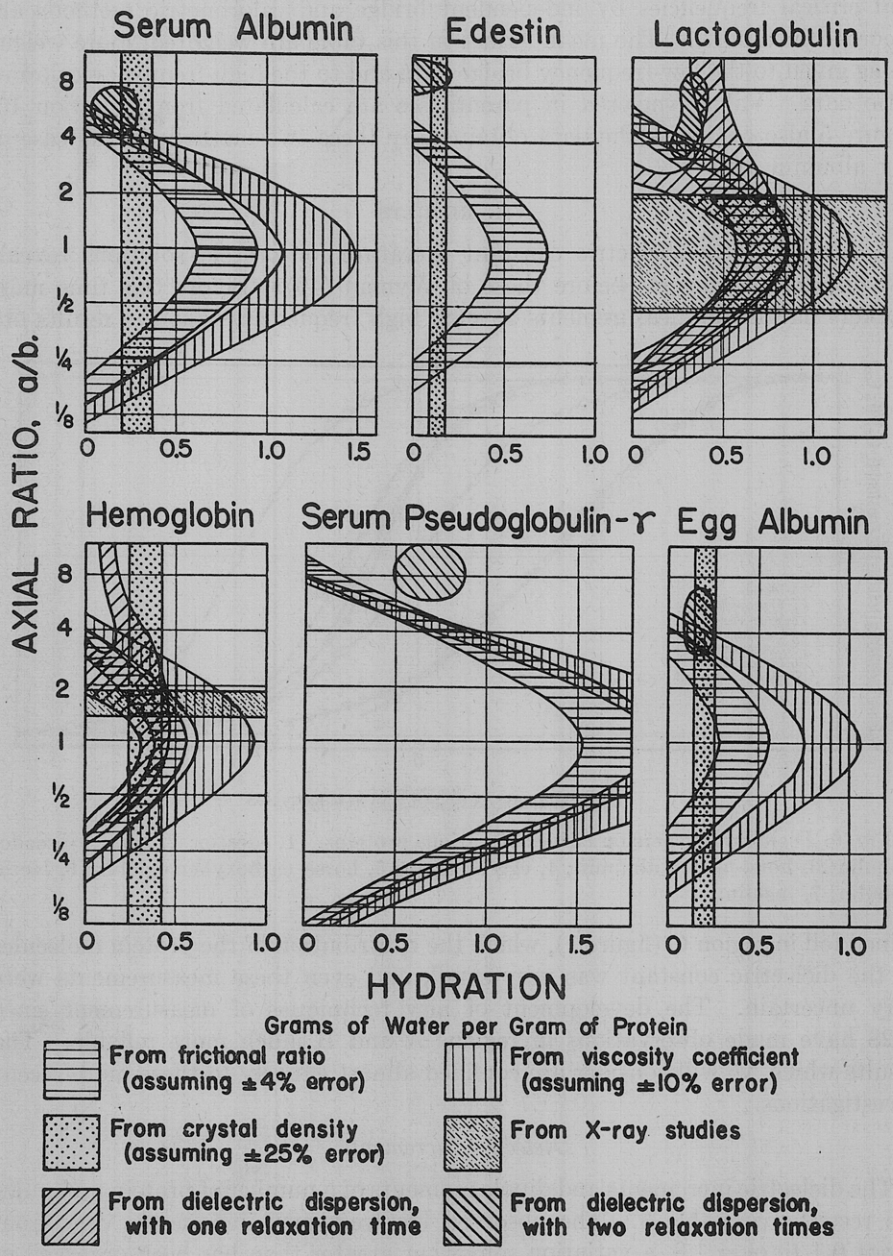

FIG. 7. Asymmetry and hydration of various protein molecules. (Taken in part from Oncley: Ann. N. Y. Acad. Sci. **41**, 121 (1941)).

the electrical symmetry of molecules of widely differing size can be made by calculating the diameter, R, which the molecule would have if it were spherical

and unhydrated, and then computing the number of unit charges, z, which must be located at each end of a dipole of this length in order to give the observed dipole moment.[9] Values obtained in this way are all much smaller than half the total anionic and cationic charges, which is roughly the maximum possible value, $z_{max.}$. The ratio $z/z_{max.}$ obtained for serum albumin is about 0.02, for hemoglobin 0.03, and for edestin 0.01. These molecules would accordingly have moments many times those reported here if they were very unsymmetrical electrically, and these measurements can be taken as proof of a fairly high degree of electrical symmetry.

Relaxation times

The observed critical frequencies and relaxation times of most of the proteins which have been studied are recorded in table 4. These values have been obtained from dispersion curves, such as illustrated in figure 6. In the cases where two critical frequencies have been observed, the data have been interpreted in terms of the geometrical asymmetry a/b, the dipole angle θ, and the relaxation time τ_0 of a sphere of equal volume. These values can be expressed in terms of the asymmetry a/b and the hydration of the protein molecules, w, and these quantities can be compared with those obtained by diffusion, ultracentrifuge, x-ray, and other methods. Figure 7 illustrates this comparison, the results being recorded as areas which represent probable combinations of asymmetry and hydration. The agreement is fairly good in most cases, and all of the molecules recorded here would appear to be more or less elongated ellipsoids with hydration values from 0.2 to perhaps 0.6.

When these results are compared with similar studies on other macromolecules, they appear to indicate a characteristic degree of rigidity in protein structure which is not found in most materials.

REFERENCES

(1) ANDREWS, A. C.: J. Am. Chem. Soc. **62,** 942 (1940); see also Ph.D. dissertation, University of Wisconsin, 1938.
(2) ARRHENIUS, S.: J. Chem. Phys. **5,** 63 (1937).
(3) ARRHENIUS, S.: Physik. Z. **39,** 559 (1938); Nova Acta Regiae Soc. Sci. Upsaliensis [4] **12,** No. 5 (1941).
(4) CARR, W., AND SHUTT, W. J.: Trans. Faraday Soc. **35,** 579 (1939).
(5) COHN, E. J.: Naturwissenschaften **20,** 44 (1932).
(6) COHN, E. J.: Ann. Rev. Biochem. **4,** 122 (1935).
(7) COHN, E. J.: Chem. Rev. **19,** 241 (1936); Harvey Lectures, p. 124 (1938-39).
(8) COHN, E. J., FERRY, J. D., LIVINGOOD, J. J., AND BLANCHARD, M. H.: Science **90,** 183 (1939).
(9) COHN, E. J., MCMEEKIN, T. L., FERRY, J. D., AND BLANCHARD, M. H.: J. Phys. Chem. **43,** 169 (1939).

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[9] We have used the equation

$$z = \mu/(eR) = 0.41\ [(\Delta\epsilon_t/g)^{1/2}M^{1/6}/\bar{v}^{1/3}] = 0.41\ [\delta_t^{1/2}/V^{1/3}]$$

where M is the molecular weight, $(\Delta\epsilon_t/g)$ the total dielectric increment per gram per liter, and $\bar{v}$ the partial specific volume of the protein, and δ_t and V are the corresponding molal total increment and volume.

(10) COLE, K. S., AND CURTIS, H. J.: Rev. Sci. Instruments **8,** 333 (1937).
(11) DANIELS, F., MATHEWS, J. H., AND WILLIAMS, J. W.: *Experimental Physical Chemistry,* 3rd edition, p. 378. McGraw-Hill Book Company, Inc., New York (1941).
(12) DEBYE, P.: *Polar Molecules,* Chap. V. The Chemical Catalog Company, Inc., New York (1929).
(13) DEBYE, P.: Trans. Faraday Soc. **30,** 679 (1934).
(14) DRUDE, P.: Z. physik. Chem. **23,** 267 (1897); **40,** 635 (1902).
(15) DUNNING, W. J., AND SHUTT, W. J.: Trans. Faraday Soc. **34,** 467, 479 (1938).
(16) ELLIOTT, M. A., AND WILLIAMS, J. W.: J. Am. Chem. Soc. **61,** 718 (1939).
(17) ENTRIKIN, P. P.: J. Am. Chem. Soc. **63,** 2127 (1941).
(18) ERRERA, J.: J. chim. phys. **29,** 577 (1932).
(19) FERRY, J. D., COHN, E. J., ONCLEY, J. L., AND BLANCHARD, M. H.: J. Biol. Chem. **128,** Proc. xxviii (1939).
(20) FERRY, J. D., AND ONCLEY, J. L.: J. Am. Chem. Soc. **60,** 1123 (1938).
(21) FERRY, J. D., AND ONCLEY, J. L.: J. Am. Chem. Soc. **63,** 272 (1941).
(22) FRICKE, H., AND CURTIS, H. J.: J. Phys. Chem. **40,** 715 (1936); **41,** 729 (1937).
(23) FUOSS, R. M.: J. Am. Chem. Soc. **56,** 1031 (1934); Physik. Z. **35,** 59 (1934).
(24) FÜRTH, R.: Z. Physik. **22,** 98 (1924); Handbuch der biologischen Arbeitsmethoden IIIB, 835 (1929).
(25) HEMINGWAY, A., AND MCCLENDON, J. F.: Physics **2,** 396 (1932).
(26) JONES, G., AND CHRISTIAN, S. M.: J. Am. Chem. Soc. **57,** 272 (1935).
(27) KIRKWOOD, J. G.: J. Chem. Phys. **2,** 351 (1934).
(28) KIRKWOOD, J. G.: J. Chem. Phys. **7,** 911 (1939).
(29) MALSCH, J.: Physik. Z. **33,** 19 (1932); Ann. Physik **12,** 865 (1932).
(30) MARTIN, G.: Physik. Z. **37,** 665 (1936).
(31) MÜLLER, F. H.: Physik. Z. **34,** 688 (1933); Trans. Faraday Soc. **30,** 729 (1934).
(32) ONCLEY, J. L.: J. Am. Chem. Soc. **60,** 1115 (1938).
(33) ONCLEY, J. L.: J. Phys. Chem. **44,** 1103 (1940).
(34) ONCLEY, J. L.: Ann. N. Y. Acad. Sci. **41,** 121 (1941).
(35) ONCLEY, J. L.: Unpublished data.
(36) ONCLEY, J. L., FERRY, J. D., AND SHACK, J.: Ann. New York Acad. Sci. **40,** 371 (1940).
(37) ONSAGER, L.: J. Am. Chem. Soc. **58,** 1486 (1936).
(38) PERRIN, F.: J. phys. radium **5,** 497 (1934).
(39) SCATCHARD, G., AND PRENTISS, S. S.: J. Am. Chem. Soc. **56,** 2314 (1934).
(40) SCHMELZER, C.: Physik. Z. **37,** 162 (1936); Ann. Physik **28,** 35 (1937).
(41) SHACK, J.: Ph.D. dissertation, "Dielectric Absorption of Protein Solutions," Harvard University, 1939.
(42) SHUTT, W. J.: Trans. Faraday Soc. **30,** 893 (1934).
(43) VAN VLECK, J. H.: J. Chem. Phys. **5,** 320, 556 (1937).
(44) WILLIAMS, J. W.: Trans. Faraday Soc. **30,** 723 (1934); J. Franklin Inst. **219,** 211 (1935).
(45) WILLIAMS, J. W., AND ONCLEY, J. L.: Physics **3,** 318 (1932).
(46) WILLIAMS, J. W., AND WATSON, C. A.: Cold Spring Harbor Symposia Quant. Biol. **6,** 208 (1938).
(47) WILSON, J. N.: Chem. Rev. **25,** 377 (1939).
(48) WYMAN, J., JR.: Phys. Rev. **35,** 623 (1930).
(49) WYMAN, J., JR.: J. Biol. Chem. **90,** 443 (1931).
(50) WYMAN, J., JR.: J. Am. Chem. Soc. **58,** 1482 (1936).
(51) WYMAN, J., JR.: Chem. Rev. **19,** 213 (1936).
(52) WYMAN, J., JR., AND MARCY, H. O.: J. Am. Chem. Soc. **63,** 3388 (1941); **64,** 638 (1942).
(53) WYMAN, J., JR., AND MCMEEKIN, T. L.: J. Am. Chem. Soc. **55,** 908 (1933).