of PII than is its base combining capacity. Possessed of an excess of basic over acid groups, PII has a more nearly neutral isoelectric point than the other serum globulins studied.

5. The titration curves of these proteins from *p*H 1.6 to 12.3 are reported and analyzed in terms of apparent dissociation constants.

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF PHYSICAL CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, NO. 396, AND FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies of the Dielectric Properties of Protein Solutions. I. Carboxyhemoglobin[1,2]

BY J. L. ONCLEY

Many methods have been employed in determining the nature and structure of colloidal materials. As a first approximation the colloidal particle generally has been considered a sphere of given radius and density. Further advances have involved consideration of models of lower degrees of geometrical symmetry. At the present time there are several methods which may be employed for determining accurately the size, or size distribution, of colloidal particles or of molecules of high molecular weight, and which give consistent results for many systems. There are also methods for the study of the shapes of molecules. The next step in our attempt to understand substances of colloidal dimensions is the determination of their electrical symmetry. Such investigations upon substances of low molecular weight have revealed much concerning their structure.[3,4] The importance of the configuration of electrical charges in the interpretation of a variety of physical chemical properties has been considered among others by Scatchard, Kirkwood and Cohn.[5,6]

In the present communication the theory of dielectrics in polar solution is developed briefly, the bridge method that has been adopted is described, and an empirical method is given for correcting dielectric values at low frequency for polarization effects. Studies upon carboxyhemoglobin are reported which supplement other studies of this well-known crystalline protein from the laboratory, and demonstrate the methods of characterizing proteins by their dielectric properties. Comparable studies upon other proteins will be reported subsequently.

I. Theory

The electrical symmetry of molecules may be studied by measuring the dielectric constants of their solutions. The dielectric constant, ϵ, of any polar liquid or solution can be interpreted as being almost entirely a measure of the number of molecules oriented by an external electrical field of unit strength. These molecules are oriented by a torque depending on the field strength and the dipole moment μ, a constant for each molecular species. Orientation is hindered by the frictional forces in the solution depending on the frequency ν, and a constant τ, designated as the "relaxation time."[7] Thus we find that the number of molecules oriented at unit field strength will decrease in the frequency region where the hindering frictional forces and the orienting torque become of the same order of magnitude. At lower frequencies the orienting torque is sufficient to overcome completely the resisting forces, and we have a high dielectric constant, ϵ_0.[8] At very high frequencies the resisting forces completely overcome the orienting torque and we again have a constant but low value of ϵ.

If we now consider a binary mixture of two polar molecules (dipoles), we find a more complicated behavior. Figure 1 represents the typical

(1) A preliminary report of this investigation was presented to the Fourteenth Colloid Symposium, held at Minneapolis, Minn., June 10–12, 1937.

(2) This investigation has been supported in part by grants from the Committee of the Permanent Charity Fund, Inc., and from the Farnsworth Fund, Harvard Medical School.

(3) Debye, "Polar Molecules," Chemical Catalog Co., New York, N. Y., 1929.

(4) Smyth, "Dielectric Constant and Molecular Structure," Chemical Catalog Co., New York, N. Y., 1931.

(5) Cohn, "Annual Review of Biochemistry," Vol. IV, 1935, p. 122.

(6) Cohn, *Chem. Rev.*, **19**, 241 (1936).

(7) Other forces might be important in certain cases, but for large molecules in low viscosity solvents this effect has been shown to be the most important. The "relaxation time" is defined as 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 $t = 0$.

(8) Some workers use the term "specific inductive capacity" so that the term variable dielectric "constant" may be avoided. The phrase "specific inductive capacity" has an unfortunate connotation derived from induced dipoles, whereas the molecules with which we are concerned are permanent dipoles.

variation of dielectric constant with frequency for such a mixture when the relaxation times, τ_1 and τ_2, of the two components are quite different, τ_2 being the larger. The figure is divided into five regions, each of which exhibits a different dielectric behavior. In region A the orienting torque acting on dipoles of both types 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 dipoles with the larger relaxation time τ_2 can no longer be neglected, the orientation of molecules of type 2 is no longer independent of the frequencies, and we find a region of decreasing dielectric constant. In

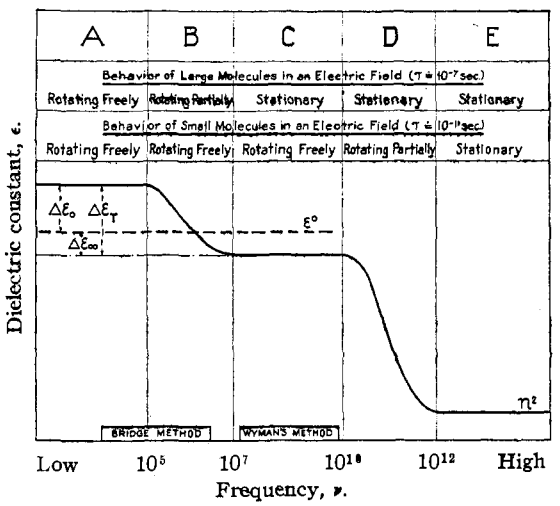

Fig. 1.—Typical dielectric behavior of a binary mixture involving two relaxation times which differ widely.

region C this frictional force has overcome completely the orienting force in the case of molecules of type 2, and they then contribute very little to the dielectric constant. The type 1 dipoles, however, are still orienting independent of the frequencies, and we have a second region of constant, intermediate dielectric constant, ϵ_∞; D represents a region of decreasing orientation of molecules of type 1, and E a region of no orientation at all of molecules of either type. The dielectric constant here is very low, differing from unity only because of some energy stored in the dielectric by the distortion of the electron and nuclear positions in the molecules (atomic and electronic polarization), and this contribution is small when compared with that of the orientation of highly polar molecules.

The interpretation of these dielectric constant values in terms of the electrical symmetry, usually measured by the dipole moment μ,[9] is a difficult problem when polar solvents are used. The equations employed involve slight modifications of those derived by a rigid application of Debye's relations.[10-12] Debye's theory[3] as derived for non-polar solvent media leads to equations adequately tested for such cases, but their application to solvents of high dielectric constant can be undertaken only with considerable apprehension. Expressing his relationship in terms of the volume polarization p,[13] we have the two equations

$$p = (\epsilon - 1)/(\epsilon + 2) \qquad (1)$$

and

$$\mu^2 = (p_0 - p_\infty)9kT/(4\pi n) \qquad (2)$$

where k is Boltzmann's constant, T the absolute temperature, and n the number of polar molecules contained in 1 cc. Equation (1), the Clausius–Mosotti relationship, gives the volume polarization, p, in terms of the dielectric constant; and equation (2), introduced by Debye, independent of (1), gives the electric moment μ in terms of the volume polarizations p_∞ and p_0 measured at frequencies just above and below the dispersion frequency (Fig. 1). Expressing the concentration of polar molecules as grams per liter, g, we have $n = gN/1000\ M$, where N is Avogadro's number and M the molecular weight. Equation (2) becomes

$$\mu^2 = \frac{9000\ kTM}{4\pi Ng}\left(\frac{\epsilon_0 - 1}{\epsilon_0 + 2} - \frac{\epsilon_\infty - 1}{\epsilon_\infty + 2}\right) = \frac{27{,}000\ kTM\ (\epsilon_0 - \epsilon_\infty)}{4\pi Ng\ (\epsilon_0 + 2)(\epsilon_\infty + 2)} \qquad (3)$$

If ϵ^0, the dielectric constant of the solvent, is large, and $\epsilon_0 - \epsilon^0$ and $\epsilon^0 - \epsilon_\infty$ small, we obtain the *approximate* equation

$$\mu^2 = \frac{27{,}000\ kTM\ (\epsilon_0 - \epsilon_\infty)}{4\pi Ng\ (\epsilon^0 + 2)^2} \qquad (4)$$

For aqueous solutions at 25° ($\epsilon^0 = 78.5$) we have

$$\mu = 0.1494 \times 10^{-18}\sqrt{M(\epsilon_0 - \epsilon_\infty)/g}$$

Studies by Wyman and others[14] upon the dielectric constant of amino acids, polypeptides, and other materials, indicate that equation (1)

(9) $\mu = Re$, where R is the distance separating the centroids of positive and negative charges, and e the absolute value of the electronic charge.

(10) Bloch and Errera, *Physik. Z.*, **33**, 767 (1932).

(11) Marinesco, *Kolloid-Z.*, **58**, 285 (1932).

(12) Williams and Oncley, *Physics*, **3**, 318 (1932).

(13) $p = P\rho/M$, where P is the more commonly used molar polarization, ρ is the density, and M is the molecular weight.

(14) Wyman, *Chem. Rev.*, **19**, 213 (1936).

cannot be applied in systems of high dielectric constant, and suggest the empirical relation

$$p = (\epsilon - a)/b \qquad (5)$$

where a and b are constants.

For binary mixtures

$$p = (\epsilon - \epsilon^0 - a)/b$$

and

$$(p_0 - p_\infty) = (\epsilon_0 - \epsilon_\infty)/b$$

Combining with equation (2) we have

$$\mu^2 = \frac{9000\, kTM\,(\epsilon_0 - \epsilon_\infty)}{4\mu Ngb} \qquad (6)$$

or

$$\mu = \alpha\sqrt{M\,(\epsilon_0 - \epsilon_\infty)/g} \qquad (6a)$$

where α is evaluated at 25° for various values of b in Table I. Kirkwood[16] estimates the moment,

TABLE I

VARIOUS EVALUATIONS OF α AT 25° FOR EQUATION (6a), AND THE CORRESPONDING VALUES FOR THE DIPOLE MOMENT OF GLYCINE

	b	$\alpha \times 10^{18}$	Glycine[a] moment
Debye theory	2160	0.149	0.78
Wyman[15]	8.5	2.4	12
Kirkwood[16]	5.8	2.9	15

[a] Expressed in Debye units (= 10^{-18} e. s. u.).

15.0 Debye units, on the basis of studies of the solvent action of neutral salts on glycine at low dielectric constants by Cohn;[17] and Scatchard and Prentiss[18] estimate the moment, 14.8 Debye units, on the basis of freezing point measurements. The agreement between these values and that calculated from the distance separating the positive and negative charges in glycine (14.3 to 15.3 Debye units) leads us to adopt the values $b = 5.8$ and $\alpha = 2.9$ until more adequate theoretical grounds are available. The moments obtained for glycine by the use of each of these values of b, taking $(\epsilon_0 - \epsilon_\infty)/g$ to be 0.36,[19] are given in Table I.

For the dielectric behavior in the dispersion region (Region B), we may refer to Debye's treatment of anomalous dispersion (3) in its most general form (avoiding the Clausius–Mosotti hypothesis).

$$p = p_\infty + (p_0 - p_\infty)/(1 + j2\pi\tau\nu) \qquad (7)$$

$$p = p' - jp'' = p_\infty + (p_0 - p_\infty)/(1 + 4\pi^2\tau^2\nu^2) - j2\pi\tau\nu(p_0 - p_\infty)/(1 + 4\pi^2\tau^2\nu^2)$$

where $j = \sqrt{-1}$. The volume polarization p is a complex function made up of a real part, p', and an imaginary part, p''. Considering only the real part, we have

$$p' = p_\infty + (p_0 - p_\infty)/(1 + 4\pi^2\tau^2\nu^2) \qquad (7a)$$

Combining equation (7a) with (5) yields

$$\epsilon' = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/(1 + 4\pi^2\tau^2\nu^2) \qquad (7b)$$

and with (1) yields

$$\epsilon' = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/\left[1 + 4\pi^2\tau^2\nu^2\left(\frac{\epsilon_0 + 2}{\epsilon_\infty + 2}\right)^2\right] \qquad (7c)$$

Introducing a "critical frequency" $\nu_c = 1/(2\pi\tau)$ in (7b) and $(\epsilon_\infty + 2)/[(\epsilon_0 + 2)2\pi\tau]$ in (7c), we have

$$\epsilon' = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/(1 + \nu^2/\nu_c^2) = \epsilon_0 - (\epsilon_0 - \epsilon_\infty)(\nu^2/\nu_c^2)/(1 + \nu^2/\nu_c^2) \qquad (7d)$$

Thus ν_c is the frequency at which $\epsilon' = (\epsilon_0 + \epsilon_\infty)/2$ and its determination is equivalent to a determination of the so-called rotary diffusion constant which we call Θ.[20] We may write $\Theta = RT/N\zeta$ (similar to $D = RT/Nf$ for linear diffusion), where ζ is the "inner-frictional constant," evaluated by Stokes to be $8\pi r^3\eta$ for a sphere of radius r (similar to $f = 6\pi r\eta$ for linear diffusion), or better written $\zeta = 8\pi r^3\eta\Psi$, where Ψ is a "shape factor" to include other than spherical particles.[21] Debye has shown that τ equals $\zeta/2kT$, and hence

$$\pi\nu_c = kT/\zeta = RT/N\zeta = \Theta \qquad (8)$$

if we use equation (7b), or

$$\pi\nu_c\frac{\epsilon_0 + 2}{\epsilon_\infty + 2} = \Theta \qquad (8a)$$

if we use (7c). Since equation (3) represents the behavior of polar liquids and solutions more adequately than (1), we have chosen equations (7b) and (8) for the calculation of τ and Θ. Values calculated by means of (7c) and (8a) differ only by a few per cent. in case $(\epsilon_0 + 2)$ is nearly equal to $(\epsilon_\infty + 2)$, which is the case in most of our measurements.

II. Method of Measurement

The further study of protein solutions demanded the development of a reliable method for the measurement of the dielectric constant and conductivity of conducting systems over a wide range. Such a method should also be capable of operation with considerable speed, and as a routine and convenient procedure. A bridge method fulfills these requirements, and the desired reliability at high frequencies can be ob-

(15) Wyman, THIS JOURNAL, **58**, 1482 (1936); see also Onsager, *ibid.*, **58**, 1486 (1936).

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

(17) Cohn, *Naturwissenschaften*, **20**, 44 (1932).

(18) Scatchard and Prentiss, THIS JOURNAL, **56**, 2314 (1934).

(19) Estimating -0.06 for $(\epsilon_\infty - \epsilon^0)/g$, and taking $(\epsilon_0 - \epsilon^0)/g$ as 0.30 [Wyman's value at 25°, THIS JOURNAL, **56**, 908 (1933)].

(20) See Williams and Cady, *Chem. Rev.*, **14**, 186 (1934).

(21) See Perrin, *J. phys. radium*, **5**, 510 (1934), for equations expressing various types of ellipsoids of revolution.

tained by making a substitution measurement rather than a direct one, thus eliminating all errors in the bridge itself (such as inequality of the ratio arms, inductance effects, and stray capacities). The problem of obtaining a suitable resistance unit has been overcome by the use of a "compensated" resistance of constant and known inductance rather than the so-called "non-inductive" resistance sometimes described.[22] The effect of inductance and capacitance in resistance units was therefore the first step in the solution of our problem. As a first approximation a resistance box may be represented as a resistance R and inductance L_R in parallel with a capacity C_R. We have measured the magnitude of these inductances and capacitances, as well as those of the standard condenser and cell. Suitable corrections are then applied to all of the readings, and we are able to make fairly accurate determinations of dielectric constant of solutions with specific conductivities as high as 10^{-4} ohms^{-1} cm^{-1}.

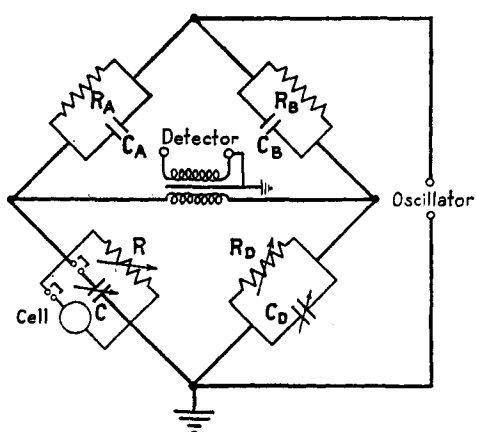

Fig. 2.—Diagram of the radio frequency bridge used.

The bridge used was the type 516-C radio-frequency bridge manufactured by General Radio Company.[23] It was modified in several ways. A 1-ohm slide wire resistor was used to replace the tenths-ohm decade in order to make a more accurate resistance balance. The variable resistance in the bridge was then placed in series with a 100-ohm resistance, and this combination made up the resistance R in Fig. 2, which could then be varied from 100 to 211 ohms by manipulation of the decades and the slide wire. The conductance of this unit was then calculated by means of a reciprocal table, and the values so obtained were used in subsequent calculations. The inductance of this resistance was found to be 1.13 ± 0.05 μh. This measurement was made by assuming that potassium chloride solutions of varying conductivity had the same dielectric constant as water, and calculating the inductance from a series of measurements of various conductivities.[24] It was also checked by measurements on various types of fixed resistors, and the agreement was satisfactory in all cases.

The condenser C was a Leeds and Northrup[25] type 1188 standard condenser, variable between 50 and 1500 μμf, and read to 0.1 μμf by means of two dials. A series of difference calibrations gave small corrections which were applied when necessary. Its inductance was measured by a method essentially the same as that used by Fields and Sinclair,[26] and was found to be 0.100 ± 0.005 μh.

The cell used in most of these measurements was a "water" conductivity cell consisting of three concentric platinum cylinders separated by means of ground quartz rods. It has been described by Scatchard, Prentiss, and Jones.[27] The cell was calibrated with water, alcohol, air, and several potassium chloride solutions. The inductance of the cell and leads was determined by measurement of its increase in apparent capacitance at high frequencies when filled with conductivity water, and was found to be 0.202 ± 0.010 μh.

The units C_D and R_D can be of almost any type. We used the condenser contained in the 516 Bridge for C_D, and a low inductance variable resistance adjustable from 350 to 50 ohms for R_D. The oscillator used was a Glough-Brengle[28] type OC radio-frequency oscillator modified to give a frequency range from 25,000 to 20,000,000 cycles The maximum output voltage was approximately 0.1 v. The detector was a Sargent[29] Model 11-UA all-wave radio receiver covering the frequency range from 15,000 to 34,000,000 cycles. At frequencies below 100,000 cycles use was often made of a General Radio type 814A amplifier to increase the amplification.

Measurements were made by connecting the cell and resistance R (set at 200 or 211 ohms) in parallel in one bridge arm, and balancing R_D and C_D. The cell was then removed and the condenser C was put in its place, and C and R then adjusted until a balance was again obtained. The various units were corrected for inductive effects, and the capacitance and conductance readings thus obtained were used to calculate the dielectric constant and specific conductivity of the solution under test. The equations used in the inductance corrections were

$$\Delta C_1 = 4\pi^2\nu^2 L_c C^2/(1 - 4\pi^2\nu^2 L_c C) \quad (9a)$$

$$\Delta C_2 = -L_R[G_2^2/(1 + 4\pi^2\nu^2 L_R^2 G_2^2) - G_1^2/(1 + 4\pi^2\nu^2 L_R^2 G_1^2)] \quad (9b)$$

$$\Delta C_3 = -4\pi^2\nu^2 L_x C_x^2/(1 + 4\pi^2\nu^2 L_x C_x) \quad (9c)$$

(22) Hague, "Alternating Current Bridge Methods," third edition, Pitmans, London, 1932.

(23) General Radio Company, 30 State Street, Cambridge, Mass.

(24) The values of $\epsilon_0 - \epsilon^0$ for these solutions as calculated from the Debye–Hückel equation $(\epsilon_0 - \epsilon^0) = 0.0473\ \epsilon^0\sqrt{\gamma}$ where γ is the concentration in mole per liter, gave deviations of less than 0.1%.

(25) Leeds and Northrup Company, 4924 Stenton Avenue, Philadelphia, Pa.

(26) Fields and Sinclair, *Proc. Inst. Radio Eng.*, **24**, 255 (1936).

(27) Scatchard, Jones and Prentiss, THIS JOURNAL, **54**, 2686 (1932).

(28) Glough-Brengle Co., 2815 West 19th Street, Chicago, Ill.

(29) E. M. Sargent Co., 212 Ninth Street, Oakland, Cal.

$$\Delta C_4 = L_x\left[\frac{G_2}{1+4\pi^2\nu^2L_R^2G_2^2} - \frac{G_1}{1+4\pi^2\nu^2L_R^2G_1^2}\right]^2 \quad (9d)$$

$$\hat{C}_x = C + \Delta C_1 + \Delta C_2 \quad (9e)$$

$$C_x = C + \Delta C_1 + \Delta C_2 + \Delta C_3 + \Delta C_4 = \hat{C}_x + \Delta C_3 + \Delta C_4 \quad (9f)$$

$$G = G_2 - G_1 \quad (9g)$$

where L_c = inductance of standard condenser (C) and leads = 0.287×10^{-6}h

L_R = inductance of resistance (R) and leads = 1.13×10^{-6}h

L_x = inductance of cell and leads = 0.389×10^{-6}h

C = reading on standard condenser (expressed in $\mu\mu$f)

G_1 = conductance of initial resistance setting (expressed n μmhos)

G_2 = conductance of final resistance setting (expressed in μmhos)

ν = frequency (expressed in megacycles).

These relations were found to represent adequately the behavior of the various units although they are all approximations which become less precise at high frequencies and for high conductivities. There is a further complication when the frequency range is extended to the lower end of the scale. Figure 3a shows this effect. The points represent actual measurements of the capacitance (C_x) of a cell filled with solution, after correction has been made for the effects of inductance and capacitance of the units. The behavior expected from our previous discussion is noted, but another effect is superimposed upon it. Further study has indicated that this second effect is due to polarization at the electrodes. Since this phenomenon is not eliminated easily, a correction for it has been adopted tentatively. When capacitances are plotted against $G^2\nu^{-3/2}$ (Fig. 3b), instead of log ν, a straight line is obtained for solutions of constant ϵ. This relationship may be derived from consideration of a polarization capacity in series with the cell, and inversely proportional to the square root of the frequency.[30,31] When the measurements on protein solutions are plotted in this manner, the high frequency points exhibit dispersion. By subtracting the capacity due to polarization (ΔC_5) we obtain the curves in Fig. 3a (broken lines). Values of the dielectric constant are thus calculated by the equation

$$\epsilon' = (C_x - \Delta C_5)/10.95 = (C_x - AG^2\nu^{-1.5})/10.95 \quad (10a)$$

where the constant 10.95 is the change in cell capacitance caused by a change of unity in the dielectric constant (cell constant) and where A (with units megohms$^{1/2}$ $\mu\mu$f$^{-1/2}$) is the slope of the curve used in the polarization correction. Since we are usually interested in the dielectric increment $\Delta\epsilon = \epsilon' - \epsilon^0$, we more commonly use the equation

$$\Delta\epsilon = (C_x - AG^2\nu^{-1.5} - C^0)/10.95 \quad (10b)$$

where C^0 is the capacitance of the cell when filled with water and corrected in a manner similar to that just described. Corrections to the observed conductances G for inductances and polarization effects can be made and are small for the lower frequencies. At frequencies above one megacycle there are errors of unknown magnitude caused by "skin effects" and other complications. Since the observed conductances at frequencies under 0.2 megacycle change but little, we have calculated specific conductivities from the equation

$$\kappa = 8.1 \times 10^{-9} G \quad (10c)$$

where G is the observed conductance at low

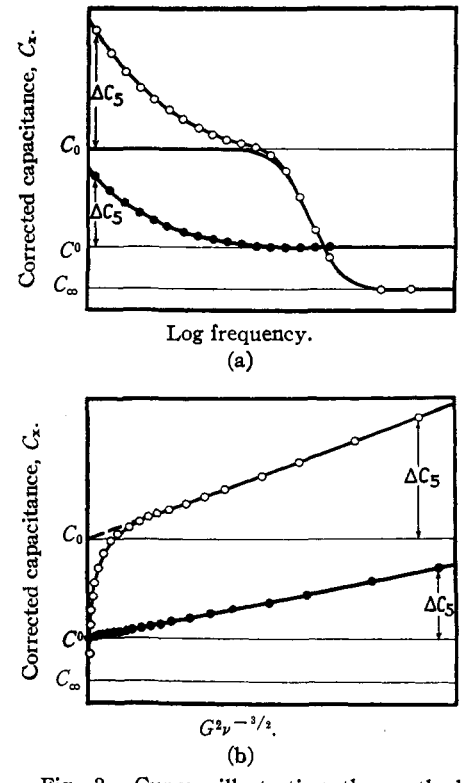

Fig. 3.—Curves illustrating the method for obtaining the polarization correction, ΔC_5: ○, typical protein solution; ●, typical potassium chloride solution.

(30) Newman, "Electrolytic Conduction," John Wiley and Sons, Inc., New York, N. Y., 1931, p. 333.

(31) Warburg, *Ann. Physik*, **6**, 125 (1901).

frequencies, expressed in μmhos,[32] and have not attempted to interpret the higher frequency data at this time.

This method of calculation (equations 9 and 10) has as its justification the fact that (a) measurements on aqueous electrolyte solutions give dielectric constants equal to that of water; (b) measurements on glycine and urea solutions give dielectric increments in good agreement with those of Wyman;[14] and (c) measurements on protein solutions of various conductivities (and hence different A and G values), or on the same protein solutions when electrolytes are added give consistent dielectric increment values.

III. Results

The carboxyhemoglobin used in this investigation was prepared from normal horse cells by the method usually used in this Laboratory.[33,34] A number of preparations were crystallized further by the addition, to saturated aqueous solutions of hemoglobin, of alcohol at −5°, as described by Ferry, Cohn and Newman.[35] Concentrations were determined by evaporation to constant weight at 105°. All of the solutions were at or near the isoelectric point. All measurements were made at a temperature of 25°, the water being distilled from block tin and of low conductivity. A large number of different hemo-

TABLE II

DIELECTRIC MEASUREMENTS ON CARBOXYHEMOGLOBIN SOLUTIONS AT 25°

Frequency megacycles, ν	Conductance μmhos, obsd., G	Capacitance μμf Obsd. C	Capacitance μμf Corr.[a] C_x	Polarization corr. μμf $-C_p$	Dielectric increment Obsd.[b] $\Delta\epsilon$	Dielectric increment Calcd.[c] $\Delta\epsilon^*$
Prepn. A; Concn. (g) = 7.0 g./liter; ρ = 0.999; η/η_0 = 1.02; $\kappa \times 10^6$ = 11.1 mhos/cm.; $A \times 10^6$ = 0.132; G_1 = 5000 μ mhos; C^0 = 880 μμf						
0.025	1358	986	969	61	2.5	2.3
.032	1360	965	948	43	2.3	2.3
.040	1361	952	936	30	2.3	2.3
.050	1362	944	928	22	2.3	2.3
.063	1363	938	921	15	2.4	2.3
.080	1365	934	917	11	2.4	2.3
.100	1366	931	914	8	2.4	2.3
.125	1369	929	912	6	2.4	2.3
.160	1371	927	909	4	2.3	2.3
.200	1375	925	908	3	2.3	2.3
.250	1382	924	907	2	2.3	2.3
.320	1396	923	905	1	2.2	2.2
.400	1402	921	904	1	2.1	2.2
.500	1418	920	902	1	1.9	2.1
.630	1443	919	900	0	1.8	2.0
.800	1480	919	899	0	1.7	1.8
1.00	1527	919	897	0	1.5	1.6
1.25	1596	918	894	0	1.2	1.4
1.60	1707	918	890	0	0.9	1.0
2.00	1849	920	886	0	0.6	0.7
2.50	2084	928	885	0	0.5	0.4
Prepn. B; Concn. (g) = 8.3 g./liter; ρ = 0.999; η/η_0 = 1.03; $\kappa \times 10^6$ = 14.3 mhos/cm.; $A \times 10^6$ = 0.112; G_1 = 5000 μ mhos; C^0 = 880 μμf						
0.025	1745	1020	998	86	2.9	2.7
.032	1748	992	970	60	2.7	2.7
.040	1750	973	951	43	2.6	2.7
.050	1752	962	940	31	2.7	2.7
.063	1755	954	932	22	2.7	2.7
.080	1758	948	926	15	2.8	2.7
.100	1760	943	921	11	2.8	2.7
.125	1763	940	918	8	2.7	2.7
.160	1768	938	916	6	2.7	2.7
.200	1773	936	914	4	2.7	2.7
.250	1781	935	912	3	2.7	2.7
.320	1790	933	910	2	2.6	2.6
.400	1805	932	909	2	2.5	2.6
.500	1825	931	907	1	2.4	2.5
.630	1854	929	904	1	2.2	2.4
.800	1900	929	903	1	2.1	2.2
1.00	1964	929	901	0	1.9	2.0
1.25	2051	928	897	0	1.6	1.6
1.60	2188	927	893	0	1.2	1.2
2.00	2366	929	889	0	0.8	0.8
2.50	2651	935	885	0	0.5	0.4
Prepn. A; Concn. (g) = 13.0 g./liter; ρ = 1.000; η/η_0 = 1.04; $\kappa \times 10^6$ = 15.2 mhos/cm.; $A \times 10^6$ = 0.120; G_1 = 5000 μ mhos; C^0 = 880 μμf						
0.025	1868	1062	1038	106	4.7	4.3
.032	1870	1025	1002	73	4.4	4.3
.040	1870	1003	979	52	4.3	4.3
.050	1871	988	965	38	4.3	4.3
.063	1872	978	954	26	4.4	4.3
.080	1873	970	946	19	4.4	4.3
.100	1876	965	941	13	4.4	4.3
.125	1879	962	938	10	4.4	4.3
.160	1883	959	935	7	4.4	4.3
.200	1888	957	933	5	4.4	4.2
.250	1897	957	933	3	4.4	4.2
.320	1911	955	930	2	4.4	4.1
.400	1930	953	928	2	4.2	4.0
.500	1957	952	926	1	4.1	3.9
.630	1997	949	923	1	3.8	3.7
.800	2053	947	919	1	3.5	3.4
1.00	2133	943	913	0	2.9	3.0
1.25	2246	940	907	0	2.4	2.6
1.60	2419	940	902	0	2.0	1.9
2.00	2646	940	895	0	1.4	1.3
2.50	2991	944	890	0	0.8	0.7

[a] Corrected by equations (9). [b] Calculated from equation (10b). [c] Calculated from the equation $\Delta\epsilon^* = g[0.33 - 0.44(\nu^2/1.9^2)/(1 + \nu^2/1.9^2)]$.

(32) The constant 8.1×10^{-9} is obtained from the change in cell capacitance caused by the change of unity in the dielectric constant (10.95 μμf) by the equation $0.0885 \times 10^{-6}/10.95 = 8.1 \times 10^{-9}$.

(33) Ferry and Green, *J. Biol. Chem.*, **81**, 175 (1929).

(34) Green, *ibid.*, **93**, 495 (1931).

(35) Ferry, Cohn and Newman, THIS JOURNAL, (in press).

globin preparations were studied, and all yielded consistent and satisfactorily reproducible results. Solutions were kept in a cold room when not in use, and all measurements were made within a few days after the preparation of the crystalline hemoglobin.

Table II gives the results of dielectric constant measurements on three carboxyhemoglobin solutions. The first three columns give the frequency, the observed conductance, and the observed capacitance. These values, corrected in accordance with equations (9) yield the capacitances, C_x, recorded in column 4. The correction due to polarization effects, ΔC_5, is recorded in the fifth column,[36] and observed dielectric increments, $\Delta\epsilon$, are given in column 6. Table III summarizes the data for the low frequency dielectric increments, $\Delta\epsilon_0$, on these solutions and others not recorded in detail. Measurements of the increment at high frequencies, $\Delta\epsilon_\infty$, were kindly carried out by Professor J. Wyman with his resonance method.[37] These data are graphically represented in Fig. 4 and compared with those of Errera[38] carried out at 13°. This earlier work and that now reported agree to about 10% at higher concentrations. Our results yield a value for $\Delta\epsilon_0/g$ of 0.33.

TABLE III

LOW FREQUENCY DIELECTRIC INCREMENTS FOR CARBOXYHEMOGLOBIN SOLUTIONS

Prepn.	Concn. g./liter g	Sp. cond. $\kappa \times 10^6$	Dielectric increments $\Delta\epsilon_0$	$\Delta\epsilon_0/g$
C	4.0	9.7	1.3	0.32
C	5.8	14.2	1.9	.33
A	6.6	9.3	2.3	.34
A	7.0	11.1	2.3	.33
B	8.3	14.3	2.8	.33
A	13.0	15.2	4.4	.33
C	14.3	23.5	4.5	.32

These measurements thus lead to a fairly accurate determination of the low frequency dielectric increment, $\Delta\epsilon_0/g$. The critical frequency, ν_c, and hence the relaxation time τ or rotary diffusion constant Θ, we shall report more accurately at a later time when improvements in the accuracy over the higher frequency ranges have been effected. A preliminary value of ν_c, obtained by finding the frequency at which $\Delta\epsilon = (\Delta\epsilon_0 + \Delta\epsilon_\infty)/2$, gives 1.78, 1.92, and 1.99 megacycles, respectively, for the solutions of concentration 7.0, 8.3, and 13.0, and we may take 1.9 megacycles as its approximate value.

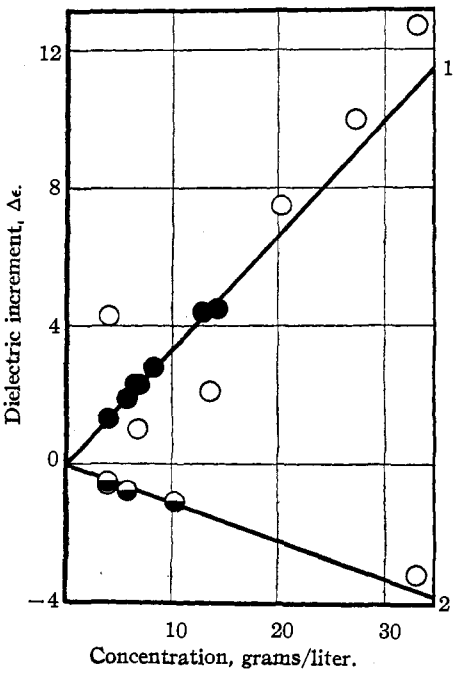

Fig. 4.—Low (curve 1) and high (curve 2) frequency dielectric increments for carboxyhemoglobin: ○, Errera[38]; ◒, Wyman[37]; ●, this paper, Table III.

IV. Discussion

Dielectric Increment.—The dielectric behavior of carboxyhemoglobin at the lower frequencies is similar to that of amino acids and peptides. The dielectric increment per gram, $\Delta\epsilon_0/g$, was 0.33, a value of the same order of magnitude as obtained for glycine, 0.30.[19] It also can be compared with values measured for other proteins. Wyman reports $\Delta\epsilon_0/g$ for zein to be about 0.3.[39] Errera[38] reports results for carboxyhemoglobin already referred to, and values of about 0.3 for egg albumin, 0.5 for serum albumin, and 1.5 for glutine. Shutt[40] reports 0.12 for $\Delta\epsilon_0/g$ for egg al-

(36) The correction for polarization is very large at the lower frequencies, so that those points are used to evaluate the constant A. In the region where the dielectric increment begins to decrease with increasing frequency, we find the correction due to polarization amounts to less than 10% in most cases. The use of cells of smaller capacitance would decrease this correction, and such cells are being designed.

(37) Professor Wyman found $\Delta\epsilon_\infty/g$ values of −0.11, −0.14, and −0.11 for solutions of concentrations 4.0, 5.8, and 10.2 grams per liter, and the value $\Delta\epsilon_\infty/g = -0.11$ is used in our calculations.

(38) Errera, *J. chim. phys.*, **29**, 577 (1932).

(39) Wyman, *J. Biol. Chem.*, **90**, 443 (1931). See also ref. 14. This value was obtained at temperatures from 50 to 70°. At lower temperatures this value became much smaller, a result which seems to be clarified by the work of Watson, Arrhenius and Williams [*Nature*, **137**, 322 (1936)] on the basis of a dissociation at the higher temperatures. These later workers state that their dielectric constant studies are consistent with the work of Wyman, but give no values for $\Delta\epsilon_0$ as a function of the concentration.

(40) Shutt, *Trans. Faraday Soc.*, **30**, 893 (1934).

bumin and Arrhenius[41] about 0.12 for gliadin. Expressing our increment for carboxyhemoglobin on a mole basis, $\Delta\epsilon_0/m$, often called δ, the value 22,000 is obtained if we take 66,700 as its molecular weight. The dipole moment estimated from this value, using the equation $\mu = 4.77 \times 10^{-10}\sqrt{\delta/2.3}$,[42] is 470 Debye units. In order to estimate the dipole moment from our equation (6b) with $\alpha = 2.9$, we must also use $\Delta\epsilon_\infty/g$,[43]

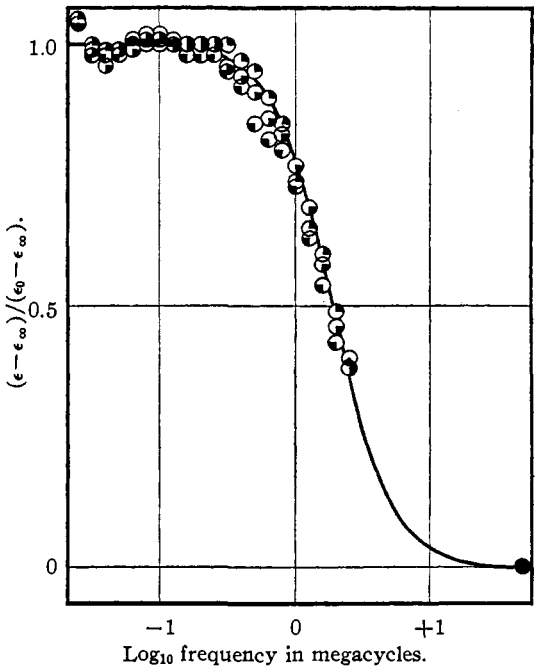

Fig. 5.—Dispersion curves for carboxyhemoglobin, results from Table II. The curve represents dielectric constant values calculated from the equation $\Delta\epsilon = g(0.33 - 0.44(\nu^2/1.9^2)/(1 + \nu^2/1.9^2))$: ○, g = 7.0 g./liter; ◑, g = 8.3 g./liter; ●, g = 13.0 g./liter.

(41) Arrhenius, *J. Chem. Phys.*, **5**, 63 (1937).

(42) Cohn, ref. 5, p. 106, equation 11.

(43) The value $\Delta\epsilon_\infty/g$ might be used to calculate the amount of water associated with the protein molecule if we assumed that the dielectric constant at high frequencies, ϵ_∞, contributed entirely by solvent dipoles, was proportional to the concentration of these dipoles.[15] Then

$$\epsilon_\infty - 1 = (\epsilon^0 - 1)(1 - v g/1000)$$

where v is the volume of water (in cc.) displaced by one gram of anhydrous protein. This yields

$$\Delta\epsilon_\infty/g = -\frac{\epsilon^0 - 1}{1000} v; \quad v = \frac{1000}{\epsilon^0 - 1}\left(-\Delta\epsilon_\infty/g\right)$$

and $1000/(\epsilon^0 - 1)$ for water at 25° is 12.9. We can set $v = v_p + w/\rho_0$, where ρ_0 is the density of the solvent, v_p is the partial specific volume of the anhydrous protein, and w the number of grams of water which appear to be associated with each gram of anhydrous protein. Then

$$w = \left[\frac{1000}{\epsilon^0 - 1}\left(-\frac{\Delta\epsilon_\infty}{g}\right) - v\right]\rho_0$$

and the partial specific volume of the hydrated protein

$$v'_p = (v_p + w/\rho_0)/(1 + w) = v/(1 + w)$$

Taking $\Delta\epsilon_\infty/g = -0.11$ and $v_p = 0.75$, we obtain w as about 0.6, found to be −0.11 for carboxyhemoglobin.[37] The value so obtained is 500 Debye units, agreeing well with the value calculated above. Although there is a considerable amount of uncertainty in this calculation, it seems to be a much better estimate than the low figure of about 25 Debye units obtained by application of equation (6a) with $\alpha = 0.149$. Errera calculated a value of about 50 Debye units in still another manner.

Relaxation Time.—The dielectric increments recorded in the last two columns of Table II demonstrate that within the experimental accuracy of the method (about ±0.3% in ϵ) the observed values are calculatable by equation (7d) where $\Delta\epsilon_0/g = 0.33$, $\Delta\epsilon_\infty/g = -0.11$, and $\nu_c = 1.9$ megacycles. These results are reproduced graphically in Fig. 5, the curve representing the value calculated by equation (7d). The critical frequency, $\nu_c = 1.9$ megacycles, corresponds to a relaxation time of $\tau = 8.4 \times 10^{-8}$ sec., and a rotary diffusion constant Θ of 6.0×10^6 sec.$^{-1}$ (calculated from equation (7b)). It can be compared with the value 2.1 megacycles calculated for a protein of molecular weight 66,700 and a specific volume (of hydrated protein) of 0.81[44] if we assume a shape factor, Ψ, of unity.

In closing I wish to express my indebtedness to Professor E. J. Cohn for his interest and aid throughout this investigation, to Mr. R. F. Fields and Dr. D. B. Sinclair of the General Radio Company for many helpful discussions regarding modifications of the bridge used, to Dr. J. D. Ferry and Professor J. Wyman for their collaboration in making certain of the measurements, to Professor George Scatchard for the use of his cell and for many helpful suggestions, and to Professor R. M. Ferry and Mrs. E. S. Newman for the preparation of the hemoglobin used in this work.

Summary

The dielectric behavior of protein solutions is discussed from a partially theoretical and partially empirical basis. It is found that there are three important quantities necessary for the description of this behavior: (1) the dielectric increment at low frequencies, $\Delta\epsilon_0/g$, which depends upon the difference in polarity between the protein and the solvent molecules; (2) the dielectric

larger but of the same order of magnitude as that obtained by Adair [*Proc. Roy. Soc.* (London) **B120**, 422 (1936)], Sørensen [*Compt. rend., trav. lab. Carlsberg*, **19**, No. 11 (1933)], and others (0.2 − 0.4) under somewhat different conditions.

(44) Adair and Adair, *Proc. Roy. Soc.* (London), **B120**, 422 (1936).

increment at high frequencies, $\Delta\epsilon_\infty/g$, which depends chiefly upon the volume and hydration of the protein molecules; (3) the critical frequency, ν_c, which depends chiefly upon the size and shape of the molecules and upon the viscosity of the solvent.

Methods are presented for the determination of these quantities by measurements of the dielectric constants of these solutions with a radio frequency bridge. The frequency range covered at present is from 25,000 to 2,500,000 cycles per second, and solutions with specific conductivities up to about 1×10^{-4} mhos/cm. can be studied.

Measurements upon a series of solutions of crystallized carboxyhemoglobin have yielded consistent and reproducible results, and give $\Delta\epsilon_0/g = 0.33$, $\Delta\epsilon_\infty/g = -0.11$, and $\nu_c = 1.9 \times 10^6$ cycles/sec., interpreted as indicating a dipole moment for the carboxyhemoglobin molecule of about 500 Debye units, on the basis of an orienting dipole with a molecular weight of 66,700. The method is now being applied to a number of other proteins.

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Studies of the Dielectric Properties of Protein Solutions. II. The Water-Soluble Proteins of Normal Horse Serum[1,2]

BY JOHN D. FERRY AND J. L. ONCLEY

The dielectric constant of a solution of dipolar ions affords an estimate of the dipole moment of the solute, and is an important quantity in the interpretation of the interaction of such a solute with electrolytes and other dipolar ions.[3] Studies of the dielectric constants of solutions of amino acids, peptides, and other dipolar ions of low molecular weight[4] have contributed greatly to knowledge of the physical chemistry of these substances. A method recently described[5] now makes it possible conveniently to measure the dielectric constants of electrolyte-free solutions of proteins over a wide range of frequencies. Data for these multipolar ions of colloidal dimensions may be interpreted by considerations similar to those which hold for the smaller dipolar ions. In addition, from the dispersion of the dielectric constant may be calculated the relaxation time of a protein in solution, which is related to the shape and size of the molecule. Studies with the well-characterized protein, carboxyhemoglobin, already have been reported. The present paper extends this work to certain of the proteins of blood serum.

The protein components of serum usually have been separated by fractional precipitation from concentrated salt solutions. They have been characterized by their chemical compositions, molecular weights, isoelectric points, acid and base binding capacities, and cataphoretic mobilities, and by various physical properties of their solutions.

Albumins.—The albumins of serum are crystallized readily from ammonium sulfate solutions of concentration more than half saturated. Their molecular weight, as determined by osmotic pressure measurements in a variety of solvents, is about 73,000;[6,7] and ultracentrifugal studies, which give almost the same molecular weight, have shown that, whether several times recrystallized or not, they are essentially monodisperse.[8] Although the serum albumins have the same molecular weight, they can be separated by fractional crystallization into portions of widely different solubilities in concentrated salt solutions.[9] The chemical compositions of such fractions also have been shown to differ considerably with respect to content of carbohydrate and certain amino acids.[10]

(1) A preliminary report of this investigation was presented to the Division of Physical and Inorganic Chemistry at the 94th Meeting of the American Chemical Society, September 6–10, 1937.

(2) This investigation has been supported in part by grants from the Committee of the Permanent Charity Fund, Inc., and from the Farnsworth Fund, Harvard Medical School.

(3) Cohn, *Chem. Rev.*, **19**, 241 (1936).

(4) Wyman, *ibid.*, **19**, 213 (1936).

(5) Oncley, THIS JOURNAL, **60**, 1115 (1938).

(6) Adair and Robinson, *Biochem. J.*, **24**, 1864 (1930).

(7) Burk, *J. Biol. Chem.*, **98**, 353 (1932).

(8) (a) Svedberg and Sjögren, THIS JOURNAL, **50**, 3318 (1928); (b) von Mutzenbecher, *Biochem. Z.*, **266**, 250 (1933); (c) McFarlane, *Biochem. J.*, **29**, 407, 660, 1209 (1935).

(9) Sörensen, *Compt. rend. trav. lab. Carlsberg*, **18**, No. 5 (1930).

(10) Hewitt, *Biochem. J.*, **30**, 2229 (1936); **31**, 360 (1937).