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DIELECTRIC CONSTANTS OF PROTEINS

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ABSTRACT. The dielectric constant data of the aqueous solutions of carboxyhaemoglobin, albumins, zein gliadin, myoglobin etc., have been used to calculate dipole moments from the new equation $(\epsilon - n^2)M/d = 4\pi N\mu^2/kT$ The moments previously evaluated by Oncley using an empirical equation are systematically higher than that evaluated by the new equation.

Molecular radii of these proteins have been evaluated using the new expression for the dispersion of polar binary mixtures. The identity of Oncley's expression to calculate the critical frequency f ; $\epsilon'_{12} = \epsilon_{1(0)} + (\epsilon_{1(0)} - \epsilon_{1(\infty)})/(1 + f^2/f_c^2)$ and hence the molecular radius has been shown to be due to fortuitous coincidence arising out of certain approximations which hold good in dilute aqueous solutions

The molecular radii (a) evaluated from dispersion data on the basis of the simple picture of the huge molecule as a rotating sphere, and characterised by a single relaxation time are related to the dipole moment (μ) by the relation $\mu = 2a \times e$ (where e is the electronic charge) being actually the moment of a huge dipole with the dipolar distance equal to the diameter of the spherical cavity scooped out by the rotating giant molecule.

INTRODUCTION

The dielectric constants of the aqueous solutions of a number of proteins ; carboxyhaemoglobin, albumins, zein, gliadin, myoglobin and etc., have been investigated by a number of workers. Oncley (1938) calculated the dipole moment of the dissolved protein molecules, using a semi empirical relationship based on Wyman's empirical equation. The dispersion data on the proteins have also been utilized to determine the relaxation time by using the theoretical expression of Perrin (1934). The ratios of the axes of an assumed molecular ellipsoid have been derived from Perrin's theory for pseudoglobulin gliadin and other proteins which do not conform to the simple picture of the molecule considered as a rotating sphere.

In the present paper, dipole moments of these protein molecules have been calculated by the application of the new equation $(\epsilon - n^2)M/d = 4\pi N\mu^2/kT$ to the dielectric constant data on aqueous solutions. The moments so obtained have been discussed in relationship with the molecular radius which has been calculated by applying the new relationship to dispersion and absorption data of these proteins.

The case of dispersion of binary mixtures in which both the components are polar is depicted in Fig. 1.

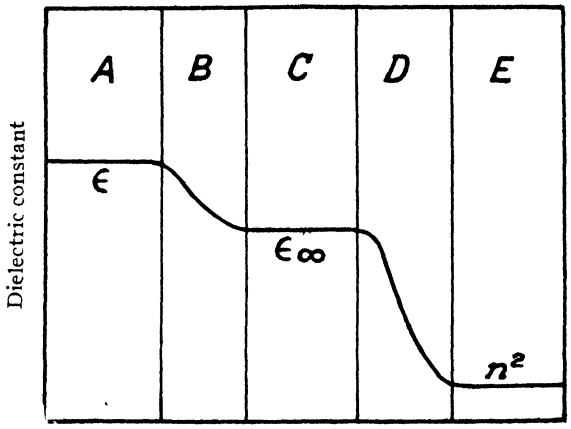


FIG. 1

In region 'A' at low frequencies, the orienting torque is sufficient to overcome all frictional forces so that the molecules of both the components are completely oriented. Consequently this region is characterised by a high dielectric constant. In region B the frictional forces of the molecule of higher relaxation time are considerable and the dispersion is characterised by a gradual decline of the dielectric constant. In region C the component of higher relaxation time has completely ceased to respond to the field and thus contributes very little to the dielectric constant although the other component is still completely able to follow the field. Thus in this region the dielectric constant has a constant intermediate value ϵ_∞. The region 'D' which corresponds to the dispersion of the second component of lower relaxation time is characterised by a decline of the dielectric constant. Finally the region E where neither of the two components is orienting, is characterised by a low dielectric constant, equal to the square of refractive index. With this picture in mind and considering that the dispersion of the dissolved protein molecule is completely defined by the region ABC, Oncley (1938) derived an equation :

$$\mu^2 = (p_0 - p_\infty)9kT/4\pi n \qquad \ldots \quad (1)$$

where p_0 and p_∞, are the polarisations per c.c. of the solution in the low frequency region (region A) and the high frequency region (region C) respectively and n, the number of polar molecules per c.c. being equal to $gN/1000M$ where g=gms. per liter of the solute of molecular weight M.

Using, for polarisation per c.c. an equation of the type $p=(\epsilon-a)/b$, suggested by Wyman (1936) the equation (1) becomes

$$\mu^2 = 9000\ kTM(\epsilon_0 - \epsilon_\infty)/4\pi Ngb$$

or $$\mu = \alpha\sqrt{M}(\epsilon_0 - \epsilon_\infty)g \quad \text{where} \quad \alpha = \sqrt{\frac{9000\ kT}{4\pi Nb}}$$

α was evaluated as 2.9 by assuming for glycine the established value of μ in the above equation. With this value of 'α' the moments of the proteins have been calculated since the factor $(\epsilon_0 - \epsilon_\infty)/g$ is experimentally observed as the sum of the low frequency and high frequency dielectric increments

For the dielectric behaviour in the dispersion region (B C in figure 1) Oncley has followed Debye's treatment in its most general form (replacing the D.C.M. polarisation law $\frac{(\epsilon-1)}{(\epsilon+2)}$ by $\frac{\epsilon-a}{b}$ and gets for ϵ' the dielectric constant at γ frequency the expression $\epsilon' = \epsilon_\infty + \frac{(\epsilon_0 - \epsilon_\infty)}{1+\gamma^2/\gamma_c^2}$, γ_c being the critical frequency. The critical frequency is given by $2\pi\gamma_c\tau = 1$ where τ is the relaxation time.

In this paper a simple extension of the new equation

$$\frac{(\epsilon - n^2)M}{d} = \frac{4\pi N\mu^2}{kT}$$

to binary systems has been utilized to calculate the moment. In actual calculations two approximations have been made; (1) density of solutions$\simeq$ density of solvent$\simeq$1, as the solutions studied are very dilute; (2) P_E the electronic polarisation is not taken into account since it becomes absolutely negligible when compared with the enormous values for P_2 the total polarisation of the solute.

TABLE I

	M. W.	$f_2 \times 10^6$	$P_2 \times 10^{-4}$	μ new	μ lit
Myoglobin [Marcy and Wyman (1942)]	17000	0 249	333	131	163
Zein [Elliot and Williams (1939)]	40000	2 917	2364	356	400
Gliedin [Entrikin (1941)]	40000	9 365	336	130	177
Carboxyhaemoglobin [Oncley (1938)] ..	68000	3 555	2785	386	470' 500
Albumin (1) } [Ferry and	73000	7.993	1275	262	280
Albumin (2) } Oncley (1938)] } ...	73000	5.471	3327	422	510
Pseudoglobulin [Ferry and Oncley (1938)] ..	150000	9.919	15230	903	1000' 1200
Edestin [Cohn and Edsall (1935)] ...	300000	...	...	1080*	1400

* Calculated by an approximate relation

$$P_2 = \frac{\Delta\epsilon}{\omega_2} = \frac{4\pi M\mu^2}{kT},$$

$\Delta\epsilon'\omega_2$ being the dielectric increment per gram. This relationship follows from the new relationship applied for solutions

$$p_{12} = \frac{\epsilon_{12}-1}{d_{12}} = \frac{(\epsilon_1 - 1)\omega_1}{d_1} + p_2\omega_2$$

when the approximation $d_{12} \simeq d_1 \simeq 1$ and $\omega_1 \simeq 1$ is made.

'e'=moments calculated by Cohn's (1935) relationship, $\mu=4.77\times10^{-10}\sqrt{\delta/2.3}$, where δ=dielectric increment per mole. The other values in the column μ_{ob} are calculated by Oncley's equation, $\mu=2.9\sqrt{\frac{M\Delta\epsilon}{g}}$, where, $\frac{\Delta\epsilon}{g}$=dielectric increment per gm.

From the observed critical frequencies the relaxation time of the proteins have been calculated by $\tau=\frac{1}{2\pi\gamma_c}$ (this relation being the result of applying the new equation to the dispersion theories). Molecular radii 'a' have been calculated by using Debye's expression $\tau=\frac{4\pi\eta a^3}{kT}$ based on the consideration of the molecule as a sphere rotating in a viscous medium of viscosity η. The results are tabulated in Table I.

TABLE II

Protein	γ_c (m.c.s)	$\tau\times10^8$	'a' Å	2 a c	μ new
Myoglobin	5.54	2.87	21.3	203	163
Zein	...	30.0	37.5	358	358
Gliadin *	...	43.5	18.4	177	130
Carboxyhaemoglobin	1.9	8.4	31.1	300	386
Albumin	0.85	19.0	41.1	392	422
Pseudoglobulin	0.24	66.1	62.2	600	900† 800 700

* τ was calculated from the relation $\epsilon'=\epsilon_\infty+\frac{(\epsilon_0-\epsilon_\infty)}{1+\omega^2\tau^2}$. In other cases $\tau=\frac{1}{2\pi\gamma_c}$.

† With increasing concentration.

DISCUSSION

It is necessary at the very outset to deal with the semi-empirical equation of Oncley which has been widely used to calculate the moment of the proteins. Assuming that the dispersion is defined by the region ABC (Fig. 1) he writes

$$p_2=\frac{4\pi N\mu^2}{9kT}\frac{g}{1000}=\frac{(\epsilon_0-\epsilon_\infty)}{b}$$

where 'b' is the constant in Wyman's relation $p=\frac{(\epsilon-a)}{b}$. A rigorous

application of Wyman's equation to binary system would give

$$\frac{(\epsilon_{12}-a)}{bd_{12}} = \frac{(\epsilon_1 - a)}{bd_1}\omega_1 + p_2\omega_2$$

ω_1 and ω_2 being weight fractions. Since in dilute solutions $d_{12}\simeq d_1\simeq 1$

$$p_2 = \frac{4\pi N\mu^2}{9kT} = \frac{(\epsilon_{12}-\epsilon_1)}{b}\omega_2 = \frac{(\epsilon_{1\infty}-\epsilon_1)}{b}\frac{g}{1000}$$

This is very nearly the same as Oncley's relation except that he writes ϵ_∞ (corresponding to region C in Fig. 1) in place of ϵ_1 the dielectric constant of solvent. It is found in experiments however that $\epsilon_\infty \simeq \epsilon_1$. Oncley's equation suffers from the disadvantage that it is based on Wyman's relation which is purely empirical.

Similarly in the dispersion case the relation $\epsilon' = \epsilon_\infty + \frac{(\epsilon_0-\epsilon_\infty)}{1+\gamma^2/\gamma_c^2}$ which has been derived again on the basis of Wyman's polarisation as applied to the region of dispersion ABC (Fig. 1), follows only under the assumption $d_{12}\simeq d_1\simeq 1$ and $\omega_1\simeq 1$. It is strange that Oncley does not mention this approximation.

An expression for the dispersion of a binary system of two polar components, has been derived in a previous paper on the basis of the new equation $(\epsilon-n^2)M/d = 4\pi N\mu^2/kT$ which has been found applicable to polar liquids and solids.

According to it ϵ'_{12}, the dielectric constant of the solution at 'ω' frequency is given by

$$\epsilon'_{12} = \epsilon_{12(\infty)} + \frac{(\epsilon_{1(0)}-\epsilon_{1(\infty)})}{1+\omega^2\tau_1^2}\frac{\omega_1}{d_1}d_{12} + \frac{(\epsilon_{2(0)}-\epsilon_{2(\infty)})}{1+\omega^2\tau_2^2}\frac{\omega_2}{d_2}d_{12}$$

$$= \epsilon_{12(\infty)} + \frac{(\epsilon_{1(0)}-\epsilon_{1(\infty)})}{1+\omega^2\tau_1^2}\frac{\omega_1}{d_1}d_{12} + \left(\frac{d_{12}}{1+\omega^2\tau_2^2}\right)\left(\frac{(\epsilon_{12(0)}-\epsilon_{12(\infty)})}{d_{12}} - (\epsilon_{1(0)}-\epsilon_{1(\infty)})\frac{\omega_1}{d_1}\right)$$

where τ_1 and τ_2 are the relaxation times and $\epsilon_{1(0)}$ and $\epsilon_{2(0)}$ the static dielectric constants of the components of the binary mixture.

Coming to the specific case of the dispersion of the dilute aqueous solutions of proteins the following approximations could be made—

(1) $\omega\tau_1 \simeq 0$ since in the region in which the measurements are done ω and τ_1 the relaxation time of solvent (water) are individually so small that their product becomes $<< 1$. $\omega\tau_1$ becomes considerable only at higher frequencies when the dispersion of water starts.

(2) In aqueous dilute solutions $d_{12} \simeq d_1 \simeq 1$ and $\omega_1 \simeq 1$

Making these approximations

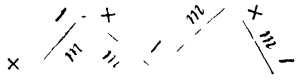
$$\epsilon'_{12} = \epsilon_{1(0)} + \frac{(\epsilon_{12(0)}-\epsilon_{1(0)})}{1+\gamma^2/\gamma_c^2}$$

This equation is identical with the one derived by Oncley, except that in place of $\epsilon_{1(0)}$ he writes ϵ_∞ which is found experimentally very nearly equal to $\epsilon_{1(0)}$. Thus Oncley's expression has fortuitously turned out to be a particular case, involving the above-mentioned approximations, of the most general equation of dispersion obtained by the application of the new equation. In general it is found that the moments calculated by the new equation are of the same order as obtained by Oncley's relationship. The latter values are systematically higher.

The calculations made for pseudoglobulin have to be considered with a certain amount of caution since this was a singular case amongst the proteins which showed marked deviations from the expected linearity of the dielectric constant with concentration in dilute regions. It shows a rapidly declining value of dielectric increment with increasing concentration, Ferry and Oncley actually took an extrapolated value of the increment for calculating the moment. In the case of gliadin, which has been studied in water-alcohol systems, the new equation was applied in the form usually written for a binary system except that for M_1 the molecular weight of the solvent the expression $M_a f_a + M_w f_w$ was used, where M_a, M_w, f_a and f_w are the molecular weights and molefractions of the alcohol and water respectively. It is of interest to note that there has been considerable controversy regarding the value of the molecular weight of gliadin. An ultracentrifugal study of gliadin conducted by Svedberg and Krejci (1935) has revealed that the protein is homogeneous with respect to molecular weight. According to them there is probably a mixture of whole and half molecules of weight 34500 and 17250 at 20°C while at higher temperatures the dissociation into half molecules is complete. The diffusion constants given by Lamm and Polson (1936) reveal a molecular weight of 27000 for the predominant constituent. Arrhenius (1937) estimates the molecular weight as 27000. On the other hand accurate determinations of the amount of certain amino acids in gliadin give a molecular weight 42000, and osmotic pressure measurements in concentrated urea and in water give values ranging from 40000 to 44000. A value of 40000 has been assumed in calculating the moment by the new equation. The assumption of the other value 27000 would reduce the moment by 20 per cent.

No attempts have been made so far to interpret the dipole moments of these proteins on the basis of molecular structure. A globular protein is now best considered as a coiled polypeptide chain. Very little is known as to how these peptides of a large variety of amino acids are arranged with respect to one another. In view of this it is not surprising that the dipole moments have not been quantitatively studied in the light of the structure of the molecules. However, Marcy and Wyman (1942) have attempted to make a comparative study of the structure of the proteins, haemoglobin and myoglobin since both these are made up of 'heme' units. Myoglobin is known to contain one heme while haemoglobin contains 4 hemes. The authors point

out that if there were a considerable degree of stabilization of these heme units with electric moments parallel and pointing in the same direction the polarisation and hence the dielectric increment per gram of haemoglobin should be greater than that of myoglobin. In the limiting case of complete parallel alignment the increase should be four-fold. On the other hand if the 4 units were oriented independently the increment would be the same for both. The experimentally observed fact, *viz.*, that haemoglobin has a dielectric increment twice as great as that of myoglobin suggests that in haemoglobin there is a "partial stabilization" of these units with their electric moments parallel. In this discussion it is obvious that Marcy and Wyman have assumed that the increment of dielectric constant gives a measure of the dipole moment whereas it is the dielectric increment per gram *multiplied by the molecular weight* that gives a measure of the polarisation (and hence of the square of the moment). The moments calculated by the new equation shows that haemoglobin has nearly thrice the moment of myoglobin. This can be explained on the basis of a zigzag arrangement of the 4 hemes

(assuming a mutual tetrahedral angle) 

whence if 'm' is the moment of each heme the resultant of the 4 hemes will be equal to the result of two moments of magnitude 2 m at 71° *i.e.*, 3.1 m. or nearly 3 times the moment of a heme.

In Table II, are recorded the values for the critical frequency γ_c; the time of relaxation $\tau = \frac{1}{2\pi\gamma_c}$ and finally the molecular radius 'a' calculated as $\sqrt[3]{\frac{\tau k'T}{4\pi\eta}}$ based on the assumption that the molecules are rotating spheres and are characterised by a single unique value of relaxation time. It has been postulated by Perrin that an ellipsoidal molecule could be characterised by at least two relaxation times. The main reason for abandoning the idea of a single relaxation time in favour of multiple times of relaxations seems to be that the calculated curves for dispersion, based on a single τ do not fit in with the experimental data. It may be pointed out here that the data for carboxyhaemoglobin, myoglobin and albumin are calculable on the basis of a single relaxation time. Even in the case of pseudoglobulin the deviation between the theoretical and experimental curves is indeed very small, in fact too small to warrant the abandonment of the postulate of a single unique value of relaxation time. Further, it is to be pointed out that the postulate of two critical frequencies is not justified or confirmed by any marked breaks or irregularities in the dispersion curves which for instance in the case of gliadin and zein are smooth, asymptotic thus characteristic of molecules having a single relaxation time. It has, in fact, been found necessary to calculate

the values of the two critical frequencies by resolving the experimental dispersion curve into two hypothetical dispersion curves. The molecular radii (a) that have been calculated on the basis of the simple picture of the molecule as a rotating sphere and characterised by a single τ show a close relationship with the dipole moment 'μ' previously calculated from static dielectric constants using the new equation. Table II, shows that the product $2a \times e$ (where 'e'=electronic charge) is equal to the dipole moment. Considering the limitations involved in dispersion measurements at the low frequencies, the agreement should be considered very satisfactory. The rather large deviation in gliadin may be due to the fact that the relaxation time which has been calculated in this case by the equation $\epsilon' = \epsilon_\infty + \frac{(\epsilon_0 - \epsilon_\infty)}{1 + (2\pi\nu\tau)^2}$ is subject to large errors since ϵ', ϵ_0 and ϵ_∞ are of very nearly the same magnitude so that the factors $(\epsilon' - \epsilon_\infty)$ and $(\epsilon_0 - \epsilon_\infty)$ magnify any small error in measuring ϵ', ϵ_0 and ϵ_∞. The quantity $2a \times e$ is actually the moment of a huge dipole with the dipolar distance equal to the diameter of the spherical cavity scooped out by the rotating molecule. Thus the spherical protein molecule can be considered to be from the electrical point of view, equivalent to a dipole along its diameter.

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