Journal of the Nigerian Association of Mathematical Physics
Volume 10 (November 2006), **15 - -20**

Theoretical model analysis of molecular orientations in liquid protein dielectrics

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Abstract

In this study, some theoretical model functions have been used to explain the molecular behaviour of four different types of proteins; human haemoglobin, Insulin, egg-white lysozyme and β - globulin molecules in solution. The results of the computational fitting procedures showed that the dielectric dispersion of the protein molecules generally followed the Debye and Cole-Cole functions. The dielectric parameters obtained from the dispersions, relating to the structural and electrical properties of the molecules were tabulated. The relationships between the dispersion amplitude Δ and the molecular dipole moment μ of the proteins and also between the relaxation time τ and the energy of activation ΔH of the molecules have been highlighted. The molecular interpretation of the polarization effects responsible for the dielectric dispersions have been discussed.

1.0 Introduction

It is well known that the type of material between the plates of a capacitor increases its capacitance by a factor called the dielectric constant or relative permittivity. Most substances used for this purpose have a dielectric constant lying between 2 and 10 and are good insulators. Such materials are termed non-polar because their constituent molecules do not bear a permanent dipole moment capable of rotating in an electric field. This implies that the distribution of both positive and negative charges on the molecule is symmetrical with respect to the centre of the molecule and therefore there is no dipole moment. If however, the distribution of charge on the molecule is asymmetrical, the molecule bears a permanent dipole, i.e. it becomes polar. Owing to the interaction of the dipole moment with an electric field, a polar substance has a dielectric constant which is larger than that of a non-polar material.

Previous research works in this area [1,2,3,4] have shown that the dielectric constant of a polar molecule is strongly dependent on various physical parameters such as the temperature, pressure and frequency of the applied electric field, whereas the dielectric properties of a non-polar material are independent of them.

The accuracy with which theoretical functions fit the experimental dielectric relaxation data is of considerable importance in the development of adequate molecular orientation models in liquid dielectrics. Using this technique, the dielectric behaviour of a wide range of aqueous protein solutions has been studied over a frequency region extending from a few kilohertz to tens of gigahertz. The dielectric behaviour of a number of those molecules has been represented analytically, either in terms of the single Debye equation, involving only one relaxation time and a semi circular locus in the complex dielectric plane, or in terms of the equation due to Cole and Cole [5] which is an arc of a circle in the complex plane and involves a logarithmically symmetrical distribution of relaxation times about a most probable value. A third representation proposed by Cole and Davidson [6] corresponds to an asymmetrical distribution of relaxation times and involves a skewed-arc locus.

Most protein molecules exist in solution in their natural environment. Their molecular behaviour can therefore be studied directly in solution. However, crystalline protein molecules, especially in the powdered form can also be studied by dissolving them in appropriate solvents. Most of them are found to be readily soluble in water at room temperature and normal pH, while some are soluble only at acidic pH, and others are soluble in other liquids such as propylene glycol and ethylene glycol. The purpose of this work is to describe some theoretical functions used in elucidating the behaviour of protein molecules such as insulin, lysozyme, β-globulin and haemoglobin in solution. The importance of the work is to understand the basic dielectric behaviour of such molecules in their natural liquid environment.

2.0 Experimental consideration

Three methods of sample preparation were used in this study. The haemoglobin protein solutions studied were prepared directly from fresh human blood obtained from subjects with haemoglobin genotype AA, SS and AS using the method of Laogun et al [7,8]. The protein concentration in each solution was diluted to 5%.

The second batch of protein solutions studied was egg-white lysozyme and gamma globulin which are soluble in water. The solutions were prepared from powdered egg-white lysozyme [Grade 1; Lot 102F -8075] and gamma-globulin [Chon Fraction 2] purchased from Sigma Chemical Company, UK. The samples were prepared by dissolving an appropriate amount of the powder in distilled water to produce 5%, 10%, 20% and 30% solution concentration. A solution of the insulin powder which was not soluble in water were prepared from an acid mixture of propylene glycol which readily dissolved the insulin at a pH of around 3 samples were also prepared in different concentrations of insulin and propylene glycol respectively, using the method of Laogun and Sheppard [9]. The dielectric measurements were carried out on each sample using experimental techniques which had been described previously [10]. Relative permittivity ε' and the loss factor ε" were measured using a series of dielectric bridges involving a Boonton's 75C Capacitance Bridge, a Wayne Kerr B201 Bridge and a Marconi dielectric bridge working over the frequency range from around 10 KHz to 100 MHz. The measured parameters ε' and ε" generally have an error of less than ±5%. The parameters were later fitted to relevant mathematical dielectric functions using a least square curve fitting routine [11], depending on the assumed shape of the molecule rotating in the applied electric field.

3.0 Theoretical analysis of dielectric data

The initial approach used was that of Peter Debye who considered protein molecules in solution as spheroids of revolution under an applied electric field. Thus, their behaviour is often explained using the Debye function [12] given by $\varepsilon^*(\omega) = \varepsilon_\circ[\varepsilon' - j\varepsilon''] = \left[\varepsilon_\infty + \Delta\int \varphi(t)e^{-j\omega t}dt\right]$. The real and imaginary parts of the function are given by: $\varepsilon' = \varepsilon_\infty + \dfrac{\Delta}{(1+\omega^2\tau^2)}$, $\varepsilon'' = \dfrac{\sigma-\sigma_\circ}{\omega} = \dfrac{\Delta\omega\tau}{1+\omega^2\tau^2}$ where $\Delta = \varepsilon_s - \varepsilon_\infty$ with the subscripts s and ∞ indicating the limit of ε' at very low and high frequencies respectively. τ is the relaxation time and ω is the angular frequency.

The real and imaginary components of the general Debye function gives a parametric equation of a circle in the $\varepsilon'' - \varepsilon'$ plane. On eliminating the parameter $\omega\tau$ and rearranging, one obtains $\left(\varepsilon' - \dfrac{(\varepsilon_s+\varepsilon_\infty)}{2}\right)^2 + \varepsilon''^2 = \left(\dfrac{\varepsilon_s+\varepsilon_\infty}{2}\right)^2$. This is the equation of a circle with centre, $\left(\left(\dfrac{\varepsilon_s+\varepsilon_\infty}{2}\right), 0\right)$ and radius $\dfrac{\varepsilon_s-\varepsilon_\infty}{2} = \dfrac{\Delta}{2}$. Therefore, for many simple liquids, a plot of ε'' against ε' in the argand diagram lies on a semicircle.

Certain protein molecules are made up of long-chain amino acids. Such long-chain molecules and polymers show broader dispersion curves and lower maximum dielectric loss than would be expected for a single Debye dispersion. To account for the expected distribution of relaxation times in such molecules, Cole and Cole [5] modified the Debye function to the form:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1+\left(\dfrac{jf}{f_R}\right)^{1-\alpha}} - \frac{j\sigma_\circ}{2\pi f\varepsilon_\circ}$$

where α is the spread parameter for the relaxation times ($0 < \alpha \leq 1$). For a single Debye relaxation α = 0, while for an infinite distribution of relaxation times, α = 1. Unlike the semicircle argand diagram of the Debye dispersion, the Cole-Cole function gives an arc which is symmetrical about a line through the centre of the circle parallel to the ε'' axis.

Certain materials are found to exhibit neither the Debye nor the Cole-Cole symmetry. Thus, for such liquids the plot on the $\varepsilon'' - \varepsilon'$ argand plane gives a skewed arc. The function for the skewed arc locus which

corresponds to an asymmetrical distribution of relaxation times was proposed by Cole and Davidson [5, 13], given by: $\frac{\varepsilon^* - \varepsilon_\infty}{\varepsilon_\circ - \varepsilon_\infty} = \frac{1}{(1+ j\omega\tau)^\alpha}$ where α is again the spread parameter ($0 < \alpha < 1$).

Cole and Davidson found that for certain materials such as glycerol and propanediol, the plot of ε'' -ε' exhibited a skewed arc. It is worthy of note that in our studies, none of the samples used exhibited the Cole-Davidson skewed dispersion behaviour. As shown in figure 1, the $\varepsilon'' - \varepsilon'$ plot of our haemoglobin (*Hb.*) data shows that the loci for the Hb AA and Hb SS samples followed the Debye and the Cole-Cole interpretations only.

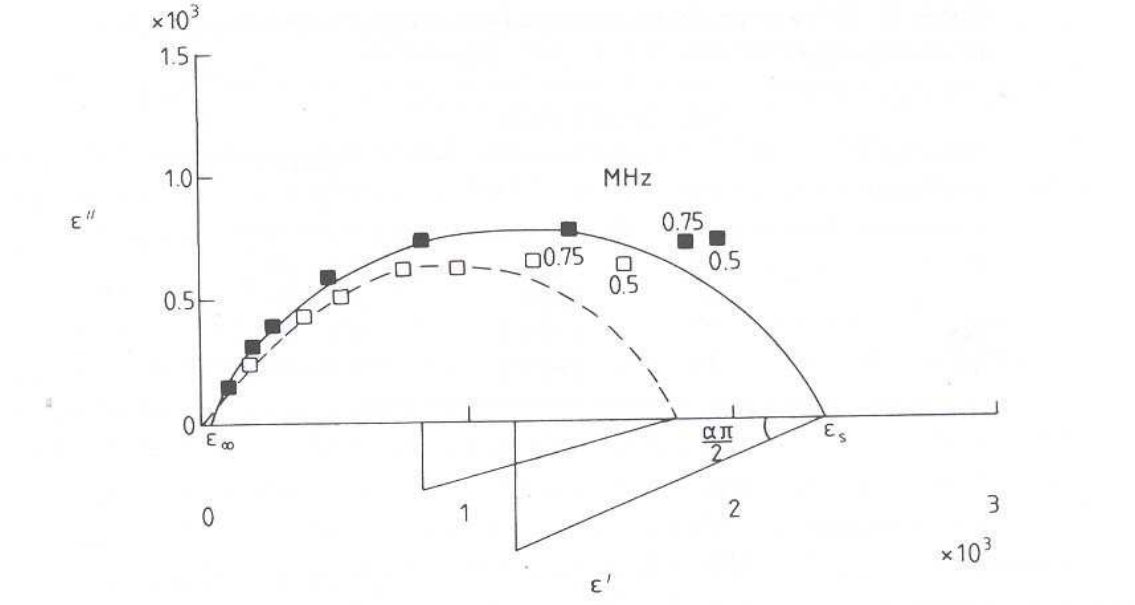

Figure 1: The ($\varepsilon'' - \varepsilon'$) plot for haemoglobin (Hb) AA and Hb SS (open)

For the haemoglobin data, we also plotted ε" against frequency, f and (ε_s-ε')/($\varepsilon - \varepsilon_\infty$) against ω^2 to obtain the dispersion amplitude Δ, τ and α. Table 1 shows the computer fitted values of the dispersion parameters for both the Debye and Cole-Cole model functions.

Table 1: Computer fitted values of ε_s, ε_∞, dispersion amplitude Δ and relaxation time τ for the single Debye and Cole-Cole dispersions of human haemoglobin (Hb) AA (adult haemoglobin), AS (sickle cell haemoglobin trait) and SS (abnormal sickle cell haemoglobin) in solution.

Haemo-globin Genotype	Fitted dielectric parameters								
	ε_s		ε_∞		Δ		α	τ x 10^{-7} s	
	Debye	Cole-Cole	Debye	Cole-Cole	Debye	Cole-Cole	Cole-Cole	Debye	Cole-Cole
AA	2351	2330	80	40	2271	2290	0.26	1.9	0.80
AS	2311	2260	75	40	2236.5	2220	0.23	2.1	0.80
SS	1863	1760	65	30	1798.2	1730	0.18	2.5	1.33

The data for egg-white lysozyme was used to investigate changes in the dielectric parameters at different solution pH using both the Debye and Cole-Cole model functions. Table 2 shows the computer fitted dielectric parameters for a 5% aqueous solution of egg-white lysozyme at a pH of 3.5, 7.0 and 11.0 and a temperature of 20^0C . The dielectric dispersion of the lysozyme samples, at each pH level, showed that the relaxation times for the Debye function was shorter than that of Cole-Cole, due to the longer relaxation amplitudes of the Cole-Cole model.

Table 2: Computer–fitted dielectric parameters for aqueous solutions of egg white lysozyme at a temperature of 20^0C.

pH	Model function	ε_∞	Δ ($\varepsilon_s - \varepsilon_\infty$)	τ x 10^{-7}sec.	α	δ_0 mSm^{-1}
3.5	Debye	77.1 ± 2.7	54.8 ± 3.0	1.13 ± 0.07	-	1.64 ± 0.06
	Cole-Cole	75.9 ± 1.6	63.2 ± 3.5	1.22 ± 0.06	0.06 ± 0.02	1.61 ± 0.06
7.0	Debye	83.6 ± 6.1	127.7 ± 7.6	1.36 ± 0.13	-	1.36 ± 0.13
	Cole-Cole	70.5 ± 3.8	179.5 ± 8.1	1.89 ± 0.15	0.20 ± 0.05	1.89 = 0.15
11.0	Debye	94.3 ± 7.1	211.4 ± 15.0	1.79 ± 0.12	-	1.82 ± 0.07
	Cole-Cole	69.5 ± 6.8	382.4 ± 10.2	2.49 ± 0.19	0.34 ± 0.02	1.76 ± 0.05

Table 3 shows the computer–fitted parameters obtained from the dispersion of different concentrations of gamma-globulin protein molecules at a temperature of 25^0C. It would be observed that the root-mean-square errors (RMSEs) for the Cole-Cole model were generally much lower than the Debye, showing that the Cole-Cole was a more superior model for the analysis. The values of the parameters quoted in the table are therefore from the Cole-Cole fit.

Table 3: Variation of the dielectric dispersion parameters Δ , τ , α and the model root-mean-square errors with concentration of gamma-globulin.

Concentration kg m$^-$	Δ	τ (μs)	α	RMSE	
				Cole-cole	Debye
122	106.1	3.4	0.26	0.90	3.65
102	85.7	3.1	0.33	0.96	3.64
83	71.6	2.5	0.36	0.59	3.32
64	50.3	1.7	0.35	0.50	2.50
45	33.0	1.0	0.30	0.28	1.67
25.6	19.2	0.5	0.30	0.24	1.00

4.0 Discussion

4.1 Dielectric dispersion amplitude Δ and dipole moment μ

In general the dipole moment of a protein molecule in aqueous solution is fluctuating with time as protons move to and from the ionisable sites of the molecular surface. The consideration of the dipole moment can therefore be split into two parts, first the permanent dipole moment (i.e the time or ensemble average) and secondly the mean square fluctuation dipole. The possible contributions of each of these to the dielectric dispersion has been discussed by Scheider [14] making use of the kinetic properties of the system. The permanent dipole moment may be written as $\mu = \int_v \rho(r) r d\nu$ where ρ(r) is the average volume charge density of any point r in the molecule and the integral is taken over the whole volume of the molecule, v'.

This integral may be divided into two parts, i.e , $\mu = e \sum \langle X_i \rangle r_i + \int_v \rho(r) r d\nu$ where e is the protonic charge, and x_i has the value -1, 0 or 1 according to the charge on site i. The first of these terms arises from the charged groups on the surface of the molecule, while the second term describes the charge distribution on the remaining part of the molecule of volume, v'.

Avoiding the complication of accurately obtaining molecular dipole moment using Scheider's method, Oncley [15] treated protein molecules in aqueous solution as rigid dipoles in a viscous medium. He then derived the equation relating the dipole moment μ of the protein molecules to the dielectric dispersion amplitude Δ and the relative molecular mass M using the generalised Kirkwood-Onsager theory [16,17], showing that μ is given by: *i.e* $\mu = \left[\frac{2\varepsilon_0 MRT\Delta}{N}\right]^{\frac{1}{2}} g^{-\frac{1}{2}} \frac{3}{n_p^2 + 2}$, where g is the Kirkwood correlation parameter and n_p is the Onsager internal refractive index for the protein. However, it has been the common practice to calculate protein dipole moment using the approximations g = 1, n_p = 1, in which case the function reduces to $\mu = \left[\frac{2\varepsilon_0 MKT\Delta}{N}\right]^{\frac{1}{2}}$. The basic unit is in coulomb.meter which converts to the more familiar unit of Debye (D), [1D =3.33 x 10^{-30} C-m].

From this study, the variation of the dipole moment with the dispersion amplitude of insulin molecules was investigated at different temperatures as shown in table 4. The table shows a fluctuation ithe value of μ due to the fluctuation in the dispersion amplitude with temperature. In general, it may be said from our result, that the dispersion amplitude and dipole moment of the insulin molecules are independent of temperature up to 25^0C. Their relatively high values at 40^0C may be due to increased molecular agitation at that temperature.

Table 4: Variation of dielectric dispersion amplitude and dipole moment of a solution of crystalline insulin molecules with temperature.

Temperature ^{0}C	ε_∞	Dispersion amplitude Δ	Dipole moment μ (Debye unit)
3	49.7±0.3	6.1 ± 0.1	121 ±1
10	47.3 ± 0.1	6.2 ± 0.2	124 ±2
18	44.7 ± 0.1	5.6 ± 0.4	120 ± 4
25	41.6 ± 0.1	5.2 ± 0.4	117 ±4
40	38.2 ± 0.3	6.7 ± 0.7	137 ± 7

4.2 Dipole relaxation time, τ and energy of activation, ΔH.

Dielectric relaxations are rate processes, and the relaxation time τ is the reciprocal of a mean rate coefficient, χ. Such molecular rate processes usually follow the Arrhenius temperature law. Thus, using that concept, Eyring [18] expressed the molecular rate coefficient,χ in terms of the relaxation time,τ as: $\chi = \frac{1}{\tau} = A^1 \bullet \exp\left(\frac{-\Delta H}{RT}\right)$ or $\tau = \exp\ A\left(\frac{\Delta H}{RT}\right)$ where A = $1/A^1$ is a numerical constant and ΔH is Arrhenius activation enthalpy per mole. Thus, a plot of log τ against 1/T gives:

$$\frac{d\ (\log\ \tau\ )}{d\left(\frac{1}{T}\right)} = \frac{\Delta \mathrm{H}}{2.303\ R}$$

where ΔH is in Joule mol^{-1}. The value of ΔH for water molecules is known to be equal to 16.5 kJ mol^{-1} while those of insulin and γ-globulin investigated in this study gave values of 25± 2 kJ mol^{-1} and 14.9± 2.7 kJ mol^{-1} respectively.

4.3 Molecular interpretation of the dispersion mechanisms

From the proteins we have studied, the dispersion amplitude Δ is generally proportional to the concentration of the solution. However, each individual protein exhibits its characteristic dispersion which may be attributed to some additional polarization effects arising from a number of processes. The most prominent of these include permanent dipole rotation, proton fluctuation, the Maxwell Wagner double layer mechanism and Schwartz's iron mobility effects. The permanent dipole moment theory [12, 16, and 17] assumes that protein molecules possess permanent dipole moments, which will experience an orientational force when subjected to an electric field. If an alternating electric field of increasing frequency is applied, the relative proportion of the orienting force to the viscous resistance of the solution drops with frequency, resulting in a characteristic dielectric dispersion. Debye [12] showed that a spherical molecule rotating in a viscous medium may be described by a single relaxation time. The low values of the relaxation spread parameter, α obtained in the Cole-Cole analysis of the proteins we studied indicates that the process of Debye rotation plays an important role in the dispersions observed.

The proton fluctuation theory [19] supports the existence of acidic and basic sites on the surface of protein molecules, thus making protons to continually bind and dissociate, causing the dipole moment of the molecule to fluctuate with time. It may be observed from the Kirkwood equation that the dispersion amplitude is proportional not to μ but to μ^2, showing that it is possible to obtain a dispersion even if the average dipole moment is zero, as long as there are sufficient fluctuations to provide a substantial mean square dipole moment. However, the determination of the relaxation time is usually complicated by the kinetic nature of the process. The non-zero permanent dipole moment exhibited by the samples measured in our study supports the strong contribution of proton fluctuation in the dispersions.

The Maxwell Wager effects [20, 21] occur in an inhomogeneous mixture of dielectrics owing to the build up of charges on the boundaries between the different materials. This process appears to be quite favorable in the

dispersions observed in our studies, since both the protein molecules and the solvents have their own different conductivities and permittivities. The Cole-Cole function has been found to be well-suited for describing such a complex process containing a distribution of Debye relaxation times with a non-zero spread parameter α. This therefore, is in support of the superiority of the Cole-Cole function over the Debye established from our results.

Lastly, the contribution of Schwarz's ion cloud model [22] cannot be underestimated in the dispersion processes, since the motion of the ion cloud existing around protein molecules may create some additional substantial polarization effects on the application of an electric field.

5.0 Conclusion

Some model functions have been used in describing the characteristic dielectric dispersions of four different types of proteins at the molecular level. The functions helped in unfolding the mechanisms of protein-protein and protein-solvent molecular interactions in their liquid environment. The least squares curve-fitting technique used helped in determining the appropriate functions in terms of the root-mean-square errors of fit. The dielectric parameters obtained from the fitting procedures relate to the molecular structure and electrical properties of the proteins and are of great importance in understanding their functional behaviours and also in general protein engineering.

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