CHAPTER FIVE

THEORY OF DIELECTRIC RELAXATION AND OPTICAL ROTATION DISPERSION
The following elucidates the principle of dielectric relaxation experiment. Electric polarizibility and relaxation times of molecules or side chains are normally probed through this technique. While the polarizibility essentially quantifies the electric dipole moment suitably averaged over the entire configurational space, the physical dimension of the molecules or side chains manifest themselves in the relaxation time measurements. These two dielectric parameters are not necessarily related to each other, nonetheless, the combination of these two can be very powerful in the measurement of macromolecular structure.

The following is adapted from the excellent review article of Takashima [1969].

The electric polarization $P$ comprises of two parts namely the atomic and electronic polarization $P_\infty$ and the orientational polarization of the electric dipole $P_{\text{dlp}}$, hence

$$ P = P_\infty + P_{\text{dlp}} $$

(V.1)

when the field is switched on, $P_\infty$ appears instantaneously but $P_{\text{dlp}}$ takes a finite time more or less increasing in an exponential fashion to a saturation value. Expressing the dielectric constant of the material $\varepsilon$ as a complex function of real and imaginary parts $\varepsilon'$ and $\varepsilon''$

$$ \varepsilon = \varepsilon' - i\varepsilon'' $$

(V.2)

and designating the zero frequency and infinite frequency dielectric constants as $\varepsilon_0$ and $\varepsilon_\infty$, the frequency dependence ($\omega$) of $\varepsilon$ can be written in the familiar Debye equation as
\[ \varepsilon(\omega) = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + i\omega \tau} \]  

(V.3)

where '\(\tau\)' is the relaxation time of the dipoles. This can be shown to reduce to

\[ \varepsilon'(\omega) = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + \omega^2 \tau^2} \]  

(V.4)

and

\[ \varepsilon''(\omega) = (\varepsilon_0 - \varepsilon_\infty) \frac{\omega \tau}{1 + \omega^2 \tau^2} \]  

(V.5)

\(\varepsilon''(\omega)\) peaks at a critical frequency \(\omega_c\) so that \(\omega_c \tau = 1\) and this corresponds to maximum dielectric loss. The above equations are referred to as Debye equations.

Debye interpreted the relaxation time '\(\tau\)' at absolute temperature '\(T\)' in terms of the rotation of the polar molecules in a continuum medium with friction and using Stokes law for rotation of an equivalent sphere of radius '\(a\)', '\(\tau\)' comes out as

\[ \tau = \frac{f}{2k_B T} = \frac{4\pi \eta a^3}{k_B T} \]  

(V.6)

where the rotational frictional coefficient \(f = 4\pi \eta a^3\) and \(k_B\) is the Boltzmann's constant.

Normally in long chain polymers one has to take into account a distribution of relaxation times originating from molecular interactions, variations in the lengths of side chains and groups, etc. These effects are accumulated for in the relaxation time distribution function.

If \(G(\tau)\) is the distribution of relaxation times, \(\varepsilon'(\omega)\) and \(\varepsilon''(\omega)\) can be expressed as

\[ \varepsilon'(\omega) = \varepsilon_\infty + (\varepsilon_0 - \varepsilon_\infty) \int_0^\infty \frac{G(\tau)d\tau}{1 + \omega^2 \tau^2} \]  

(V.7)

and

\[ \varepsilon''(\omega) = (\varepsilon_0 - \varepsilon_\infty) \int_0^\infty \frac{G(\tau)\omega \tau d\tau}{1 + \omega^2 \tau^2} \]  

(V.8)
using these equations for determining \( \tau \) from the measurements of \( \varepsilon''(\omega) \) is a formidable task, since \( G(\tau) \) needs to be exactly known apriori or it has to be assigned a given distribution. Because of this problem a modified form of Debye equations were proposed by Cole and Cole expressed as

\[
\varepsilon(\omega) = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + (i\omega\tau)^{1-\beta}}
\]  

where \( 0 \leq \beta \leq 1 \). This is further reducible to

\[
\left( \varepsilon' - \frac{1}{2} (\varepsilon_0 + \varepsilon_\infty) \right)^2 + \varepsilon''^2 = \left( \frac{1}{2} (\varepsilon_0 - \varepsilon_\infty) \right)^2
\]

Implying a plot of \( \varepsilon' - (1/2) (\varepsilon_0 + \varepsilon_\infty) \) against \( \varepsilon'' \) will yield a circle with radius of \( (1/2) (\varepsilon_0 - \varepsilon_\infty) \). This is an ideal situation and corresponds to systems that obey Debye equations in full, where distribution of relaxation times are ignored. When these effects are included, one obtains a depressed semicircle with the center on the abscissa. From the depression one can estimate the Cole-Cole parameter \( \beta \) quite exactly. The semicircle is normally referred to as a cole-cole plot in literature.

It is also possible to calculate the enthalpy (\( \Delta H \)) and entropy (\( \Delta S \)) of activation of the dielectric processes using Eyring's rate theory of elementary processes. If \( k_0 \) is the rate constant for molecules or side chains undergoing transformation from an oriented state to the random distribution, the electric polarizibility can be expressed through the rate theory of Kauzmann as

\[
P(t) = P_0 \exp(-k_0 t) \]  

where \( k_0 \) relates to the relaxation time as \( k_0 = 1/\tau \). This finally yields

\[
\tau = \frac{h}{K_B T} \exp\left( \frac{\Delta H}{RT} \right) \exp\left( \frac{-\Delta S}{R} \right)
\]

where 'R' is universal gas constant and 'h' is Planck's constant. Hence from the study of \( \tau \) versus \( 1/T \) it is possible to derive both \( \Delta H \) and \( \Delta S \) from the experimental data.
V.B OPTICAL ROTATORY DISPERSION

Optically active molecules are known to rotate the plane of polarization of polarized light. The variation of magnitude of this rotation with wavelength is termed as Optical Rotatory Dispersion (ORD). For a molecule in solvent to exhibit ORD behavior, the molecule should not have a center of inversion, plane of symmetry or an alternating rotation reflection axis of symmetry. This should be applied not only to the isolated chromophore under study but to the whole system of the molecule plus its environment, including the solvation sphere. The only inherently asymmetric chromophore accessible in proteins is disulphide bond (-S-S-). Such systems normally display very intense optical activity than dosymmetric chromophore in asymmetric fields.

In the visible incident light, protein solutions exhibit optical rotation effects which are due to the electronic transitions involving delocalized 'π' electrons of the peptide bond or of some lateral groups [Walton, A.G. and Blackwell, J., 1973]. If the protein undergoes coil to helix (helix to coil) transition, then the specific interactions between the peptide units will modify the nature of the electronic transitions. The ORD generally provides valuable information about possible conformations of the chains.

In an ORD experiments the specific rotation \([\theta]_{\lambda}\) at the excitation wavelength '\(\lambda\)' is written as

\[
[\theta]_{\lambda} = \frac{\theta_{\text{meas.}}}{c \cdot l}
\]  

(V.13)

where \(\theta_{\text{meas.}}\) is the measured rotation angle in degrees, 'c' is polymer concentration in gm cc\(^{-1}\) and 'l' is the typical optical path in the cell in deci-meter (dm). The dispersive behavior is described by Drude equation

\[
[\theta]_{\lambda} = A \frac{\lambda_0^2}{\lambda^2 - \lambda_0^2}
\]  

(V.14)

where the polymer conformation is accumulated for by the parameter 'A'. The wavelength \(\lambda_0\) corresponds to the wavelength where collagen exhibits a strong
negative cotton effect. For collagen $\lambda_0 = 210 \pm 5$ nm. For a collagen solution denatured by heating, the amplitude of the cotton effect decreases significantly, but $\lambda_0$ remains almost constant. Von Hoffel and Wong [1963] have shown that at any wavelength $> 300$ nm the specific rotation of collagen or gelatin solutions can be analyzed through Drude's equation (Eq.(V.14)). This relation also stands for partially renatured solutions [Blout, E.R., et.al., 1963]

All these relations are rigorously established for dilute solutions. But Djabourov, et.al., [1988b] checked its validity for moderately concentrated solutions of gelatin, which correspond to gel formation conditions.

From the above Drude’s equation, Djabourov, et.al., [1988b] deduce an expression for helix content and this is given as,

$$\chi = \frac{[\theta]_{\text{meas.}} - [\theta]_{\text{coll.}}}{[\theta]_{\lambda}^{\text{collagen}} - [\theta]_{\lambda}^{\text{coll}}}$$  \hspace{1cm} (IV.15)

Where '$\chi$' is the amount of helix present at a particular instant of time (at a particular temperature) and $[\theta]_{\lambda}^{\text{collagen}}$ and $[\theta]_{\lambda}^{\text{coll}}$ are the specific rotation of the system in collagen and coil state respectively.