DIELECTRIC RELAXATION OF MOLECULES WITH FLUCTUATING DIPOLE MOMENT

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ABSTRACT When a dissolved macromolecule is in chemical equilibrium with a free ionic species, the charge configuration, and hence the dipole vector, of the macromolecule is fluctuating. Expressions for the static dielectric constant and the relaxation spectrum of such a mixture are here derived in terms of the components of the mean moment and the root mean square fluctuation moment, the molecular relaxation time constants, and the chemical rate constants of the ionic binding reaction. Contrary to a previous treatment of this problem by Kirkwood and Shumaker (1), it is shown that fluctuations introduce no independent components into the relaxation spectrum.

INTRODUCTION

The dielectric properties of compounds having fixed composition have been extensively studied. But, when the dielectric sample consists of a mixture in chemical equilibrium such that the dipole moments may change their instantaneous magnitude and orientation by binding or releasing a charged or polar species, it can be expected that the bulk dielectric behavior may depend on the chemical process.

Indeed, since a complete energetic description of an element of the sample requires both its chemical and its spatial characterization, the application of an external electric field admits of the possibility of supplying energy to the chemical-orientational sequence, and of obtaining in the sample as a whole a non-equilibrium steady state. It follows that the resultant dielectric properties in the mean may depend on the rate of the chemical reaction. In fact, this is the case.

Kirkwood and Shumaker, who first dealt with this problem for the special case of static fields in 1952 (1), obtained a result which is inconsistent with the present analysis, even in the restricted case, because of the implicit use of assumptions valid only for statistical systems in equilibrium. The same consideration makes such general theories of dielectric behavior as those of Cole (2) based on the statistical mechanical framework of Kubo (3) inapplicable to the present problem, since these require that a Hamiltonian may be validly written for the elements of

the sample, or at least that point density in phase space is conserved in the sense of the Liouville equation.

Molecules in chemical equilibrium may be classified by the number of equivalent binding sites per molecule. Where this number is small, each site must be treated individually. Where this number is large, the fluctuation dipole moment may be treated as a continuous variable, characterized by a Gaussian probability distribution in each of 3 orthogonal directions, with transition probabilities well approximated by a Markov process.

The present paper will deal with the latter category, an adequate approximation when the molecular species is a rigid macromolecule of protein size and the sites are the basic sites for binding of hydrogen ion.

ASSUMPTIONS

For the purpose of this discussion, we consider the simplest model which possesses the essential features of the charge fluctuation phenomenon.

(*a*) We assume a solution of identical, rigid molecules, in which there is no chemical or electrostatic interaction between solute and solvent, nor among solute molecules, nor among the ion-binding sites of any molecule. We assume that the effective field acting on a molecule is identical with the field of external charges.

None of these is strictly true. But no qualitative character in the result is lost through these assumptions, and great complexity is avoided. Furthermore, for macromolecular systems, to which the fluctuation problem is most immediately pertinent, such idealization is commonly and successfully used (4).

(*b*) We assume weak applied fields, neglecting all second and higher order field terms.

(*c*) We assume that the concentration (or, equivalently, the chemical potential) of the free ionic species is independent of any macroscopic coordiates. This is assured if the electrode-solution interface is not blocking to charge transfer.

(*d*) Relaxation is statistically a Langevin process (see equations [13*a*] and [13*b*] below), giving the classical Debye dispersion relations (5), or equivalently, a response function of the form of equation [5] below. It will be here further assumed that the relaxation of elemental transient moments may also be treated as a Langevin process. This assumption is valid if at any instant we can deal with subsamples composed of a statistically large number of molecules whose histories of charge configuration and spatial orientation are identical in all significant respects. Since the significant history of a molecule in this respect is of the order of several relaxation times, this requirement is easily met.

DEFINITIONS

(*a*) All macroscopic quantities, such as the dielectric constant, D, are under-

stood to be incremental values, the reference value being that which is measured for the same solution at a frequency which is high for the relaxation of solute, and low for the relaxation of solvent.

(*b*) The long time average of a quantity, x, shall be designated by $\bar{x}$, the ensemble average by $\langle x \rangle$, and the expectation value by $\langle x \rangle_{exp}$.

(*c*) Two coordinate systems shall be distinguished: the first, fixed in space, having local symmetry about the unit vector $\mathbf{e}$ in the direction of the imposed field; and the second, fixed in a given solute molecule, and defined by unit vectors $\mathbf{a}_i (i = 1, 2, 3)$ along the principal axes of a generalized ellipsoid representing the molecule. In the first, only components along $\mathbf{e}$ will be of interest, so that the transformation from molecular to space coordinates can be accomplished through 3 time-dependent direction cosines between the vectors $\mathbf{a}_i$ and $\mathbf{e}$, designated by $A_{i*}(t) \equiv \mathbf{a}_i(t) \cdot \mathbf{e}$.

DERIVATION

For an isotropic, homogeneous medium in the presence of an applied alternating electric field, $\mathbf{E} = \mathbf{E}_o e^{i\omega t} = \mathbf{e} E_o e^{i\omega t}$ of angular frequency ω, the fundamental electrostatic relation between the (incremental) complex dielectric constant, D, and the polarizability α, gives:

$$D = 4\pi\alpha = 4\pi n \frac{\overline{\langle \mathfrak{y} \rangle \cdot \mathbf{E}^*}}{\overline{(\mathrm{Re}\, E)^2}} = \frac{4\pi n}{\frac{1}{2}E_o^2} \frac{1}{Y} \int_0^Y \langle \mathfrak{y}(t) \rangle \cdot \mathbf{E}_o e^{-i\omega t}\, dt$$

$$= \frac{8\pi n}{E_o} \frac{1}{Y} \int_0^Y \langle \mu_*(t) \rangle e^{-i\omega t}\, dt \qquad [1]$$

where Re means "real part of," E^* is the complex conjugate of E; n is the number of solute molecules per cubic centimeter; $\mathfrak{y}$ is the molecular dipole vector in space coordinates, and μ_* its inner product with $\mathbf{e}$; Y is to be large compared with ω^{-1}; $i = \sqrt{-1}$.

From Definitions (*c*), $\mathfrak{y}(t)$ and its component $\mu_*(t)$ can be related to the components $\mu_i(t)$ along molecular coordinates, through the direction cosines $A_{i*}(t)$,

$$\mu_*(t) = \mathfrak{y}(t) \cdot \mathbf{e} = \sum_{i=1}^{3} [\mu_i(t) \mathbf{a}_i(t)] \cdot \mathbf{e} = \sum_{i=1}^{3} \mu_i(t)\, A_{i*}(t). \qquad [2]$$

We separate $\mathfrak{y}(t)$, the instantaneous molecular dipole vector, into its mean value and a fluctuating quantity. The time average of the latter is by definition, zero.

$$\mathfrak{y}(t) = \bar{\mathfrak{y}} + \delta\mathfrak{y}(t) \qquad [3a]$$

$$\mu_i(t) = \bar{\mu}_i + \delta\mu_i(t) \qquad [3b]$$

$$\overline{\delta\mu_i(t)} = 0 \qquad [3c]$$

From the classical Debye theory (5), we know that in the presence of an applied field $E = E_o \mathrm{Re}\, e^{i\omega t}$, a molecule with fixed dipole moment will have a time-dependent expectation value for the axis alignment cosines A_{i*} given by

$$\langle A_{i_e}(t)\rangle_{\text{exp}} = \frac{1}{3kT} E_o \mu_i \text{ Re } [e^{i\omega t}/1 + i\omega\tau_i]. \quad [4]$$

The response function, γ, to a unit impulse of applied field at time, t', $E = \delta(t - t')$, is then the Fourier transform

$$\gamma(t, t', \tau_i) = \text{F.T.}\left[\frac{\mu_i}{3kT}\frac{1}{1 + i\omega\tau_i}\right] = \frac{\mu_i}{3kT}\frac{1}{\tau_i}\exp[-(t - t')/\tau_i], \qquad t \geq t', \quad [5]$$

where τ_i is the relaxation time appropriate to the rotary diffusion of the molecule about the axes perpendicular to $\mathbf{a}_i$ (6), and $\delta(t - t')$ is the delta function.

We extend this concept now, by Assumption (*d*) above, to molecules whose dipole moments are not fixed in time.

The magnitude of the alignment is linear in both the applied field intensity and the component magnitude of the dipole vector, and hence we can write a response function, Γ, to a unit impulse in the product of applied field and moment at time t', $E\mu_i = \delta(t - t')$, which can characterize the response of a molecule with variable dipole vector:

$$\Gamma(t, t', \tau_i) = \frac{1}{3kT}\frac{1}{\tau_i}\exp[-(t - t')/\tau_i], \qquad t \geq t'. \quad [6]$$

It follows then that the expectation value of axis alignment for a molecule depends on the history from $-\infty$ to t of the product $E(t')\mu_i(t')$ according to the superposition integral:

$$\langle A_{i_e}(t)\rangle_{\text{exp}} = \int_{-\infty}^{t} \text{Re } E(t')\mu_i(t')\Gamma(t, t', \tau_i)\, dt', \quad [7]$$

or

$$\langle A_{i_e}(t)\rangle_{\text{exp}} = \frac{1}{3kT}\frac{1}{\tau_i}\int_{-\infty}^{t} \text{Re } E(t')\mu_i(t')\exp[-(t - t')/\tau_i]\, dt'. \quad [8]$$

Then, by equation [2], summing over all molecules, and replacing the expectation value by an ensemble average,

$$\frac{1}{n}\sum_{\substack{\text{all}\\ \text{molecules}}} \langle \mu_e(t)\rangle_{\text{exp}} = \langle \mu_e(t)\rangle$$

$$= \frac{1}{3kT}\sum_{i=1}^{3}\frac{1}{\tau_i}\left\langle \mu_i(t)\int_{-\infty}^{t} \text{Re } E(t')\mu_i(t')\exp[-(t - t')/\tau_i]\, dt'\right\rangle. \quad [9]$$

With equation [3*b*], we obtain,

$$\langle \mu_e(t)\rangle = \frac{1}{3kT}\sum_{i=1}^{3}\frac{1}{\tau_i}\left\langle [\bar{\mu}_i + \delta\mu_i(t)] \cdot \int_{-\infty}^{t} \text{Re } E(t')[\bar{\mu}_i + \delta\mu_i(t')]\exp[-(t - t')/\tau_i]\, dt'\right\rangle. \quad [10]$$

This equation, expanded, gives 4 terms,

$$\langle \mu_s(t) \rangle = \frac{1}{3kT} \sum_{i=1}^{3} \frac{1}{\tau_i} \left\{ \left\langle \bar{\mu}_i^2 \int_{-\infty}^{t} \text{Re } E(t') \exp\left[-(t-t')/\tau_i\right] dt' \right\rangle \right. \quad (i)$$

$$+ \left\langle \bar{\mu}_i \int_{-\infty}^{t} \text{Re } E(t') \delta\mu_i(t') \exp\left[-(t-t')/\tau_i\right] dt' \right\rangle \quad (ii)$$

$$+ \left\langle \bar{\mu}_i \delta\mu_i(t) \int_{-\infty}^{t} \text{Re } E(t') \exp\left[-(t-t')/\tau_i\right] dt' \right\rangle \quad (iii)$$

$$\left. + \left\langle \delta\mu_i(t) \int_{-\infty}^{t} \text{Re } E(t') \delta\mu_i(t') \exp\left[-(t-t')/\tau_i\right] dt' \right\rangle \right\}. \quad (iv) \qquad [11]$$

Term (*iii*) vanishes by equation [3*c*]. Term (*ii*) has as a factor the ensemble average correlation of a fixed quantity, $\bar{\mu}_i$, with a variable of vanishing mean, $\delta\mu_i(t')$, and hence vanishes as well.

By standard methods of integration, term (*i*), with equation [1], yields the classical Debye dispersion equation for the mean dipole moment,

$$D_m(\omega) = \frac{4\pi n}{3kT} \sum_{i=1}^{3} \bar{\mu}_i^2 [1/1 + i\omega\tau_i]. \qquad [12]$$

Term (*iv*) is the fluctuation term, involving the ensemble average correlation of the instantaneous dipole vector at a time, t, with the dipole vector at time t'. To obtain an expression for this correlation, the assumptions governing interactions [Assumptions (*a*)] and the spatial distribution of free charge (ion) density [Assumptions (*c*)] are used.

With these assumptions, the dipole moment fluctuation along a given molecular axis is a Markov process. More specifically, from a given instantaneous charge configuration, the fluctuation probability is biased toward the mean configuration in proportion to the deviation from the mean at that instant. This condition is described by the Langevin equations:

$$\frac{d\mu_i}{dt} = -\frac{\mu_i(t) - \bar{\mu}_i}{\tau_\delta} + z(t) \qquad [13a]$$

$$\left\langle \frac{d\mu_i}{dt} \right\rangle_{\text{exp}} = -\frac{\mu_i(t) - \bar{\mu}_i}{\tau_\delta}, \qquad [13b]$$

where τ_δ is a fluctuation time constant whose relation to the ionization reaction rate for the charge-binding sites is derived below, and where $z(t)$ is a purely random quantity which by definition has a vanishing expectation value.

For such a process, the desired correlation is known (7) to be,

$$\langle [\mu_i(t') - \bar{\mu}_i][\mu_i(t) - \bar{\mu}_i] \rangle_{\text{exp}} = \overline{(\mu_i(t) - \bar{\mu}_i)^2} \exp\left[-(t-t')/\tau_\delta\right]. \qquad [14]$$

Since $[\mu_i(t) - \bar{\mu}_i]$ is just $\delta\mu_i(t)$, term (*iv*) of equation [11] becomes,

$$\langle \mu_{e\delta}(t) \rangle = \frac{1}{3kT} \sum_{i=1}^{3} \frac{1}{\tau_i} \overline{\delta\mu_i^2(t)}$$

$$\cdot \int_{-\infty}^{t} \mathrm{Re}\, E(t') \exp\left[-(t - t')/\tau_\delta\right] \exp\left[-(t - t')/\tau_i\right] dt'. \quad [15]$$

For convenience, we define a new time constant

$$1/\tau_i' \equiv 1/\tau_i + 1/\tau_\delta. \quad [16]$$

Then, equation [15] with equation [1], by standard methods of integration, yields the fluctuation dielectric increment,

$$D_\delta(\omega) = \frac{4\pi n}{3kT} \sum_{i=1}^{3} \overline{\delta\mu_i^2} \left(\frac{1}{1 + \tau_i/\tau_\delta}\right)\left(\frac{1}{1 + i\omega\tau_i'}\right). \quad [17]$$

Combining equations [12] and [17], we obtain finally,

$$D(\omega) = \frac{4\pi n}{3kT} \sum_{i=1}^{3} \left[\bar{\mu}_i^2 \left(\frac{1}{1 + i\omega\tau_i}\right) + \frac{\overline{\delta\mu_i^2}}{1 + \tau_i/\tau_\delta} \left(\frac{1}{1 + i\omega\tau_i'}\right)\right]. \quad [18]$$

DISCUSSION

It is of interest to examine equation [18] in two extreme cases. It is clear that if $\tau_\delta \ll \tau_i$, there will be practically no detectable fluctuation dielectric increment at any frequency, even if the fluctuation mean-square moments are large. This corresponds to the situation where the state of ionization of the charge-binding sites on a molecule is in such rapid fluctuation that, during the period of one molecular relaxation, the molecule acts as if the sites were truly "partially ionized."

On the other hand, if $\tau_\delta \gg \tau_i$, then, from equation [16], $\tau_i' = \tau_i$, and

$$D(\omega) = \frac{4\pi n}{3kT} \sum_{i=1}^{3} \left[(\bar{\mu}_i^2 + \overline{\delta\mu_i^2}) \left(\frac{1}{1 + i\omega\tau_i}\right)\right] \quad [19]$$

which corresponds to the situation where a given instantaneous charge configuration remains fixed on a molecule for a period of many relaxations.

It is seen that, although the magnitude of the total dielectric increment may be strongly dependent on fluctuation moments, in all cases the measured relaxation times are primarily related to τ_i, the molecular relaxation times, being at the most diffused somewhat toward the shorter relaxation time end of the spectrum (to an extent given by equation [16]) in the intermediate case where τ_δ and τ_i are comparable.

FLUCTUATION TIME CONSTANT

It remains to relate τ_δ, the fluctuation time constant, to the ionization reaction rates at the charge-binding sites, and, if possible, to estimate its value for a typical macromolecule in relation to its molecular relaxation times.

The ionization reaction for a group, S, which binds hydrogen ion is written,

$$S + H^+ \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} SH^+ \qquad [20]$$

Thus, the elementary fluctuation probability is governed by the rate constants k_{12} and k_{21} and the pH of the solution. (In neutral or basic solutions, the reactions involving OH^- must also be included. If several types of ionizable groups are involved, several fluctuation time constants will be calculated.)

τ^+, the mean lifetime of the non-ionized state (when hydrogen ion is bound), is just k_{21}^{-1}, and τ^-, the mean life of the ionized state, depends on the pH of the solution,

$$\frac{\tau^+}{\tau^+ + \tau^-} = \frac{1}{1 + K/[H^+]} = f \qquad [21]$$

where $K = k_{21}/k_{12}$, $[H^+] = 10^{-pH}$, and f is the proton occupation expectation value. Thus, of n similar sites, fn are on the average occupied by a hydrogen ion.

If at some instant $(fn + \delta)$ sites are occupied, the probability that in an infinitestimal time interval, dt, a hydrogen ion will be dissociated is,

$$dp^- = (fn + \delta)\frac{dt}{\tau^+} \qquad [22]$$

and the probability that one will be bound is

$$dp^+ = [(1 - f)n - \delta]\frac{dt}{g\tau^+} \qquad [23]$$

where $g = \tau^-/\tau^+ = (1 - f)/f$. Then the Langevin time constant is obtained by comparing

$$\left\langle \frac{d\delta}{dt} \right\rangle_{exp} = (p^+ - p^-) = \frac{-\delta}{(1 - f)\tau^+} \qquad [24]$$

with equation [13b]; and immediately,

$$\tau_\delta = \frac{\tau^+}{1 + [H^+]/K} = \frac{1}{k_{21} + [H^+]k_{12}} \qquad [25]$$

The typical groups on the surface of proteins, which are titratable near the middle of the pH range, are the free carboxyl groups and the imidazole groups of histidine. Since acetic acid and imidazole in water solution dissociate with approximately the same K as the corresponding groups on protein molecules, it is reasonable to expect that the rate constants determined by Eigen (8) for the reactions,

$$CH_3COO^- + H^+ \rightleftharpoons CH_3COOH \qquad [26]$$

$$Im + H^+ \rightleftharpoons Im\ H^+ \qquad [27]$$

are approximately correct for the titration reactions on protein.

Using as an example an isoionic solution of serum albumin at 0°C, and at a pH of 5.0, one obtains, approximately,

$$\tau_\delta(\mathrm{Im}) = 1.2 \times 10^{-5} \text{ sec.}$$
$$\tau_\delta(\mathrm{COO}^-) = 0.6 \times 10^{-5} \text{ sec.} \qquad [28]$$

The longest major molecular relaxation time measured for such a solution is about 0.5×10^{-6} seconds. For this protein, then, $\tau_\delta > 10\ \tau_i$, and equation [19] may be used.

CONCLUSION

It has been shown that charge fluctuations do not introduce essentially new relaxation times into the dielectric dispersion spectrum. Equation [18] shows how the magnitude of the total dielectric increment and the broadening of the relaxation spectrum are related to the fluctuation parameters. These parameters are computed for a typical protein solution.

Experimental evidence to support these conclusions, and showing as well that ionic conduction relaxation may be eliminated from some experimental systems, has been obtained by Oncley (9).

APPENDIX 1

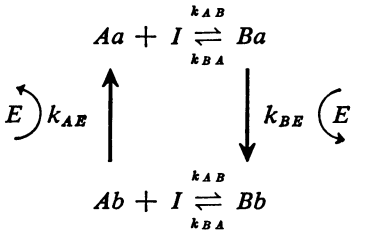
ENERGY AND THE STEADY STATE

To demonstrate the possibility of a non-equilibrium steady state in a dielectric experiment, let us consider a solution containing the molecular species A and an ionic species I. Suppose that A has a single site for binding I, and let us refer to AI as B. Chemically, the interaction is completely described by

$$A + I \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B \qquad [29]$$

In the presence of an external field, it is evident that A and B, having different dipole vectors in relation to molecule-fixed axes, will approach different asymptotic mean spatial orientations, which we may call a and b, respectively. Given enough time, each would actually reach its appropriate mean orientation. Kauzmann (10) has suggested the parallel between orientation in an electric field and a chemical rate process, and has used a terminology similar to this description.

If the concentration of I is independent of any macroscopic coordinates, (see Assumptions (c) above), then the chemical reaction equation, [29], proceeds independently of the state of orientation of the species. The microscopic transition from chemical species A to B is followed by, but is not simultaneous with, the transition in the expectation value of the state of orientation from a to b. Thus, the speices A in orientation a, which we may refer to as Aa, upon binding I becomes Ba, not Bb. Though Ba and Bb are chemically the same, they are not energetically the same. A complete chemical and spatial description then, requires a cyclic process, which may be represented schematically by



[30]

This cycle is driven clockwise by energy supplied by the electric field, the "vertical" reactions being coupled to the field. The over-all mean polarizability depends on the relative concentrations of *Aa, Ab, Ba,* and *Bb,* which in turn are evidently functions of the ratios of the orientation rate constants to the chemical rate constants.

In an alternating field, some dissipation of energy is associated with the imaginary component of dielectric constant. The coupling energy involved in [30] is not of this sort, and occurs as well with static fields. It is the energy represented by the difference in potential of *I* in the external field between its mean point of binding on a given molecule and its mean point of dissociation, neglecting electrophoretic movement of the molecule.

If the electrodes are blocking to charge transfer, which is neither usual nor practical, then the scheme of [30] must be further altered to account for the chemical potential gradient of *I*. In this case the total potential (chemical and electrical) of *I* is uniform, the total coupling energy vanishes, and an equilibrium dielectric measurement could conceivably be made. However, in such an experiment most of the electric potential drop is at the electrodes, and meaningful data are hard to obtain.

APPENDIX 2

ALTERNATE DERIVATION FOR A SPECIAL CASE

A more basic and direct approach to the present problem is to expand upon the method used by Debye (5) in which he obtains for spherical molecules with fixed dipole moment a distribution function $f(\varphi)$ representing the probability of finding a given molecule at a given instant with its dipole vector making the angle φ with respect to the electric field vector.

For the general case of fluctuating dipoles, the Debye method is prohibitively complex. However, it is instructive to work it out for a special case to a result identical with that from the more general development given above.

Debye's method begins by counting the net number of dipoles, Δ_1, which enter a solid angle of orientation $d\Omega(\varphi)$ in a time interval δt, due to orientation produced by the torque of the imposed effective field, F; and the number Δ_2, which enter the same solid angle due to Brownian rotational movement. The change of the distribution, $\delta f(\varphi)$ in δt, is then related to the sum $(\Delta_1 + \Delta_2)$.

We must now obtain a twofold distribution function, $f(\mu, \varphi)$, representing the probability of finding a given molecule at some instant with a dipole vector of magnitude μ and with orientation φ.

To Debye's expressions for Δ_1 and Δ_2, modified for the two-dimensional element $d\mu d\Omega$, we must now add a quantity, Δ_3, which represents the net number of dipoles which enter $d\mu d\Omega$ as a result of chemical fluctuations.

The special case we consider is a spherical molecule with vanishing mean dipole moment. The instantaneous moment is furthermore restricted to a single axis fixed on the molecule, so that fluctuations are restricted to the instantaneous dipole axis.

For mathematical simplicity, the distribution of moments along the molecule-fixed axis is assumed to be Gaussian and to be the result of a Markovian random walk process of equal steps of magnitude U, with central tendency. Again for mathematical simplicity, the intervals, $-\infty < \mu < +\infty$, and $0 \leq \varphi \leq \pi/2$, are used; orientations $\pi/2 < \varphi \leq \pi$ are given by negative values of μ.

From elementary considerations, then, the probability of a fluctuation step $\pm U$ for a dipole of instantaneous value $\mu(t)$, in a time interval δt, is given by

$$p(\mu, \pm U) = \frac{\delta t}{\tau}\left[\frac{1}{2} \mp \frac{U\mu(t)}{4\Delta}\right] \qquad [31]$$

where Δ is the mean square fluctuation moment, $\overline{\mu^2}$, and $1/\tau$ fluctuations occur per second, in the mean.

It is then easily shown that the Langevin fluctuation time constant is given by,

$$\tau_\delta = \frac{2\Delta}{U^2}\tau \qquad [32]$$

If a time interval, δt, is chosen which is small compared with τ, the probability that a single molecule will experience 2 fluctuations may be neglected, and $\Delta_3(\mu, \varphi)$ is made up only of those dipoles going from μ to $(\mu \pm U)$, and those coming from $(\mu \pm U)$ to μ. Thus,

$$\begin{aligned}\Delta_3(\mu, \varphi) = d\Omega \{ &-f(\mu, \varphi)[p(\mu, +U) + p(\mu, -U)] \\ &+ f(\mu + U, \varphi)p(\mu + U, -U) \\ &+ f(\mu - U, \varphi)p(\mu - U, +U)\}. \end{aligned} \qquad [33]$$

Expanding,

$$f(\mu \pm U, \varphi) = f(\mu, \varphi) \pm \frac{\partial f}{\partial \mu} U + \frac{1}{2}\frac{\partial^2 f}{\partial \mu^2} U^2 + \mathrm{O}\, U^3 \qquad [34]$$

and using equations [31] and [33], we obtain,

$$\Delta_3 = d\Omega \frac{\delta t}{\tau}\frac{U^2}{2}\left\{\frac{f(\mu, \varphi)}{\Delta} + \frac{\mu}{\Delta}\frac{\partial f}{\partial \mu} + \frac{\partial^2 f}{\partial \mu^2}\right\} + \mathrm{O}\, U^3 \qquad [35]$$

Using Debye's calculation of Δ_1 and Δ_2, we then obtain

$$\begin{aligned}\frac{\partial}{\partial t} f(\mu, \varphi) &= (\Delta_1 + \Delta_2 + \Delta_3)/d\Omega\, \delta t \\ &= \frac{1}{\sin \varphi}\frac{\partial}{\partial \varphi}\left[\sin \varphi\left(\frac{kT}{\zeta}\frac{\partial f}{\partial \varphi} + \frac{\mu F}{\zeta}\sin \varphi \cdot f\right)\right] + \frac{U^2}{2\tau}\frac{\partial}{\partial \mu}\left[\frac{\mu}{\Delta} f + \frac{\partial f}{\partial \mu}\right] \end{aligned} \qquad [36]$$

where ζ is the inner frictional constant.

Using the molecular relaxation time,

$$\tau_i = \frac{\zeta}{2kT} \qquad [37]$$

and the relation [32] for τ_δ; and making the substitution, $\cos\varphi = \Phi$, we obtain finally,

$$\frac{\partial}{\partial t} f(\mu, \varphi) = \frac{1}{2\tau_i} \frac{\partial}{\partial \Phi}\left[(1 - \Phi^2)\left(\frac{\partial f}{\partial \Phi} - \frac{\mu F}{kT} f\right)\right] + \frac{1}{\tau_\delta} \frac{\partial}{\partial \mu}\left[\mu f + \Delta \frac{\partial f}{\partial \mu}\right] \quad [38]$$

where F is the effective external field.

Solving first for static fields, $F = F_o$, and $\partial f/\partial t = 0$, we obtain the solution,

$$f_o(\mu, \varphi) = Je^{-\mu^2/2\Delta}\left[1 + \left(\frac{1}{1 + \tau_i/\tau_\delta}\right)\frac{\mu F_o}{kT}\cos\varphi\right] + O\left(\frac{\mu F_o}{kT}\right)^2 \quad [39]$$

where J is a normalization constant.

Then, assuming separability of the time dependence, we seek a solution for the time-dependent equation to show the relaxation of polarization upon removal of a fixed field F_o at time $t = 0$, by trying a solution of the type,

$$f(\mu, \varphi, t) = Je^{-\mu^2/2\Delta}\left[1 + \left(\frac{1}{1 + \tau_i/\tau_\delta}\right)\frac{\mu F_o}{kT}\cos\varphi\psi(t)\right] \quad [40]$$

which yields,

$$\psi(t) = \exp\left[-\left(\frac{1}{\tau_i} + \frac{1}{\tau_\delta}\right)t\right]. \quad [41]$$

Then solving for the space average dipole moment in the direction of the external field,

$$\langle\mu_e\rangle = \frac{\iint f\mu\cos\varphi\, d\mu\, d\Omega}{\iint f\, d\mu\, d\Omega} \quad [42]$$

we obtain,

$$\langle\mu_e\rangle = \frac{\overline{\mu^2}F_o}{3kT}\left(\frac{1}{1 + \tau_i/\tau_\delta}\right)\exp\left[-\left(\frac{1}{\tau_i} + \frac{1}{\tau_\delta}\right)t\right]. \quad [43]$$

A Fourier transformation (11) immediately yields the frequency-dependent behavior of equation [17].

APPENDIX 3

"PROTON MIGRATION"

The term "proton migration" refers to the movement over the surface of a macromolecule by a hydrogen ion which has been dissociated from an ionizable group, but which remains bound to the molecule as a whole. Whether such movement actually takes place is not known. Its effect on dielectric relaxation measurements cannot be determined from fluctuation theories relating to the acid-base equilibrium properties of titratable groups, and hence neither this nor Kirkwood's analysis includes proton migration. More needs to be known about the protein-water surface before proton migration can be either dismissed or satisfactorily analyzed.

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