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Dielectric Behavior of Biological Polymers

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1. INTRODUCTION

We discuss the electrical impedance chracteristics of biological cells and membranes in part I. The theory of inhomogeneous dielectrics and the theory of counterion polarization are the most important mathematical tools to explain the polarization of cells and cell membranes.

The dielectric properties of biopolymers such as proteins and nucleic acids are the topic of part II. It has been debated for some time what relaxation theory is the most pertinent in order to explain the mechanism of the polarization of charged macromolecules. The classical polar molecule theories have proven suffient to account for the dipolar structure of small globular protein molecules. However, the polar theory was found unable to explain the dipolar structure of highly charged fibrous biopolymers such as DNA and polyamino acids. It is now generally agreed that the theory of counterion polarization is most appropriate for these cases. Since the counterion polarization theories were disussed already in part I, we will not go into that subject. However, this does not mean that we consider the counterion polarization theories less important than the polar theory. As a matter of fact, the counterion polarization theories play a role more important than the polar molecule theory for the elucidation of the origin of the unusual dipole moment of highly charged biological polyions.

2. PERMANENT DIPOLE EFFECTS (DEBYE)

Relaxation mechanism responsible for dispersive behavior of biological materials may be caused either by permanent or field induced dipole moments. Here, our discussion is confined to the theory of permanent dipole moment. Many molecules possess electrical dipoles produced by an asymmetric distribution of positive and negative charges. These polar molecules tend to orient with an applied field, while thermal motion opposes preferential orientation. The development of the theory of polar molecules was a breakthrough at that time and had a far reaching impact. Debye (1929) introduced the concept of orientational polarization. He derived an equation for the static electric polarization of molecules so dilute that intermolecular interaction can be neglected.

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$$\frac{\varepsilon_o-1}{\varepsilon_o+2}V=\frac{4\pi N_A}{3}\left(\alpha+\frac{\mu^2}{3kT}\right) \tag{1}$$

Here ε_o is the static (low frequency) dielectric constant, V the molar specific volume, N_A Avogadro's number and μ the molecular dipole moment. The term α represents electronic and atomic distortions. It determines the high frequency limit ε_∞ since these processes are very fast. For dilute molecules, the dielectric constant ε is not much larger than 1 and under these conditions does eq.(1) yield satisfactory results. It soon became apparent that this theory has limited applicability for most polar substances. The shortcoming was found to stem from an insufficient analysis of internal fields in Debye's theory. Subsequently, Onsager (1936) introduced the concept of a 'direction field' and successfully eliminated the internal inconsistency of Debye's equation. Onsager's equation is

$$\frac{(\varepsilon-n^2)(2\varepsilon+n^2)}{\varepsilon(n^2+2)^2}=\frac{4\pi N_A\,\mu_0{}^2}{9VkT} \tag{2}$$

where n is the refractive index and μ_0 the dipole moment. Onsager's equation predicts correctly the dielectric constant of many polar substances. However, Onsager's equation was not able to account for the electric polarization of polar liquids with large dielectric constant such as water. The distribution of polar molecules in liquids is more ordered than in gases. Consequently, the mean dipole moment of liquids is not necessarily zero in the absence of an electrical field. Under these conditions, two reference axes are needed to define the orientation of individual molecules in liquids. One axis is parallel to the net dipole moment of the liquid with zero field and the other the vector of the applied field. Kirkwood (1939) derived

$$\frac{(\varepsilon-1)(2\varepsilon+1)}{9\varepsilon}V=\frac{4\pi N_A}{3}\left\{\alpha+\frac{g\mu^2}{3kT}\right\} \tag{3}$$

where g is the "correlation parameter" defined by

$$g=1+z<\cos\theta> \tag{4}$$

z is the number of nearest neighbors and θ the angle between adjacent molecular axes. For example, in liquid water with tetrahedral structure, $z=4$ and $\theta=45-50°$. Satisfactory agreement with experimental results, however, demands that we must include not only the nearest neighbors but also molecules in outer shells. Further refinements assume bending (Pople 1951) or breaking of hydrogen bonds (Haggis et al, 1952) between adjacent water molecules.

For charged polar molecules such as amino acids and proteins, some empirical equations are more effective and yield better agreements with experiments. A semi empirical equation by Kirkwood has been used frequently for the calculation of the dipole moments of amino acids

$$(\mu\mu_0)^{1/2}=0.127\sqrt{(P_2-P_{20})T} \tag{5}$$

where P_2 and P_{20} are given by $P_2=(2/9)(1000\delta+\varepsilon_1 V_2)$ and $P_{20}=(4\pi N/3)\alpha_2$ where δ is dielectric increment/mol, ε_1, V_2 and α_2 are the permittivity of solvent, specific volume of solute and the polarizability of solute. However, for protein molecules, another semi-empirical equation by Oncley is most commonly used.

$$\mu^2=(9000kT/4\pi Nh))\,(M\delta) \tag{6}$$

where M is molecular weight of proteins and δ is dielectric increment per gram protein per 1000ml. h is an empirical parameter which has a numerical value of 5.8 that gives the best result.

Dielectric relaxation of polar molecules is caused by the phase lag between the applied sinusoidal field and the dipole orientation. Dipolar molecules are unable to follow instant changes in the direction of the applied periodic field. This causes phase shift and energy dissipation, resulting in a decrease in dielectric constant. If the relaxation is characterized by only one relaxation time, the dispersion of the dielectric constant is given by equation (7) (Debye 1929),

$$\varepsilon^*=\varepsilon_\infty+\frac{\varepsilon_o-\varepsilon_\infty}{1+j\omega\tau} \tag{7}$$

where τ is a cubic function of molecular radii. Note that this equation is identical with eq.(1) in part I. Distributions of relaxation times are often caused by molecular inhomogeneity and nonspherical shape. It is fairly common with synthetic polymers and biopolymers such as DNA. The origin of the distribution of relaxation times has been discussed by various authors.

The time constant of dielectric relaxation is, as mentioned before, proportional to the cube of the radii of molecules. For example, the dielectric dispersion is oberved at 15−20GHz for water, 400−500MHz for simple amino acids. The dispersion curves of some spherical proteins fit nicely the Debye theory of single relaxation time. However, the dispersion curves of other proteins deviate from it, indicating the presence of more than one relaxation time. For ellipsoidal molecules, there are separate orientations along major and minor axes. Then, eq.(7) must be rewritten using two dispersion terms (Oncley, 1943).

$$\varepsilon-\varepsilon_\infty=\frac{\Delta\varepsilon_1}{1+(\omega\tau_1)^2}+\frac{\Delta\varepsilon_2}{1+(\omega\tau_2)^2} \tag{8}$$

where $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ are the dielectric increments due to the orientation along the major and minor axes. τ_1 and τ_2 are relaxation time associated with these orientations. The rotation of ellipsoidal molecules can be analyzed using the theory of rotary diffusion developed by Perrin (see Oncley, 1943). This theory relates the rotary diffusion constants of ellipsoidal particles to their axes a and b. The relaxation time is calculated as the reciprocal of the rotary diffusion constant. Therefore, for a certain value for a/b, the ratio of τ_1/τ_2 can be calculated. The ratio of $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ is determined by dipole angle, i.e., the angle between dipole vector and molecular axes. Therefore, eq.8 can be

solved from assumed axial ratio and dipole angle θ. By best fit, we can obtain satisfactory agreement between calculation and experiment, and determine the shape of the protein molecule. This approach is useful for proteins with large axial ratios, i.e., 5−10. However, the theory is of limited use for protiens with an axial ratio of less than 2−3.

3. EXPERIMENTAL DATA

3.1 Electrolytes

Water is an inorganic substance, yet it is one of the most important substances for living organisms. Water is in many ways unique; It has high heat capacity, a high boiling point and a large dielectric constant. The dipole moment of water is 1.84 D and its vector bisects the angle between two OH bonds. Formation of intermolecular hydrogen bonds makes water a highly ordered liquid. Calculations of its dipole moment using Kirkwood's equation (1939) did not yield good results. Better agreement was obtained by Pople (1951) by allowing the bending of hydrogen bonds. Even better results were obtained by Haggis et al. (1952) by allowing the breaking and reforming of hydrogen bonds and consideration of outer shell molecules. These results indicate that water is a highly associated liquid, with extensive hydrogen bonding. It was also found experimentally that the energy barrier of dipole rotation of water is about 3−4 Kcal/mol (Hasted et al. 1953). This observation indicates that the orientation of a water molecule requires a breakage of only one hydrogen bond.

The dielectric relaxation of water is characterized by only one time constant corresponding to a characteristic frequency $f_o=1/2\pi\tau$ near 20 GHz at room temperature. The dielectric constant decreases from about 78 to 4 as the frequency increases. The sharpness of the dispersion of water remains a mystery, since interaction between neighboring molecules would tend to broaden the dielectric relaxational response. However, it is well accepted that the dielectric relaxation of liquid water is characterized by only one time constant. The high frequency term ε_∞ is about 4. The dielectric response of electrolytes is quite similar, except for a small decrease of ε_o and a larger conductivity. The low frequency limiting conductances is caused by the mobile ions and does not contribute to the dipole moment of solutes.

The behavior of water in biological cells has been debated by many investigators (Whipple 1965, Hazelwood 1984). In spite of the gel-like nature of cytoplasm, the dielectic behavior of intracellular water is amazingly similar to that of bulk water except for those tightly bound to macromolecules and perhaps membranes. Protein bound water appears to display an entirely different behavior (Schwan 1957 and 1965, Grant 1965). The relaxation frequency is much lower than that of normal water. In addition, the behavior indicates a broad spectrum of relaxation times, corresponding to frequencies ranging from some hundred MHz to a few GHz. Permittivity limit values ε_o and ε_∞ are comparable to those of water and ice.

3.2 Biological Macromolecules

3.2.1. Polypeptides, Amino Acids.

Polypeptides, proteins and nucleic acids are the most investigated biopolymers.

Early dielectric constant measurements were performed with amino acids and simple peptides. It was found that amino acids posses large dipole moments compared to those of other organic acids. This dipole moment is due to the charge pair of COO^- and NH_3^+ groups, separated by about 3.33 A in α-amino acids and 4.16A for β-amino acids (Wyman, 1936). The formation of peptides increases the distance between positive and negative charges, thus increasing the dipole moment. There are two known conformations for highly polymerized polypeptides and/or polyamino acids. These are the random coil and alpha helix structures (Pauling et al. 1951). The former is an amorphous structure without internal regularity and the peptide chain configuration is random and flexible. The net dipole moment of random coil polypeptides is usually very small if their carboxyl side chains are blocked with non-polar groups such as benzyl groups. Removal of these non-polar groups transforms them into highly charged free acids. These charged polyamino acids have, even in the random coil conformation, a very large dipole moment which is, perhaps, due to the polarization of counterions (Takashima, 1963, Muller et al. 1974). The second form of polyamino acids is a crystalline helical state characterized by a highly ordered secondary structure having polar groups aligned in one direction along the helical axis. Of the polar groups having a large dipole moment, the C=0 groups in the main chain and also in side chains are most prominent. Although the dipole vectors of the C=0 groups in side chains partially cancel those in the main chain, neverthless, the sum of the dipole vectors turns out to be very large and proportional to the degree of polymerization (Wada, 1962). The dielectric relaxation is believed to be due to the rotary diffusion of the entire molecule along the major axis and they are observed at very low frequencies.

3.2.2 Proteins.

Protein molecules are vital to the living organisms. There are two types of protein molecules, a) fibrous proteins such as collagen and muscle proteins and b) globular proteins such as hemoglobin, albumin and insulin. Both fibrous and globular proteins have large molecular weights (the smallest protein has a M.W. of 13.000) and consist of a variety of amino acids. Although the internal structure of these proteins is complex, the coordinates of their amino acids are accurately known for many proteins from X-ray crystallography. The folding of the peptide chains in proteins is irregular or amorphous and often appears to be structureless. The conformation of protein molecules is now known to be the result of an intricate interplay among hydrophobic and electrostatic forces. Protein molecules carry positive and negative charges on the surface due to the presence of polar side chains and also due to α-carboxyl and amino groups at both ends. Their locations are well known as shown in Figure 1 The dipole moment of proteins is largely determined by the distribution of these fixed charges. The distribution of surface charges has been found to be rather uniform in many proteins and the net moment is smaller than expected for their large size. Calculation of the net dipole moment based on the knowledge of the distribution of surface charges have been conducted for many proteins as shown in Table 1 (Schlecht 1969, South et al. 1972, Barlow et al., 1987, and Takashima and Asami, Unpublished). The calculations consist of two parts, the dipole moment due to fixed surface charges (Column 3) and

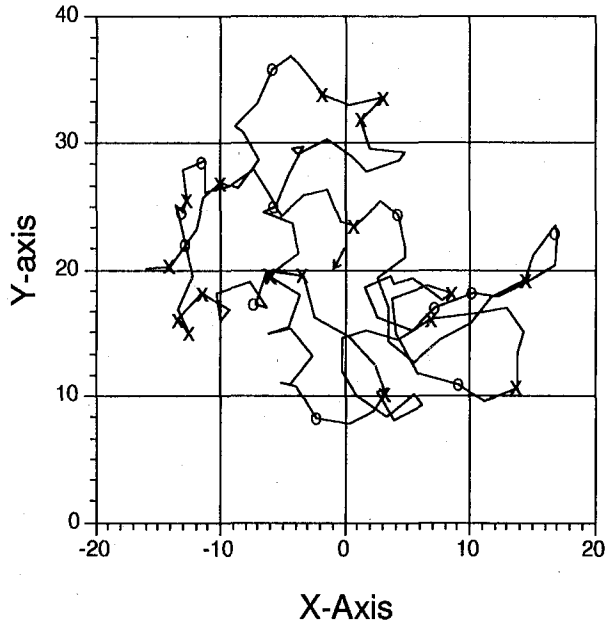

Fig. 1. Two dimensional presentation of a protein molecule, Lysozyme in the X-Y plane. Crosses represent positive charges and open circles represent negative charges. The small arrow indicates the vector of the dipole moment and its length is the distance between positive and negative charge centers.

Table 1. Calculated and Measured Dipole Moment of Proteins

Name	MW	Calculated Moment					Measured
		Charge	⟨r⟩	Core	Total	⟨θ⟩	
MBN*	17,000	170		0	170		167*
		(247)	(2.1)	(57.5)	(200)	(148°)	
CYT	13,000	310		−20	290		
		(255)	(4.1)	(72)	(203)	(142°)	(227)
RNS	13,700	481		−80	401		
		(330)	(5.7)	(19)	(331)	(85°)	(280)
LYZ	14,300	111		89	200		
		(130)	(2.1)	(61)	(141)	(92°)	(122)
HBN(α)	16,000	235		0	235		183*
		(203)	(2.8)	(54)	(210)	(90°)	
CPA	34,000	408		87	495		
		(435)	(3.3)	(152)	(575)	(2°)	(760)
TRP	23,000	356		0	356		
		(295)	(4.4)	(62)	(297)	(95°)	(345)

MBN··Myoglobin, CYT··Cytochrome C, RNS··Ribonuclease, LYZ··Lysozyme, HBN··Hemoglobin, CPA··Carboxypeptidase, TRP··Trypsin

*: By Schlecht, Unmarked: By Barlow and Thornton

( ): by Takashima and Asami.

the vector sum of bond dipole moments of individual amino acids (core moment, column 5). The moment due to fixed charges is calculated using eq.(9).

$$\mu=(\Sigma n_j L_j)\cdot e\cdot R_{n-p} \tag{9}$$

where R_{n-p} is the distance between the centers of positive and negative charges, e is elementary charge. n_j is the number of polar amimo acids such as glutamic acid, lysine and others. The parameter L_j is given by

$$L_j=\frac{1}{1+B} \text{ for positive charges} \tag{10}$$

$$L_j=\frac{B}{1+B} \text{ for negative charges} \tag{11}$$

where the parameter $B=10^{\mathrm{PH}-\mathrm{pK}}$ with pK the ionization constant of individual amimo acids.

The core moment is calculated by summing up vectorially the moment of C=0 groups in the main chain and in side chains. The magnitude of core moment turns out to be considerably smaller in magnitude than that due to fixed charges and its vector often opposite to that of the fixed charges. Column 4 shows the distance between the positive and negative charge centers. Note that the distance is unusually small for some proteins indicating that fixed charges are distributed nearly randomly. Column 7 shows the angle between dipole vectors due to fixed charges and core moments. The last column shows the results of the measurements of the dipole moments of these proteins. In general, agreements are reasonably good for small proteins (see myoglobin and cytochrome C, e.g.) and poor for large and complex proteins which often have subunits. A striking fact is that the net dipole moment of proteins is small considering their large sizes. The small arrow shown in Fig. 1 illustrates the net dipole vector due to fixed charges. Comparing its length with the size of entire molecule, we are able to conclude that the net dipole moment of globular proteins is unexpectedly small. This indicates that the distribution of fixed charges in proteins is highly randomized.

The dielectric relaxation of protein solutions has been observed between 100KHz and 10MHz (see Fig. 2) depending on their molecular weight. The relaxation time is found to be proportional approximately to the cube of molecular weight indicating that the polarization of proteins is mostly due to the orientational diffusion. The shape of their dispersion curves do not fit the Debye equation as expected. This indicates that a simple spherical model is not sufficient to account for the dielectric relaxation behavior of these proteins.

3.2.3. Dielectric Properties of DNA.

It has been known for some time that charged large biological and synthetic polyions posses a large mean square dipole moment although structural considerations preclude the presence of a mean dipole moment or a permanent moment. It has been

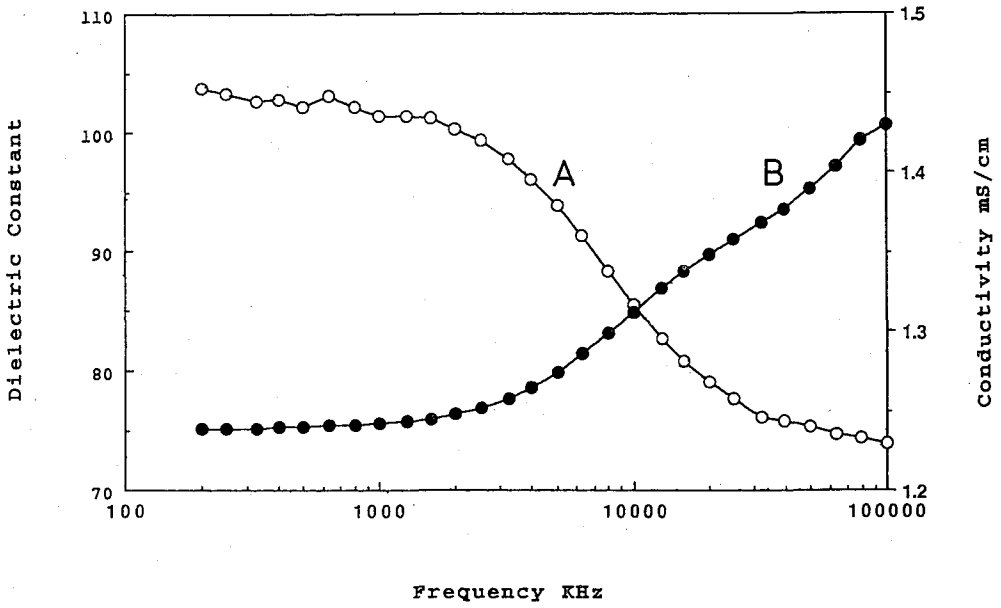

Fig. 2. Dispersion behavior of the permittivity ε and conductivity κ of cytochrome c solution as function of frequency (Curves A and B)

postulated that the origin of the dipole moment of polyelectrolytes stems from the fluctuation of mobile counterions caused by an electrical field. Some exemplary dielectric constant results obtained with DNA solutions are presented below. DNA is a very large fibrous polymer which stores all genetic codes using unique sequences of its base molecules. Because of the double helical structure, DNA molecules are fairly rigid even in solution. Moreover, polynucleotide chains are negatively charged because of the presence of phosphate groups. Because of their large sizes, the dielectric dispersion occurs at very low frequencies namely around 5-8 Hz. Figure 3 shows the frequency profile of the dielectric constant of a dilute solution (0.05%) of a highly polymerized DNA (molecular weight about 3×10^6) (Hayakawa, et al. 1975). We first point out that the static dielectric constant of DNA solution reaches a high value of 4×10^4 at a concentration of only 0.05%. This corresponds to a dipole moment of 1.5×10^6 D.U. This large dipole moment was found to be proportional to the degree of polymerization, indicating that the dipole moment of DNA lies along the major axis of the double helix. This conclusion is contrary to the early concept that DNA has a transverse moment (Allgen, 1949).

Structural consideraions, i.e., the antiparallel double helical configuration, preclude the possibility of the DNA molecule having a large permanent dipole moment. This is because of cancellation of possible dipole moments in each strand. Left for consideration is counterion polarization.

The theory above uses a model in which charged molecules are surrounded by a continuous distribution of counterions. This model does not consider the specific interaction between ions and charged sites. Binding of counterions to charged sites on the polyion's surface has a profound effect on the dielectric behavior of

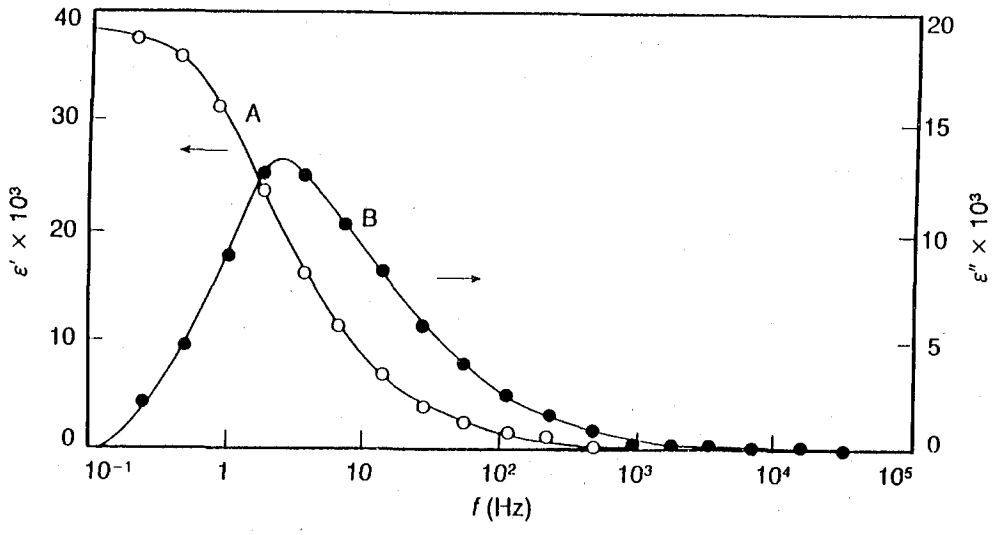

Fig. 3. Dielectric dispersion of the DNA solution measured with a four terminal technique. A is dielectric constant and B is dielectric loss: dissipation of energy.

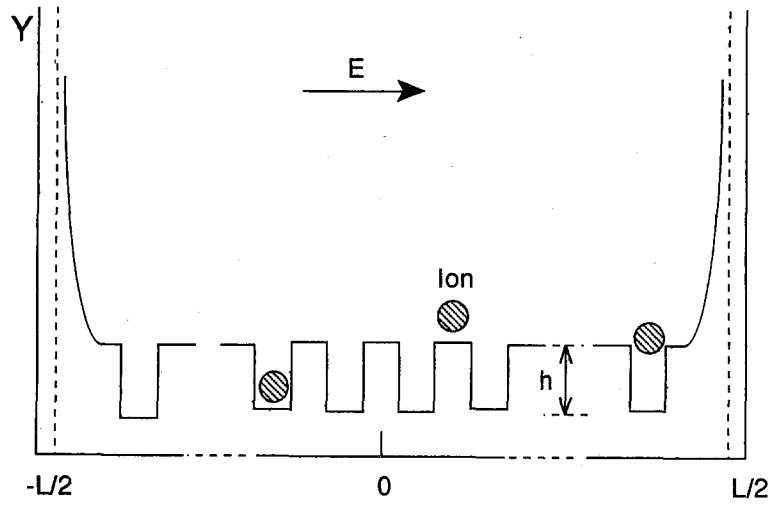

Fig. 4. Schematic presentation of one dimensional discrete potential well model. h is the height of potential barriers and shaded circles show counterions. L is the length of linear polymers.

polyelectrolytes. For example, binding of counterions causes a drastic reduction of the dielectric constant of polyelectrolytes. In order to account for this fact, the model must have a feature which may explain the observed effects of ion binding. A model which may be termed 'a discrete potential well model' has been used for some time for linear charged polymer molecules. Figure 4 is a schematic presentation of this model (Mandel, 1961). It consists of an array of potential wells which are separated from one another by an energey barrier h. If ions are found in these potential wells, they are considered bound to charged sites, i.e., neutralization of the charge, otherwise they are

considered unbound. The dipole moment of linear polyions is calculated by computing the distribution of unbound as well as bound counterions along the fiber in the presence of an electrical field. This discrete potential well model has been used by several investigators (Mandel, 1961, McTague and Gibbs, 1966, Warashina et al., 1973 and Minakata, et al., 1972. Accoding to these investigations, the binding of counterions causes an increase in dielectric constant when charged sites are only partially occupied. However, further ion binding causes a marked decline of the dielectric constant (Minakata, et al. 1972). This prediction is in agreement with the results with synthetic poly-electrolyte molecules. Counterion binding to charged DNA-phosphate groups causes a marked decrease in dielectric constant. However, the initial rise in $\Delta\varepsilon$ which is predicted by aforesaid theories has not been observed. Macromolecular solutions display a variety of responses. They all share the γ-response due to the presence of water. In addition they display a weak δ-response which is probably mostly caused by bound water. The amount of bound water is about 0.3 g bound water per g protein for globular proteins. Macromolecular solutions frequently display a γ-dispersion which is shifted to slightly lower frequencies and involves a broadening of dispersion curve, i. e., a small distribution of relaxation times. The mechanism of this effect remain unclear. It can result from the interaction of the high frequency tail of the δ-spectrum with the γ-dispersion. We support this by two arguments: a) The effect apparently increases with macromolecular concentration. b) It appears to be unlikely that all free water shifts its properties in common as proteins are added to the pure water. Relaxation contributions depend on the distance of the water from the macromolecule, with "distant" water less affected. The δ-dispersion of macromolecular solutions is about one dielectric units per g protein 100cc, i.e. of fairly small magnitude. Its cause is mostly due to bound water as discussed above. But partial rotation of macromolecualr subunits probably also contributes. The high frequency limits ε_∞ of the δ-dispersion are not precisely known since the dispersion extends into the γ-range. However, limit values ε_∞ of the δ-dispersion are about as expected from mixture equation if the bound water surrounding protein is of low permittivity compared with that of the non-bound water.

As discussed earlier, protein solutions display a dispersion of dielectric constant at 0.1 to 10MHz. The dipole moment of protein molecules is believed to originate from the permanent dipole due to fixed charges and core bond moments. However, an alternate model was proposed by Kirkwood and Shumaker (1952). They pointed out the importance of the role of mobile protons as the origin of the large dipole moment of protein molecules and that there are more dissociated amide groups on the protein surface than loosely bound protons. Therefore, protons can migrate from one amide group to another when an electrical field is applied. The fluctuation of the proton distribution can produce a non-vanishing mean square dipole moment even if the mean permanent dipole moment of the protein is zero, namely,

$$\langle\mu^2\rangle = \langle\mu\rangle^2 + \Delta\mu^2 \tag{12}$$

Here $\Delta\mu^2$ is the moment produced by the fluctuating protons and given by

$$\Delta\mu^2 = e^2 f^2 b^2 \sum_i \frac{n_i}{2 + K_i/[H^+] + [H^+]/K_i} \tag{13}$$

where e, b, n_i are elementary charge, radius of equivalent sphere and number of ith charge. f is a shape factor and K_i is the dissociation constan of the ith group. This equation indicates that $\Delta\mu^2$ increases when the pH of the protein solution approaches the pK value of the ith group on the surface of protein molecules. Numerical calculations by the authors demonstrate that the dipole moment of proteins can be accounted for by the proton fluctuation mechanism. However, theoretical and experimental analyses (Scheider, 1965, Takashima,1965) demonstrated that the proton fluctuation moment either can be observed only if certain conditions are met or is negligibly small for proteins with a molecular weight below 10^5. Kirkwood et al. were, nevertheless, the first to point out the importance of the fluctuating proton distribution for generating a non-vanishing mean square dipole moment. This mechanism is now considered unimportant for small proteins as the origin of their dipole moments. However, the concept of non-vanishing mean square dipole moments due to the fluctuation of mobile surface ions played an important role in the development of counterion polarization theories for highly charged polyelectrolytes as discussed earlier. Amino acids display a dispersion in a few hundred MHz. Because of this, the dispersion should be termed a δ-dispersion. Their δ-behavior is of polar origin, rather than the relaxation of bound water. Neither amino acids for proteins appear to show any α-effect, i.e., their permittivities are frequency independent in the LF-range.

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