MICROWAVE DIELECTRIC BEHAVIOUR OF SOME BIOMOLECULES

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Master of Philosophy

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Dedicated to

My Supervisor

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CERTIFICATE

This is to certify that, the research work embodied in this dissertation entitled **"MICROWAVE DIELECTRIC BEHAVIOUR OF SOME BIOMOLECULES"** has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, for the partial fulfilment of the award of Master of Philosophy. This work is original and has not been submitted so far, in part or full, for any other degree or diploma of any University.

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Kundan

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CONTENTS

CHAPTER 1

INTRODUCTION

Microwaves are a part of electromagnetic spectrum and their band extends from 300 MHz to 300 GHz. These nonionizing radiations have relatively short wavelengths and high frequencies compared to extremely low frequency fields. Microwave radiations are emitted by the air traffic control systems, defence, radar, earth satellite, television broadcast systems to name a few. This has led to the introduction of a newfound dimension to understand the effect and mechanism of EMF- biointeraction. While this issue is still in a mode of serious discussion microwaves has found its way into medication, communication and domestic use. Medical diathermy devices, microwave ovens and above all the mobile cell phones are now in ever increasing use. To understand the basic mechanism of interaction knowledge of dielectric parameters of biomolecules under physiological conditions is one of great concern. DNA damage have been reported which may be due to (1) signal transduction (2) free radical production (3) shifts in electrons within DNA molecules (4) and stress response.

Biomolecules are responsible for various important biological functions of living body. DNA, Cytochrome, Ribonucrease, Lysozyme, Trypsin, Pepsin, Albumin etc, are some important biomolecules. Under constant voltage, molecular dipoles in the dielectric tend to orient themselves along the electric field E and it is characterized by the dielectric constant ε.

The dielectric constant of a substance varies with (i) its composition (ii) physical structures (iii) temperature and (iv) frequency. Since different biological molecules have different structure, the complex dielectric constants at different frequency can be used for its characterization.

AIMS & OBJECTIVE

The primary purpose of this work is to measure the concentration and temperature dependence on dielectric parameters of biomolecules in solution. The effect of changes in the dielectric constant of the solvent and on the dielectrics properties of solution are expected to provide us information about the mechanism of dielectric relaxation. In the present work we have studied two biomolecules DNA and Lysozyme. Some details about their structure and functions are mentioned below.

DNA:

Deoxyribonucleic acid is the acronym of a biomolecule DNA. Nucleic acids are high molecular mass polymers formed of pyrimidine and Purina bases, a sugar, and phosphoric acid backbone as shown in Fig (1). Nucleic acids are built up of nucleotide units which are composed of sugar, base and phosphate groups in helical conformation. Nucleotides are linked by three phosphates groups which are designated α, β and γ. The phosphate groups are linked through the pyrophosphate bond. The individual nucleotides are joined together

by groups of phosphates that form the phosphodicster bond between the 3′ and 5′ carbon atoms of successive sugars **Fig. (2).**

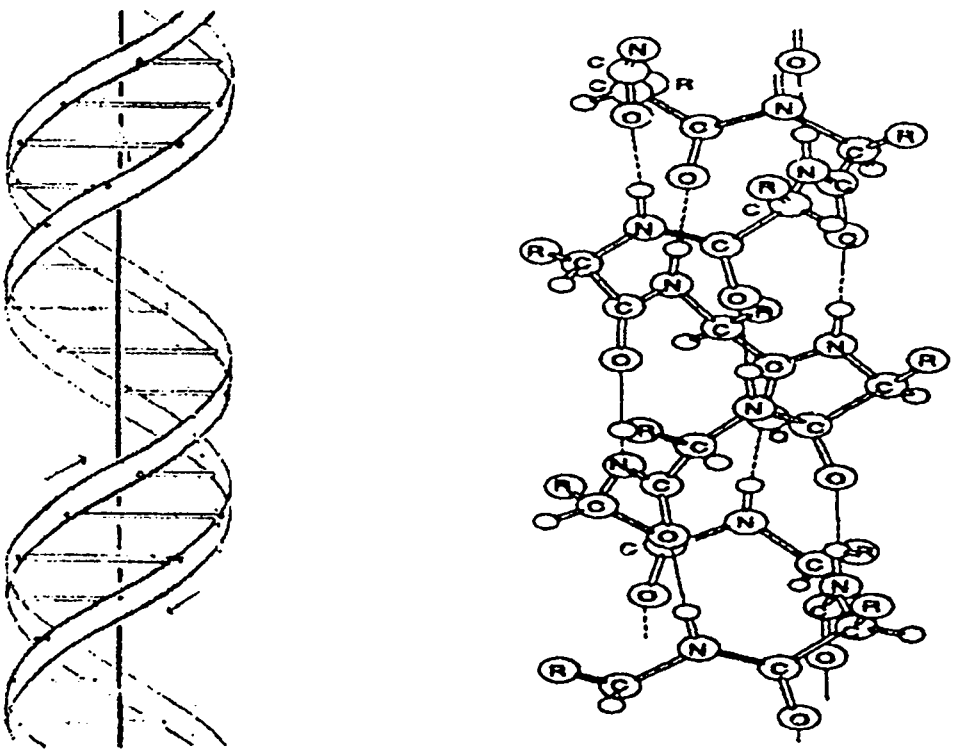


Figure 1 The right-handed helical conformation of DNA.

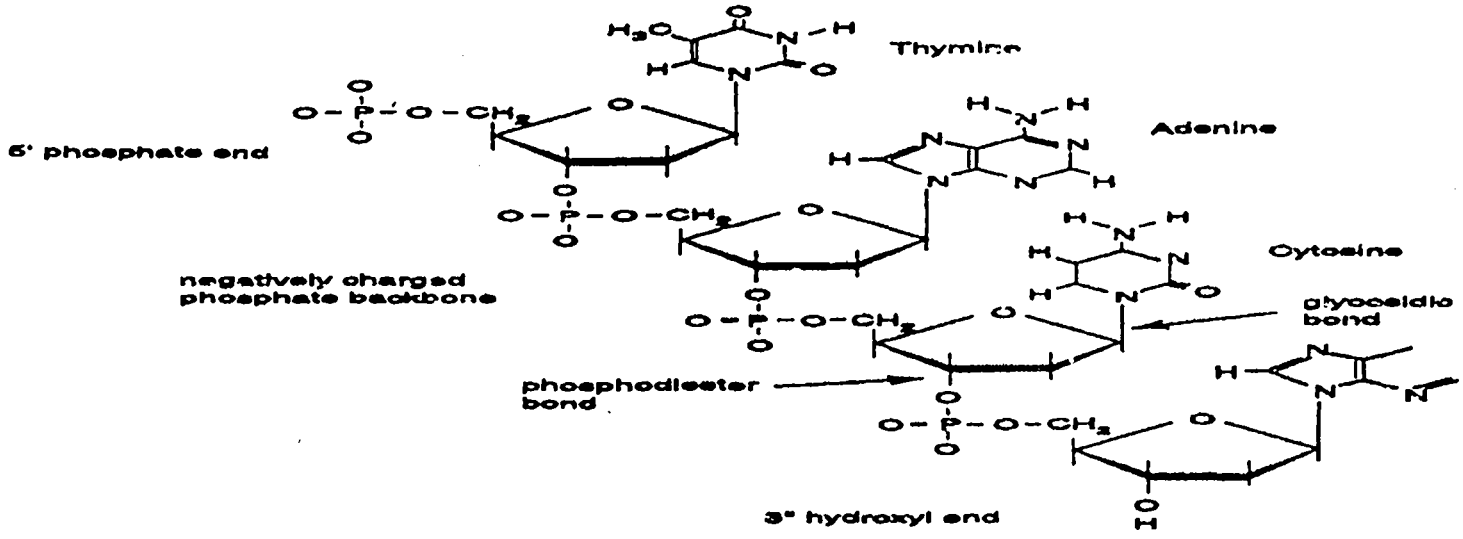


Figure 2 Single strand of DNA and the phosphodiester and glycosidic bonds.

These phosphate groups are acidic. Polynucleotides have a hydroxyl group at one end and a phosphate group on the other end. Nucleosides are subunits of nucleotides and contain a base and a sugar. The bond between the sugar and base is called the glycosidic bond as shown in Fig. (3). The base can rotate only in sterically permissible orientations about the glycosidic bond.

The helix is formed from two strands. The bases in adjacent strands combine by hydrogen bonding, an electrostatic interaction, with a pyrimidine on one side and purine on the other. In DNA the purine adenine (A) pairs with the pyrimidine thymine (T). The purine guanine (G) pairs with the pyrimidine cytosine (C). A hydrogen bond is formed between a covalently bonded donor hydrogen atom that is positively charged and a negatively charged acceptor atom. The A-T base pair are associated by two hydrogen bonds, whereas C-G base pairs associate by three hydrogen bonds. The base pair sequence is the carrier of genetic information.

The DNA molecule has a net negative charge due to the phosphate backbone. When dissolved in a cation solution, some of the charge of the molecule is neutralized by cations. The double stranded DNA molecule has little intrinsic permanent dipole moment as shown in Fig. (4). The reason is that the two strands that compose the helix are oriented so the dipole moment of one strand cancels the other.

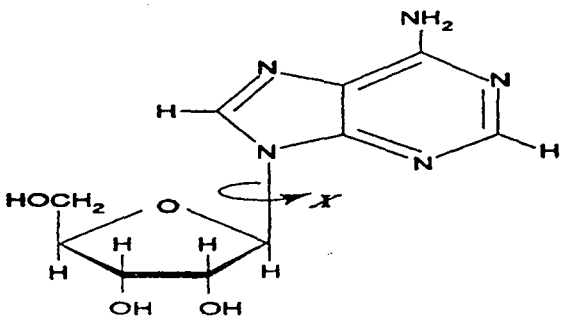


Figure 3 Glycosidic bond denoted by X.

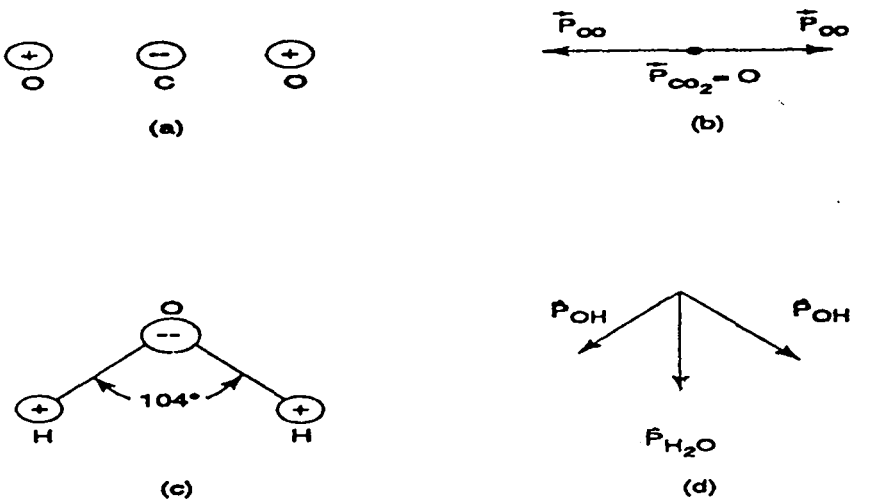


Figure 4 Permanent dipole moments of molecules. In (a) the charge in the carbon dioxide molecule is depicted. (b) depicts the cancelation of the dipole moments in carbon dioxide. (c) and (d) describe the dipole moment of the water molecule.

When DNA is dissolved in a solvent, such as saline solution, an induced dipole moment forms due to reorganization of charge into a layer around the molecule called the counterion sheath.

By using X-ray diffraction Watson and crick studied that the structure of DNA is in the form of a double stranded helix. Also nuclear magnetic resonance (NMR) experiments reveal a lot about its structure. Types A and B DNA are in the form of right-handed helices. Type Z DNA is in a left-handed conformation. A transition from type A to type B DNA occurs when DNA is dissolved in a solvent. Double-stranded DNA is not a rigid rod, rather a meandering worm-like chain. The DNA molecule as a whole is very stable.

The helical form of the DNA molecule produces major and minor grooves in the outer surface of the molecule. There are also bound water molecules in the grooves. Many interactions between proteins or protons with DNA occur in these grooves.

The interaction of the counterions with the DNA molecule has been a subject of investigations. Some of the counterions bind to the phosphate backbone with a weak covalent band. Other counterions are more loosely bound and some may penetrate into the major and minor grooves. The ions become bound near charges in the DNA molecule and a double layer forms. The ions

attracted to the charged DNA molecules forms a counterion sheath that shields some of the charge of the DNA as shown in **Fig. (5)** (Gulbrand et. al 1989).

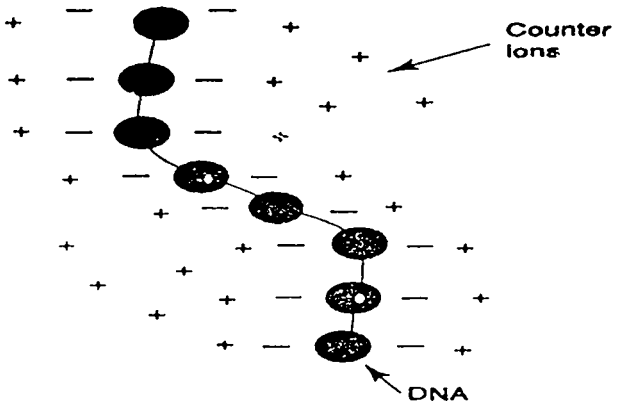


Figure 5 Counterions and DNA molecule.

The counterion sheath around a DNA molecule is composed of cations such as Na or Mg, which are attracted to the backbone negative phosphate charges. These charges are mobile and oscillate about phosphate charge centres in an applied electric field. A portion of these counterions is condensed near the surface of the molecule whereas the vast majorities are diffusely bound. The condensed counterions bind with the phosphate backbone charges in a weakly covalent bond. In this bond, the outer S-shell of the counterion may hybridize with the S and P shells of the phosphate oxygen (Saxena et al. 1992). The dielectric relaxation of DNA in ethanol also showed the counterion-dipole theory of relaxation (Sakamoto et al. 1979). Here the microwave dielectric study of DNA provides valuable information regarding solute-solvent

interactions. The magnetic fields due to microwaves interact with conducting electrons in the stacked bases of the DNA. Such interaction may alter the gene function by altering the conformation of the DNA.

The dominant charges on the DNA molecule are from oxygen atoms in the phosphate groups located on the outer surfaces of the helix. Renugopalakrishnan and Lakshminarayanan, (1971) studied the charges on DNA.

Bases are stacked vertically by vander waals forces, which originate in the attraction of induced dipole moments between atoms as shown **in Fig. (6).**

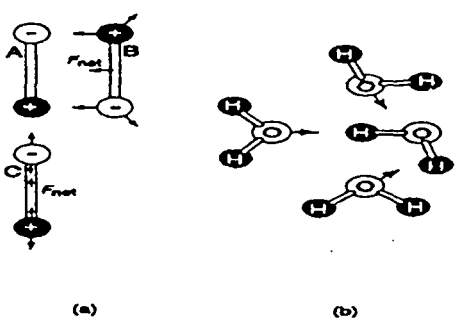


Figure **6** Origins of hydrogen and van der Waals bonds. In (a) the attractive force between dipoles is depicted. In (b) the attractive force between water molecules due to charge separation is shown.

Hydrogen bonding between bases originates from the attractive force between dipole moments and requires the dissociation of a water molecule. The bond strengths of various bonds are shown in the table given below.

Bond Type	Binding energy (J/mol)
Covalent bond	-2.8×10^5
Ionic bond	-1.8×10^5
Hydrogen bond	-1.8×10^4
Vander Waals	-2.8×10^3

The helix strands can be either separated by heating the DNA solute followed by quenching or by decreasing the ionic strength of the solvent. The reaction of strand separation is called melting denaturation.

The modeling of the helix-coil transition requires analysis of polyelectrolyte theory coupled with free-energy considerations. If the strands separate, they will coil. Single stranded DNA in a rigid oriented state has a permanent dipole moment due to the alignment of the positively charged amide groups. Takashima, S. (1966), in his study of dielectric dispersion of deoxyribonucleic acid showed that coiling severely decreases the net permanent dipole moment. Single stranded DNA has a permanent dipole moment of approximately 20 D per base and bases are separated by 0.34 nm. (1D $=3.33 \times 10^{-30}$ Cm.)

On the other hand Double stranded DNA possesses a large induced dipole moment due to counterion atmosphere of the order of thousands of Debye. Hogan et al. (1978) also confirmed the above result.

The induced dipole moment μ in an electric field E in defined in terms of the polarizability $\mu = \alpha E$.

Oosawa, (1970) from statistical mechanical means calculated the mean-squared dipole moment $<\mu^2>$ and α as:

$$\alpha = \frac{d\langle\mu\rangle_{E=0}}{dE} = \frac{\langle\mu^2\rangle_{E=0}}{k_B T}$$

The above expression clearly shows that polarizability is a result of fluctuations in the dipole moment. Hanss, and Bernengo, (1973) in his study of dielectric relaxation and orientation of DNA molecules have measured a small permanent dipole moment for DNA. When alternating field is applied, the symmetry of the molecule may be deformed slightly to produce a small permanent dipole moment. Plum and Bloomfield, in his study showed that small permanent dipole moment is due to attached charged ligands such as proteins or multivalent cations. These ligands produce a net dipole moment on the DNA molecule by breaking the symmetry.

It has been proved that DNA molecule possesses both induced dipole moment and permanent moment. Obviously the response of these dipole moments are different in an external applied field.

Hogan et al. (1978) in his study of transient electric dichroism of DNA molecules calculated how much of the relaxation of the DNA molecule is due to induced dipole moment and permanent dipole moment.

The potential energy of a permanent dipole moment at an angle θ to the E is given as

$$U = -\mu E \cos\vartheta$$

where as for the induced dipole moment, potential energy is given as U= -$\Delta\alpha/2$ ($E^2 \cos^2 \theta$) where $\Delta\alpha$ is the difference in polarizability along anisotropy axes of the molecule. The majority of the moment was induced rather than permanent.

Crothers, (1994) studied the effect of charge imbalance in DNA strands for DNA bending. Single stranded DNA bends when attached proteins neutralize one side of the DNA molecule. Repulsions between negative phosphate charges in the rest of the helix cause bending. This bending is responsible to cause a permanent dipole moment as the phosphate charge balance is changed.

Our interest lies in dielectric behaviour of DNA in solution. A lot of study has been undertaken on dielectric properties of bound water and polyelectrolytes around DNA. The region close to the DNA molecule has a low dielectric constant and a fixed charge. The region far from the molecule has a dielectric constant close to that of water. Lamm, (1997) studied the variation of dielectric constant in the grooves, near the surface, and far away from the DNA molecule. The permittivity depends on:

(i) Solvent Concentration

(ii) Distance from the molecule

(iii) Boundary effects and

(iv) Dielectric field saturation

The variation of dielectric constant with position significantly alters the predictions for the electric potential in the grooves regions. Besides the basic H_2O triad structure of the water molecule there are also hydrogen-bonded networks created by dipole –dipole interactions which form hydroxyl (OH^-) and hydronium (H_3O^+) ions. The dielectric constant of water is about 80, whereas biological water contains ions which affect both the real and imaginary parts of the permittivity. Water bound in proteins and DNA has a decreased permittivity. The reason may be due to constraints on the movement of the molecules when they are attached to biomaterials.

Maleyev et al. (1993) in his study of physical properties of the DNA water system estimated that DNA contains 5 to 20 bound water molecules per base pair. Some of the bound water in the minor and major grooves of DNA located on the charged nitrogenous bases and forms an aqueous bridge Fig. (7). This water is tightly bound and rotational motion is constrained in response to oscillating electric fields and hence has a lower dielectric constant. Water molecules are also bound to the negatively charged phosphate groups on the exterior of the helix.

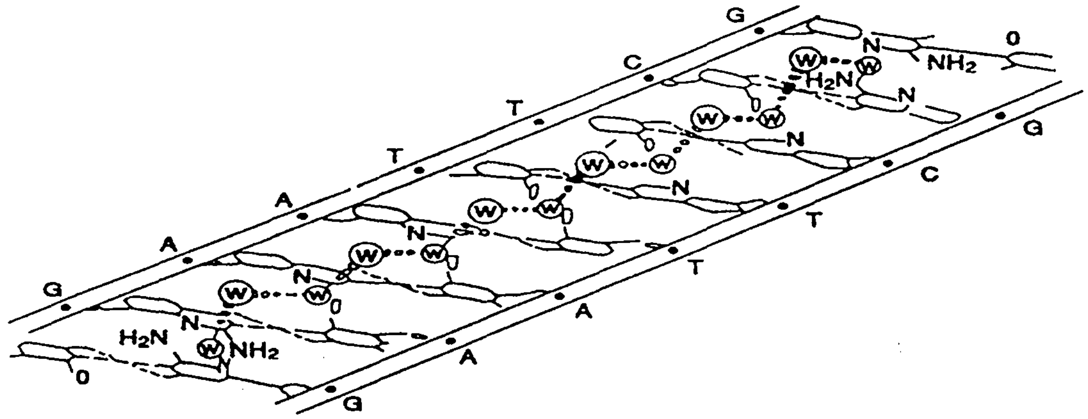


Figure 7 Water backbone in the minor groove of B-DNA. W denotes water molecule, dashed lines used for hydrogen bonds in base pairing. The water molecules are around the phosphate groups.

Lysozyme:

Lysozyme is the lytic enzyme that cleaves the glycosidic bond. It is a small protein, basic (isoelectric point) and stable. It was the first enzyme containing all the 20 usual amino acids to be sequenced. This enzyme is of paramount importance in biochemistry and biophysics. Lysozyme is widely distributed in nature, so some biological functions other than its primary anti-bacterial action are expected. Lysozyme is effective against bacterial and viral infections and also shows antiphlogistic activity in a number of pathological conditions. Lysozyme is also responsible for immune-stimulating actions, hence used as a medicine and digestive enzyme in certain cases.

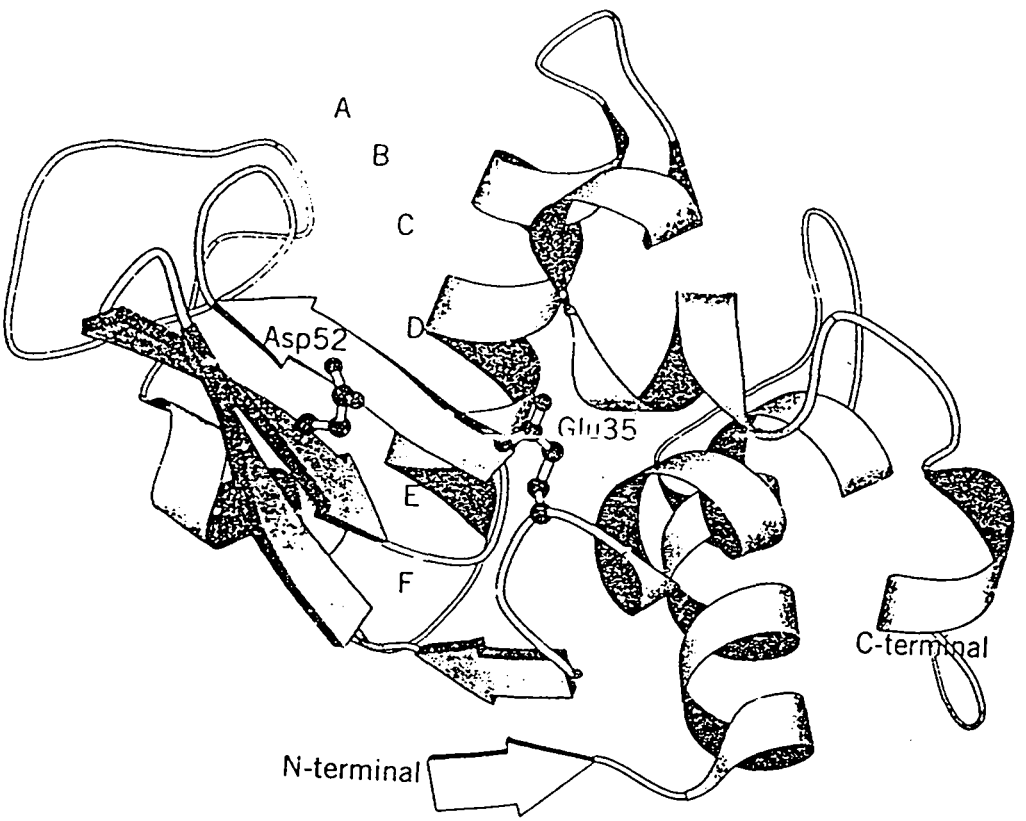


Fig. 8(a) the three-dimensional structure of hen lysozyme. Resi Glu35 and Asp52 are shown as the catalytic groups.

The three dimensional structure of lysozyme is shown diagrammatically in Fig 8a. The structure adopts a mixed α/β fold. A deep active site cleft divides the molecule into two domains; one is almost entirely β sheet structure, the other is α – helix rich and consists of the N- and C-terminal segments. The two domains are linked by an α-helix.

Lysozyme hydrolyses β-1, 4-glycosidic bonds between N-acetylglucosamines, and chitin derivative can be used as substrate. This also hydrolyses the β-1, 4-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine residues in polysaccharides.

The X-ray crystal structure of lysozyme has been determined in the presence of a non-hydrolysable substrate analog. This analog binds tightly in the enzyme active site to form the ES complex, but ES cannot be efficiently converted to EP. It would not be possible to determine the X-ray structure (**Fig** 8 b.) in the presence of true substrate, because it would be cleaved during crystal growth and structure determination.

The active site consists of a crevice or depression that runs across the surface of the enzyme. Many hydrogen-bonding contacts between the substrate and enzyme active site that enables the ES complex form. There are 6 subsites within the crevice, each of which is where hydrogen-bonding contacts with the sugar are made. In site D, the conformation of sugar is distorted in order to make the necessary H-bonding contacts. This distribution raises the energy of the ground state, bringing the substrate closer to the transition state of hydrolysis.

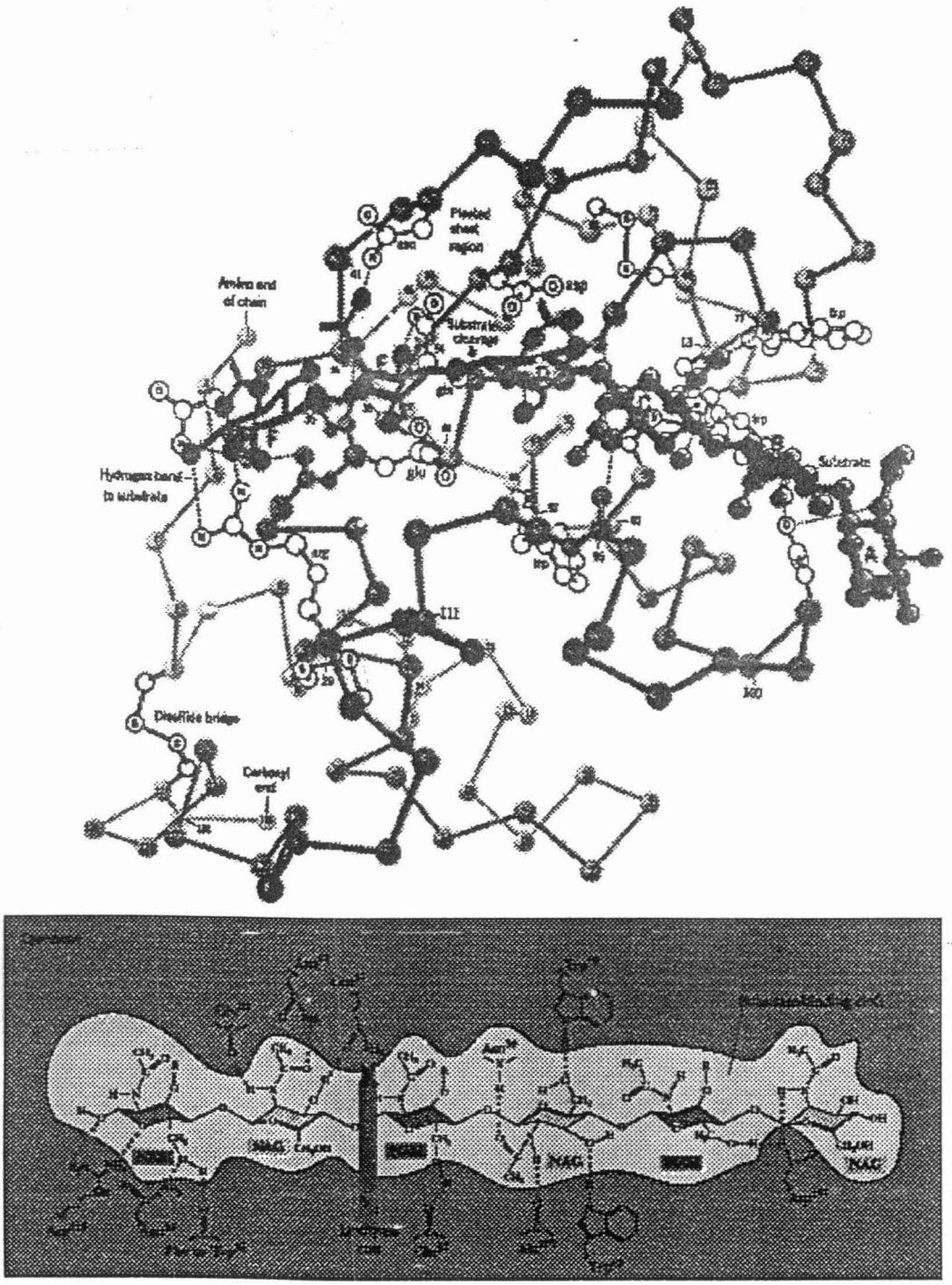


Fig. (8b) X-ray crystal structure of lysozyme

CHAPTER II

LITERATURE REVIEW

Ise, et al. (1963) in his study on the electrostatic potential of B-DNA has predicted by numerical modeling that the dielectric constant of solvent (water) has a critical role. This model shows that small ions can penetrate into the minor and major grooves. Hence there is a strong relationship between dielectric behaviour and solvent concentration. In another study Bonincontro, et al. (1988) analysed that the phosphate groups are far apart and the water molecules are arranged independently around the phosphates. In type A DNA the water molecules form a continuous water bridge between the phosphates. Similarly, in type Z DNA, water molecules form bridges between amino groups in bases and oxygen in the phosphate groups.

The dielectric relaxation in DNA occurs in the range of 1 to 100 Hz, another relaxation was found in megahertz region. This is primarily due to movement of condensed counterions bound to individual phosphate groups. There are many types of motion of DNA molecule in the electromagnetic field such as propeller twist occurs when two adjacent bases in a pair twist in opposite directions. In breather mode, two bases oscillate in opposition as hydrogen bonds are compressed and expanded. Other motions of the base pairs of the DNA molecules are roll, twist and slide.

In the adjoining fig 2.1, the dielectric relaxations of DNA are shown in whole frequency range.

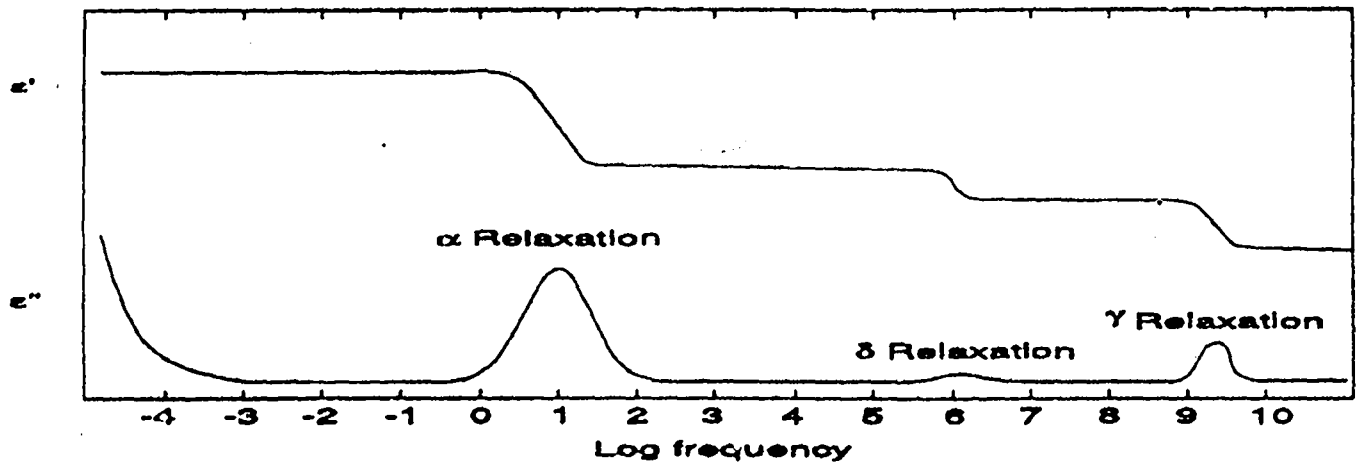


Figure 2.1 Dielectric relaxations of DNA.

Takashima, S. (1984) in his study of dielectric dispersion of DNA has proved that denatured DNA tended to coil. As a result there is decrease in effective length and hence also dipole moment. As single stranded DNA is considered to be rigid, relaxation would occur in the low megahertz frequencies.

Let us discuss the mechanism involved in the polarization relaxation. Torque experienced when dipole is placed in electric field in given as $\Gamma = \bar{P} \times E$. Longitudinal rotation relaxation time is given as

$$t_1 = \frac{kL}{2k_B T}$$

where k is a constant,

k_B is Boltzmann's constant

L is length of the molecule.

So, t_1 is found to be proportional to L. Considering the case when the dipole moment is parallel to the major axis. This will occur when there is excess charge on the dipole ends. This is shown in Fig. 2.2. Now in this case end over end rotation will occur. Here relaxation time t is given as

$$t_2 = \frac{\pi \eta l^3}{6kT[ln(l/b) - r]}$$

where η is viscosity and b is radius. Here we observe that relaxation time varies as L^3. Here we find that dielectric relaxation is dependent on molecular mass. However, this is not in good agreement with the experimental result as in reality DNA a molecule is never found in a rigid rod structure.

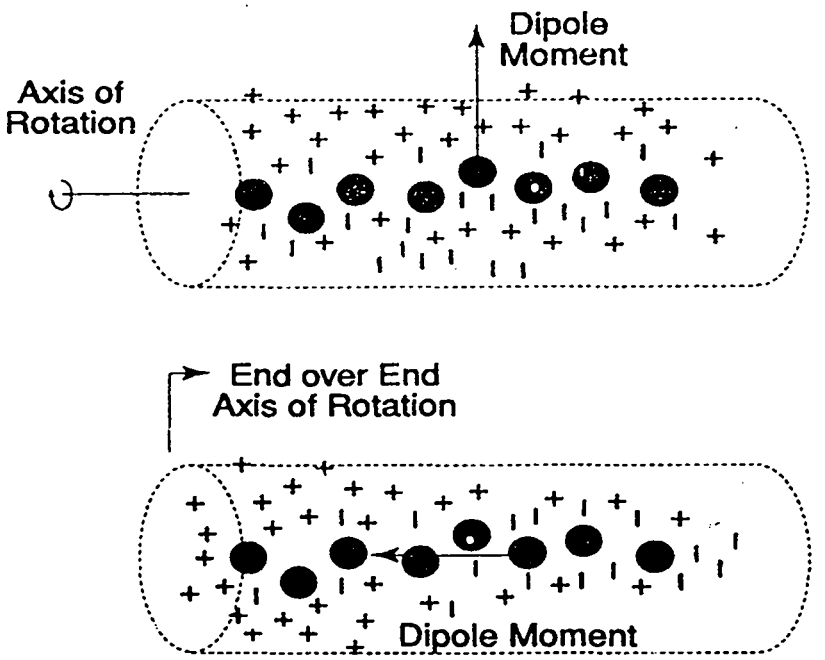


Figure 2.2 Torques on dipolar, rigid rod.

Sakamoto et al. (1976) in his study of dielectric relaxation of DNA in aqueous solutions found that the dielectric relaxation time varies in proportion to the square of the length of the molecule. This is quite in good agreement with experimental result as obtained by other researchers. Hence here contradiction occurs.

Edwards et al. (1994) in his study of structure in the electric potential emanating from DNA molecule had considered the charges to be resided only on the sugar phosphate backbone and bases. Here molecule was represented in there concentric cylinders. The inner one was molecule, the middle one counterions and the outer layer represented the solvent. The charges from the phosphate backbone were assumed to reside on the outer surface of the inner layer. In this study the skin depth was found to be of the order of 0.5 nm.

There is a great impact of solvent concentration and cations on the dielectric constant of DNA. With the increase in solvent concentration, phosphate charges get neutralized. Hence dielectric increment decreases.

There are a lot of study on the use of both monovalent and divalent metal cations in DNA solvent such as Nacl, $cucl_2$, dyes, pb, cd, zn etc. Duguid and Bloomfield, in his study found that the dielectric increment decreased for divalent cations. Dyes neutralize charge and stability depends on the cation concentration. Its concentration determines the dissociation of the DNA molecule. Sakamoto el al (1980) analysed the impact of concentration of salts and dyes on dielectric relaxation of DNA solutions as described in the adjoining fig 2.3(a, b,c)

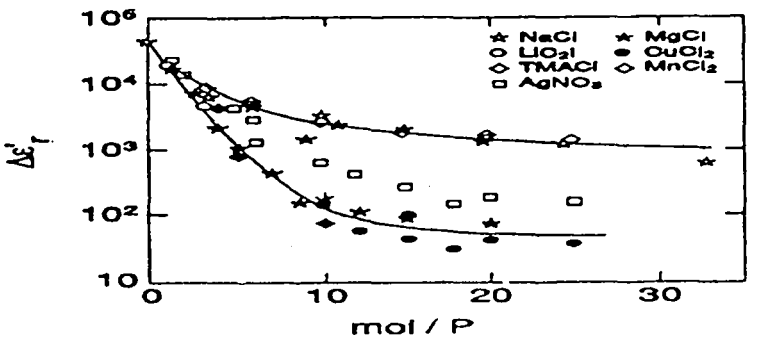


Figure 2.3a Dielectric increment versus mol/P where P is number of phosphate residues

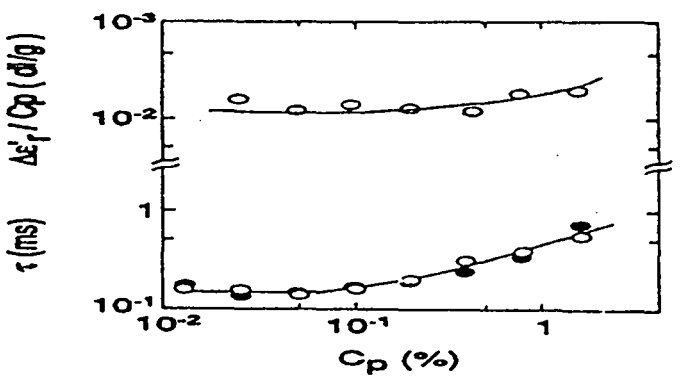


Figure 2.3b Dielectric increment and relaxation time vs concentration of DNA C_p.

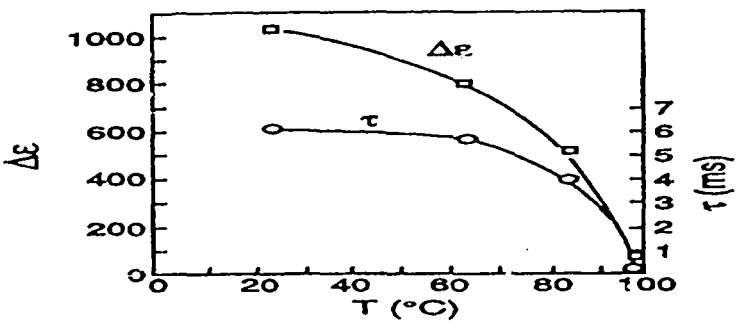


Fig 2.3c Effect of cations on relaxation time and dielectric increment

Nucleohistones and protamine-DNA complexes occur in somatic-cell nuclei of mammals. So its study is of paramount importance. A lot of research was done on the dielectric properties of nucleohistones and DNA. Histones and protamines are tightly bound in the major groove of the DNA molecule. They produce stability in the double helix by neutralizing some of the phosphate charges. Hence melting temperature is increased. As studied there is tight binding of protamine in the major groove. Thus the denaturing temperature of DNA in protamine increases. One of the applications of protamine to be used as a cation in DNA solution is due to its capability to stabilize DNA.

Galindo and Sokoloff, (1996) in his study found a mathematical expression for helix melting temperature in saline solution in degree Celsius as

$$T = 100.3 + 14.8 \ln C_P$$

where *Cp* is concentration in moles per litre.

DNA in a saline solution melts at a lower temperature than in protamine solution.

Bonincongtro, et al. (1989) have found the effects of protamine sulphate and arginine on dielectric relaxation both as a function of temperature and a over a large frequency large. They also observed the dielectric relaxation behaviour. The sulphate decreased the induced polarization by neutralizing phosphate charges. It was observed that the binding of protamine to DNA reduced the number of water molecules in the DNA complex. As protamine is a major-groove binder it tends to exclude bound water from the groove. **(Fig 2.4)**

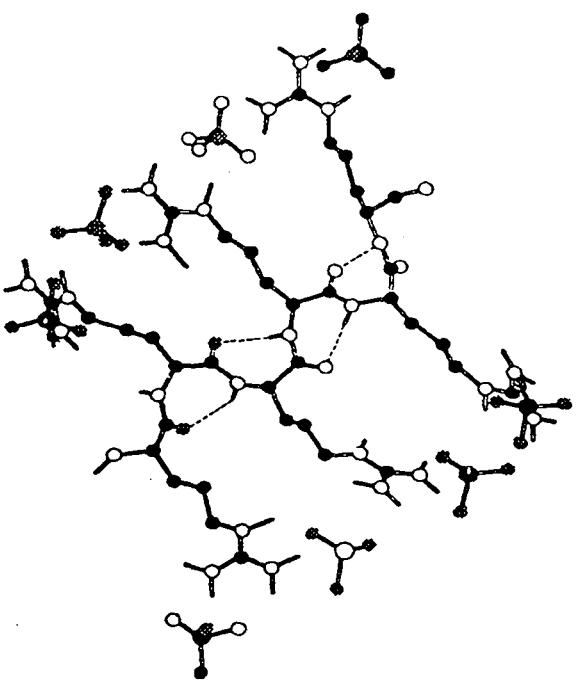


Figure 2.4 Six arginine residues bound to DNA; only the phosphate groups of the DNA molecule are shown.

Activation enthalpy was also estimated by the mathematical expression:

$$\frac{1}{\tau} = \frac{k}{\tau}\exp(\Delta S / R)\exp(-\Delta H / RT)$$

where ΔH = enthalpy of activation energy.

ΔS = molar entropy of activation energy.

K is a constant.

Galindo, et al. (1996) in his study on DNA also concluded that dielectric properties depend on cation concentration and temperature of the solvent. Stronger concentrations yields a higher dissociation temperature. The reason behind this is the screening of the phosphate charges by the saline solution. The molecule is having different free energy in different configurations. In thermal denaturation counterions are released from the molecule as the strand separate. Hence conductivity of solvent increases.

So far as conductivity of Biomolecule is concerned they have semi-conducting properties. Bakhashi, (1994) studied electronic conduction in protein and DNA and elaborated a lot about their properties. The semiconducting properties originate due to internal charge transfer mechanism. Two types of periodicity exist in DNA molecule (1) the phosphate backbone is periodic (2) the base- pair sequences are aperiodic. DNA also contains counterions and bound water. Periodicity promotes semi conductivity. In DNA the periodic component of the base pairs produce band gaps and the aperiodic component of the base pairs acts

as impurities in the band gap found by the phosphate backbone. The valance and conduction band gaps are between 0.3 e.v. to 0.8 e.v.

Risser and Beratan, (1993) in his study have proved electron transfer single-stranded DNA. Similar studies were conducted by other researchers too. Stemp et al. (1995) in his study confirmed the above results. Investigations were carried out in other biomolecules too. Bakshi in his study (1994) on biomolecule had analysed the whole phenomenon lucidly. He found in proteins that there were two main areas of charge transport. The charge transport is in the main polypeptide chains. The conduction through the main polypeptide chain dominates over the hydrogen bond mechanism. Electron transfer phenomenon in biomolecule is of prime importance as it is directly related to the damage at molecular level.

Hanlon et al. (1996) in their study found a larger density of protons in the minor groove than in the counterion solution surrounding the molecule. Many proteins attached to the DNA would not function normally with large charge transfer. If high charge transfer occurs then there will be permanent molecular damage.

Recently, Blank and Goodman, (1997) studied the phenomenon of interaction of DNA with electromagnetic field. They found electromagnetic fields stimulate biosynthesis in biomolecules especially in DNA. Recent studies shows that magnetic fields play a role in gene activation. This may be due to strong charge transport in DNA interacting with applied magnetic fields. The gene segment can be localized through charge transfer by bending of base pairs and also by enzyme interaction with base pairs.

Bonioncontro et al. (1988) studied Water induced conductivity of DNA. They found that conductivity was increased due to increase in water content in the solvent. The temperature dependence on the conductivity of biomolecules is governed by this equation:

$$\sigma = \sigma_0 \exp(-E / K_B T)$$

where K_B is Boltzmann's constant

T is the absolute temperature.

This is found to be independent of molecular mass. Pedone, (1999) in his study on dielectric relaxation on DNA found that the reason for this is due to the motion of condensed ions within a subunit of the DNA molecule. The γ relaxation is found to be around 18 GHz. This may be due to water molecule.

There are significant differences between the permittivity of the solvent and solutions containing DNA.

Takashima, S. (1984) found that DNA in its stretched state possessed a dipole moment oriented transverse to the axis. The phosphate group produces a permanent transverse dipole moment of about 20D per 0.34 nm base pair section. Hence net dipole moment is significant. As the base pair twist, the dipole moment decreases.

Poisson-Boltzmann Applied to Model the DNA Helix

It is possible to use the Poisson-Boltzmann equation to study the counterion atmosphere around the DNA helix by assuming a charge distribution or

potential on the helix surface. A number of models have been developed to study the helix and helix coil transition. For example, the model by Galindos assumes a counterion atmosphere and a helix of infinite length with constant charge density distributed evenly on the surface. In this study they assumed two cylindrical layers. The first layer contained the condensed counterions and in the second layer, the diffuse counterions. This is an approximation since most of the charge actually occurs in the phosphate groups in the backbone. Each phosphate sugar group was assumed to have one electron of extra charge. The boundary conditions on the surface of the DNA at r = a are Gauss' law

$$\frac{d\psi}{dr}(a) = \frac{2qt}{a},$$

and the vanishing of the field at $r \rightarrow \infty$

$$\psi(\infty) = 0$$

$qt = L_b/b$, where b is the length of the cylinder over which the charge is distributed and L_b is the Bjerum length. The solution in this case for the potential around the helix is

$$\psi(r) = \frac{k_B T}{q} In \frac{k^2 r^2}{2(\varepsilon_s / \varepsilon \beta_o^2)} \sinh^2 \beta_o In(Ar),$$

where ε_b and ε_s are the dielectric constants of the bulk solvent and solute and A and β_0 are constants which are determined by the boundary conditions. It is

important to realize that the dielectric constant near the surface is different than it is far away from the surface. The state of conformation is determined by minimizing the free energy.

According to the model, repulsive electrostatic forces from the excess of charge on one face of the helix act spontaneously to create and maintain compression of the opposite helix face and hence bending of the double helix.

DNA molecules that are bent or curved are anomalous in electrophoretic mobility. Curved molecules run more slowly than straight ones. Strauss and Maher exploited the technique of comparative electrophoresis to determine the direction and magnitude of the DNA bend that results from selective charge neutralization.

There are a number of models for DNA polarizability. The model developed by Mandel overestimated the counterion-induced polarizability. Since it neglects counterion repulsion. The model of Manning predicts an increase in polarizability with increasing solvent concentration in contradiction to experimental result. Polarizability decreases with increasing solvent strength. Probably this is due to phosphate charge neutralization by the increased concentration of counterions. Polarization also decreases with increasing temperature.

A lot of study was done on theoretical modeling of denaturing of DNA. Yakushevich, (1989) has given a simplified model of DNA. In this model the bonds were modeled by springs. Muto, et al. (1990) in his study assumed the

helix backbone to be composed of anharmonic Tada potentials between nucleotides and Lennard-Jones potential for the hydrogen crosslinks.

Bonds were modeled by potentials and the coupled -force problem was solved. The solutions to the differential equations are thermal solitons.

When DNA is put in a solvent there arises a layer between DNA and liquid smaller electron affinity will acquire greater positive charge. The potential difference will attract ions of opposite charge to the surface and repel like charges. The potential from the surface decreases as $\psi(\chi) = \psi_0 \exp(-\chi / \lambda)$ where λ is the skin depth. The solution to the above eqn. was given by the Dukhin and Shilov. A detailed analysis of the counterion atmosphere and dielectric relaxation was given.

Simple Model of Dipole Moment:

The dipole moment of polymer molecules is usually modeled by a set of charges that are connected by flexible links. In this section, we review a model for the dipole moment of a string of rigid rods. (Fig 2.5)

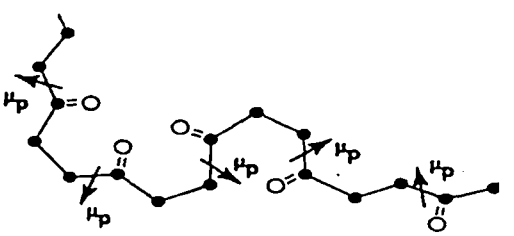


Figure 2.5 Polymer chain of dipoles of moment μ_p.

The net charge at positions $\vec{r}_1$ is $q\delta ni = q(ni - < n >)$, where q <n> is the mean value of charge distribution. The net dipole moment is

$$\vec{\mu} = \sum_i q(r_i - r_G)\delta n_i$$

where the center of mass for N links is

$$r_G = \frac{i}{N}\sum_i r_i$$

The mean-squared moment is

$$< \mu^2 >=< q^2 \sum_i \sum_j (r_i - r_G)(r_j - r_G)\delta n_i \delta n_j > .$$

This is approximately

$$< \mu^2 >= q^2 \sum_i \sum_j (r_i - r_G)(r_j - r_G) < \delta n_i \delta n_j > .$$

If we apply this model to the double stranded DNA helix and assume there is a probability α_B of an ion binding at a site, then the correlation function for the charge distribution can be approximated by:

$$< \delta n_i \delta n_j >= \delta i_{ij} \alpha B(1 - \alpha B).$$

We then have

$$\sqrt{< \mu^2 >} = q\sqrt{N\alpha B(1 - \alpha B) < L^2 >},$$

where $< L^2 >= \frac{1}{N}\sum_{i=1}^{N} < (r_i - r_G)^2 >$.

This model correctly predicts the dependence of the dipole moment on the length of the polymer but not on the temperature dependence. In the next section we will generalize this model to get correct temperature dependence.

Dipole Moment, Permittivity, and Relaxation Time:

Minakata developed a comprehensive phenomenological model for discrete counterion binding on rod like polymers. It includes three different types of counterion binding sites. This model is important as it correctly predicts a number of the important measurable quantities in the relaxation process. The mean squared dipole moment is defined as:

$$< \mu^2 >= q^2 \sum_{ij} r_i r_j < (n_i - < n >)(n_j - < n >) = q^2 \sum_i (\Delta_i^2 n r_1^2 + 2e^2 \sum_{ij} (\Delta_{ij} n) r_j r_i + j),$$

where n is the number of bound ions at a specific site, $\Delta_i^2 n =< n_i^2 >^2$, and $\Delta_{im} n =< n_i n_{i+m} > - < n^2 >$. This model yields

$$\Delta\varepsilon = \frac{\pi N B n q^2 L^2}{9 k_B T} [\Delta n^2 + 2\sum_i \Delta_i n]$$

where B is the ratio of the internal to external fields, L is length of the molecule, and N is number of polyions per unit volume. The important result of this

model is that it correctly predicts the dielectric increment to be proportional to L^2 and temperature dependence to be of the form 1/T.

Another model developed by Sakamoto relates dipole moment of the counterion sheath to dielectric increment from counterion fluctuation theory and agrees well with experiment.

$$\Delta\varepsilon = \frac{4\pi N < \mu^2 >}{3k_B T}$$

Sakamoto defined the dielectric relaxation time in terms of the maximum frequency f_m in the loss spectrum by $T_D = 1/2\pi f_m$. Fro double stranded calf thymus DNA, T_D was proportional to the square of the molecular mass of water M_W and the mean dipole moment was proportional to $M_W^{3/2}$ and the mean dipole $T_D \approx 2\ T_Z$, where $T_Z = 0.42\ M_w\ \eta_{red}/RT$ is the Zimm viscoelastic relaxation time. Here η_s is solvent viscosity and η_{red} is the reduced viscosity.

Levstik et al, (1999) examined the dielectric relaxation of hydrated proteins. The hydration water of proteins is essential to biological activity. Proteins are inactive in the dehydrated state. They begin their biological activity when their hydration level reaches approximately 0.2 g of water per g of protein. Water molecules are hydrogen bonded to the protein, which induces some drastic departure from the properties of bulk water. Dielectric measurements provide important information about this bound water. Water

molecules are highly polar and they form a network on the surface of the protein along which electrons can be transferred. The formation of this network with long-range connectivity has been detected as a perlocation transition when the water content approaches 0.5 g/g of protein.

When temperature is reduced, the proton mobility slows down. Careri et al, 1985 studied hydrated lysozyme powders in the frequency range 10 KHz to 10 MHz. In this frequency range protonic conduction is at the origin of the dielectric relaxation. Kunst, et al 1991also found that the proton mobility is dependent on temperature in ice. When temperature is reduced, the proton mobility slows down. The building block of a model for hydration water is a description of the water molecule in order to investigate the structure of water patches on the surface of a weakly hydrated protein. The interaction between two water molecules is described by a lennard-jones potential between the molecule centres and electrostatic interactions of effective charges associated to the protons and the oxygen.

The results of the dielectric measurements which exhibit long-range charge transport on the protein surface suggest that in the studies of protein activity and folding, the rate of charge transport should not be neglected.

Theoretical Background

The dielectric properties of a biological system determine the coupling and absorption of non-ionizing electromagnetic energy into the system. Although a complete description of the dielectric charge distributions in the molecule is not available, but they have advantage that they are accessible albeit indirectly from the experiment. These properties can be calculated from the wave functions of the molecules from the quantum mechanical approach and it is expected that dielectric information will be cross check for the quality of this approach (Hill et al. 1961). Theoretically it is now possible to estimate the dipole moments of protein.

Dielectric studies of materials have been a powerful tool in assessing the structure and behavior of molecular materials. The response of a material to an applied electromagnetic field is determined by the electrical and magnetic properties of the medium. For a non-magnetic system, the significant property which determines the impedance offered to the incident wave is the dielectric constant of the medium. If the medium is lossy, energy is absorbed as the radiation penetrates into the material. The amplitude of the wave decreases i.e., attenuation occurs as energy is absorbed. This is accompanied by a shift in phase. The attenuation and the phase shift are dependent on the dielectric properties of the medium characterized by complex dielectric constant ε^* of the medium denoted as ε'-jε''. Here ε' is the real permittivity, the dielectric constant

of an equivalent loss less dielectric and ε'' denotes the loss factor. The complex permittivity is frequency dependent.

Our present understanding of the dielectric properties of materials is based on the Debye theory (Smyth 1995, Bottcher 1952) of relaxing dipole interacting with an applied electric field. Early work showed that a set of exactly equivalent, non-interacting dipoles characterized by a single relaxation time (Γ) adequately explained the behaviour of weak dipolar solutions or dipolar molecules in the gas phase, but was insufficient to account for the broader frequency range over which dispersion was observed in solids and liquids in the frequency range below around 10^{10} Hz. This difficulty was circumvented by a consideration of distribution of relaxation times, like Cole-Cole, Fuoss-Kirkwook, Cole-Davidson and Willams-Watts treatments (Hill et al. 1969; Bottcher, 1978) applied to the case of more interactive media. Such approaches involve interpretation of the experimental measurement in terms of degree of fit to empirical functions.

An empirical characterization of loss in solids and liquids was proposed by Jonscher (1977) and a more generalized expression was proposed by R. H. Hill (1978). The various types of dielectric response have been summarized by Ngai et al. (1979). At one extreme is the case of non-interacting system characterized by Debye behavior. Increasing nearest neighbour interactions lead to behavior as postulated by Cole and Cole or Cole and Davidson.

Finally, as the interactions tend to be more complicated as in the case of solids and solid like substances universal dielectric response suggested by Jonscher (1977) seems to be applicable.

The Debye Equation: Let a system consists of dipolar molecules. If the system were placed in a static electric field, then polarization will be in equilibrium with the field. If the electric field is alternating at low frequencies, the polarization will still be in phase with the electric field. If the frequency is increased and is sufficiently large then polarization will lag behind the applied field leading to absorption of energy and fall in permittivity.

If the field is removed and there is polarization even in the absence of an electric field, then there is decrease in orientation polarization. This decrease of the orientation polarization depends only on the value of the orientation polarization at that instant. Let us assume the rate of change of polarization to be proportional to the polarization, the differential equation for the orientation polarization in the absence of an electric field is given as:

$$\frac{dp_{\infty}(t)}{dt} = \frac{-1}{\tau} p_{\infty}(t) \qquad \text{..... (3.1)}$$

The solution of the above eq. is given as

$$p(t) = p(0)e^{-\frac{t}{\tau}} \qquad \text{..... (3.2)}$$

In the reverse situation when the polarisation is built up due to application of a constant external field.

$$p_{or}(t) = p(\infty)\left(1 - e^{\frac{-t}{\tau}}\right) \qquad \text{..... (3.3)}$$

The total polarisation P_S in a static field E may be divided in two parts

$$P_S = P_\infty + P_{or} \qquad \text{..... (3.4)}$$

where p_{or} indicates the part of P_S due to dipole orientation and P_∞ the part due to the polarizability of the particles. Neglecting the time required to establish $P\infty$ relative to the time required to build up P_{or}, we may consider P_{or} to be built up in the time in which P_∞ changes to P_S. The electric displacement D is related to the applied field E and polarisation P by the relation

$$D = E + 4\pi P. \qquad \text{..... (3.5)}$$

Since in the static case $D_s = \varepsilon_s E$ where ε_s is the static permittivity of the medium

$$P_s = \frac{\varepsilon - 1}{4\pi} E \qquad \text{..... (3.6)}$$

Similarly the refractive index is defined as

$$P_\infty = (n_\infty{}^2 - 1)\frac{E}{4} \qquad \text{..... (3.7)}$$

With

$$n^2{}_\infty = \varepsilon_\infty$$

The theory of dielectric relaxation is based on the assumption that eq. (3.3) is also valid for an alternating field. Representing the alternating field by E (t) eq. (3.4) with the help of eqs. (3.6) and (3.7) becomes

$$p_{or} = \frac{\varepsilon_s - 1}{4\pi} E(t) - \frac{\varepsilon_\infty - 1}{4\pi} E(t) \qquad \text{..... (3.8)}$$

If the field is alternating with an angular frequency ω, it can be represented by

$$E(t) = Ee^{jwt} \qquad \ldots\ldots\ldots\ldots \quad (3.9)$$

The differential eqn for the build up of polarisation would then become

$$\frac{dp_{or(t)}}{dt} = \frac{1}{\tau}\left(\frac{\varepsilon_s - \varepsilon_\infty}{4} E_o e^{jwt} - P_{or}(t)\right) \qquad \ldots\ldots\ldots\ldots \quad (3.10)$$

The general solution of this equation is:

$$p_{or}(t) = ce^{-\frac{t}{\tau}} + \frac{\varepsilon_s - \varepsilon_\infty}{4\pi(1 + jwt)} E_o e^{jwt} \qquad \ldots\ldots\ldots\ldots \quad (3.11)$$

The first term on the R.H.S. would decrease to an infinitesimal small value after some time and therefore can be neglected.

Sinusoidal function of time with the same frequency as the applied field but lagging in phase with request to E.

The complex permittivity $\varepsilon^* = \varepsilon' - j\varepsilon''$

This leads to

$$\varepsilon' = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (wt)^2} \qquad \ldots\ldots\ldots \quad (3.12)$$

And

$$\varepsilon'' = \frac{(\varepsilon_s - \varepsilon_\infty)}{1 + (wt)^2} wt \qquad \ldots\ldots\ldots \quad (3.13)$$

According to eqs. (3.12) will decrease from to whereas will become maximum at ωt =1

Also plot of ε′ and ε″ against log (w) would be symmetrical. A method for checking eqs. (3.12) was proposed by Cole and Cole. From eqs. (3.12) it is

evident that a plot of ε″ versus ε′ should be a semicircle with radius $(\varepsilon_s - \varepsilon_\infty)/2$ and centre being on the abscissa at a distance of $(\varepsilon_s - \varepsilon_\infty)/2$ from the origin.

Departure from Debye Behavior:

Eqs. (3.12) Provides insight into behavior of the orientation on polarization for a large number of condensed systems. However marked deviations are observed for many systems. For example when we plot graph of ε″ as function of frequency then there is more than one maximum. Also peak is broader in the graph. The deviations occur due to a distribution of relaxation times either distinct from each other or closely spaced so that they are not directly evident.

This leads to depressed semicircular plot of ε″ against ε′. The factor h is related to the depression of the centre from ε′ axis.

Another generalized expression was given by Davidson and Cole.

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{(1 + jw\tau)^\beta}$$

Cole-Cole plot for this equation is asymmetric. And this plot is often called skewed arc. At low frequencies this plot cuts ε′ axis perpendicularly. However, at high frequency this cut occurs at an angle πβ/2.

The maximum of ε″ occurs for $W > \tau^{-1}$. Some of the descriptions of relaxation behaviour have been proposed for experimentally measurable function other than ε* (W). Fuoss and Kirkwood, Jonscher suggested

expressions for loss factor ε''. Williams and Watts proposed expressions for step response function. Bottcher and Bordeqijk (1978) and Hill et al. (1969) gave detailed description of the various functions proposed for explaining the departure from pure Debye behavior.

EXPERIMENTAL SECTION

1. Experimental techniques

A variety of experimental procedures for determination of the dielectric parameters of various samples of differing size and shape have been summarized by different authors (Von Hippel, 1961; Roberts et al. 1946; Brunfldt, 1987; Dalton et al. 1986; Buck Master et al. 1985; to mention a few). These different measurement techniques can be broadly classified into two categories:

(1) Time Domain Techniques, and

(2) Frequency Domain Techniques

Of there two classes, the Time Domain Technique is of a more recent origin. It has been employed by several investigators for the dielectric properties as well as moisture content measurement of liquids. Especially popular in this class of techniques is the Time Domain Reflectometry (TDR) method. In Time Domain Techniques the permittivity of the sample is calculated from the measured resonance frequency and Q-factor resonant methods.

The frequency domain techniques can be further classified into the following subgroups:

(i) Free Space Technique

(ii) Cavity Perturbation Techniques (Sucher et al. 1963; Musil et al. 1986).

(iii) Transmission Techniques in wave guides/ coaxial lines, and

(iv) Reflection Techniques in wave guides/coaxial lines.

Free Space Technique:

This method is suitable for the measurement of permittivity of medium and high loss materials. This method is employed when two different purely reactive terminations of a sample are available. Suber and Crounch (1948) developed a method where the sample is enclosed within a section for which the wave ratio are measured for both short circuit and open circuit and $Y_{\varepsilon} = Y_{i1} \times Y_{i2}$ where Yi1 and Y_{i2} are the corresponding measured impedance. The greatest advantage of this method is that it is relatively simpler and does not involve solution of a transcendental equation.

Cavity Perturbation Techniques:

The method is used for measurement of dielectric constants of low loss samples of small quantity which can be put into any convenient form. Measurements are recorded for two situations; cavity without sample and cavity with sample. The difference between these two manifests itself in a small difference in the observed complex frequency (real frequency + Q factor). This is then used to calculate the dielectric constant of the sample. The method has been successfully employed by many investigators, (Bethe et al., 1943; Birenbaum et al., 1969; Aruna and Behari, 1981; Behari et al., 1982; Bayser et al, 1992; Biofot et al., 1992).

Transmission Techniques in Wave Guides/Coaxial Lines:

This method is particularly suitable for high loss samples. In such cases measurement is based on infinite sample length (when most of the energy entering the sample gets absorbed, the sample is termed as an infinite sample).

The normalized input impedance at the interface of two dielectrics, when the second one is of infinite length, is directly related to the dielectric properties of the two. In a wave-guide with (TE_{10}) mode, the relative complex dielectric constant is,

$$\varepsilon = \frac{1}{1+\left(\frac{\lambda_c}{\lambda_g}\right)} + \frac{1}{1+\left(\frac{\lambda_g}{\lambda_c}\right)^2} \quad \frac{\tau - j\tan\{k(D-D_R)\}}{1-j\tau\tan\{k(D-D_R)\}} \qquad \ldots\ldots (4.1)$$

Where, λ_g is the guide wavelength, and λ_C is the cut off wavelength, Γ is the VSWR and (D-D_R) is the shift in minima position when an infinite sample is replaced with short circuit. A number of investigators have been successful with this method (Behari et al., 1982; Bayser et al., 1992; Biofot et al., 1992; Karolkar et al., 1985; to name a few).

Reflection Techniques in Coaxial Lines/Wave Guides:

Reflection methods are usually adopted in a coaxial line sample holder where the reflection coefficient or scattering parameter is determined at a redefined reference plane from the sample holder. The reflection coefficient can be

determined very easily with the use of a network analyzer, using swept frequency technique or slotted line apparatus or using a resonator terminated by the sample (Stuchly et al., 1979, 1980). The resonator method uses an infinite sample where changes in the resonating frequency and Q factor produced by the sample are measured. For the TEM mode, magnitude of the reflection coefficient is, $|\Gamma| = \exp[-n(1/Q_\varepsilon - 1/Q_o)]$ and phase angle $\theta = 2\pi n(1 - f_c/f_o)^n$ where resonant frequency with sample is f_c and resonating frequency of the resonator without the sample is f_o. n is dependent on resonator length = $(n_c/21)$; the dielectric parameter ε is given by

$$\varepsilon_r = \left(\frac{1-\tau}{1-\tau}\right)^2 \quad \text{where } \tau = |\tau| e^{i\theta} \quad \ldots\ldots\ldots\ldots \quad (4.2)$$

This method can be applied for a lossy material with a high dielectric constant (e.g.: a biological tissue) but it is limited to a discrete frequency.

Resonator methods are not suitable for high loss liquids because the resonance peak becomes so broad that ε cannot be measured correctly. As an alternative, open cavity methods with high loss liquids filled in a capillary can be applied. A method has been devised by Van Loon and Finsey (1975) involving computer analysis of the reflected power profile. The wave propagation constant is determined by fitting an analytical curve to the reflection profile; this then, yields the value of the complex permittivity.

In their recent investigations, Stabell and Misra (1990) designed a new procedure for in vivo dielectric measurement using an open-ended line probe.

Any system imperfection is completely bypassed.In this method since the calibration is carried out with four materials of known dielectric constants and then the reflection coefficients of these materials are then used in the final calculations along with the reflection coefficient of the sample.

In case of reflection methods in wave-guides, the reflection coefficient from a defined reference plane is put to use for permittivity measurements. The reflection coefficient itself may be measured by slotted line or a network analyzer or by simply forming a resonator terminated by the sample (Stuchly et al., 1978).

Two popular techniques involving measurement of reflection coefficient in a wave guide are:

(a) The Two Point Method and,

(b) The Single-horn Reflectometry Method. (Arcone et al., 1988).

Both these methods are suitable for either loss less dielectrics or dielectric with medium loss. .

Two point method was adopted for the present work.

The Two Point Method:

The underlying theory of this method can be understood by a consideration of Fig. 4.1 (a), which shows an empty short-circuited waveguide with a probe located at a voltage minimum D_R. The next fig. 4.1 (b) shows the same wave-guide, containing a sample length I_ε with the probe located at a new

voltage minimum D. The sample is placed adjacent to the short circuit. Looking from $T_{\varepsilon 1}$ towards the right and the left, one can write the impedance equation as:

$$Z_o \tan k_I = -Z_\varepsilon \tan k_\varepsilon I_\varepsilon \quad \ldots\ldots\ldots\ldots \quad (4.3)$$

Similarly in fig. 4.1 (a), looking toward the right, we have,

$$Z_o \tan k (I_R + I_\varepsilon) = 0 \quad \ldots\ldots\ldots\ldots \quad (4.4)$$

Now,

$$\tan k (D_R - D + I_\varepsilon) = \tan k [(I_R + I_\varepsilon) - (l + I_\varepsilon) + I_\varepsilon]$$

$$= \tan k [(I_R + I_\varepsilon) - l]$$

Ezpanding the tangent and using eq. (4.4) and substituting into eq.(4.3) we get,

$$Z_o \tan k (D_R - D + I_\varepsilon) = Z_\varepsilon \tan k_\varepsilon I_\varepsilon \quad \ldots\ldots\ldots\ldots \quad (4.5)$$

As $Zo/Z_\varepsilon = k_\varepsilon/k$, we can rewrite (4.5) as,

$$\frac{\tan k (D_R - D + I_\varepsilon)}{k l_e} = \frac{\tan ke\ le}{k_e\ l_e} \quad (4.6)$$

All the quantities associated with the left hand side of eq. 4.6 are measurable, while the right hand side is in the form (tan Z)/Z. So once the measurement has been performed, the complex number, $\dot{Z} = k_\varepsilon I_\varepsilon$, can be found by the solution of the transcendental equation and from it we can calculate k_ε. The relative permittivity ε_r follows directly from k_ε.

$$k = 2\pi \frac{\sqrt{\varepsilon_r \mu_r - \left(\frac{\lambda}{\lambda_c}\right)^2}}{\lambda} = \frac{2\pi}{\lambda_g} \quad \ldots\ldots\ldots\ldots (4.7)$$

Considering the fact that the tangent function is periodic in nature, there exist an infinite number of solutions for ε_r. Therefore it becomes necessary either to know approximately the right solution or to perform a second identical experiment with a sample of different length $l_{2\varepsilon}$. The proper solution will then be the one common to the two sets of solution. We thus get an "intersection point", as illustrated in fig. (4.2) for a particular case. In the complex case, like the present one, equation (4.6) has been recast to achieve a more practical form (Sucher and Fox, 1963).

Procedure:

1. All the equipments were connected as shown in fig. 4.3.
2. With no sample dielectric in the short-circuited line, D_R, the position of the minimum in the slotted line with respect to an arbitrarily chosen reference plane (D = 0), was found out.
3. The guide wavelength, λ_g. was measured by measuring the distance between alternate minima in the slotted line.
4. The sample dielectric was inserted in the short circuit to give a sample length of around $\lambda_g/4$ for best accuracy.
5. The position of the minimum in the slotted line, D with the sample in the short circuit and with respect to a reference plane (D = 0) was measured.

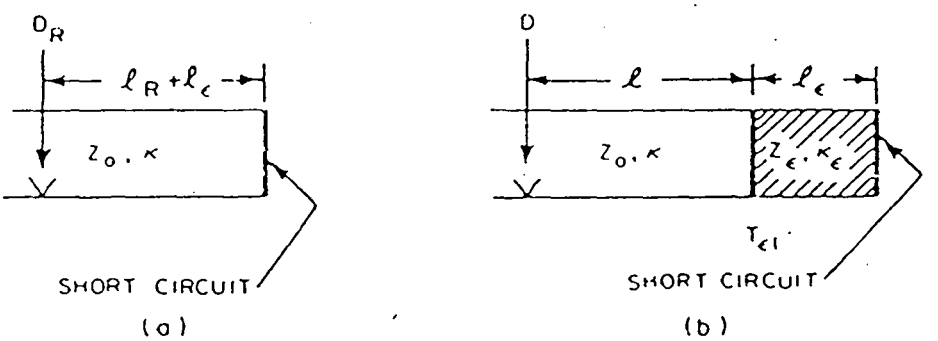


Fig 4.1 Dielectric constant measurement with short-circuited wave guide.

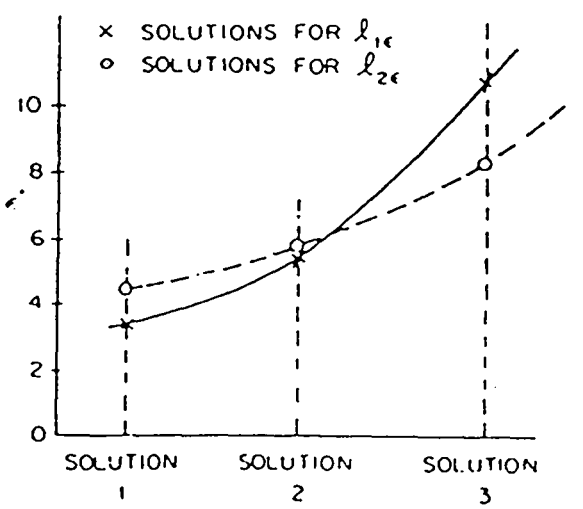
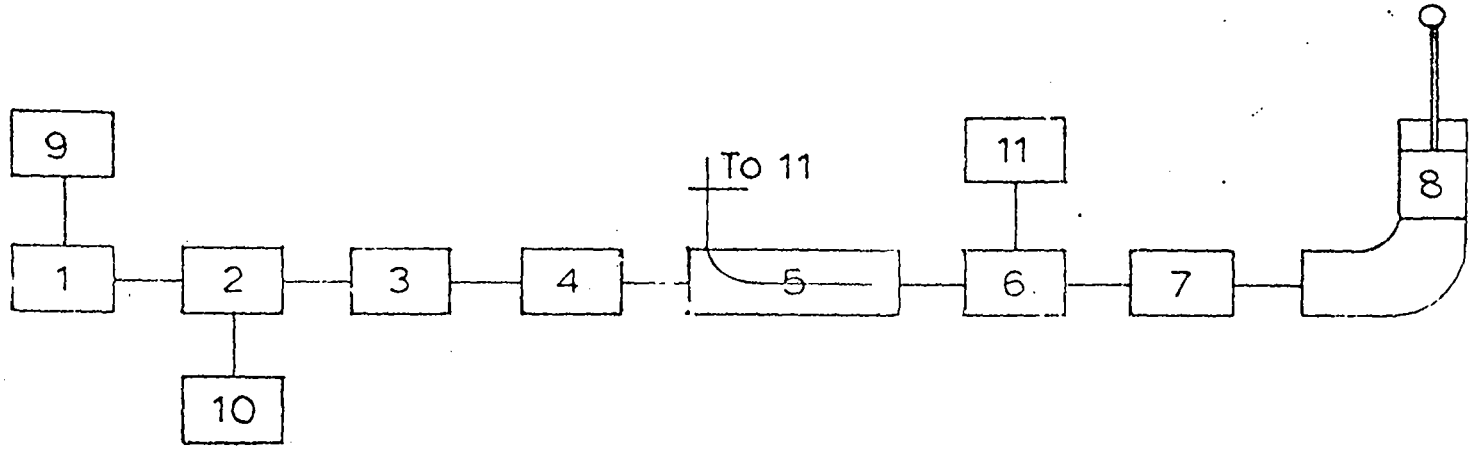


Fig 4.2 Solutions for ε ' obtained from the measurement of Two Different length samples of the same material.



1 Generator (Klystron)
2 Isolator
3 Frequency meter
4 Slide Screw Tuner
5 Directional coupler
6 Slotted section
7 E-H. Tuner
8 Liquid cell
9 Klystron power supply
10 Matched load
11 V.S.W.R. meter

Fig 4.3 Schematic diagram of set up for measurements on liquids

Calculations:

For the complex dielectric constant, the calculations were as follows:

(1) Determination of the wave number

$$K = \frac{2\pi}{\lambda_g} \qquad \ldots\ldots\ldots\ldots \quad (4.8)$$

(2) Determination of phase constant

$$\phi = 2K(D - D_R - l_e) \qquad \ldots\ldots\ldots\ldots \quad (4.9)$$

(3) Determination of reflection coefficient

$$\Gamma = \frac{r-1}{r+1} \qquad \ldots\ldots\ldots\ldots \quad (4.10)$$

(4) Determination of the complex number

$$C\angle -\psi = \frac{1}{jkl_e}\left(\frac{1-|\Gamma|e^{J\phi}}{1+|\Gamma|e^{J\phi}}\right) \qquad \ldots\ldots\ldots\ldots \quad (4.11)$$

In order to solve eq. (4.11), the C - ψ graph was used and the corresponding x and θ values noted directly.

These values of x and θ were then used in the calculation of Y with eq. (4.12). For the same sample, two closest possible values of Y for different lengths were chosen for further calculations,

$$y = \left(\frac{X}{Kl_e}\right)^2 \angle 2(\phi - 90) \qquad \ldots\ldots\ldots\ldots\ldots \quad (4.12)$$

$$G = \left(\frac{X}{Kl_e}\right)^2 \cos\phi' \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.13)$$

$$B = \left(\frac{X}{Kl_e}\right)^2 \sin\phi'$$

where $\phi' = 2(\phi - 90)$

$$\varepsilon' = \frac{G + \left(\frac{\lambda_g}{\lambda_c}\right)^2}{1 + \left(\frac{\lambda_g}{\lambda_c}\right)^2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.14)$$

$$\varepsilon'' = \frac{-B}{1 + \left(\frac{\lambda_g}{\lambda_c}\right)^2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.15)$$

2. Sample preparation

Samples: The experiments were performed on two Biomolecules. Salmon sperm DNA and Lysozyme (from chicken egg white). These were purchased from Sigma Co. Ltd.USA.

DNA - Sample solutions were prepared in EDTA and ethanol respectively.

Sample No 1: First EDTA of 1 mM (pH=8), Tris of 10 mM (pH=8) and 1 mM Nacl were seperately prepared and mixed together. A volume of 1 ml was obtained and DNA was dissolved in 1 ml of above solution to obtain a concentration of (1 mg/ml).

This solution was diluted to 20 % and 40 % by mixing (EDTA+Tris+Nacl solution) as mentioned above.

Sample No. 2 Ethanol+ aq NaCl was mixed. The solution was prepared by dissolving 1 mg powder of DNA in 1ml of ethanol+ aq. NaCl. From this other solutions of desired concentrations were prepared.

Sample No. 3 Lysozyme: 1 mg powder of above (Sigma Chemicals, USA) was dissolved in acetone (3 ml) to obtain a concentration of 0.33mg/ml.

All these samples were kept at low temperature (about 4°c).

From this we varied concentration of the solution at the desired level.

RESULTS AND DISCUSSION

Dielectric parameters are measured at X-band frequency (9.2 GHz). Experimental results are summarized in the attached table. The graphs were drawn between concentration and dielectric constants for the given samples at three different temperatures. From the graphs it is clear that they follow some regular trends.

We measured the complex dielectric constant (ε^*) by measuring the properties of the bulk solvent with and without DNA.

In the sample No. 1 containing EDTA+NaCl+DNA, it was found that the dielectric constant depends on concentration and type of cations. With increase in concentration of solvent, there is slight decrease in real part of dielectric constant (ε') of the solution. When temperature is increased gradually this change is more significant. The value of dielectric loss ($\tan \delta$) is found to be increasing with increase in temperature.

Samlple No.2, First data for solution containing Ethanol and DNA was analyzed. At temperature of 20° C, there is slight decrease in the real part of dielectric constant of the solution with increasing concentration of solvent. The value of dielectric loss ($\tan \delta$) is found to be increasing with increase in temperature. When temperature is increased gradually to 40°C there is further increase in $\tan\delta$.

Table shows variation in Dielectric Constant (ε′) of different samples with Temperature.

	Temp.in °C	Ethanol	Ethanol + DNA + aq. NaCl at 10 % Conc.	Ethanol + DNA + aq. NaCl at 20 % Conc.	Ethanol + DNA + aq. NaCl at 30 % Conc.	EDTA + Tris	EDTA + Tris + DNA at 20 % concentration	EDTA + Tris + DNA at 40 % concentration	Acetone	Acetone + Lysozyme
ε′	20	25.9	25.8	25.6	25.5	15.5	15.5	15.4	21.2	23.4
	30	25.2	24.8	24.3	24.0	15.1	14.8	14.6	19.8	21.6
	40	24.5	24.4	23.7	23.1	14.6	13.9	13.5	19.1	20.9
ε″	20	5.0	5	5.2	5.2	4.9	3.9	3.9	6.2	5.4
	30	5.3	5.4	5.7	5.7	5.2	4.3	4.4	6.4	5.6
	40	5.9	6	6.2	6.3	5.8	4.9	4.5	6.9	6.0
tan δ = ε″/ ε′	20	0.193	0.194	0.203	0.316	0.316	0.251	0.253	0.292	0.230
	30	0.210	0.217	0.237	0.344	0.344	0.290	0.301	0.323	0.250
	40	0.240	0.243	0.272	0.397	0.397	0.352	0.334	0.361	0.282

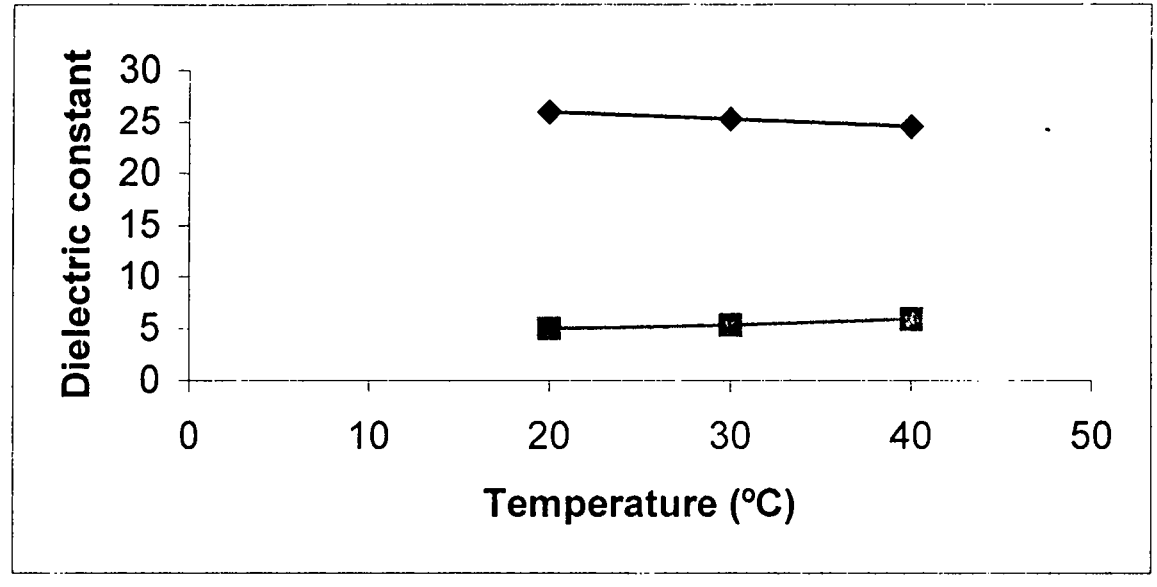


fig- 5.1 Variation in dielectric constant of ethanol with temperature.

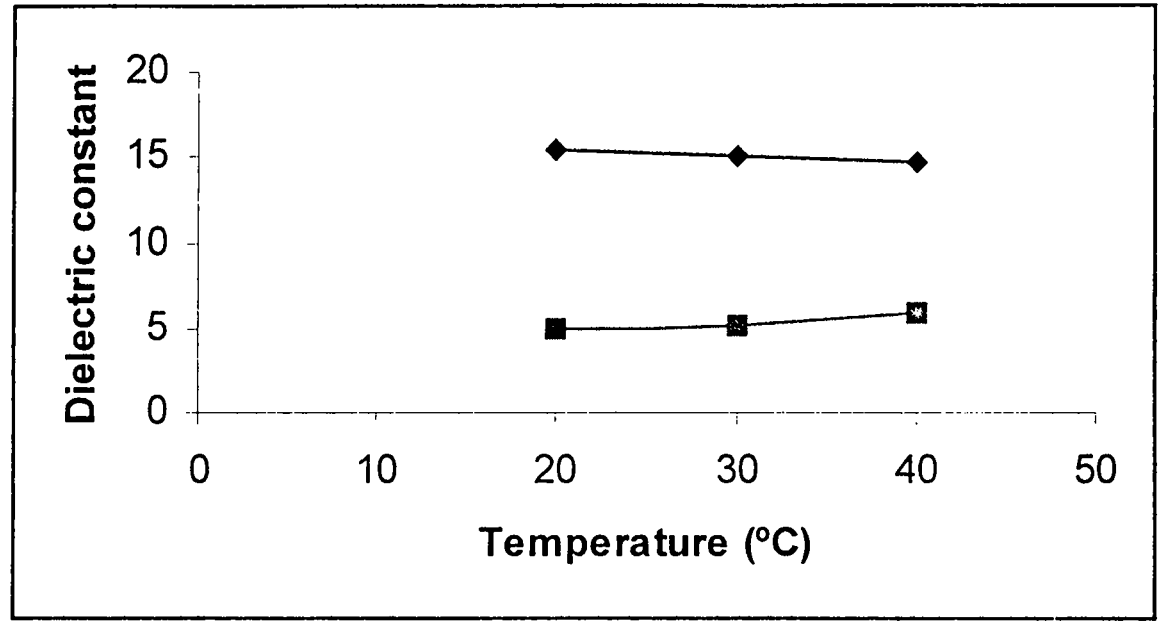


fig- 5.2. Variation in dielectric constant of sample (EDTA + Tris) with temperature.

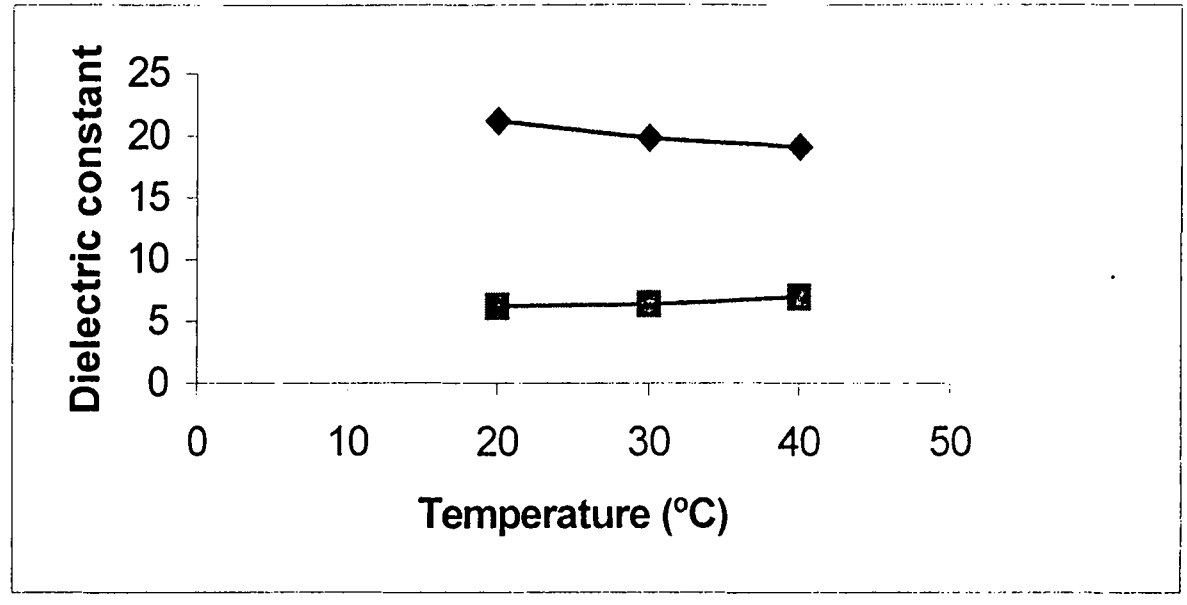


fig-5.3. Variation in dielectric constant of Acetone with temperature.

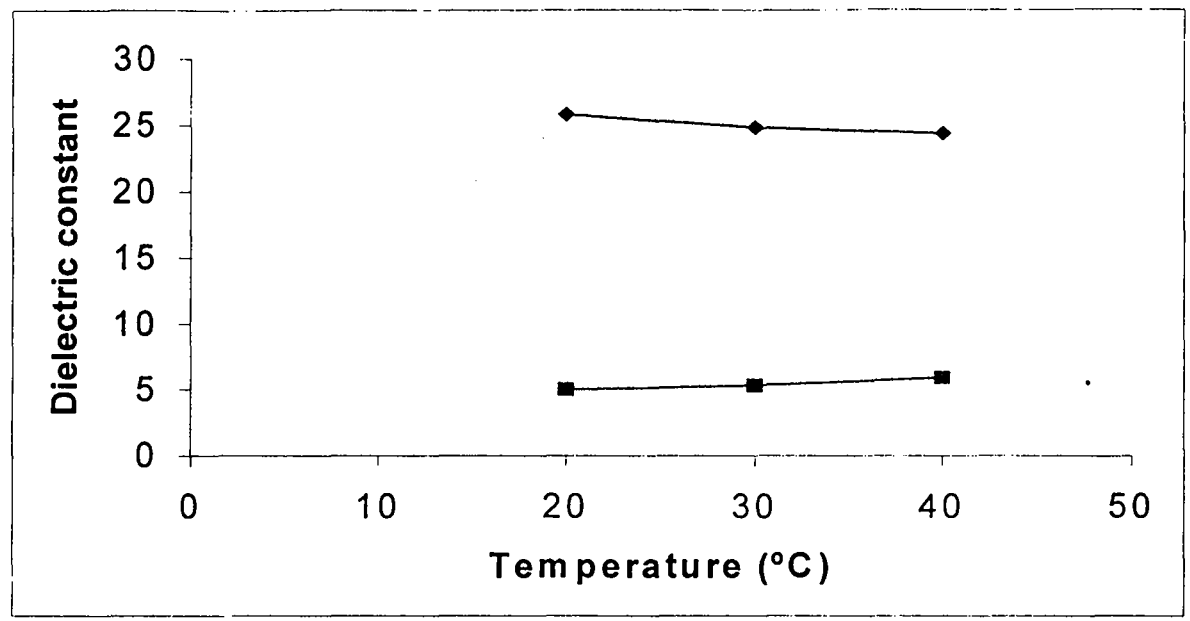


fig-5.4. Variation in dielectric constant of sample (Ethanol + DNA + aq. NaCl at 10 % Conc.) with temperature.

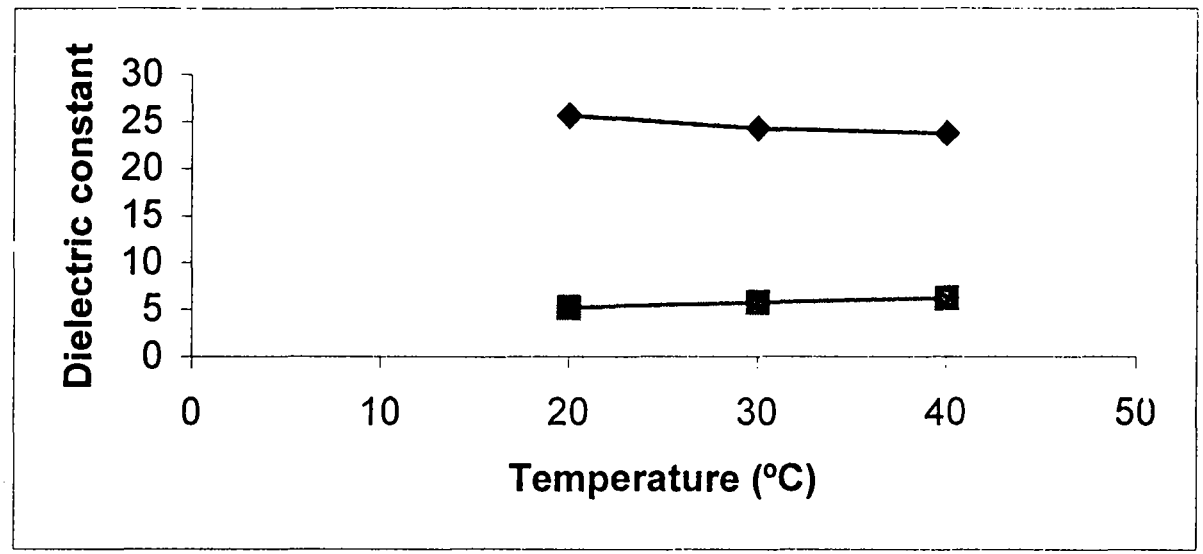


fig-5.5. Variation in dielectric constant of sample (Ethanol + DNA + aq. NaCl at 20 % Conc.) with temperature.

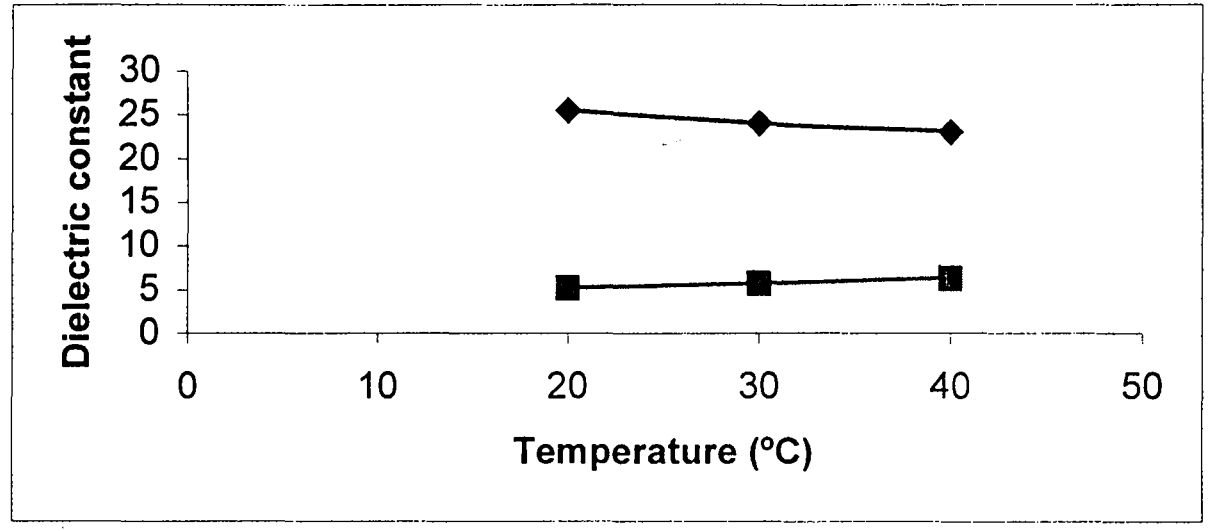


fig-5.6. Variation in dielectric constant of sample (Ethanol + DNA + aq. NaCl at 30 % Conc.) with temperature.

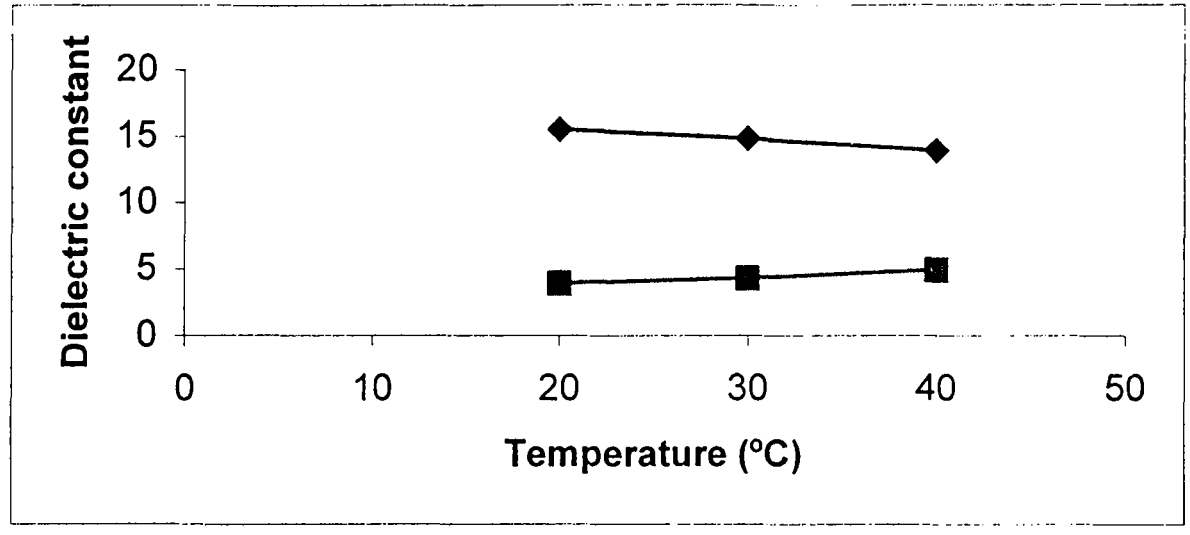


fig-5.7. Variation in dielectric constant of sample (EDTA + Tris + DNA at 20 % concentration) with temperature.

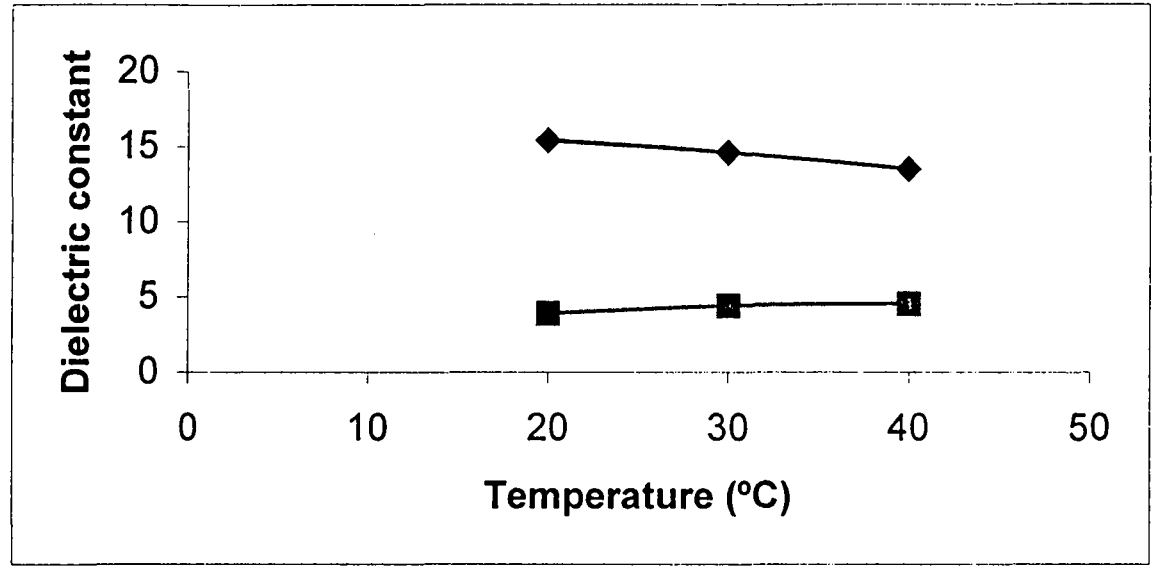


fig-5.8. Variation in dielectric constant of sample (EDTA + Tris + DNA at 40 % concentration) with temperature.

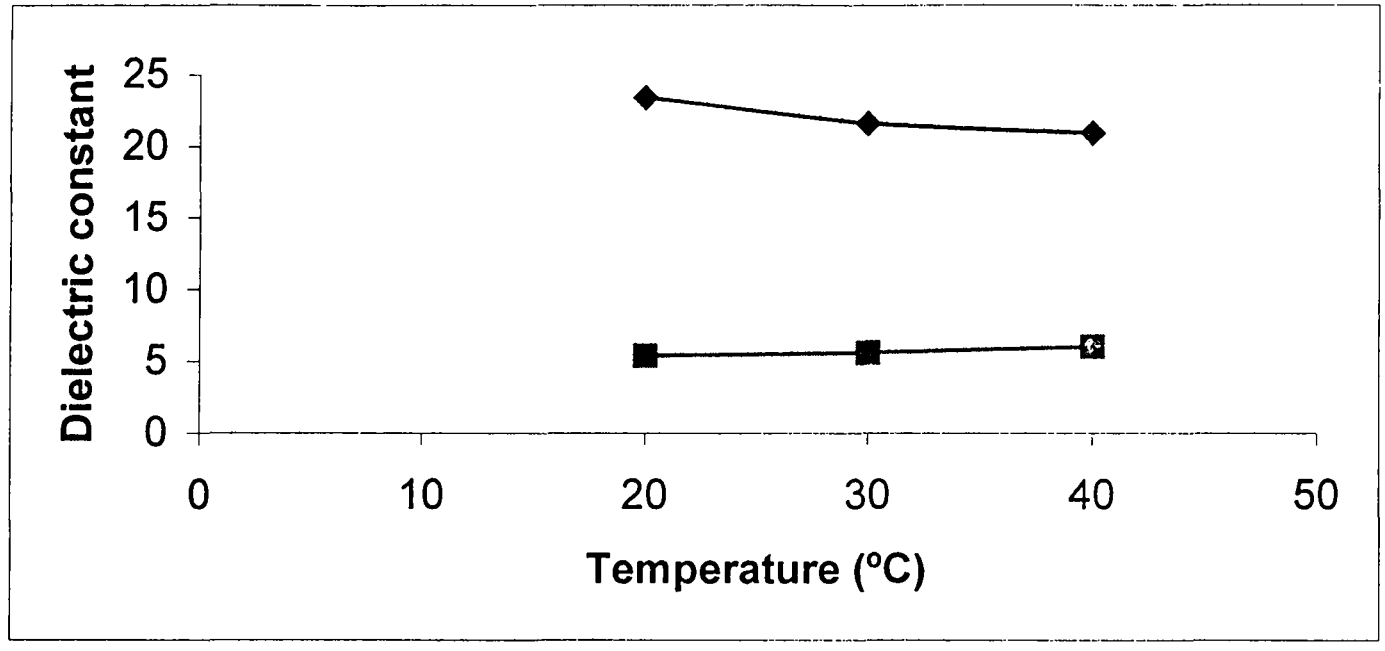


fig-5.9. Variation in dielectric constant of sample (Acetone + Lysozyme) with temperature.

There are marginal differences between the permittivity of the solvent and solutions containing DNA.

When an external field is applied to charged molecules such as DNA a dipole moment is induced arising from the migration of ions over individual subunits and over the length of the molecule. The dipole formation and alignment processes require a finite time to occur and thus give rise to the observed dielectric relaxations.with increasing concentration, it is suggested that DNA expands first on account of the increase of electrostatic repulsion between charges on DNA which arises from the decrease of dielectric constant of solvent. The decrease in the dielectric constant of solvent then makes DNA to contract.

From the analysis of the above solution No 1 and No. 2 it is clear that the binding of counterions on DNA is affected by the change of solvent. Hence there is different ($\Delta\varepsilon$) for different solutions. The mechanism of absorption of microwave energy may be considered as the main part of primary interaction.

With increase in the concentration of solvent phosphate charge gets neutralized and the dielectric constant decreases. Decrease of dielectric constant ($\Delta\varepsilon$) is seen with increase of temperature and concentration of added salt. This can be partially explained by change in the length of major axis of DNA as studied earlier in the flow-birefringence experiments.

In earlier study it has been shown that aqueous solution of DNA exhibit a dielectric relaxation covering a wide range of frequency. It is still

controversial at present whether the relaxation might involve a single mechanism or more.

From the data of sample No. 3, solution containing lysozyme + Acetone, it is clear that there is increase in the real part of dielectric constant (ε') of the solution. This is found to decrease with increasing temperature. This may be due to the strong bonding between active site of lysozyme and of acetone. As a result there is tendency of whole rotation of the molecule. Hence there is increase in the dielectric constant.

The forces between protein molecules in solution are in large part electrostatic in origin. These forces have been attributed to fixed constellation of electric charge which impart to the molecules permanent electric multipole. The electrostatic interaction between protein molecules, which arises from fluctuations in number, and configuration of the protons bound to the molecules.

The dipole moment fluctuations, arising from configurations fluctuations of the protons, may be accounted by the variations in the dielectric constant.

In the fluctuating charges multipole moments of two proteins molecules makes a significant contribution to the intermolecular force by a relatively single mechanism. The fluctuating electromagnetic field of each molecule alters the distribution of fluctuations in the charge constellation of the mobile protons of the other.

In favourable orientations, steric matching of a constellation of basic group on one molecule with a complementary constellation in the other could conceivably produce a redistribution of protons leading to a strong specific attraction depending upon the local structural details of the complementary constellations.

Biologicai systems are highly complex and it is unlikely that a single mechanism could explain the effects of microwaves. Hence it is reasonable to expect that more than one interaction mechanism may be responsible for their biological effects.

The possibility of microwave interacting with physical/chemical or biological system is now a matter of growing concern. Under the present circumstances, there is need for better understanding of the mechanism of action of microwaves with the biological systems and further research needed.

BIBLIOGRAPHY

Alex, Z.C.; Behari, J. and Zaidi, Z.H. Biological tissue characterization at microwave frequencies: A review. Technical Review, IETE, Vol. II. No. 1.1994.

Antosiewicz, J.; Porschke, D. Anunusual electrooptical effect observed for DNA fragments and its apparent relation to a permanent electric moment associated with bent DNA. Biophysical Chemistry 33: 19-30; 1989.

Baker-Jarvis, J.; Janezic, M.D.; Stafford, R. Analysis of an open circuited sample holder for dielectric and magnetic measurements of liquids and powders. Natl. Inst. Stand. Technol. Tech. Note 5001; 1992.

Bakhshi, A.K. Investigation of electronic conduction in proteins and DNA. Prog. Biophys. Molec. Biol. 61: 187-253; 1994.

Bayser, A. and Kuerter, J.Z. Dielectric property measurements of materials using the cavity technique. IEEE Trans. Micro. Theo. Tech. MTT. 40: 2108-2110.

Bianco, B.; Drago, G.P.; Marchesi, M.; Martini, C.; Mela, G.S. and Ridella, S. Measurements of complex dielectric constant of human sera and erythrocytes. IEEE Trans. Instrum. Meas. IM-28: 4-290; 1979.

Biofot, A.M. Broad band method for measuring dielectric constant of liquids using an automatic network analyzer. IEEE Proc. 136, pt. H. 492-498; 1992.

Blank, M., Goodman, R. Do electromagnetic fields interact directly with DNA? Bioelectromagn. 18: 111-115; 1997.

Bonincontro, A.; Caneva, R.; Pedone, F.; Romano, T.F. Complex dielectric constant of arginine-DNA and protamine-DNA aqueous systems at 10 GHz. Phys. Med. Biol. 34: 609-616; 1989.

Bonincontro, A.; Careri, G.; Giansanti, A., Pedone, F. Water induced conductivity of DNA: A dielectric-gravimetric study. Phys. Rev. A 38 (12): 6446-6447; 1988.

Buckmaster, H.A.; Hansen, C.H. and Zaghloul, H. Complex permittivity instrumentation for high loss liquids at microwave frequencies. IEEE Trans. Microwave Theory Tech. MTT-33: 822-824; 1985.

Bussey, H.E. Dielectric measurements in a shielded open circuit coaxial line. IEEE Trans. Instrum. Meas. IM-29: 120-124; 1980.

Cantor, C.R.; Schimmel, P.R. Biophysical Chemistry: Part III: W.H. Freeman. 1971.

Careri G,Geraci M., Giansanti A. and Rupley J.A., Protonic conductivity of hydrated lysozyme powders at megahertz frequencies. Proc. Natl. Acad. Sci. USA 82 (1985), 5342-5346.

Crothers, D.M. Upsetting the balance of forces in DNA. Science 266: 1819-1820; 1994.

Dee, D.; Baur, M.E. Charge and excitation migration in DNA chains. J. Chem. Phys. 60: 541-559; 1974.

Duguid, J.G.; Bloomfield, V.A. Electrostatic effects on the stability of condensed DNA in the presence of divalent cations. Biophys. J. 70 2838-2846; 1996.

Edwards, G.; Hochberg, D.; Kephart, T.W. Structure in the electric potential emanatin g from DNA. Phys. Rev. E 50: R 698-R701; 1994.

Elias, J.G.; Eden, D. Polarizability and saline concentration. Macromolecules 14: 854-865; 1981.

Fixman, M. Charged macromolecules in external fields. Macromolecules 13: 711-716; 1980.

Foster, K.R.; Epstein, B.R. and Gealt, M.A. Resonances in the dielectric absorption of DNA? Biophys. J. 52: 421; 1987.
Foster, K.R.; Epstein, B.R.; Gealt, M.A. Resonances in the dielectric absorption of DNA. Biophys. J. 52: 421-425; 1987.

Frank-Kamenetskii, M.D.; Anshelevish, V.V.;Lukashin, A.V. Polyelectrolyte model of DNA. Bioopolymers 30: 317-330; 1987.

Friedman, R.A.; Honig, B.A free energy analysis of nucleic acid base stacking in aqueous solution. Biophys. J. 69, 1528-1535; 1995.

G. Careri, G consolini and F. Bruni, Dielectric relexation of a proton glass in hydrated protein powders. Solid state Ionics 125 (1999), 257-261.

Gabriel, C.; Grant, E.H.; Tata, R., Brown P.R.; Gestblom, B.; Noreland, E. Microwave absorption in aqueous solutions of DNA. Nature 328: 145-146; 1987.

Galindo, C.E.; Sokoloff, J.B. Uncoiling transition for DNA in solution. Phys. Rev. E 54: 691-705; 1996.

Ganchev, S.I.; Bakhtiari, S. and Zoughi, R. A novel numerical technique for dielectric measurement of generally lossy dielectrics. IEEE Trans. Instru. Measurements, 41: 361-365; 1992.

Georgakilas, A.G.; Haveles, K.S.; Sideris, E.G. Dielectric study of the double helix to single coil transition of DNA. IEEE Trans. Dielec. Insul. 5: 26-32; 1998.

Grant, E.H. and South, G.P. Dielectric relaxation of proteins in aqueous solutions. Adv. Mol. Rel. Proc. 3: 355; 1972.

Grant, E.H.; Sheppard, R.J.; South, G.P. Dielectric behavior of biological molecules in solution: Clarendon Press. 1978.

Gueron, M.; Demaret, J.P. A; simple explanation of the electrostatics of the B to Z transition. Biophys. 89: 5740-5743; 1992.

Gulbrand, L.E.; Forrester, T.R., Lynden-Bell, R.M. Distribution and dynamics of mobile ions in systems of ordered B-DNA. Molecular Phys. 67: 473-493; 1989.

Hanlon, S.; Wong, L.; Pack, G.R. Proton equilibrium in the minor groove of DNA. Biophys. J. 72: 291-300; 1996.

Hanss, M.; Bernengo, J.C. Dielectric relaxation and orientation of DNA molecules. Biopolymers 12: 2151-2159; 1973.

Hill, P.N.; Green, H.E. In situ measurement of soil permittivity and permeability. J. Elect. Elect. Eng. Australia 2 (4): 205-209; 1982.

Hippel, V. Dielectric materials and Applications.; Cambirdge, MA: M.I.T. Press; 1954.

Hochberg, D.; Kephart, T.W.; Edwards, G. Structure information in the local electric field of dissolved B-DNA. Phys. Rev. E 49: R 851-R 867; 1994.

Hogan, M.; Dattagupta, N.; Crothers, D.M. Transient electric dichroism of rod like DNA molecules. Proc. Natl. Acad. Sci. 75: 195-199; 1978.

Honig, B.; Nicholls, A Classical electrostatics in biology and chemistry. Science 268: 1144-1149; 1995.

Inoue, S.; Ando, T. Interaction of clupeine with deoxyribonucleic acid I: Thermal melting and sedimentation studies. Biochem. 9: 388-394; 1970.

Ise, N.; Eigen, M.; Scharz, G. The orientation and dissociation field effect of DNA in solution. Biopolymers 1: 343-352; 1963.

Jayaram, B.; Sharp, K.A, Honig, B. The electrostatic potential of B-DNA. Biopolymers 28: 975-993; 1989.

Jesch, R.L. Dielectric measurements of oil shales as functions of temperature and frequency. IEEE Trans. Geosci. Remote Sensing GE-22 (2): 99-105; 1984.

Karolkar, B. and Behari, J. Biological tissue characterization at microwave frequencies. IEEETrans. Micro. Theo. Tech. Vol. MTT-33: 64-66, 1985.

Karrtze, U.; Schafer, M. and Pottel, R. The complex dielectric spectrum of aqueous methanol and isopropanol solutions. Z. Phys. Chem. 165: 103; 1989.

Katie, F. Staebell and Devendra Mirsa. An experimental technique for in vivo permittivity measurement of materials at microwave. IEEE Trans. Microwave Theor. Tech. 38: 38-337; (1990).

Kirkwood J.L.; Shumaker, J.B. The influences of dipole moment fluctuations on the dielectric increment of proteins in solution. Proc. Natl. Acad. Sci. USA 35: 855-862; 1952.

Levstik,C. Filipic, Z. Kutnjak, G. Careri, G consolini and F. Bruni,, Proton glass freezing in hydrated powders. Phys. Rev. E 60 (1999), 7604-7607.

Maleyev V.Y.; Semenov, M.A.; Gasan, A.I., Kashpur, V.A. Physical properties of the DNA water system. Biophys. 38: 785-801; 1993.

Mctague, J.P.; Gibbs, J.P. Polyions, J. Chem. Phys. 44: 4295-4301; 1967.

Minakata, A.; Imai, N.; Oosawa, F. Dielectric properties of polyelectrolytes II. A theory of dielectric increment due to ion fluctuation by a matrix method. Biopolymers 11: 347-359; 1972.

Miura, N.; Asaka, N.; Shinyashiki, N. and Mashimo, S. Microwave dielectric study on bound water of globule proteins in aqueous solution. Biopolymers 34: 357; 1994.

Murphy, C.J.; Arkin, M.R.; Jenkins, Y.; Ghatlia, N.D.; Bossamann, S.H.; Turro, N.J.; Barton, J.K. Long-range photoinduced electron transfer through a DNA helix. Science 262: 1025-1029; 1993.

Muto, V.; Lomdahl. P.S.; Christiansen, P.L. Two-dimensional discrete model for DNA dynamics: Longitudinal wave propagation and denaturation. Phys. Rev. A 42: 7452-7458,1990.

Nanda, N.; Bagchi, B. Anomalous dielectric relaxation of aqueous protein solutions. J. Phys. Chem. A 102: 8217-8221; 1998.

Oosawa, F. Counterion fluctuation and dielectric dispersion in linear polyelectrolytes. Biopolymers 9: 677-688; 1970.

Pack, R.G.; Garrett, G.a.; Wong, L. Lamm, G. The effect of a variable dielectric coefficient and finite ion size on Poisson-Boltzmann calculations of DNA-electrolyte systems. Biophys. J. 65:1363-1370; 1993.

Pedone, F.; Bonincontro, A. Temperature dependence of DNA dielectric dispersion at radio frequency. Biochem. Biophys. Acta 1073: 58-584; 1991.

Peyrard, M.; Bishop, A.R. Statistical mechanics of a nonlinear model for DNA denaturation. Phys. Rev. Lett. 62: 2755-2758; 1989.

Plum, G.E.; Bloomfield, V.A. Contribution of asymmetric ligand binding to the apparent permanent dipole moment of DNA. Biopolymers 29: 1137-1146; 1990.

Prabhu, V.V.; Young, L.; Prohofsky, E.W. Hydrogen-bond melting in B-DNA copolymers in a mean-field self-consistent phonon approach. Phys. Rev. B 39: 5436-5443; 1989.

Priyadarshy, S.; Risser, S.M.; Beratan, D.N. DNA is not a molecular wire: protein-like electron-transfer predicted for an extended π-electron system. J. Phys. Chem. 100: 17678-17682; 1996.

Rau, D.C.; Charney, E. Polarizability of DNA. Biophys. Chem. 14: 1-9; 1981.

Reinisch L, Kaiser R D and Krim J: Measurement of protein hydration shells using a quartz microbalance, Phys. Rev. Lett. 63 (1989), 1743-1746.

Renugopalakrishnan, V.; Lasshminarayanan, A.V.; Sasisekharan, V. Stereochemistry of nucleic acids and polynucleotides III. Electric charge distribution. Biopolymers 10: 1159; 1971.

Roberts, S. and Von Hippel, A.R. A new method for measuring dielectric constant and loss tangent in the range of EM waves. J. Appl. Phys. Vol. 17: 610-645; 1946.

Rupley J and Careri G. Protein hydration and function. Advances in protein chemistry, 41(1991, 37-172.

Saif, B.; Mohr, R.K.; Montrose, C.J.; Litovitz, T.A. On the mechanism of dielectric behavior in aqueous DNA solutions. Biopolymers 31: 1171-1180; 1991.

Sakamoto, M., Fujikado, T., Hayakawa, R., Wada, Y. Low frequency dielectric relaxation and light scattering in AC electric field of DNA solutions. Biophys. Chem. 11: 309-316; 1980.

Sakamoto, M.; Hayakawa, R.; Wada, Y. Dielectric relaxation of DNA solutions. II. Biopolymers 17: 1507-1512; 1978.

Sakamoto, M.; Hayakawa, R.; Wada, Y. Dielectric relaxation of DNA solutions. IV. Effects of salts and dyes. Biopolymers 19: 1039-1047; 1980.

Sakamoto, M.; Kanda, H.; Hayakawa, R.; Wada, Y. Dielectric relaxation of DNA in aqueous solutions. Biochem. 15: 879-892; 1976.

Sakamoto, M; Hayakawa, R; Wada, Y. Dielectric relaxation of DNA solutions. III. Effects of DNA concentration, protein contamination and mixed solvents. Biopolymers 18: 2769-2782; 1979.

Saxena, V.K.; Dorfmann, B.H., Zandt, L.L.V. Identifying and interpreting spectral features of dissolved poly (dA)-poly (dT) DNA polymer in the high-microwave range.

Saxena, V.K.; Zandt, L.L. Van. Schroll, W.K. Effective field approach for long-range dissolved DNA polymer dynamics. Phys. Rev. A 39: 1474-1481; 1988.

Saxena, V.K; Zandt, L.L. Van. Effect of counterions on the spectrum of dissolved DNA polymers. Phys. Rev. A 45: 7610-7620; 1992.

Scott, W.R.; Smith, G.S. Error analysis for dielectric spectroscopy using shielded open circuited coaxial lines of general length. IEEE Trans. Instrum. Meas. IM-35: 130-137; 1986.

Somlo, P.I. The discontinuity capacitance and the effective position of a shielded open circuit in a coaxial line. Proc. Inst. Radio Elec. Eng. Australia 28 (1): 7-9; 1967.

Soumpasis, D.M. Helix-coil transition in DNA. Proc. Natl. Acad. Sci. 81: 5116; 1984.

Steven, C. Olson and Magdy, F. Iskander. A new in situ procedure for measuring the dielectric properties of low permittivity. IEEE Trans. Instrum. Meas. IM-35: 1-2; 1986.

Strauss, J.K.; Maher, J. DNA bending by asymmetric phosphate neutralization. Science 266: 1829-1834; 1994.

Stuchly, M.A. et al. Coaxial line reflection methods for measuring dielectric properties of biological tissues at radio and microwave frequencies: A Review. IEEE Trans. Instr. And Meas. Vol. IM-29 (3): 176-183; 1980.

Stuchly, M.A.; Stuchly, S.S. Coaxial line reflection methods for measuring dielectric properties of biological substances at radio and microwave frequencies-A review. IEEE Trans. Instrum. Meas. IM-29: 176-183; 1980.

Stuchly, S.S. et al. A method for measurement of the permittivity of thin samples. J. Microwave Power. Vol. 14: 7-13; 1979.

Szabo, A.; Haleem, M.; Eden, D. Theory of the transient electric birefringence of rod-like polyions: coupling of rotational and counterion dynamics. J. Chem. Phys. 85: 7472-7479; 1986.

Takashima, S. Dielectric dispersion of deoxyribonucleic acid. J. Phys. Chem. 70 (5): 1372-1379; 1966.

Takashima, S.; Gabriel, G.; Sheppard, R.J.; Grant, E.H. Dielectric behaviour in DNA solution at radio and microwave frequencies (at 20 c). Biophys. J. 46: 29-34; 1984.

Tung, M.S.; Molinari, R.J.; Cole, R.H.; Gibbs, J.H. Influence of temperature and ionic strength on the low- frequency dielectric dispersion of DNA solutions. Biopolymer 16: 2653; 1977.

Umehara, T.; Kuwabara, S.; Mashimo, S. and Yagihara, S. Dielectric study on hydration of B-, A- and Z-DNA. Biopolymers 30: 649; 1990.

Van Loon R. and Finsey, J. The precise microwave permittivity measurement of liquids using a multipoint technique and curve fitting procedures. J. Phys. D. Vol. 8: 1232-1243; 1975.

Williams, R.J. Electron transfer in biology. Molecular Phys. 68: 1-23; 1989.

Yakushevich, L.V. Nonlinear DNA dynamics: a new model. Phys. Lett. A 136: 413-417; 1989.

Yu, S.S.; Li, H.J. Helix-coil transition and conformational studies of protamine-DNA complexes. Biopolymers 12: 2777-2788; 1973.

Zakrzewska, K.; Madami, A.; Lavery, R. Poisson-Boltzmann calculations for nucleic acids and nucleic acids complexes. J. Chem. Phys. 204: 263-269; 1996.

Zandt, L.L. Van. Resonant microwave absorption by dissolved DNA. Phys. Rev. Lett. 57: 2085-2087; 1986.