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TEMPERATURE RANGE**

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ABSTRACT

Dielectric constant ϵ' and loss factor ϵ'' have been measured in bovine tendon collagen in the frequency range 30 Hz - 3 MHz and temperature range 30°C to 200°C. Frequency dependence curve of ϵ'' shows a low frequency strong α -dispersion attributed to phonon assisted proton hopping between localized sites and a weak high frequency. α_2 - dispersion attributed to reorientation of polar components of collagen molecules. Temperature dependence of the dielectric data show release of bound moisture as a three step process with discrete peaks at 50°C, 90°C and 125°C. These peaks have been attributed to release of adsorbed surface water, water bound to exposed polar sites and strongly bound internal moisture respectively. A peak observed at 160°C has been attributed to thermally induced helix-coil transition of collagen molecules.

1. INTRODUCTION

Dielectric behaviour of biomolecules in the solid state is of great importance in understanding the charge transport and various charge polarization processes at molecular and submolecular levels [1-3]. Biologically important processes like protein water-interaction and intramolecular dynamics in proteins can also be understood in terms of dielectric behaviour of hydrated protein systems [4, 5]. Dielectric data in proteins and polypeptides are mostly available for aqueous solutions [6-8]. Solid state data had been meagre due to the initial belief that the solid state conformation of protein might be very different from their native conformation, which is always in aqueous environment. Now when we have enough evidence that the solid state and the solution state conformations are very much similar, if not identical [9, 10], attention has again been diverted to solid state dielectric studies of proteins [5, 11-14]. Solid state studies have various advantages over solution studies. The two stronger dispersions due to the bulk water and molecular rotation are absent in the solid state, which overshadow the weaker and more important dispersions like those due to bound water and intra-molecular mobilities in the solution studies. The ionic conductance components and large capacitance effects, which cause problems in solution are also much smaller in the solid state studies. Internal dynamics in proteins which is being regarded as the key to the understanding of their biological behaviour, can also, in principle, be studied by solid state dielectric data. A few dielectric studies have given some information about intramolecular dynamics of proteins or polypeptide. e.g. [4, 5]. A systematic study of the solid state dielectric properties of proteins is therefore needed with special reference to their intra-molecular dynamic processes.

Collagen was particularly selected for this study because of its importance in providing structural stability to extra cellular matrix of all multicellular animals. Tendon collagen was used as it is specially cross linked to give fibers of very high tensile strength.

Apart from providing structural stability collagen molecules may also have some contribution in the hitherto unclear active and complex role of extracellular matrix towards regulating the behaviour of cells that contact it influencing their development, migration, proliferation shape and function [15]. In addition to this collagen also has potential to be used in biomaterials for medical application such as wound dressings and artificial cardiac valves etc. [16, 17].

2. MATERIAL AND METHODS

Specimens of bovine tendon were obtained from a local slaughter house. The tendon was served from the animal, the elastic sheath was removed and the tendon was immersed in ethylether to remove the fats. Finally, it was washed in distilled water and air-dried for 5 days before use. Bovine tendon is approximately 86% pure collagen and provides collagen fibrils oriented parallel to the tendon [18]. The tendon, quite rigid when air-dried was formed into rectangular slabs suitable for measurement by slowly shaping with various grade of abrasive papers. In order to estimate the role of moisture content in air dried samples vacuum dried samples were also prepared for comparison, by evacuating the samples for 72 h at 105°C in a vacuum oven. Dimensions of air and vacuum dry samples were 2.4cm x 1.9cm x 0.371cm and 2cm x 1.9cm x 0.235cm respectively.

All measurements were made using electrode SE-70 with guard electrode placed in a thermostatic oven (TO-19) in combination with dielectric measuring set TR-10C, null detector (BDA-9) and oscillator (WBC-9). The complete set was of ANDO Electric Co. Japan. Measured quantities were C_x , the capacitance and G_x , the conductance of the specimen under test. Static relative permittivity ϵ' and loss factor ϵ'' were obtained as:

$$\epsilon' = C_x / C_0 \dots\dots\dots(1)$$

$$\epsilon'' = G_x / (\omega C_0) \dots\dots\dots(2)$$

where ω is the angular frequency of observation and C_0 , the capacitance of the empty cell, calculated as:

$$C_0 = \epsilon_0 A/d \dots\dots\dots(3)$$

where ϵ_0 = permittivity of free space, A = Area of the electrodes and d = Distance between them.

All measurements were reproducible within 5% experimental error.

3. RESULTS AND DISCUSSION

The measured dielectric constant and loss factor in air dried bovine tendon collagen at frequencies between 30 Hz to 3 MHz and the temperature range 30°C to 195°C are shown in Figures 1-2 respectively. These curves exhibit strong dispersion at low frequencies. The dielectric loss curve (See Figure 2) shows two discrete dispersions one at the lowest observed frequencies with no observable relaxation peak in the frequency range studied and a broad peak showing very little variation in the dispersion strength with temperature in the frequency range 1 KHz to 100 KHz is observed. The lowest temperature dispersion disappeared when blocking electrodes of poly (tetrafluoroethylene) was used to inhibit charge carrier injection into the test samples. This lowest temperature dispersion is therefore attributed to polarization arising from either electro-chemical reactions or the build up ion at the electrode/samples interfaces, such a dispersion at low frequency has generally been identified as Ω -dispersion [19, 20] The flat broad loss peak observed at relatively higher frequency in the air dry sample has been identified as the so called α -dispersion peak observed in other biological systems [21].

In vacuum dried sample relatively smaller values of dielectric constant were obtained (see figure 3) and the loss peak due to α -dispersion was not observable even at the lowest frequency (see figure 4), because the peak most probably has been shifted towards lower frequencies with the decrease of moisture in the sample. Both Ω and α dispersions are known to shift towards lower frequencies with the decrease of moisture content in the samples. (5). For the α dispersions $\text{Log } \epsilon''$ was found to be approximately linearly dependent on the logarithm of frequency, f , thus:

$$\epsilon'' \propto f^{-n} \dots\dots\dots(4)$$

where n is the slope of the curves. In the loss data presented here “ n ” varied between 0.48 - 0.85. According to Jonscher if $0 \leq n \leq 1$, the relation of equation (4) indicates that the dielectric constants, ϵ' and ϵ'' , would be caused by discontinuous jumps of charge carriers between localized sites [22]. Any carrier in the system will execute jump between various pairs of sites and gradually progresses in the direction of the applied field. Steady-state conductivity and electrolysis measurements on proteins have shown that for pure samples of low or moderate hydration, the conductivity is dominated by mobile protons [20]. The mobile protons originate from the ionizable groups of the protein molecule and that the sorbed water molecules form hydrogen-bond networks along which the protons can conduct [21].

The observed α -dispersion is therefore attributed to phonon-assisted proton hopping between localized sites in the system, where dielectric relaxation time τ_α is known to be related to steady-state conductivity σ arising from long range percolation of the charges along the pathways that interconnect these sites by the following equation [23, 21].

$$\tau_{\alpha} = \frac{\epsilon_0 \epsilon_{\infty}}{\sigma} \dots\dots\dots(5)$$

In order to get an insight into the thermally induced transitions in the sample dielectric constant have been plotted as a function of temperature at 30 KHz in figure 5 for both air and vacuum dried samples.

The two most prominent peaks at 50°C and 90°C observed in the air dried sample are absent in the vacuum dried sample, which shows that these two peaks are due to presence of moisture in the sample. As at 30 KHz the dominant mechanism of dielectric relaxation is proton hoppings both ϵ' and ϵ'' are proportional to the number of jumps performed by protons between sites formed by adsorbed water molecules. From room temperature to about 50°C mobility of bound water increases which results in increase of ϵ' and ϵ'' . From 50°C release of adsorbed water molecules starts which results in gradual reduction of proton hopping sites and hence both ϵ' and ϵ'' starts decreasing with temperature. Buildup of another peak at about 90°C results due to the release of water molecules bound to polar components of the protein molecules. These peaks are essentially absent in the vacuum dried samples as this adsorbed surface water and water bound to polar species of protein surface is removed under vacuum condition. Very strongly bound internal water however is not removed even after exposure to vacuum condition for 72 hours at 105°C. As a result a third peak which is manifested only as a shoulder at the higher temperature side of the second peak is present in the vacuum and air dried samples at 125°C. Another peak observed at 160°C which is present in both the samples is probably due to helix coil transition of collagen molecules.

A very weak dispersion peak was observed in the frequency range 250 KHz to 1.3 MHz at low temperature in the frequency dependence curve of dielectric loss factor of vacuum dry sample (See Figure 4). This peak shifted toward higher frequencies with the

increase of temperature showing that the underlying cause of this dispersion is Arrhenius type rate process. This relaxation is being attributed to intramolecular dynamics of polar components of collagen molecules.

A slight increase in the strength of dispersion ($\epsilon_s - \epsilon_\infty$) has been found with increase in temperature indicating an increase in intramolecular mobilities of the polar protein components with the increase of temperature. Following the theories of Debye and Onsager [24] it can be shown that:

$$\frac{(\epsilon_s - \epsilon_\infty)(2\epsilon_s + \epsilon_\infty)}{\epsilon_s(\epsilon_\infty + 2)^2} = \frac{N \langle m \rangle^2}{9\epsilon_0 K T} \dots\dots\dots(6)$$

where $\langle m \rangle^2$ is the mean square of the total dipole moment, N is the number of Dipole per unit volume, ϵ_s and ϵ_∞ are the limiting low - frequency and high - frequency relative permittivities and kT is the Boltzmann energy. From equation 6, $N \langle m \rangle^2$ was found to vary from $3.4 \times 10^{-32} \text{ C}^2/\text{m}$ at 29°C to $5.3 \times 10^{-32} \text{ C}^2/\text{m}$ at 100°C. Keeping in view the density of the sample 0.97 g/cm^3 and average molecular weight 98 of peptide units in the collagen molecule [25], the No. of peptide units/unit volume in the vacuum dry sample was calculated to be equal $59.60 \times 10^{26} / \text{m}^3$ which gives dipole moment per peptide unit as $2.4 \times 10^{-30} \text{ C.m}$. This value of dipole moment is about one fifth of the estimated dipole moment of a peptide group $1.2 \times 10^{-29} \text{ C.m}$ [26] and therefore the α_2 dispersion could have been resulted from some sort of limited relaxation of the peptide moments in the polypeptide back bones. Ring motion of proline and hydroxyproline and other side chains component may also contribute to some sort of side chain-main chain coupled mobilities. Dielectric relaxation of similar magnitude in the same frequency region due to vibrational motions of polypeptide back bones has also been observed in lysozyme and polypeptides such as polyglycine, polylysine and polyglutamic acid [5].

4. CONCLUSIONS

The dielectric data presented here reveal the presence of two separate mechanisms of dielectric dispersion. One observed at a low frequencies which has been attributed to proton hopping between localized sites and the other observed at relatively higher frequencies to the restricted mobilities of main chain or some sort of coupled mobilities of main chain and side chains.

Observation of thermally induced transitions in the dielectric data has been explained in terms of a three step moisture desorption process followed by helix to coil transition of the collagen molecules. It is logical to believe that release of strongly bound structural water results in subsequent denaturation of the protein molecule.

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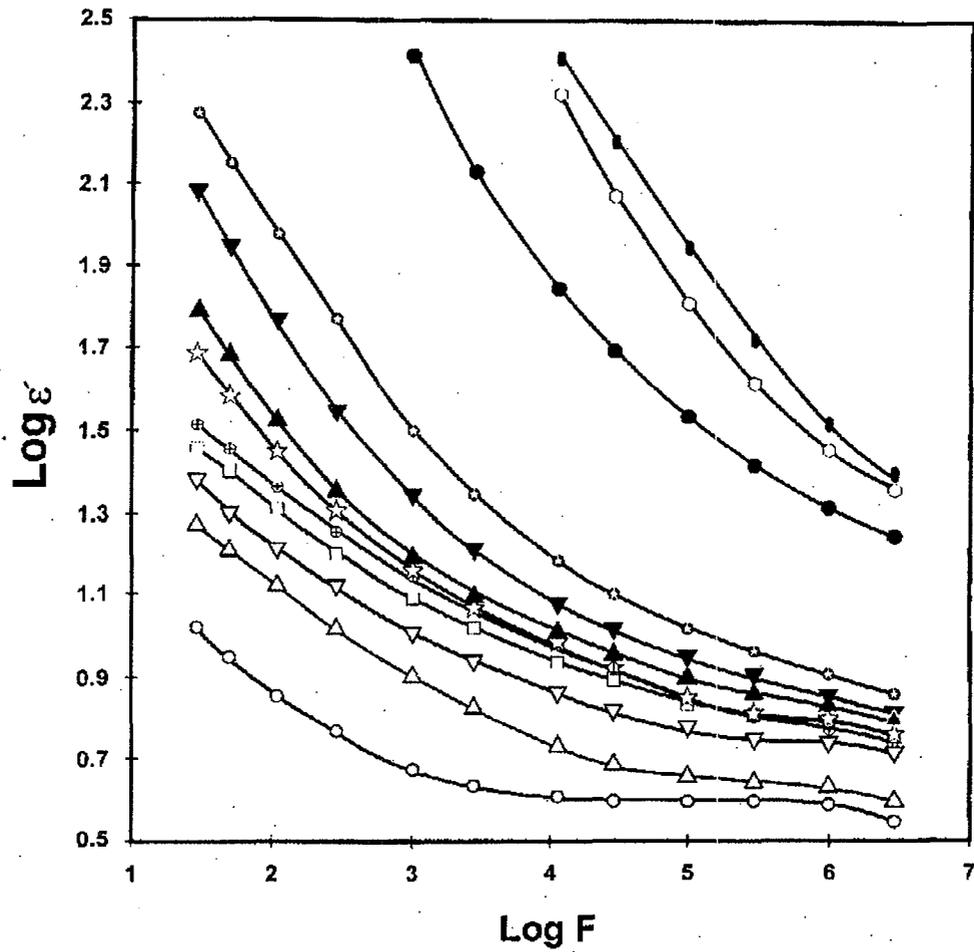
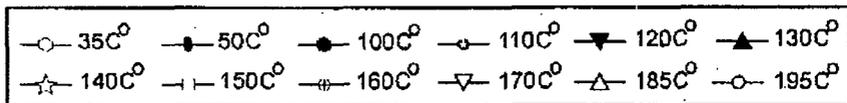


Fig. 1: VARIATION OF DIELECTRIC CONSTANT WITH FREQUENCY FOR AIR DRY BOVINE TENDON



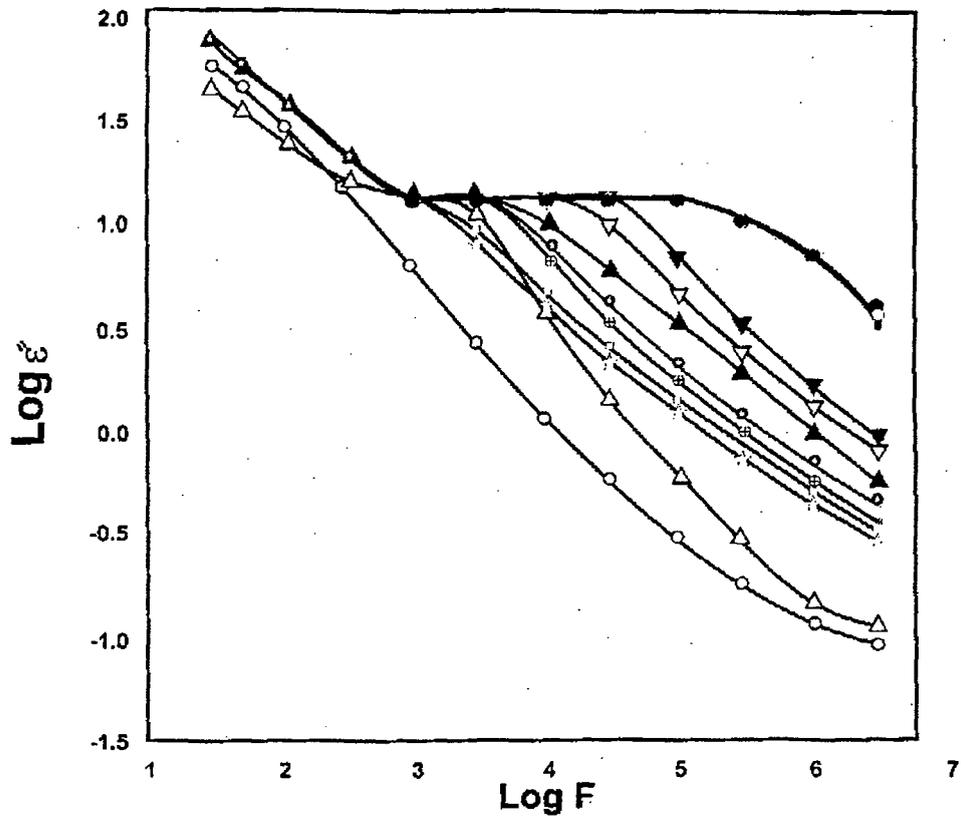
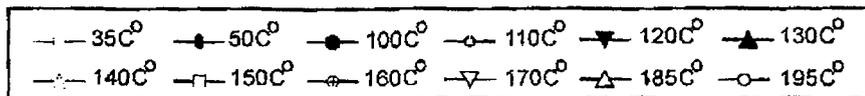


Fig. 2: VARIATION OF DIELECTRIC LOSS WITH FREQUENCY FOR AIR DRY BOVINE TENDON



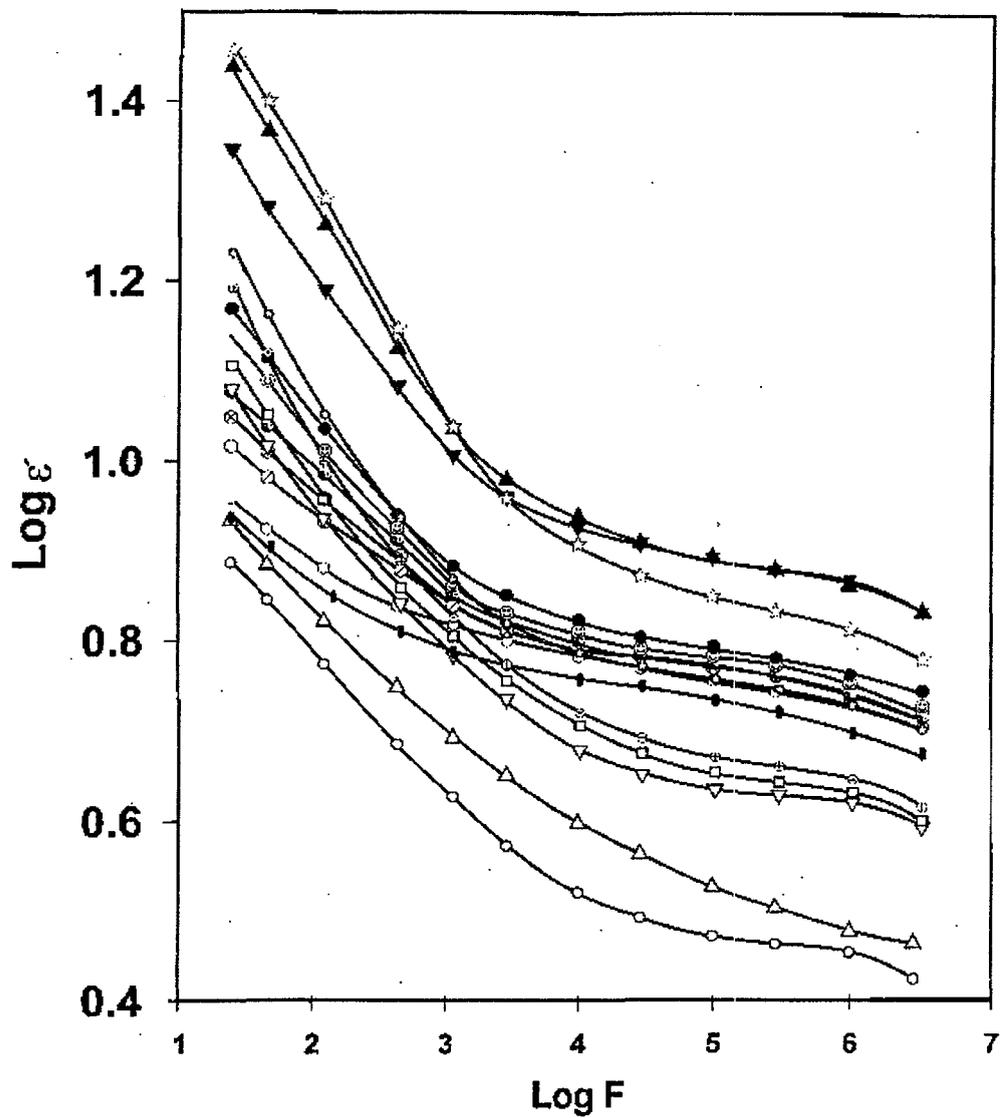
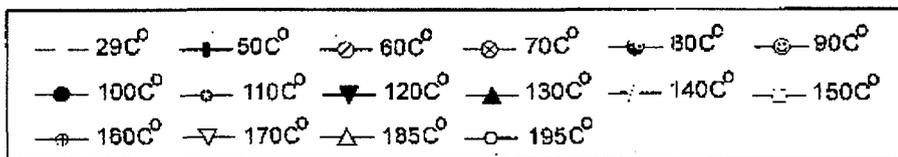


Fig. 3: VARIATION OF DIELECTRIC CONSTANT WITH FREQUENCY FOR VACUUM DRY BOVINE TENDON



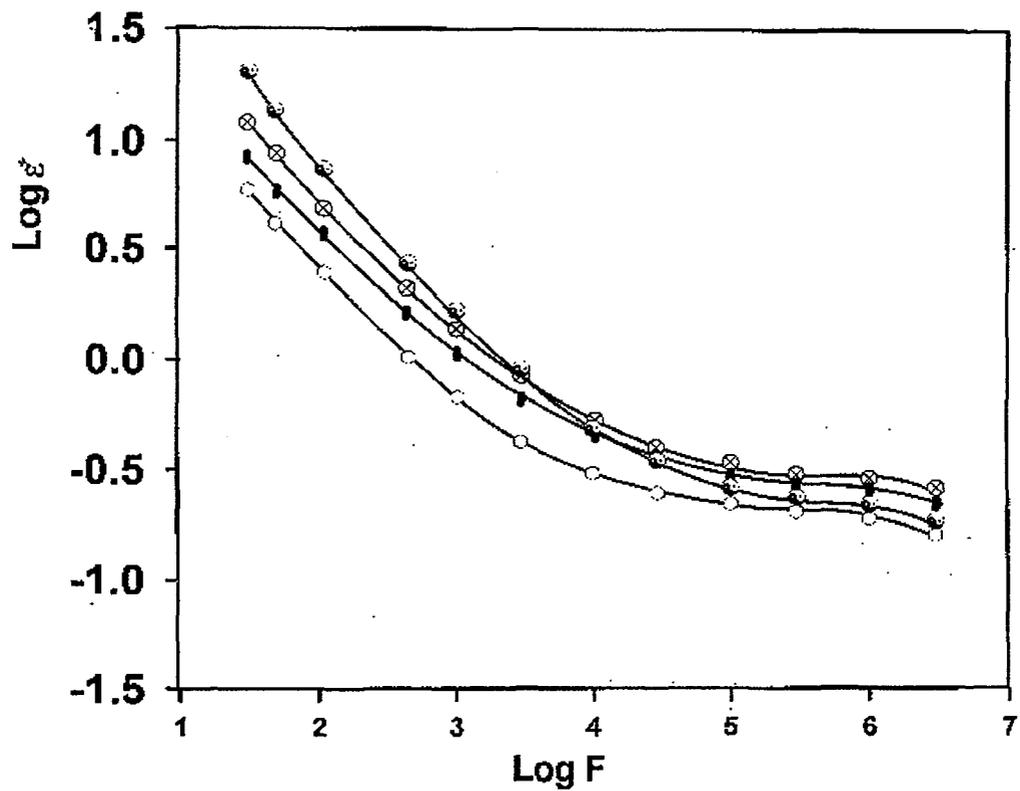
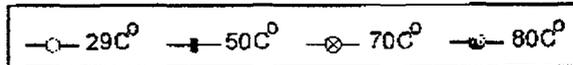


Fig. 4: VARIATION OF DIELECTRIC LOSS WITH FREQUENCY FOR VACUUM DRY BOVINE TENDON (SHOWN AT ONLY FOUR TEMPERATURES FOR CLARITY)



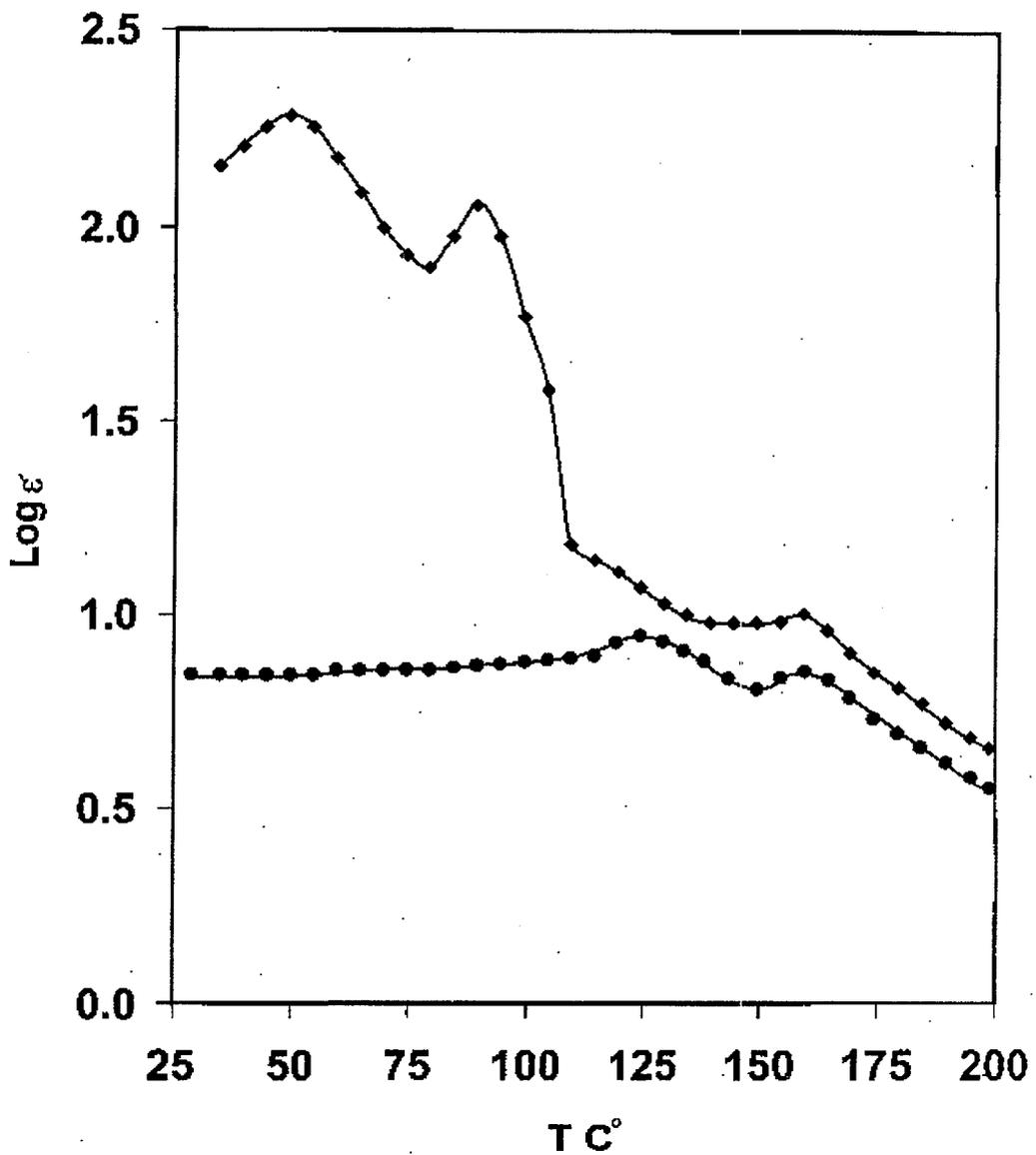


Fig. 5: TEMPERATURE DEPENDENCE OF DIELECTRIC CONSTANT FOR AIR AND VACUUM DRY BOVINE TENDON AT 30 KHz

—♦— Air Dry —♦— Vacuum Dry