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On the Dielectric Spectra of Proteins in Conducting Media

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Abstract. The effect of conductivity on the dielectric measurements of proteins is studied. For that purpose the dielectric spectra (0.03–13 MHz) of serum albumin and myoglobin in solutions of varying conductivities were recorded. The results presented confirm that Maxwell's prediction of a threshold frequency in conducting materials also holds for protein solutions. The threshold frequency of a serum albumin solution is experimentally determined and the ionic screening of the electric field when performing dielectric spectra of these samples discussed. Three distinct frequency regions must be considered: a low frequency region where the sample behaves like a conductor; an intermediate region centered around the threshold frequency where the free charges partially screen the fixed charges; and a high frequency region where the sample behaves like a good dielectric. Dielectric measurements in the low frequency region defined above, are not possible.

Key words. Proteins, dielectric spectra, Maxwell's frequency, permittivity, conductivity.

Introduction

The dielectric behaviour of biological macromolecules such as proteins and nucleic acids has been extensively studied [1–5] and the corresponding mechanisms of polarization discussed. The aqueous solutions of most of these molecules are conducting. Thus, the dielectric measurements in the low frequency range are very difficult to obtain. Great effort has been made to overcome this difficulty. By reducing the conductivity of the solutions, it was possible to go down in frequencies. Also, theoretical methods for conductivity correction have been developed [6], leading to an estimation of the dielectric constant for several biological macromolecules.

But, even when the ionic content of the media is reduced to a minimum which preserves the native state of the macromolecules, there is a low frequency range in which the dielectric measurements cannot be made. The real part of the permittivity of these samples seems to diverge when approaching the lower frequencies and no certain value of the dielectric constant can be obtained.

It was previously reported [7, 8] that conductive samples present a threshold frequency w_T (known as Maxwell's frequency) below which the screening of the probe electric fields becomes so important that it prevents the permittivity measurements being performed. At these frequencies, the free charges screen the external electric field, so that no polarization of the dipoles is produced.

Actually, three frequency regions can be distinguished. A low frequency region where the electric field is completely screened and no polarization occurs (the sample behaves like a conductor); a second region, centered around the threshold frequency, where the electric field is partially screened; and a third region at high frequencies where no screening occurs and the sample behaves like a pure dielectric.

These considerations were never taken into account when studying biological samples. To measure the extent of this phenomenon in protein solutions, in the present work we chose two proteins (bovine serum albumin and whale skeletal muscle myoglobin), which have already been extensively studied. The chosen proteins are intentionally of different sizes, isoelectric points, and amino acid composition. The concentration of both protein solutions are also purposely different.

The dielectric measurements of both proteins, in solutions of varying conductivities (10–400 μS/cm) have been performed in a frequency range of between 0.03 and 13 MHz. The results show that the dielectric measurements can only be carried out in the intermediate and high frequency ranges. An attempt to establish the lower frequency limit of this intermediate range gives an approximate value of $w_m = 0.1\ w_T$.

Also, the threshold frequency of a solution of serum albumin was experimentally obtained, thus presenting a new method for measuring the permittivity of this type of sample at this frequency.

2. Materials and Methods

Myoglobin from whale skeletal muscle (Type II M-0380) and bovine serum albumin (Fraction V A-3912) purchased from Sigma were used. The proteins were dissolved in double distilled water of a d.c. conductivity lower than 1 μS/cm and then dialyzed to obtain the minimum conductivity possible for each solution. Afterwards, the conductivity was controlled by adding NaCl.

The protein concentrations of the solutions were found from the ultraviolet absorption spectra: 80 mg/ml for serum albumin and 15 mg/ml for myoglobin.

Dielectric measurements were recorded using a Hewlett Packard 4192A LF impedance analyzer. The capacitance cell and procedure for the measurements were similar to those described by Pauly *et al.* [9].

Conductivity was measured with a Radiometer CMD3 conductivity meter. The ultraviolet spectra were recorded using a Metrolab 2500 spectrophotometer.

The threshold frequency was determined as previously described [7, 8].

All measurements were performed at 21 °C.

3. Results

In the dielectric measurements the protein solutions were exposed to a weak electric field (0.5 V/cm) varying periodically with frequency f, for which many discrete values have been chosen from the range of between 0.03 and 13 MHz.

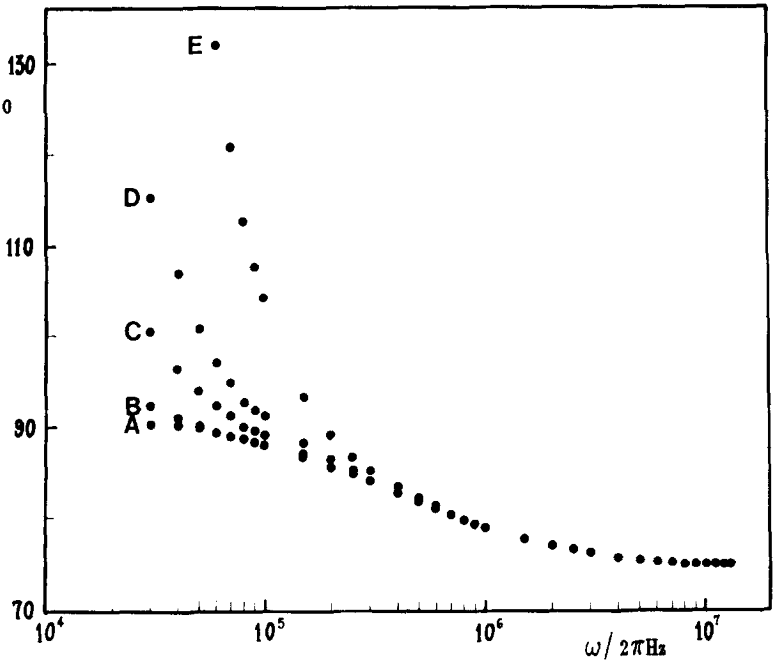

Fig. 1. Dielectric measurements of bovine serum albumin solutions, $C = 80$ mg/ml, with the increasing addition of NaCl: A. dyalized sample with no NaCl, of conductivity $\sigma = 14.6$ μS/cm; B. $\sigma = 43.5$ μS/cm; C. $\sigma = 68$ μS/cm; D. $\sigma = 120$ μS/cm; E. $\sigma = 385$ μS/cm.

Figure 1 shows the dielectric spectrum, measured by the impedance analyzer, of a dialyzed solution of serum albumin ($C = 80$ mg/ml, $\sigma = 14.5$ μS/cm) compared with some of the other spectra measured for the same protein sample with increased conductivity. The former (indicated by A) agrees with the results obtained by Essex *et al.* [4]. The other spectra show the effect of the NaCl on the dielectric measurements. It can be observed that the higher the conductivity, the higher the minimum frequency at which the permittivity is accurately measured.

In a previous work [8], it was shown that electrolyte solutions present two distinct frequency regions: a low frequency region where the sample behaves like a conductor and a high frequency region where the sample behaves like a dielectric. These two regions are separated by a threshold frequency $w_T = \sigma/\varepsilon$ (with $w_T = 2\pi f_T$).

In the present work, it is verified that a similar consideration holds for a protein solution.

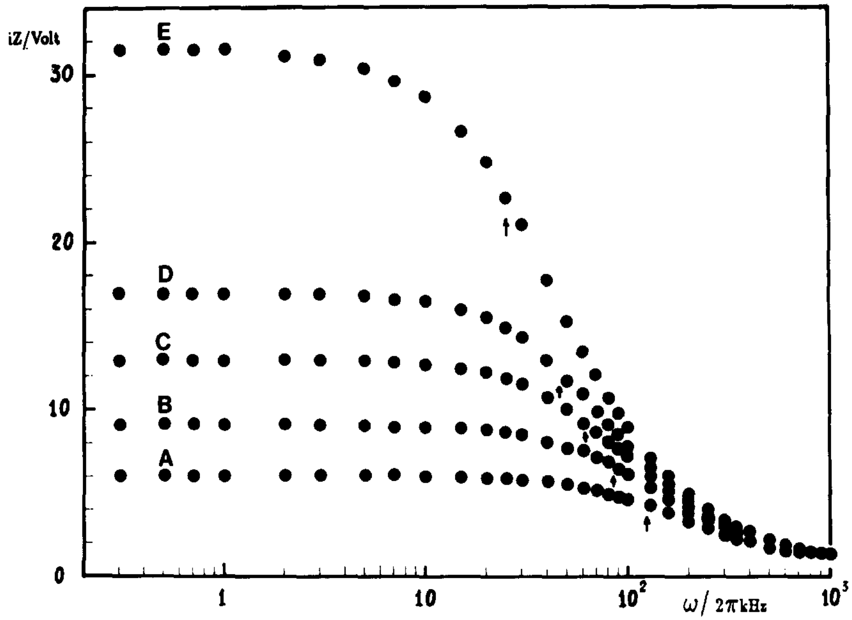

Fig. 2. Product of the impedance Z by the current i, at several frequencies. Z is the impedance across the cell filled with a dialyzed solution of bovine serum albumin, $C = 80$ mg/ml, $\sigma = 22$ μS/cm, measured at a constant current i, for different electrode distances: A. electrode distance: $d = 0.72$ mm; B. $d = 1.1$ mm; C. $d = 1.5$ mm; D. $d = 2$ mm; E. $d = 3.58$ mm. Arrows indicate the frequency at which $Z = Z(w = 0)/\sqrt{2}$ for each electrode distance. The resulting threshold frequency is $w_T = 480$ kHz.

Using the experimental method previously presented [8], the threshold frequency of a dialyzed solution of serum albumin ($C = 80$ mg/ml, $\sigma = 22$ μS/cm) was obtained. Figure 2 shows the iZ values for this solution measured at several frequencies, for different electrode distances. Here, Z is the impedance across the cell filled with the sample and measured at a constant current i. The resulting threshold frequency thus obtained was $f_T = 480$ kHz. Thus, the resulting permittivity value ($\varepsilon = \sigma/w_T$) is $\varepsilon/\varepsilon_0$ =t current i. The resulting threshold frequency thus obtained was $f_T = 480$ kHz. Thus, te resulting permittivity value ($\varepsilon = \sigma/w_T$) is $\varepsilon/\varepsilon_0 = 82$, which agrees with that measured by the bridge (from A in Figure 1).

Figure 3 shows the measured spectrum ε of a dialyzed solution of myoglobin (A in the figure; $C = 15$ mg/ml, $\sigma = 100$ μS/cm) compared with some of the spectra measured for the same protein sample with increased conductivity. The effect of NaCl is similar to that observed in Figure 1 for the serum albumin solutions.

In Figure 4, the effect of NaCl on the permittivity measurements of water, obtained by the impedance analyzer, can be observed. This effect is essentially the same as the one observed for the protein solutions (Figures 1 and 3).

For all samples studied, the minimum frequency (w_m) was determined at which the measured value of the permittivity agrees ($\Delta\varepsilon = \pm 1$) with the value measured for the lowest conductivity sample. Also, the corresponding Maxwell frequencies

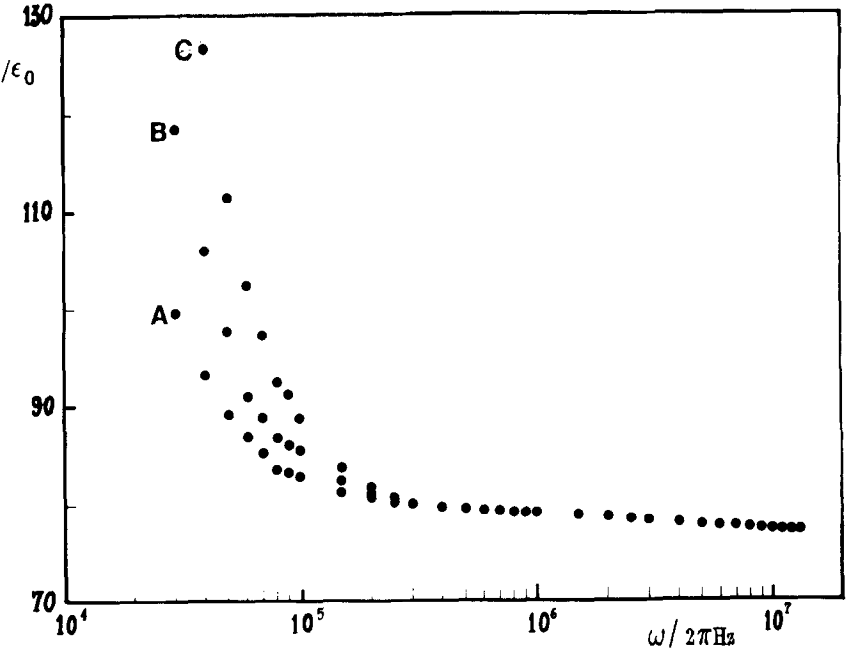

Fig. 3. Dielectric measurements of myoglobin solutions $C = 15$ mg/ml, with the increasing addition of NaCl: A. dialyzed sample with no NaCl, of conductivity $\sigma = 100$ μS/cm; B. $\sigma = 156$ μS/cm; C. $\sigma = 215$ μS/cm.

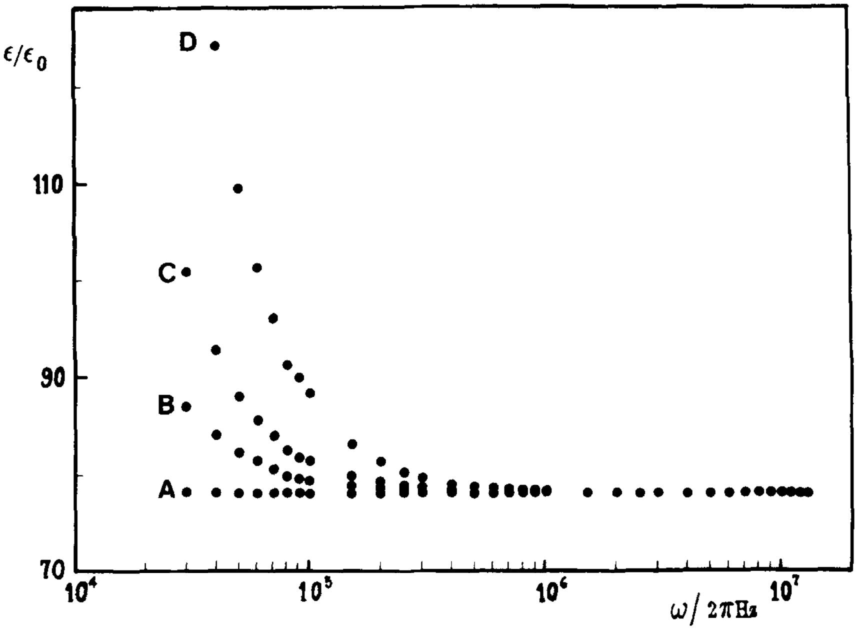

Fig. 4. Dielectric measurements of NaCl solution with increasing conductivity: A. $\sigma = 12.2$ μS/cm; B. $\sigma = 54$ μS/cm; C. $\sigma = 107$ μS/cm; D. $\sigma = 205$ μS/cm.

($w_T = \sigma/\varepsilon$) were calculated. For these calculations, the respective values of $\varepsilon/\varepsilon_0$ for serum albumin and myoglobin samples were taken as 88 and 83.5. These values are plotted in Figure 5. It can be observed that an approximated relation $w_m = 0.1\ w_T$ is valid for NaCl (full line). For the proteins the relation is always $w_m \leqslant 0.1\ w_T$. This may be because the measured permittivity of these samples is always compared with that of the lowest conductivity (i.e. 14.5 μS/cm for serum albumin and 100 μS/cm for myoglobin), which is still a conducting sample. In this plot, the repetition of points for 150, 200, and 300 kHz is due to the chosen discrete frequencies at which the measurements were taken.

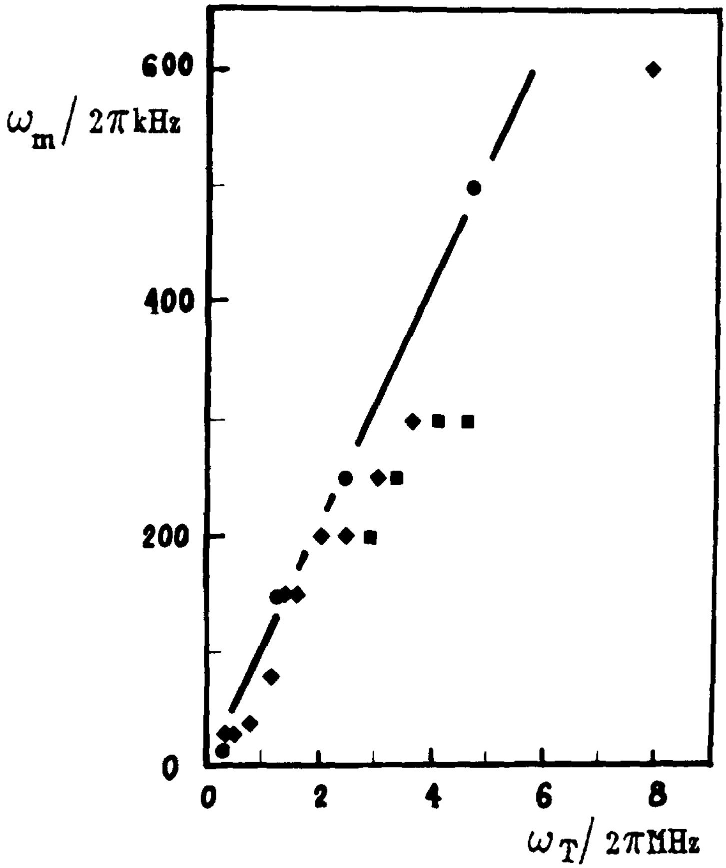

Fig. 5. Minimum frequency w_m at which the dielectric values measured ε differ by less than $\Delta\varepsilon = \pm 1$ from the corresponding value of the lowest conductivity sample. w_T is the estimated Maxwell frequency. (●) NaCl in water; (◆) serum albumin solutions; (■) myoglobin solutions. The straight line corresponds to NaCl and represents $w_m = 0.1\ w_T$.

4. Discussion

The results presented in this work for two very different proteins, confirm that Maxwell's predictions of a threshold frequency in conducting materials also holds for protein solutions.

If the dielectric spectra of protein solutions are to be measured, three distinct frequency regions can be observed: a low frequency region where the sample behaves like a conductor; an intermediate region, centered around the threshold frequency; where the free charges partially screen the fixed ones; and a high frequency region where the sample behaves like a good dielectric.

In the first region, the external electric field is completely screened, hence dielectric measurements are impossible. In considering electrode polarization, the corrections suggested by Shaw [6] using $\varepsilon = (C - C_0 - AG^2 w^{-n})d/K$, were calculated resulting, in all cases, in values of coefficient n of between 1.5 and 1.7. Although a large amount of calculation was necessary, these corrections approach the measured permittivity values of those produced for the corresponding dialyzed samples. We did not focus our discussion on the electrode polarization and its possible corrections, because this is just one of the effects produced by the free ions. Having confirmed Maxwell's prediction of a threshold frequency for protein solutions, it must be pointed out that ions produce several effects, including where conductivity is the dominant phenomenon.

Only in the second and third regions, can the dielectric measurements of the sample be obtained.

An approximation of the lower limit frequency at which the dielectric measurements can be performed is estimated for the conducting samples as $w_m = 0.1\ w_T$, which is the lower limit of the intermediate region. But, to perform measurements in this region, an adequate instrument of high conductor-dielectric discrimination is needed, for example a high-quality Schering bridge. An alternative method to determine the value of the permittivity in this region is by measuring the threshold frequency and the conductivity which, in the case of a sample with no dielectric relaxation in the interval, represents the lower frequency limit value.

Dielectric measurements in the high frequency range presents no great difficulty.

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