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DIELECTRIC SPECTRA OF PROTEINS IN CONDUCTING MEDIA

Graciela Ruderman *
International Centre for Theoretical Physics, Trieste, Italy

and

Juan R. de Xammar Oro
Instituto de Fisica de Liquidos y Sistemas Biológicos (IFLYSIB),
Departamento de Física and Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas,
Universidad Nacional de La Plata, c.c. 565, La Plata, Argentina.

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* Permanent address: Instituto de Fisica de Liquidos y Sistemas Biológicos (IFLYSIB), Departamento de Física and Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, c.c. 565, La Plata, Argentina.

ABSTRACT

Dielectric measurements of serum albumin and myoglobin in solutions of varying conductivities were performed. The results presented confirm that also for protein solutions, the Maxwell predictions of a threshold frequency in conducting materials holds. The threshold frequency of a serum albumin solution was experimentally determined. Attention should be recalled that, if the dielectric spectra of proteins solutions want to be measured, three distinct frequency regions are to 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.

INTRODUCTION

The dielectric behaviour of biological macromolecules like proteins and nucleic acids has been extensively studied (1,2,3,4) and the corresponding mechanisms of polarization discussed. The natural media of these molecules are conducting, thus the dielectric measurements in the low frequency range are very difficult to obtain. Even when the ions content of the media is reduced to a minimum which preserves the native state of the macromolecules, the conductivity is high enough to obstruct the permittivity measurements to be performed in this frequency range. 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.

To overcome this difficult theoretical methods for conductivity correction have been developed (5), leading to an estimation of this quantity for several biological macromolecules.

As previously reported (6,7), conductive samples present a threshold frequency ω_T (known as the Maxwell frequency) below which, the screening of the probe electric field becomes so important that prevents the permittivity measurements to be performed. At these frequencies the free charges screen the external electric field, so that no polarisation 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 proteins solutions, in the present work serum albumin and myoglobin were studied.

The dielectric measurements of both proteins, in solutions of varying conductivities (10μS/cm-400μS/cm) have been performed in a frequency range between 30kHz and 13MHz. The results show that the dielectric measurements can only be done in the intermediate and the high frequency ranges. An attempt to establish the lower frequency limit of this intermediate range gives an approximated value of $\omega_m=0.1\omega_T$.

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

MATERIALS AND METHODS

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

The protein concentration of the solutions was found from the ultraviolet absorption spectra.

Dielectric measurements were recorded using an LF Impedance Analyzer, Hewlett Packard 4192A. The capacitance cell and procedure for the measurements were simmilar to those described by Pauly et al. (8).

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

The threshold frequency was determined as described previously (6,7).

All measurements were performed at 21°C.

RESULTS

In the dielectric measurements the protein solutions have been exposed to weak eleotric fields (0.5V/cm) varying periodically with frequency f, for which many discrete values have been chosen between 30kHz and 13MHz.

Figure 1 shows the dielectric spectrum ε measured by the impedance analyzer, of a dyalized solution of serum albumin (c=80mg/ml, σ =14.5μS/cm) compared with some of the spectra measured for the same protein sample with increased conductivity. The former (signed by A) agrees with the results obtained by C.G. 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 truly measured.

In a previous work (7) it was shown that eleotrolyte solutions present two distinct frequency regions: a low frequency region were the sample behaves like a conductor and a high frequency region were the sample behaves like a dielectric, being these two regions separated by a threshold frequency $\omega_\tau = \sigma / \varepsilon$ (with $\omega_\tau = 2\pi f_\tau$).

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

Using the experimental method previously presented (7) the threshold frequency of a dyalized solution of serum albumin (c=80mg/ml, σ =22μS/cm) was obtained. Figure 2 shows the iZ values for this solution measured at several frequencies, for different electrode distances. There, Z is the impedance across the cell filled with the sample, measured at a constant current i. The resulting threshold frequency thus obtained was f_τ=480kHz. Thus, the resulting permittivity value ($\varepsilon = \sigma/\omega_\tau$) is $\varepsilon/\varepsilon_o$=82, which agrees with that measured by the bridge (from A in figure 1).

Figure 3 shows the measured spectrum ε of a dyalized solution

of myoglobin (A in the figure; c= 15 mg/ml, σ =100μS/cm) compared with some of the spectra measured for the same protein sample with increased conductivity. The effect of NaCl is simmilar 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, that the one observed for the proteins solutions (figures 1 and 3).

For all samples studied, it was determined the minimun frequency (ω_m) at which the measured value of the permittivity agrees ($\Delta\varepsilon_r \pm 1$) with that value measured for the lowest conductivity sample. Also the corresponding Maxwell frequencies ($\omega_\tau = \sigma/\varepsilon$) were calculated. For these calculations the respective values of $\varepsilon/\varepsilon_o$ for serum albumin and myoglobin samples were taken as 88 and 83.5. In Figure 5 these values are plotted. It can be observed that for NaCl an approximate relation $\omega_m = 0.1\omega_\tau$ is valid (full line). For the proteins is always $\omega_m \leq 0.1\omega_\tau$. This may be because the measured permittivity of these samples is always compared with that of the lowest conductivity one (i.e. of 14.5μS/cm for serum albumin, and of 100μS/cm for myoglobin), which is still a conducting sample. In this plot, the repetition of points for 150kHz, 200kHz and300kHz is due to the chosen discrete frequencies at which the measurements were done.

DISCUSSION

The results presented in this work confirm that also for protein solutions, the Maxwell predictions of a threshold frequency in conducting materials holds.

If the dielectric spectra of proteins solutions want to be measured three distinct frequency regions are 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 the dielectric measurements are impossible. In considering electrode polarization, the corrections suggested by Shaw (5) using $\hat{\epsilon} = (C - C_\infty - AG^2\omega^{-n})d/K$ were calculated, obtaining in all cases values of the coefficient n between 1.5 and 1.7. Although an important amount of calculation was needed, indeed these corrections approach the measured permittivity values to those measured for the corresponding dyalized samples. We do not focalize the discussion on the electrode polarization and its possible corrections. Being confirmed the Maxwell predictions of a threshold frequency for proteins solutions, it must be pointed out that ions produce several effects; but, these effects are hidden by the conducting behavior of the solution.

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

An approximation of the lower limit frequency at which the dielectric measurements can be performed is estimated for the conducting samples as $\omega_m = 0.1\omega_T$, which is the lower limit of the intermediate region. But, to perform the measurements in this region, an adequate instrument of high conductor-dielectric discrimination is needed, for example a high quality Shering 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.

ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

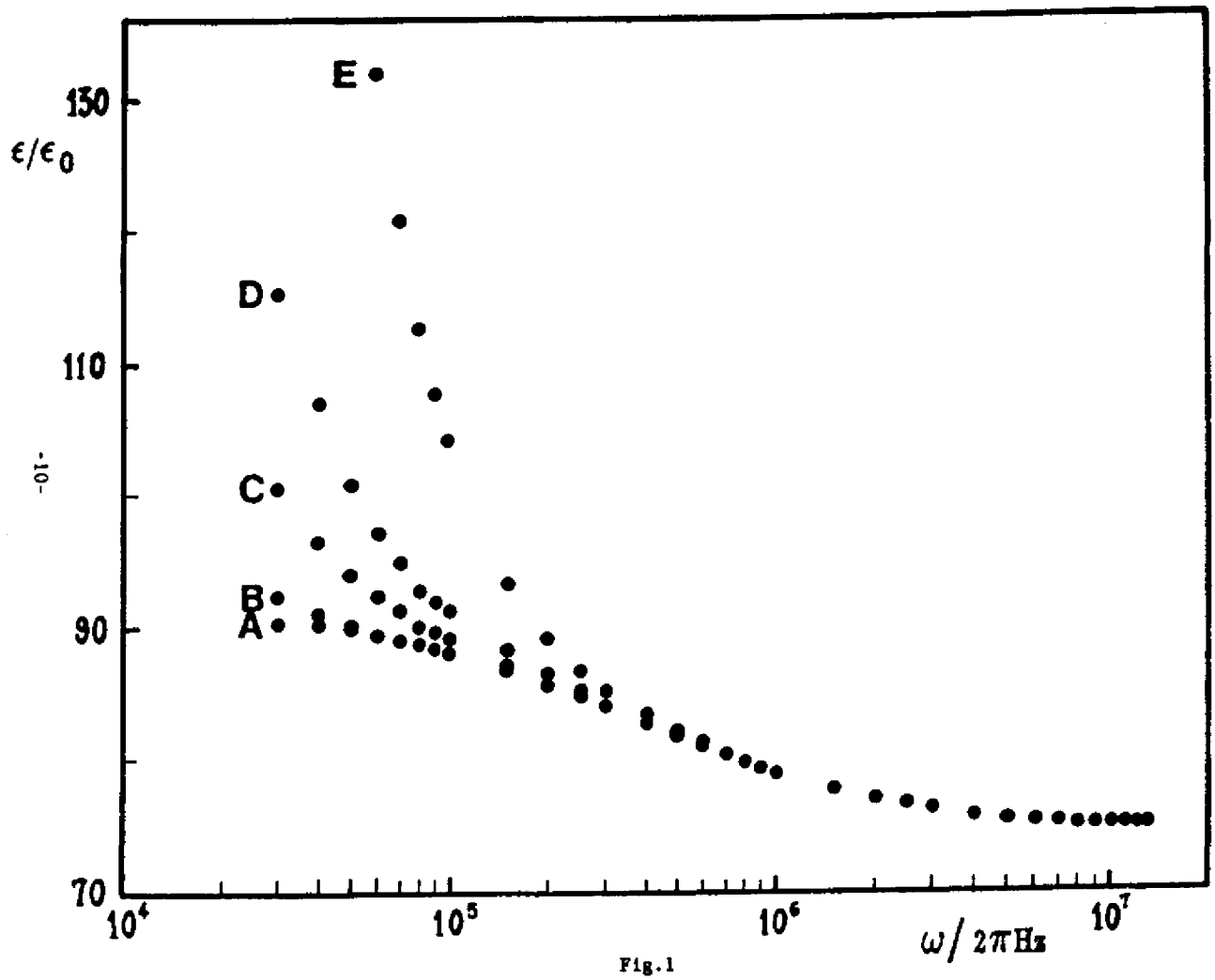
Fig.1.- Dielectric measurements of bovine serum albumin solutions, c=80mg/ml, with encreasing addition of NaCl: A. dyalized sample with no NaCl, of conductivity σ=14.6 μS/cm; B. σ=43.5 μS/cm; C. σ=68 μS/cm; D. σ=120 μS/cm; E. σ=385 μS/cm.

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

Fig.3.- Dielectric measurements of myoglobin solutions c=15mg/ml, with encreasing addition of NaCl: A. dyalized sample with no NaCl, of conductivity σ=100 μS/cm; B. σ=156 μS/cm; C. σ=215 μS/cm.

Fig.4.- Dielectric measurements of NaCl solutions with encreasing conductivity: A. σ=12.2 μS/cm; B. σ=54 μS/cm; C. σ=107 μS/cm; D. σ=205 μS/cm.

Fig.5.- Minimum Frequency ω_m at which the dielectric values measured ε differ by less than $\Delta\varepsilon=\pm1$ from the corresponding value of the lowest conductivity sample. ω_T is the estimated Maxwell's frequency. (●) NaCl in water; (◆) serum albumin solutions; (■) myoglobin solutions. The straight line corresponds to NaCl and represents $\omega_m=0.1\omega_T$.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ϵ/ϵ_0
10^4
10^5
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$\omega/2\pi$Hz

Fig.1

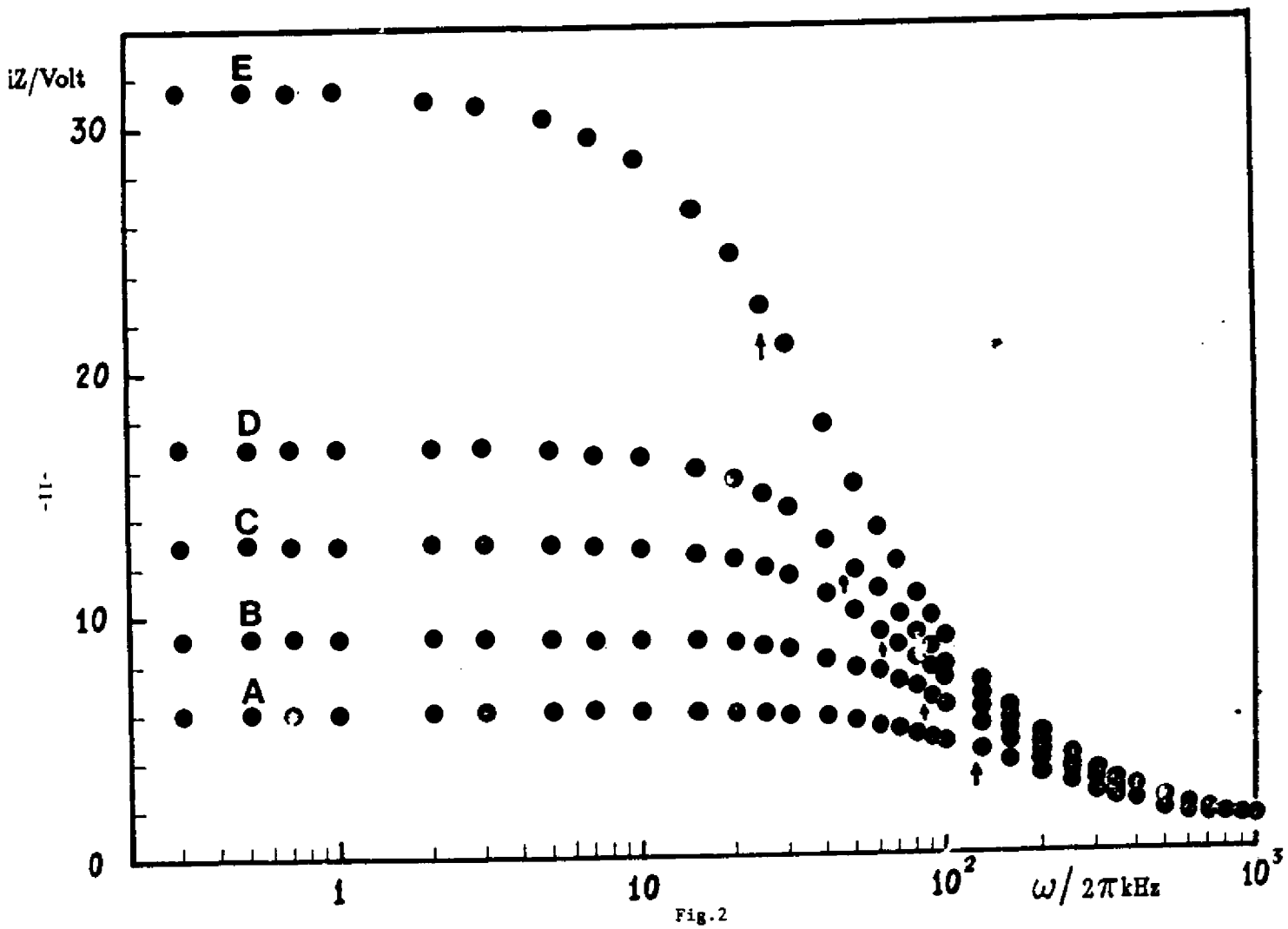
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Fig.2

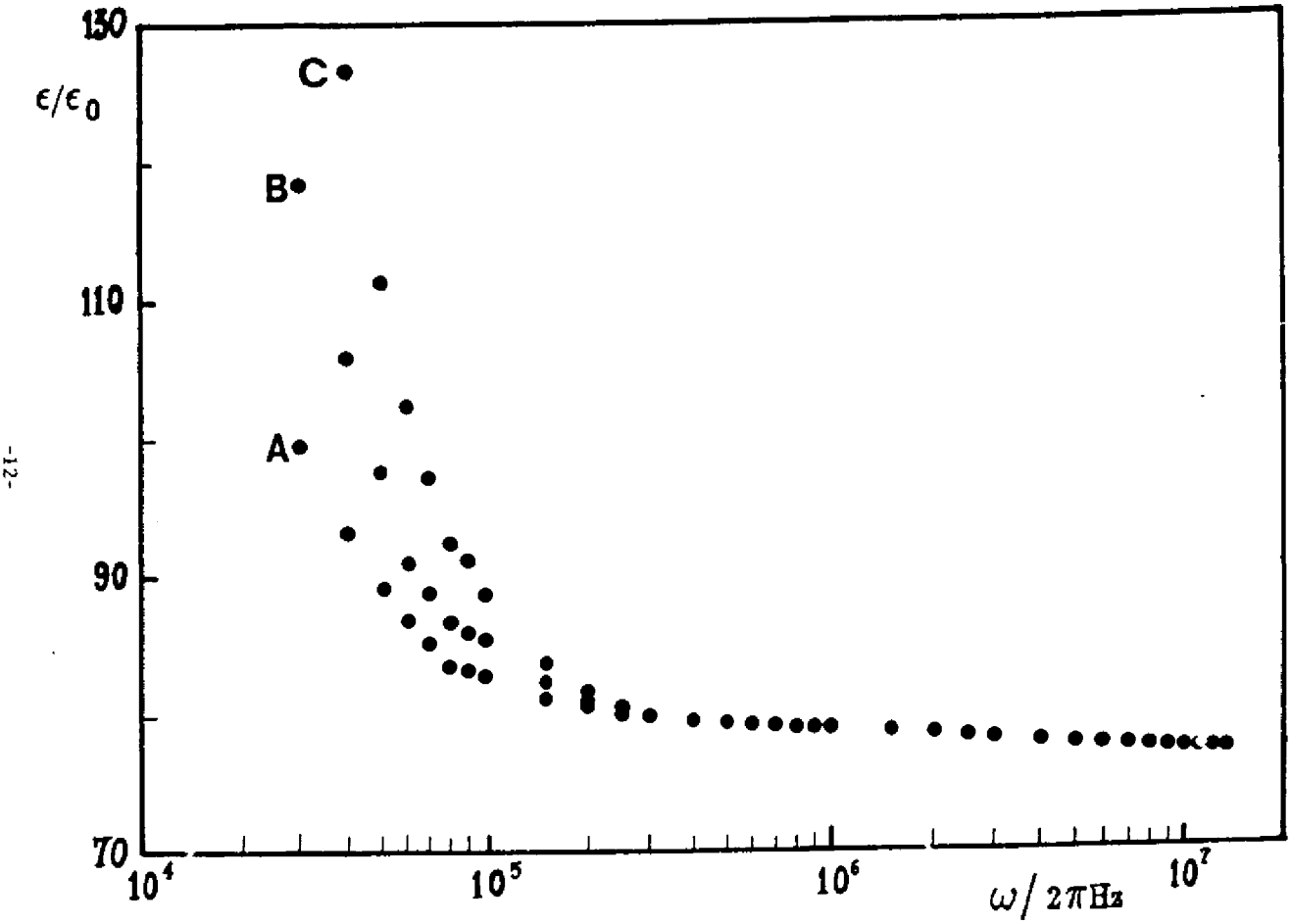
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Fig.3

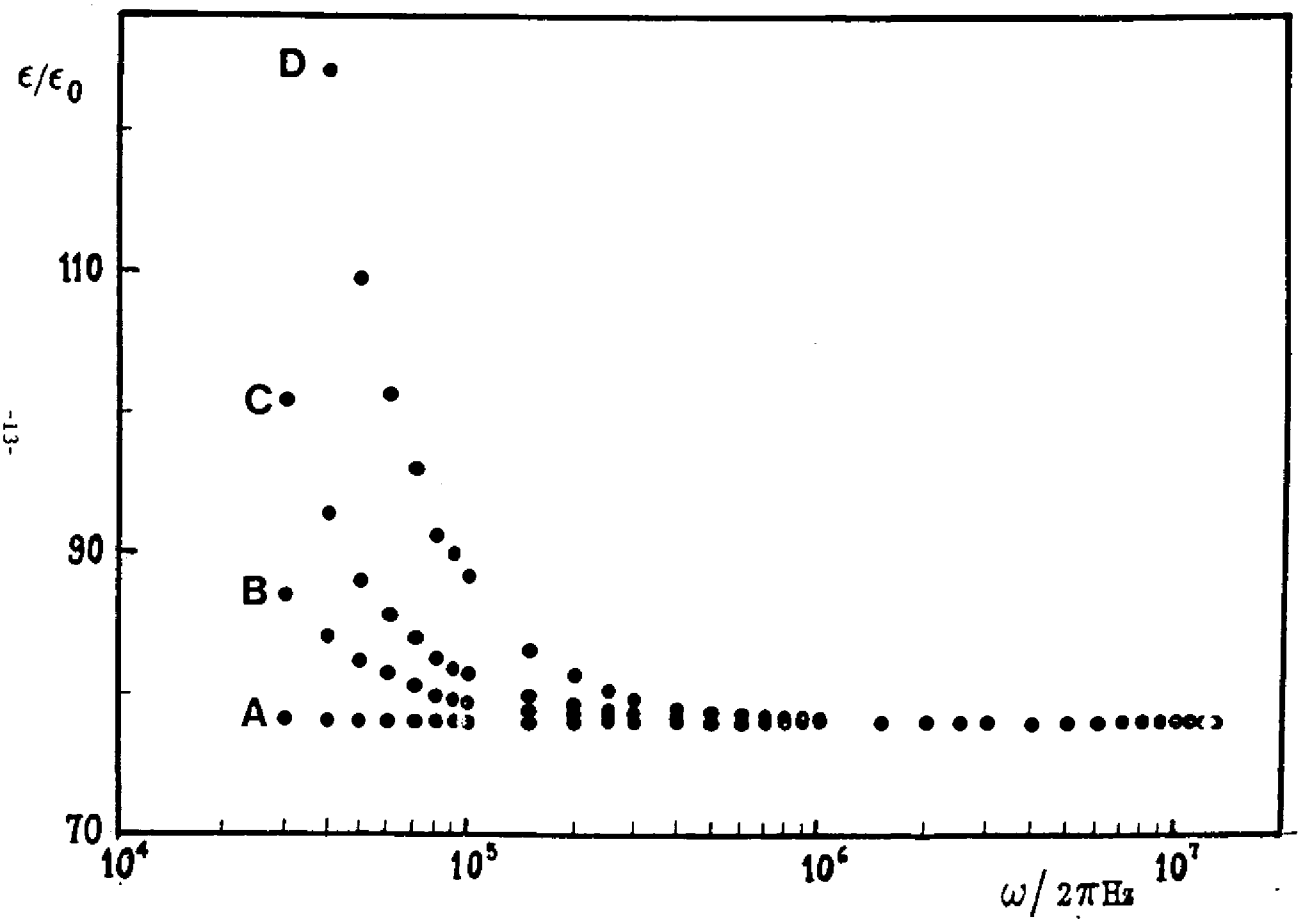
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Fig.4

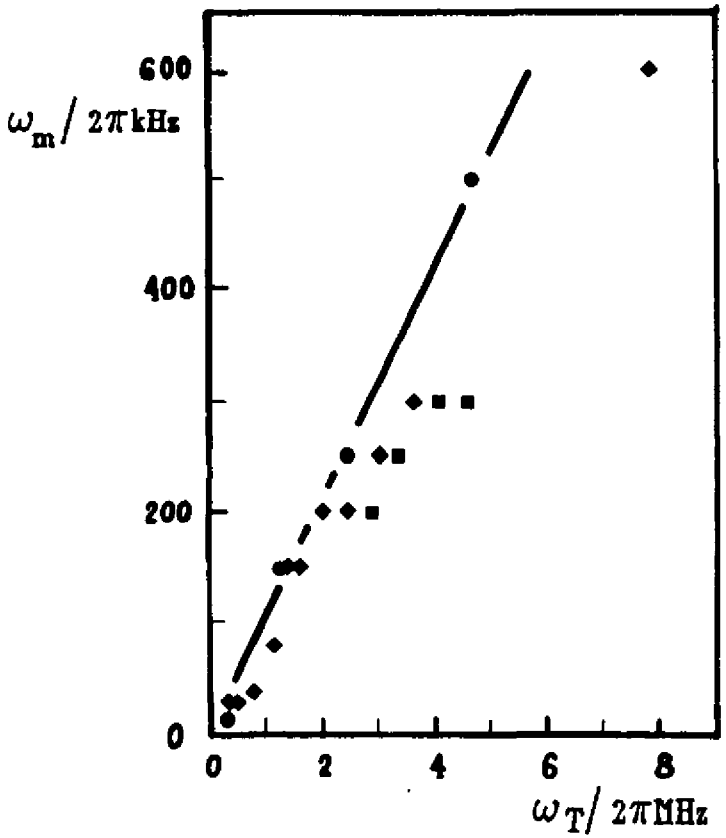

Fig.5