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A Wide-Band Dielectric Characterization System for Liquid Materials of Interest in Biology

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NOTA INTERNA B4-23

Maggio 1995

# A Wide-Band Dielectric Characterization System for Liquid Materials of Interest in Biology

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## Abstract

In many fields of applied research the interest exists for wide-band dielectric characterization of liquid materials, for example, aqueous solutions of biological materials. For this purpose a particular procedure is here proposed, based on the use of a Vectorial Network Analyzer and a suitably designed cell which contains the material under test. Fundamental features of this method are: dielectric characterization directly in the frequency domain without the use of a Fourier inverse transformation process, as it is required when TDR techniques are used; wide-band measurement in a very short time; the need of a quantity for the material to be tested far lower than those required by the actually available commercial dielectric probes.

## Keywords

Dielectric characterization, Dielectric probe, Dielectric relaxation spectra, Test of biological materials

## -1- Introduction

Research on molecular dynamics is since long time of great importance for obtaining information about physical and biological properties of biomaterials.

Research on molecular dynamics is since long time of great importance for obtaining information about physical and biological properties of biomaterials. Different experimental techniques have been proposed for this purpose, such as fluorescence, electron spin resonance, nuclear magnetic resonance, dielectric relaxation measurements [Kazuya Imamatsu, et al., 1986].

Particular reference will be made here to dielectric techniques where electric dipoles involved in the biomaterials are used as natural probes for investigation of dynamics and structure. As an example we may refer to the study of the water structure around protein molecules in aqueous solutions [Naoki Shinyashiki, et al., 1990]: in this case the water molecules attach to the oxygen, nitrogen and polar groups on the protein surface through hydrogen bonding and have an important influence on the functions of protein itself.

Such bound water can be studied by means of high resolution X-ray and neutron diffraction measurements in the crystal phase; however most of the function of proteins does not appear in crystal phase as the protein structure is stabilized by the intermolecular interaction and large structural fluctuations are restricted. In other words, the water in the crystal phase seems to be different from that in vivo so that it is of great interest to develop measuring techniques to characterize the structure of bound water in aqueous solution.

Although there are difficulties in working with solutions (the bulk liquid has a high dielectric constant and protein solutions cannot be made very concentrated so that the effects of the bound water are not easily detectable), dielectric relaxation measurements on aqueous protein solutions resulted to be quite suitable to investigate the bound water. For example, by the use of such methods a high frequency relaxation process was evidenced, around 10 GHz, due to the bulk water and a low frequency process, around 100 MHz, was found, caused by the orientation of bound water molecules on the protein surface, supplemented by fluctuation of the polar side groups on the surface itself [S. C. Harvey, et al., 1972; Satory Mashimo, et al., 1987; Nobuhiro

Miura, et al., 1994; B.Saif, et al., 1991]. It was found that such low frequency relaxation process moves little as the water content is varied, while relaxation strength directly depends on the bound water molecules and vanishes for completely dried material.

Usually dielectric relaxation data are obtained from the knowledge of material complex dielectric permittivity in the frequency domain.

Dielectric measurements can be performed by several conventional techniques; however many of them cannot be suitably used for dielectric characterization of biomaterials where presence of ionic conductivity and multiple relaxations, generally, require a short measuring period and a wide frequency range exploration. For these reasons TDR methods have been widely used for the investigation of molecular dynamics of biomaterials [Robert H. Cole, et al., 1980; Stephen Bone, 1987].

As it is well known, in TDR techniques a single step pulse is reflected from a thick sample of the material under test; the reflected signal  $r(t)$  is successively Fourier analyzed so obtaining the reflection coefficient  $R(\omega)$  in the frequency domain and the complex dielectric permittivity vs. frequency is finally calculated as

$$\varepsilon = [(1-R(\omega))/(1+R(\omega))]^2 \quad (1)$$

The valuable features of TDR techniques of allowing dielectric characterization over wide frequency range in short measuring time can now be achieved by the use of Vectorial Network Analyzers (VNA) with the further advantage of directly measuring the complex dielectric permittivity of the material in the frequency domain.

In this paper a dielectric cell, particularly designed for testing liquid biological materials, and the related measuring procedure, based on the use of a commercial VNA, is presented: the real and imaginary part of biological

material permittivity can be computed directly from the measured data of reflection scattering parameter  $S_{11}$  relative to the dielectric cell loaded by the material under test (MUT).

The characteristics of the proposed cell are of fundamental importance and are not found in the actually available commercial devices. It allows a preliminary calibration operation, a correct interaction between the tested material and the exploring signal and, particularly, it requires a small quantity of material, less than one milliliter.

For the specific VNA used in our case (HP 8753B), the swept frequency interval can range from 300 KHz to 3 GHz, while the measuring period remains extremely short (few seconds) also in the case where the highest number of measuring points (1601) in the chosen interval is used.

## -2-The Dielectric Characterization Technique

With reference to Fig.1 let it be:

$Z_0$  = Characteristic impedance of line feeding the cell

$Z_{in}$  = The input impedance of the cell at its input reference plain A-A

$S_{11}$  = The complex reflection scattering parameter at plain A-A

It is

$$S_{11} = S_{11}e^{j\Phi} = (Z_{in}-Z_0)/(Z_{in}+Z_0) \quad (1)$$

Let's now assume that the input impedance  $Z_{in}$  of the cell may be modelled as a capacitance C; in the case of empty cell, by putting  $C=C_0$ , Eq. (1) becomes

$$S_{11} = S_{011} = S_{011}e^{j\Phi_0} = S_{011}\cos\Phi_0 + jS_{011}\sin\Phi_0 = (1-j\omega Z_0 C_0)/(1+j\omega Z_0 C_0) \quad (2)$$

Eq. (2) shows that, if the real and imaginary part ( $R[S_{011}]$  and  $I[S_{011}]$ , respectively) of scattering parameter  $S_{011}$  are measured, the empty cell capacitance  $C_0$  can be obtained; it is:

$$C_0 = (R[S_{011}] - 1) / (\omega Z_0 I[S_{011}]) \quad (3)$$

or, equivalently

$$C_0 = -I[S_{011}] / (\omega Z_0 (1 + R[S_{011}])) \quad (4)$$

It is to note that the values for  $C_0$  obtained from Eqs (3) and (4) are equal provided that it is  $S_{011} = 1$ , i.e. the empty cell is a lossless system.

When the cell is loaded by the material under test its capacitance  $C$  becomes

$$C = C_0(\varepsilon' - j\varepsilon'') \quad (5)$$

where  $\varepsilon = \varepsilon' - j\varepsilon''$  is the complex relative dielectric constant of the material itself.

By substituting  $C_0$  in Eq. (2) with expression (5), by solving the resulting equation with respect to  $\varepsilon'$  and  $\varepsilon''$  and by taking into account Eq. (3), we have:

$$\varepsilon' = 2((1 + R[S_{011}])I[S_{11}]) / (I[S_{011}](1 + 2R[S_{11}] + |S_{11}|^2)) \quad (6)$$

$$\varepsilon'' = -((1 - |S_{11}|^2)(1 + R[S_{011}])) / (I[S_{011}](1 + 2R[S_{11}] + |S_{11}|^2)) \quad (7)$$

If it is  $S_{11} = S_{011}$ , from Eqs (6) and (7) we have  $\varepsilon' = 1$ ,  $\varepsilon'' = 0$ .

The dielectric characterization procedure is directly based on Eqs. (3), (6) and (7).

Firstly, in the condition of empty cell,  $S_{011}$  is measured vs. frequency over the preassigned band of interest. Successively, the cell is loaded by the material under test and  $S_{11}$  measured. It is extremely important that material completely fills the whole volume which determines the capacitance  $C_0$  so that the use of

the simple Eq. (5) is allowed to calculate the capacitance value  $C$  relative to load condition. Finally, the real and imaginary part of relative dielectric permittivity is computed according to Eqs. (6) and (7), respectively.

### **-3- The Implementation of the Procedure**

#### *a) The cell structure*

In Fig. 2 a cross-sectional view of the cell is shown and the input reference plain A-A is also indicated.

The following characteristics are to be outlined:

- a low quantity of material is required for the measure; about  $0.07 \text{ cm}^3$  in the present case;
- the structure can be easily disassembled and cleaned;
- the volume inside the cell can be filled by the tested material, accurately avoiding formation of air inclusion;
- The whole volume relative to capacitance  $C_0$  can be filled by the MUT
- A procedure exists for correctly positioning the reference plane A-A before measuring  $S_{11}$ , as it will be explained in the following part of this paragraph.

#### *b) The Measuring System*

In Fig. 3 the experimental set-up used for the measure of the reflection scattering parameter  $S_{11}$  is shown, based on the use of the VNA HP model 8753B, in the present case.

Vectorial network analyzers allow a quick and easy measure of the complex reflection and transmission coefficients of the device under test over large frequency ranges. Such instruments feed the device by an internal generated CW sinusoidal signal whose frequency is linearly swept over the band of interest; the instrument picks up a portion of the incident signal which is assumed as reference signal and automatically measures, with respect to this reference, the amplitude and phase of the signal reflected from the device or transmitted through it, so obtaining a full frequency domain characterization of the device itself. Measured data can be stored, displayed or processed according to the particular application. The accuracy allowed in measuring reflection and transmission coefficients is, generally, better than 0.1 dB for amplitude and better than  $1^\circ$  for phase angle; frequencies can be measured with 1 Hz resolution.

As explained in the previous paragraph, the first step of the procedure is the measure of  $S_{011}$  at the input plane A-A of the cell; for this purpose it is required the extension of the VNA reference plane to A-A; this an important operation which must be done as accurately as possible to avoid errors in the estimated values of  $\epsilon'$  and  $\epsilon''$ .

In the adopted structure of the cell this preliminary calibration of the reference plane can be obtained by extracting the cable from the cell and by putting a short circuit at the end of the cable itself; the reference plane of VNA is then extended until a value equal to  $180^\circ$  is obtained for  $S_{11}$  phase angle all over the explored band: the reference plane extension so obtained is characteristic of the cell and the experimental set-up used, so that it can be used in all successive measures.

The data measured vs. frequency for  $S_{11}$ , in the case of empty cell and in the case of cell loaded by the material of interest, are stored and, successively, by using Eqs. (6) and (7), the real and imaginary part  $\epsilon'$  and  $\epsilon''$  vs. frequency can be computed.



#### **-4- Experimental Results**

Here some results are reported, obtained when the measuring procedure above was applied to particular experimental conditions.

In Fig. 4 the real part of permittivity vs. frequency is reported in the case of empty cell: we can observe that the accuracy of the measure in this particular test condition is quite satisfactory.

In Fig. 5 the imaginary part of permittivity in the case where the cell is loaded by distilled water is shown: the part of the curve relative to the lower frequency range clearly shows the presence of dielectric losses due to ionic conductivity.

In Fig 6 the imaginary part of dielectric permittivity measured in the case of cell filled by a 10%w/w aqueous solution of Human Serum Albumin (HSA) is reported.

Fig. 7 gives the modified absorption spectrum is reported, obtained by applying to the data of Fig. 6 the particular procedure described in the following Appendix., to remove the influence of dc conductivity.

#### **-5- Conclusions**

Dielectric characterization vs. frequency of aqueous solution of materials is of great interest, particularly in investigating physical properties, molecular structures and dynamics of biomaterials where the use of experimental conditions as similar as possible to the physiological ones is fundamental importance.

Basic features generally required for the used measuring procedures are: wide-band exploration, short time required for the measure, small quantity of material.

In this paper the structure of a particular cell, is described with the associated measuring procedure, based on the use of a vectorial network analyzer.

The cell structure requires a quantity of tested material far less than the quantity needed by commercially available devices. The measuring procedure allows a wide-band characterization, directly in the frequency domain, in a very short time also in the case of high frequency resolution.

## Appendix

The importance has been outlined of measuring dielectric relaxation data of biological liquid materials in order to be able of characterizing the material of interest in conditions as similar as possible to the physiological ones; obviously, the data so obtained for the MUT may be in some extent modified by the solvent; moreover, spurious contribution to the absorption spectrum  $\epsilon''$  from ionic dc conductivity may also result. A method to account for the last of these two sources of error is to correct the data which directly result from the measure according to the equation [kazuya Imamatsu, et al., 1986; B. Saif, et al., 1991].

$$\epsilon''^* = \epsilon'' - J\sigma/(\omega\epsilon_0) \quad (1A)$$

where now it is:

$\epsilon''$  = Values initially obtained for the imaginary part of MUT dielectric permittivity

$\epsilon''^*$  = Corrected values for the imaginary part of MUT dielectric permittivity

$\sigma$ ,  $\omega$ ,  $\epsilon_0$  = dc conductivity of the solution, angular frequency and vacuum absolute dielectric permittivity, respectively.

The correction procedure based on equation (1A) requires that conductivity  $\sigma$  is a known quantity. We used a particular method to process measured absorption data which, under suitable assumptions, allows to remove the influence of  $\sigma$  on

the relaxation parameters (relaxation strength and relaxation frequency) also in the case of unknown conductivity.

Let us assume that the measured dielectric permittivity may be modelled, relatively to the considered frequency range, as the sum of a single relaxation Debye type term and of a conductivity absorption one; that is:

$$\varepsilon = \varepsilon'_{\infty} + (\varepsilon'_s - \varepsilon'_{\infty}) / (1 + j\omega\tau) - j\sigma / (\omega\varepsilon_0) \quad (2A)$$

where:

$\tau$  = relaxation time

$\varepsilon'_{\infty}$  = permittivity at "infinite" frequency

$\varepsilon'_s$  = permittivity at low frequency

From Eq.(2A) the absorption spectrum results

$$\varepsilon'' = \sigma / (\omega\varepsilon_0) + \omega\tau(\varepsilon'_s - \varepsilon'_{\infty}) / (1 + \omega^2\tau^2) \quad (3A)$$

Relaxation frequency and relaxation strength are defined, respectively, as

$$f_{Rel} = 1 / (2\pi\tau) \quad S_{Rel} = (\varepsilon'_s - \varepsilon'_{\infty}) / 2 \quad (4A)$$

and, for  $\sigma = 0$ , such values can be easily derived from the measured  $\varepsilon''$  vs. frequency data as, in this case,  $f_{Rel}$  is the abscissa of the maximum of  $\varepsilon''$  and  $S_{Rel}$  the value of the maximum itself. If conductivity is present,  $f_{Rel}$  and  $S_{Rel}$  cannot be directly derived from  $\varepsilon''$  vs. frequency data and a suitable correction must be introduced which, usually, requires the knowledge of  $\sigma$  (see Eq. 1A).

If this value is not available the following procedure may be of interest.

By multiplying each measured value  $\varepsilon''$  by  $\omega$  and by successively derivating with respect to  $\omega$ , according to Eq.( 3A), we obtain

$$\varepsilon''_{\text{mod}} = d(\omega\varepsilon'')/d\omega = 2\omega\tau(\varepsilon'_s - \varepsilon'_\infty)/(1 + \omega^2\tau^2)^2 \quad (5A)$$

In Fig.1A the absorption spectrum  $\varepsilon''$  relative to the case  $\sigma = 0$  (Fig 1Aa)) and the modified spectrum  $\varepsilon''_{\text{mod}}$  (Fig. 1Ab)), vs.  $x = f/f_{\text{Rel}}$ , are reported; both spectra are normalized to  $S_{\text{Rel}} = (\varepsilon'_s - \varepsilon'_\infty)/2$  (see Eq. 4A).

The analysis of  $\varepsilon''_{\text{mod}}$  allows to determine the true values of  $f_{\text{Rel}}$  and  $S_{\text{Rel}}$ , without any information about conductivity  $\sigma$ ; we have:

$$f_{\text{Rel}} = 3^{1/2} \cdot f_m \quad S_{\text{Rel}} = 4/(3 \cdot 3^{1/2}) \cdot S_m \quad (6A)$$

where  $f_m$  is the frequency where  $\varepsilon''_{\text{mod}}$  is maximum and  $S_m$  the maximum value, respectively. It is also to note that a resolution enhancement is achieved in  $\varepsilon''_{\text{mod}}$  spectrum due the derivative operator.

Although equations (6A) are valid only in the case of MUT with Debye type relaxation behaviour, similar relations can be obtained also for more general cases.

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## Legends of Figures

Fig. 1- Schematic diagram of the measure cell and its input feeding cable

Fig. 2- a) External view of the used measure cell; b) Cross-section of the used measure cell

Fig. 3- Schematic diagram of the experimental set-up used for the measure of complex reflection coefficient  $S_{11}$  from the cell

Fig. 4- Measured real part of permittivity in the case of cell loaded by air (empty cell)

Fig. 5- Measured imaginary part of permittivity in the case of cell loaded by distilled water

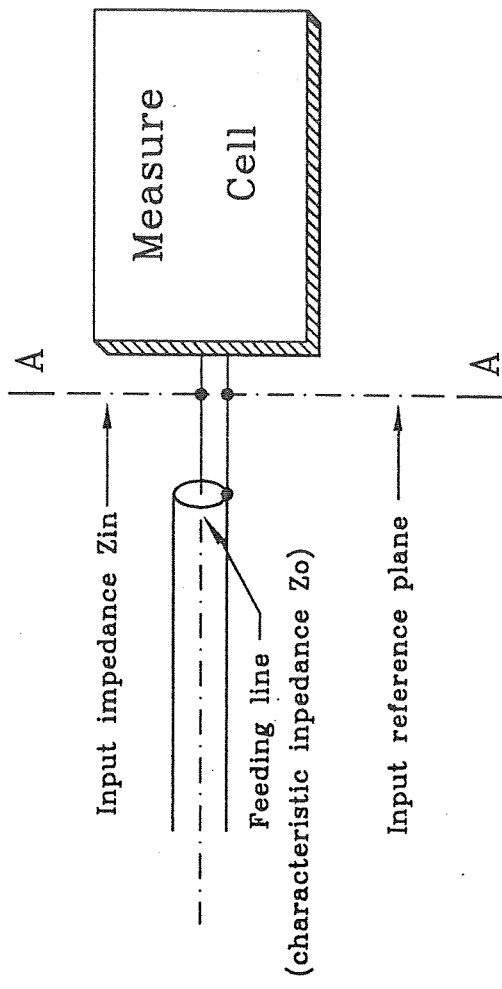
Fig. 6- Measured imaginary part of permittivity in the case of cell loaded by aqueous solution of Human Serum Albumin (HSA) (10% w/w)

Fig. 7- Data of Fig. 6 modified according to the procedure reported in Appendix which eliminates the influence of dc conductivity

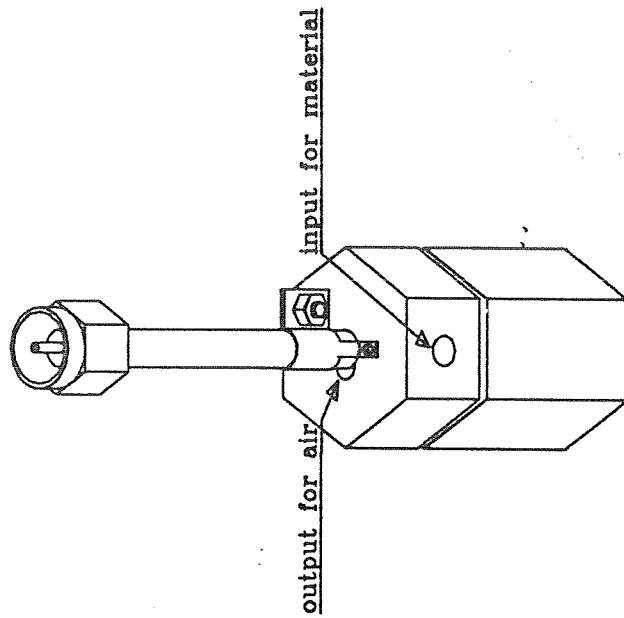
Fig. 1A- a) Diagram of imaginary part ( $\epsilon''$ ) of permittivity, normalized to its maximum  $\epsilon''_M$ , in the case of a material with Debye type dielectric behaviour and zero dc conductivity; b) Diagram of modified imaginary part ( $\epsilon''_{mod}$ ) of dielectric permittivity, normalized to  $\epsilon''_M$ , in the case of a material with Debye

type behaviour and dc conductivity different from zero, whatever its value may be

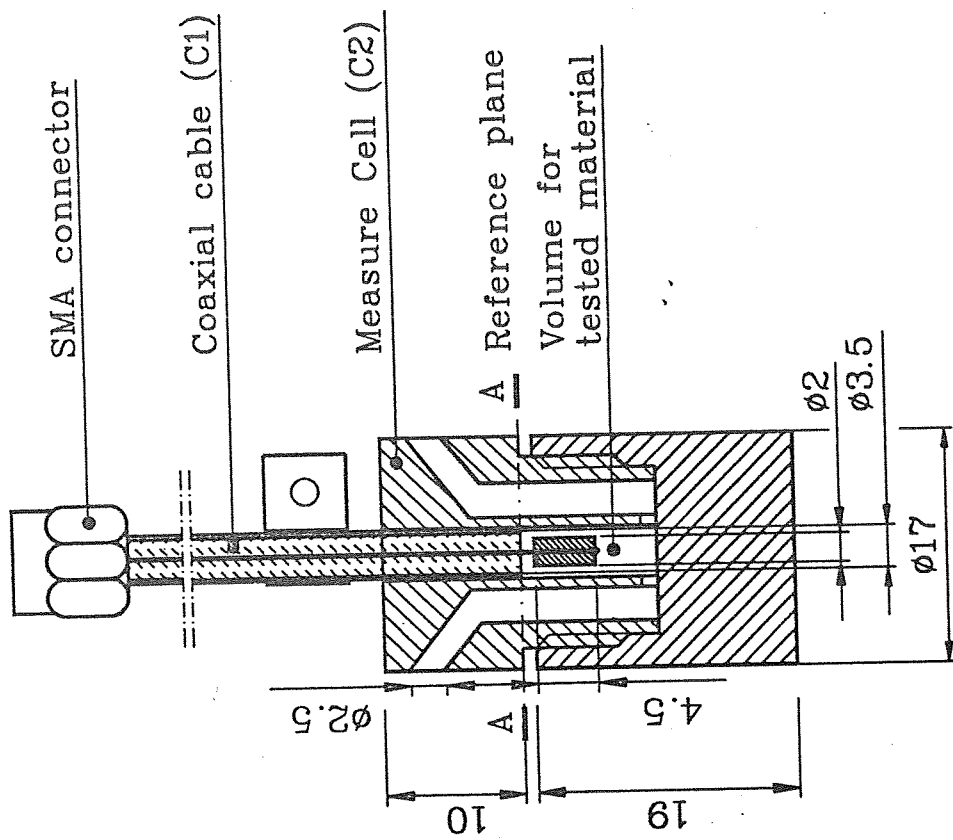




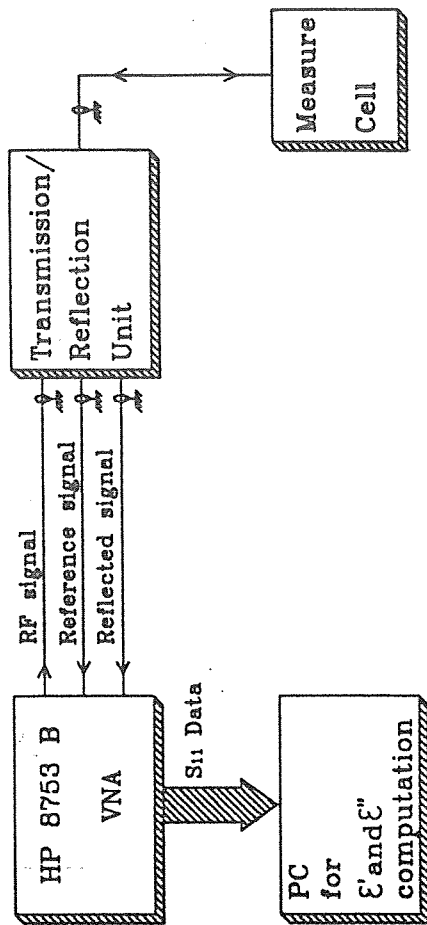
- FIG. 1 -



- FIG. 2 a) -

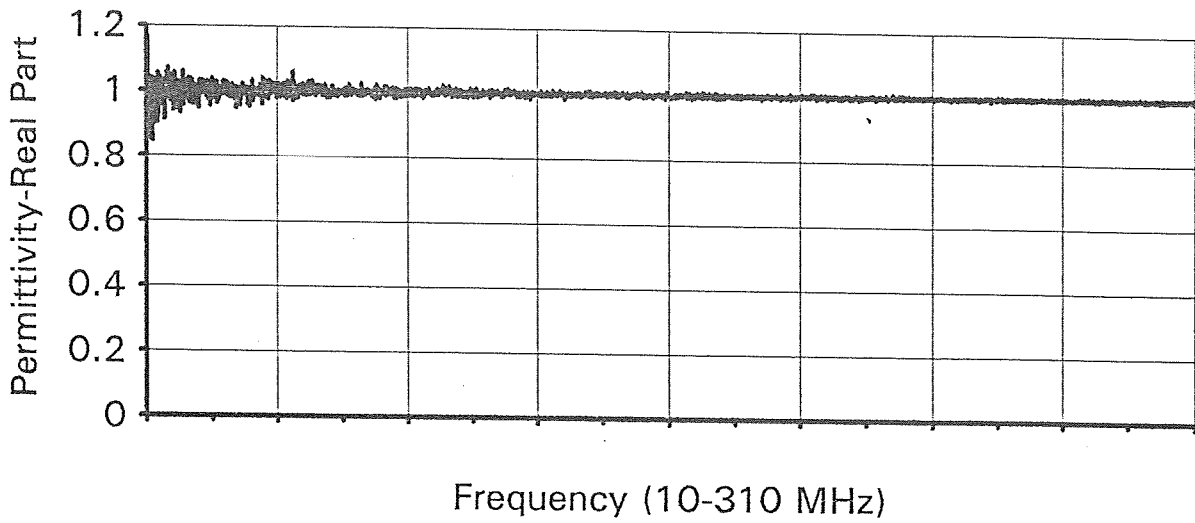


- FIG. 2.6) -



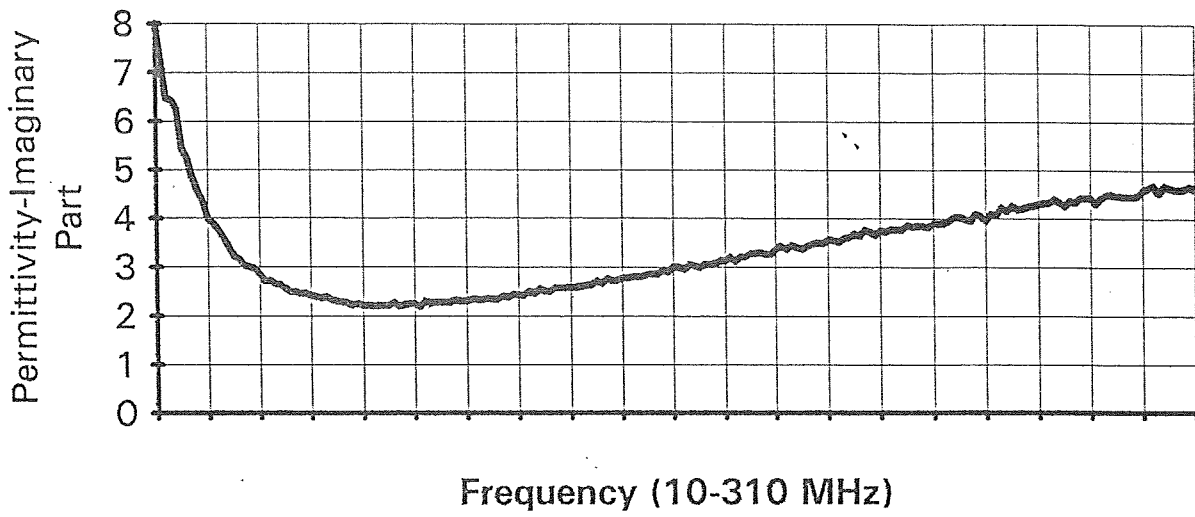
- FIG. 3 -

Air (empty Cell)



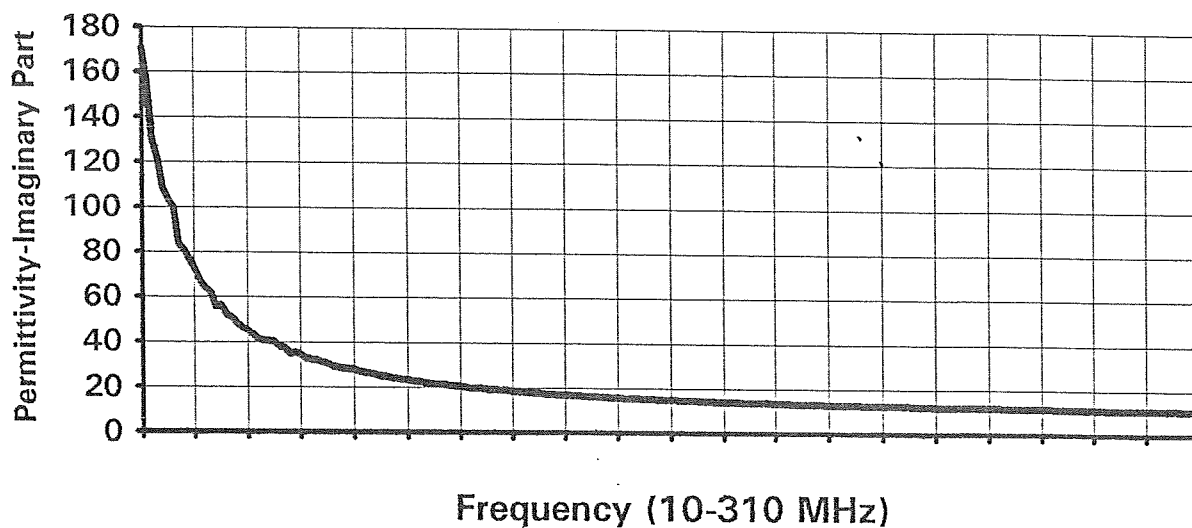
- FIG. 4 -

Distilled Water



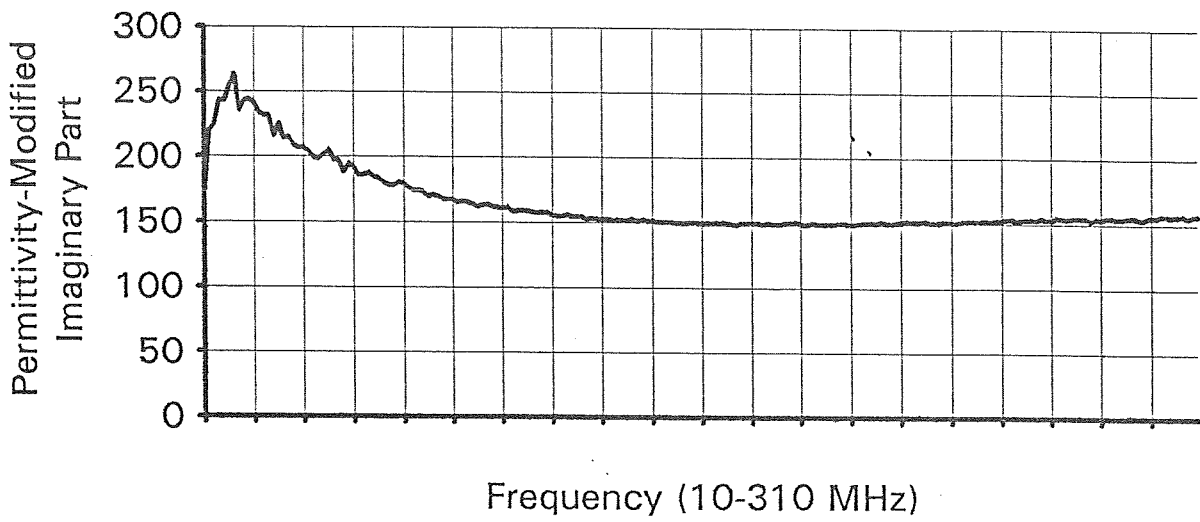
- FIG. 5 -

HSA Aqueous Solution (10% w/w)



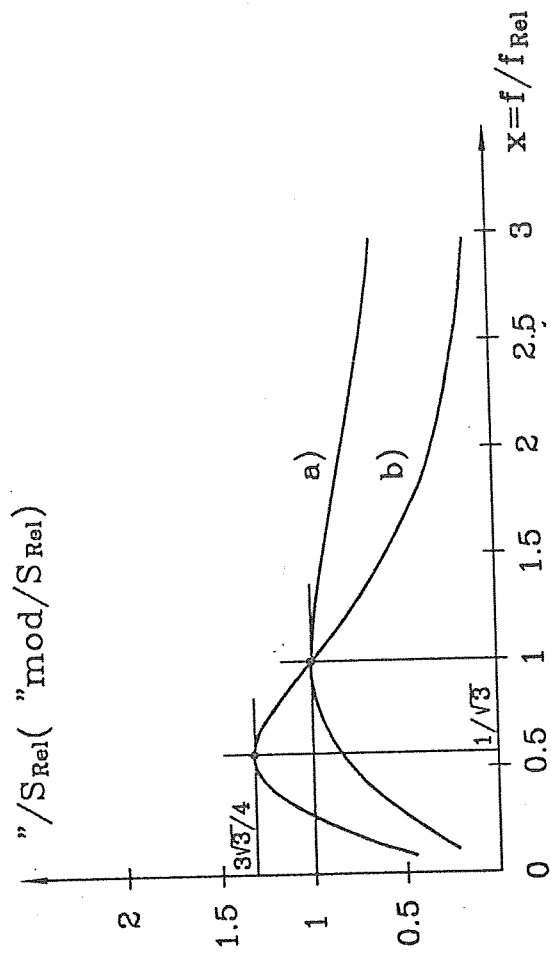
- FIG. 6 -

HSA Aqueous Solution (10% w/w)



- FIG. 7 -





- FIG. 1A -