LOW-FREQUENCY DIELECTRIC PROPERTIES OF CELL SUSPENSIONS

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ABSTRACT

In general, biological cells possess two dielectric dispersions in the range DC - 10 MHz. The higher-frequency β-dispersion is dominated by the charging of the cell membrane capacitance, whilst the lower-frequency α-dispersion is thought mainly to reflect the relaxation of ions tangential to the charged cell (membrane) surface. In many cases these dispersions overlap, and are corrupted due to the influence of electrode polarisation phenomena. We describe two computer programs which greatly assist one in obviating these problems.

INTRODUCTION

The electrical properties of tissues have been studied for at least 200 years, and during that time have yielded important information about the structure and physiology of cells. Indeed one of the first indications of the molecular thickness of cell membranes came from the work of Fricke in 1925. He studied the dielectric properties of red blood cells and derived a membrane capacitance of 0.81μF/cm^2 and a thickness of 3.3nm, values close to those accepted today. In recent years there has been a resurgence of interest in the dielectric properties of cells and tissues thanks largly to the development of automated impedance analysers and cheap microcomputers. Today dielectric methods are being used not only to study cell physiology, but are of importance in the exploitation of nonionising electromagnetic radiation (EMR) in medicine and in the evaluation of potential health hazards of such radiation. In this review we will concentrate on the "low-frequency" dielectric properties of cell suspensions (up to a few tens of MHz), emphasising the so-called α- and β- dispersions which occur in that region.

THE MEASUREMENT OF DIELECTRIC PROPERTIES

The dielectric properties of cell suspensions in this frequency range are typically measured by applying a sinusoidal current across electrodes immersed in the suspension and measuring the resultant voltage and phase angle. This is then used to calculate the values of a parallel arrangement of the idealised capacitor and conductor which give the same values of voltage and phase angle at the measuring frequency and exciting current level (i.e. one analyses in the admittance domain). These capacitance (C) and conductance (G) values are dependent upon the dimensions of the (notionally parallel-plate) electrode used to supply the exciting current and so must be normalised to values independent of the electrode geometry. This is done using

equations 1 and 2.

[1] $G = \sigma'(A/d)$

[2] $C = \epsilon'\epsilon_o(A/d)$

The electrode geometry is characterised by the cell constant (d/A), where A is the area of one electrode and d is the distance between them. Thus conductivity (σ') is equal to the conductance of an electrode of unit dimensions, and reflects the ease with which delocalised electric charge can migrate through the material under the field's influence. ϵ_o is the permittivity of free space ($8.854.10^{-12}$ F/m) and ϵ' is the permittivity of the material relative to that of vacuum. Permittivity (ϵ') relects the extent to which "localised" charge distributions can be distorted under the influence of the electric field.

THE PERMITTIVITY AND CONDUCTIVITY OF CELL SUSPENSIONS

When one measures the permittivity and conductivity of a cell suspension as a function of increasing frequency it is found that permittivity falls, and conductivity rises, in a series of steps known as dispersions. Two main types occur in the frequency range of interest; these are the α- and β-dispersions. The latter will be introduced first and used to illustrate how such dispersions are characterised.

THE β-DISPERSION

Biological membranes have conductances of the the order of perhaps 10^{-3} mS/cm^2 and may be regarded (with respect to the intracellular and extracellular spaces) as essentially nonconductors. On each side of this "insulator" are conducting ionic solutions (cell cytoplasm and suspending medium) and so a cell membrane is analogous to a classical electrical capacitor. This means that when an exciting voltage is applied across a cell suspension the membrane capacitance (C_m) is charged up by ions moving under the influence of the electric field produced, ie C_m is charged up through the conductivities of the cell medium (σ'_o) and the cytoplasm (σ'_i). Thus the membrane charging is equivalent to a resistor (reflecting σ'_i and σ'_o) in series with a capacitor (reflecting C_m and the volume fraction of cells) and like such networks it possesses a time constant (relaxation time) τ (equivalent to CR), which reflects the time taken to charge up the membrane. An equivalent circuit is given in Fig 1.

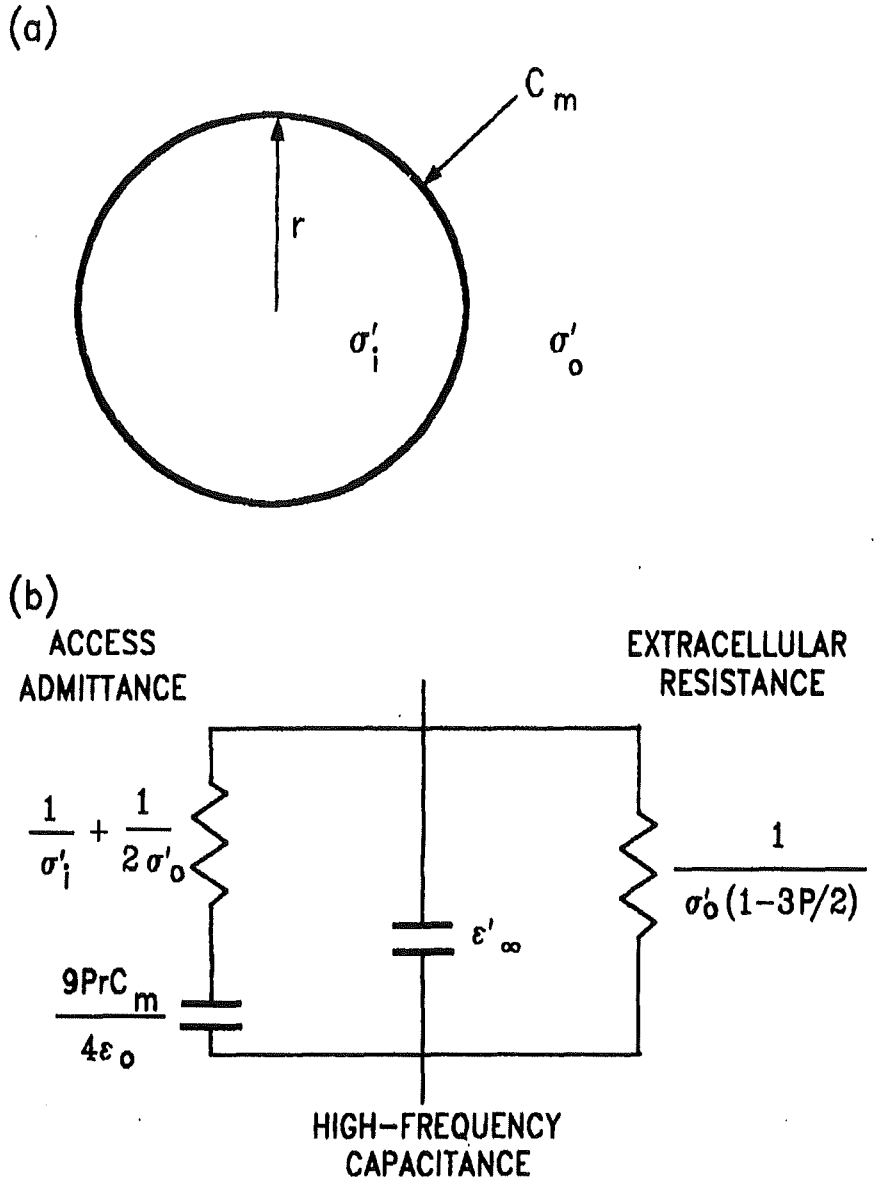

Fig 1. A model used to characterise the dielectric properties of cell suspensions. (a) A spherical cell of radius r, membrane capacitance C_m, and internal and external conductivities of σ'_i and σ'_o respectivly. (b) The equivalent circuit of a suspension of such cells present at a volume fraction P.

The physical interpretation of this is that as the frequency (f) rises, fewer and fewer ions have time to charge up the membrane(s) before the field changes direction. Thus the charge stored by the suspension for a given exciting voltage falls and the capacitance and permittivity of the suspension drops. At low frequencies the admittance (conductance to alternating current) of the cell membranes is very low and so the cells behave as non-conductors suspended in a conducting medium. This means that most of the current must flow around the cells. As the frequency

increases, the membrane's admittance rises and an increasing amount of current can flow through the membranes and the highly conducting cytoplasm of the cells; thus the conductivity of the suspension increases. This fall in permittivity and rise in conductivity with increasing frequency is illustrated in Fig 2.

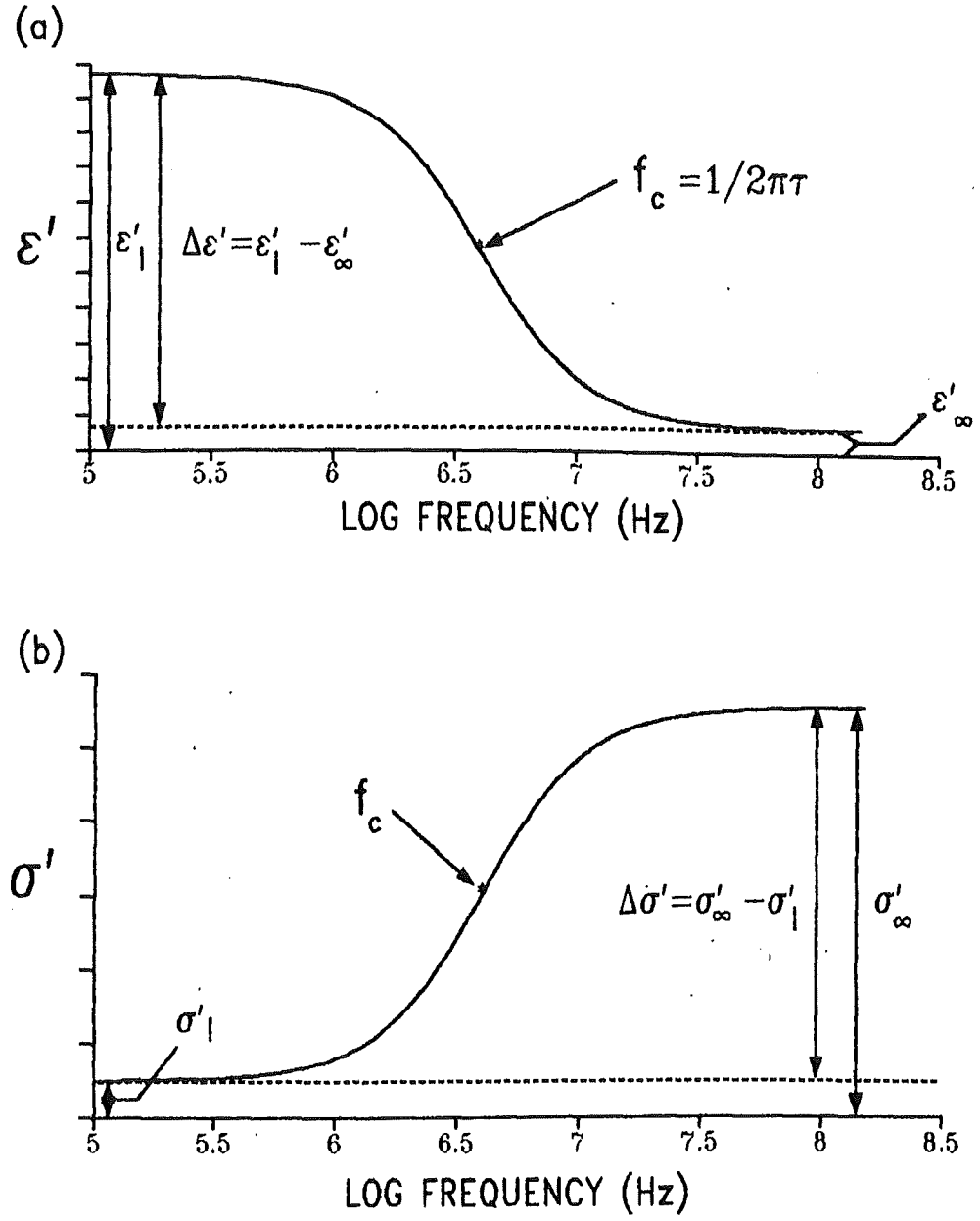

Fig 2. The changes in permittivity (ε') and conductivity (σ') with frequency are characterised by the values of ε' and σ' before the dispersion has started (ε'$_l$, σ'$_l$) and after it is complete (ε'$_\infty$, σ'$_\infty$) , the size of the changes (Δε', Δσ') and the frequency at which Δε' and Δσ' are half completed (f_c). The effects of frequency on permittivity and conductivity are shown respectively in (a) and (b).

As one can see both the permittivity and conductivity go from a low-frequency plateau to a high-frequency one, the frequency at which these transitions are half completed (i.e. at $\epsilon'_\infty+[\Delta\epsilon'/2]$ and $\sigma'_1+[\Delta\sigma'/2]$) is called the characteristic frequency (f_c). Characteristic frequency is related to the relaxation time (τ) by the equation $f_c=1/(2\pi\tau)$. The equations which describe these properties for the β-dispersion in terms of the variables shown in Figs 1 and 2 are given below.

[3] $$\epsilon'_1 = \epsilon'_\infty + 9PrC_m/4\epsilon_o$$

[4] $$\sigma'_1 = \sigma'_o(1-P)/[1+(P/x)]$$

[5] $$\sigma'_\infty = \sigma'_o[1 + 3P(\sigma'_i+\sigma'_o)/(\sigma'_i+2\sigma'_o)]$$

[6] $$\tau = rC_m[(1/\sigma'_i)+(1/2\sigma'_o)]$$

where $\Delta\epsilon'=\epsilon'_1-\epsilon'_\infty$, and x is a form factor which is 2 for a sphere. Although the β-dispersion can be seen purely in terms of the above considerations it should not be forgotten that the underlying physical process is a Maxwell-Wagner interfacial type of polarisation due to the differences in complex permittivities between the cells and the medium.

THE α-DISPERSION

Cell surfaces are generally negatively charged, but the surface charge density depends on the cell type. Thus, erythrocytes have neuraminic acid residues projecting from the glycoproteins and glycolipids of the plasma membrane. Gram-positive bacteria have a high surface charge due to the teichoic acids in their cell walls. The presence of this cell surface charge results in a diffuse counterion layer around the cells as shown in Fig 3a. When an electric field is applied to this cell the counterions move tangentially to the cell surface, resulting in an induced dipole moment being produced along the length of the cell (Fig 3b). These induced dipoles then partially neutralise some of the charge on the electrode surfaces thus allowing more charge (Q) to flow at the same applied voltage (V). Thus to the measuring system the capacitance (C=Q/V) of the electrodes has increased and so has the permittivity of the material between them (see equation 2). As it takes a finite time for the counterions on the cell surfaces to reach the end of the cells one can see that the number of ions managing to do so will increase with decreasing frequency. This means that the capacitance of the electrodes and hence the permittivity of the material between them also drops as the frequency rises. As before, the permittivity falls, and the conductivity rises, between two plateau values as frequency is increased. The conductivity rises because the energy from the electric field must either be stored (as reflected by ϵ') or dissipated (as reflected by σ') and therefore for a linear system a permittivity fall must be accompanied by a rise in conductivity (the Kramers-Kronig relation).

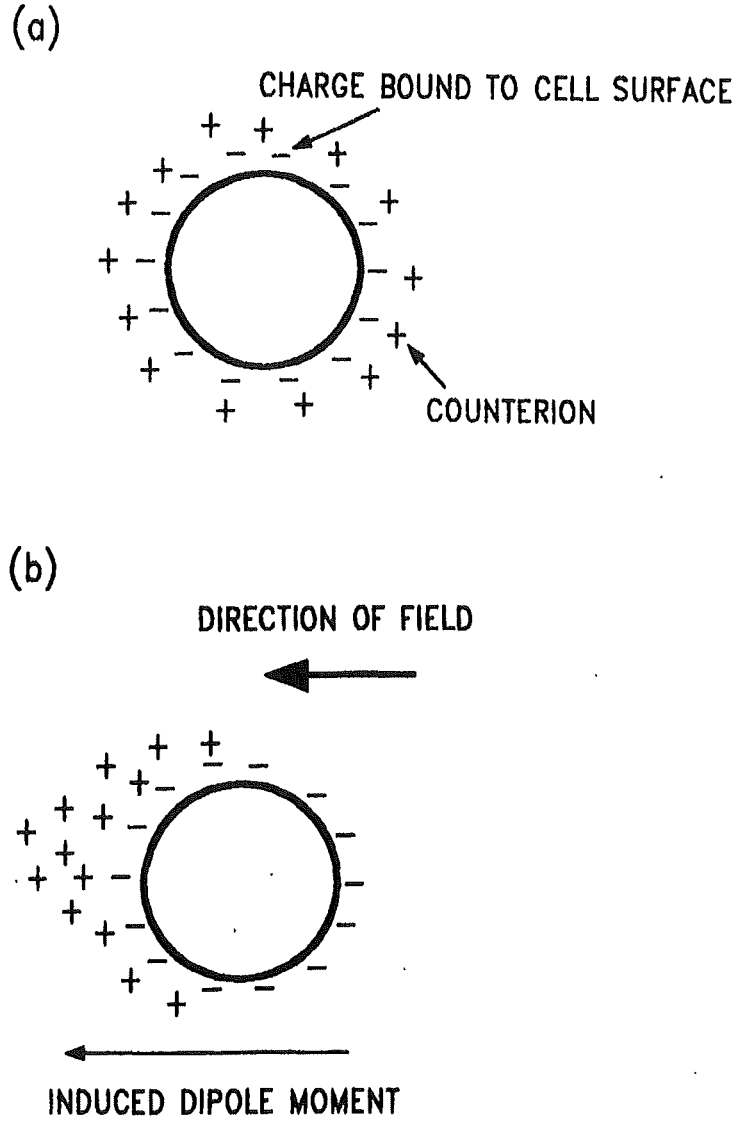

Fig 3. (a) A cell with a net surface charge surrounded by a diffuse layer of counterions. (b) The production of an induced dipole moment by the application of an electric field.

THE EFFECT OF A DISTRIBUTION OF RELAXATION TIMES (τ)

The electrical properties of individual cells in a suspension are not identical. This means that the distribution of cell sizes inevitably present must result in a distribution of relaxation times, since the τ value of the β-dispersion is proportional to the cell radius (r), while that of the α-dispersion is proportional to r^2. If a given dispersive mechanism exibits such a distribution in τ (and hence f_c) values then its fall of permittivity and rise in conductivity with increasing frequency will be less steep as is shown for ε' in Fig 4.

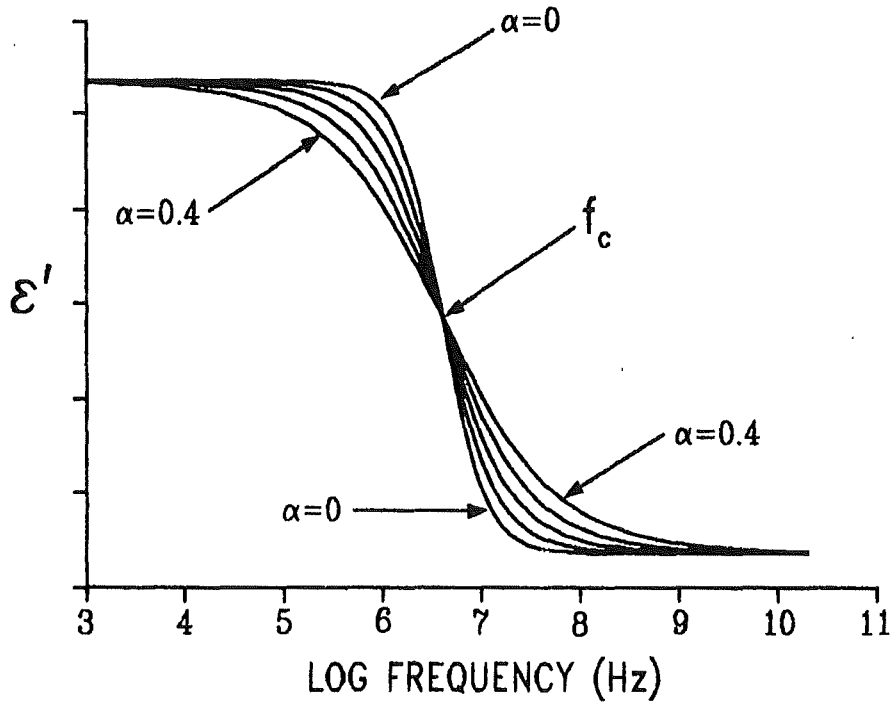

Figure 4. The effect of increasing the distribution of relaxation times, as measured by α, on the fall in permittivity with increasing frequency. α varies between 0 (no distribution) to 1 (infinite distribution), and as can be seen its effect is to make the fall in ϵ' less steep.

DECONVOLUTING DIELECTRIC SPECTRA

One of the major problems encountered in dielectric studies is that each dispersion can take several decades of frequency to run to completion. This means that if two dispersive mechanisms occur in the same frequency range, as is generally the case with the α- and β-dispersions, they will seriously overlap with each other. A further problem encountered at low frequencies is that the admittance of the electrical double layer (and other reactions occurring) at the surface of the charged electrodes (electrode polarisation) can dominate measurements in the sub-MHz region. A typical set of data for human erythrocytes is shown in Fig 5. To deconvolute these data one must minimise the contribution due to the electrodes' polarisation and in our experience this can be done by subtracting the polarisation control data from the actual cell data as is done in Fig 5.

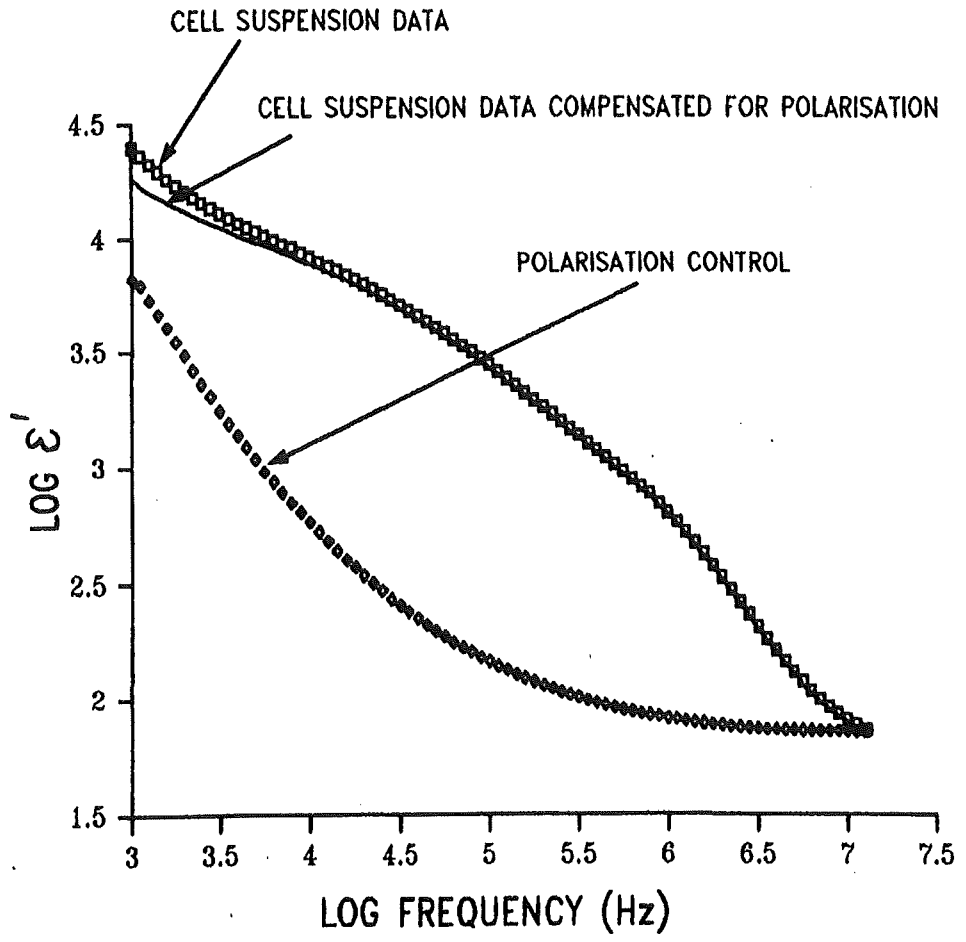

Fig 5. Dielectric properties of human red blood cells (P=47%, 37°C) suspended in a low conductivity medium (1mM NaCl, 0.5mM KCl, 20mM HEPES, 280mM D-Sorbitol, pH 7.6) in order to mimimise electrode polarisation. The data were obtained using an HP 4192A impedance analyser connected to the sample via two platinum-blacked platinum pin electrodes. The polarisation control data are for a sample of the suspension medium adjusted with double distilled water to the same low-frequency conductivity as the cell suspension. The data compensated for polarisation are generated by subtracting the polarisation data from the suspension data and then adding back ϵ'_∞ (= 70) to each point.

Once this compensation has been carried out one fits the Cole-Cole equation for two dispersions (Eq 7) to the resulting data points.

[7]

$$\varepsilon'(\omega) = \frac{\left[1+(\omega\tau_L)^{(1-\alpha_L)}\ \mathrm{SIN}\ (\alpha_L\pi/2)\right]\Delta\varepsilon'_L}{1+(\omega\tau_L)^{2(1-\alpha_L)}+2(\omega\tau_L)^{(1-\alpha_L)}\ \mathrm{SIN}\ (\alpha_L\pi/2)} +$$

$$\frac{\left[1+(\omega\tau_H)^{(1-\alpha_H)}\ \mathrm{SIN}\ (\alpha_H\pi/2)\right]\Delta\varepsilon'_H}{1+(\omega\tau_H)^{2(1-\alpha_H)}+2(\omega\tau_H)^{(1-\alpha_H)}\ \mathrm{SIN}\ (\alpha_H\pi/2)} + \varepsilon'_\infty$$

The subscripts L and H refer to the low- and high-frequency dispersions respectively, and $W=2\pi f$. The Cole α value in Eq 7 is usually taken as a measure of the distribution of τ values for each of the dispersion mechanisms. It may take a value of 0 for no distribution of relaxation times (Debye dispersion) up to a maximum value of 1 for an infinite distribution.

We have devised two computer programs (for use with IBM-PCs and compatibles) to deconvolute the permittivity data using equation 7. The first program (COLE.WKS) is written on a LOTUS 1-2-3 -type spreadsheet (VP-Planner) because of its flexibility and good graphics. This program first allows us to correct the data for polarisation as was done in Fig 5. Estimated values for the variables in equation 7 may then be entered and the computer then uses them to calculate the locus of the points generated, in the form of a 'fit' to the data. Graphs of the ϵ' data and the fit can then be presented on the computer screen in various ways (e.g. of log ϵ' vs log f, ϵ' vs log f, error vs log f) by using the function keys. This enables the extent of any remaining electrode effects to be gauged and a set of reasonable initial estimates for each of the variables in equation 7 to be generated. Our experience is that manual iteration, comparing the effects of changes in the estimates on the goodness of fit as judged by the overall (modulus of the) percentage error, allows one rapidly to obtain an excellent fit to the data of interest.

Alternatively the data compensated for polarisation may then be downloaded into a BASIC program (COLE.BAS) together with the estimates of the fit. If electrode polarisation does affect the low-frequency data then the program can be told to ignore the frequency range over which this occurs. The data are then fitted iteratively to equation 7 by the program, using the initial estimates. A BASIC program was used for the automatic iteration because this was beyond the capabilities of the spreadsheet in terms both of complexity and the speed required. Once the fitting is complete the best fit and the ϵ' data are viewed and evaluated on the spreadsheet program. As the ϵ' data range typically from 20000 at low frequencies to 70 at high frequencies it is appropriate to resort to a "percent error" method of judging the goodness of the fit, and both the BASIC and spreadsheet programs use this approach. The graphs generated on the spreadsheet can then be loaded into the graphics program VP-Graphics for further annotation and printing.

The data of Fig 5 (compensated for polarisation) are redrawn in Fig 6, along with the best fit produced using the two computer programs.

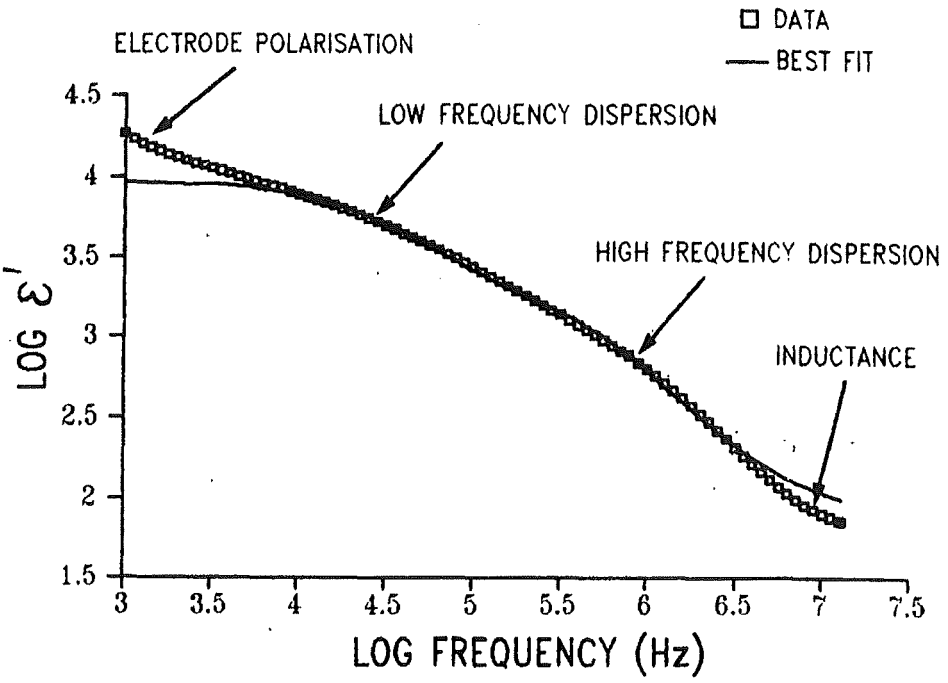

Fig 6. The data from figure 5 which have been compensated for polarisation are plotted with the fit to these data generated by the two computer programs. As one can see a much reduced contribution from the electrodes is seen at low frequencies while at high frequencies a stray inductance distorts the data and because of this only the data for frequencies between 5kHz and 6MHz were used for the fitting. The best fit was $\Delta\epsilon'_L$=8271, f_C Low=28517 Hz, α_L=0.1900, $\Delta\epsilon'_H$=1099, f_C High=691650 Hz, α_H=0.0661, ϵ'_∞=70.

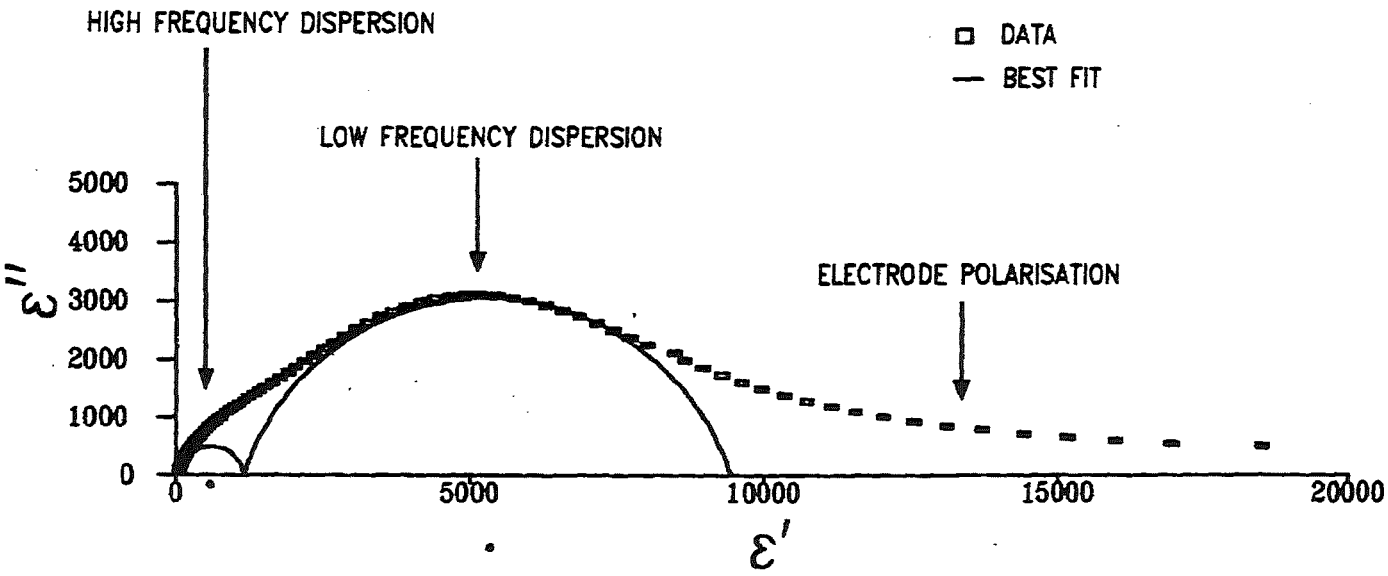

Figure 7. A complex permittivity (Cole-Cole) plot of the data in Fig 6.

One can represent such data in the complex permittivity domain by plotting ϵ'' (reflecting energy dissipation) against ϵ' (reflecting energy storage). This results in a semi-circle for each dispersive mechanism and the data from Fig 6 are replotted in this manner in Fig 7. Notice that the centres of the semi-circles are depressed below the abscissa and this is taken to reflect the distribution of τ values present. We conclude that the present approach provides a useful and convenient means for the deconvolution of dielectric spectra in lossy media.

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REFERENCES

Some recent reviews of this area of study can be found in the following references.

K.R. Foster and H.P. Schwan in C. Polk and E. Postow (Eds), CRC Handbook of Biological Effects of Electromagnetic Fields, CRC Press, Boca Raton, FL, 1986, p.27-97.

E.H. Grant, R.J. Sheppard and G.P. South, Dielectric Behaviour of Biological Molecules in Solution, Oxford University Press, Oxford, 1978.

D.B. Kell, in A.P.F. Turner, I. Karube and G.S. Wilson (Ed.), Biosensors: Fundamentals and Applications, Oxford University Press, Oxford, 1987, p.427-468.

R. Pethig, Dielectric and Electronic Properties of Biological Materials, Wiley, Chichester, 1979.

R. Pethig and D.B. Kell, Phys. Med. Biol., 32 (1987) 933-970.

O.F. Schanne and E.R.P. Ceretti, Impedance Measurements in Biological Cells, Wiley, Chichester, 1978.