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Substitution and spreadsheet methods for analysing dielectric spectra of biological systems

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Abstract. *1.* Two major problems are encountered when one wishes to fit audio- and radio-frequency dielectric spectra of biological cell suspensions (or other materials): (*a*) changes in the apparent frequency-dependent permittivity of the system due to the phenomena of electrode polarisation can dominate those due to the biological system, and (*b*) because of the overlap of different dispersions it may be very difficult to deconvolute the individual contributions of the underlying biophysical mechanisms. *2.* The extent of electrode polarisation depends substantially upon the conductivity of the medium surrounding the cells, but only marginally on the nature of the ions of a given valency contribution to it. *3.* This, and the fact that the apparent time constants of the phenomena contributing to electrode polarisation are much greater than those of biological dielectric dispersions, permits one to use a simple substitution method to extract the latter in the presence of the former. This is shown both by simulation and by experiments using suspensions of human erythrocytes. *4.* A spreadsheet method is described for the display of dielectric data and their conformance to the double Cole-Cole equation. The method provides a rapid and convenient approach, based on interactive graphical outputs, for the fitting of dielectric data to this equation. *5.* Estimates derived from the spreadsheet program may be used in a BASIC program to arrive at the optimal fit. *6.* The method is applied to the strongly-overlapping α- and β-dispersions of erythrocytes, permitting their deconvolution and providing a high level of accuracy.

Key words: Dielectric permittivity – Dielectric dispersion – Spreadsheets – Deconvolution – Electrode polarisation

Introduction

The electrical properties of biological materials have been the subject of research for many centuries (Stock 1984). Indeed the attempts to understand electrostatic phenomena during the 18th century inevitably led to speculations and experiments in medicine (Rowbottom and Susskind 1984). Today the effects of electromagnetic radiation on biological material are the subject of intense academic and applied research (e.g. Pilla 1980; Adey 1981; Illinger 1981; Konig et al. 1981; Becker and Marino 1982; Grandolfo et al. 1983; Becker and Selden 1985, Chiabrera et al. 1985; Polk and Postow 1986; Black 1987; Michaelson and Lin 1987; Marino 1988; Borgens et al. 1989). However, it is a knowledge of the underlying linear and nonlinear dielectric properties of such materials and systems which must underpin any mechanistic understanding of such effects (see e.g. Grant et al. 1978; Schanne and Ceretti 1978; Pethig 1979; Pethig and Kell 1987; Foster and Schwan 1986, 1989; Kell et al. 1988; Westerhoff et al. 1988; Davey and Kell 1989, 1990).

A knowledge of tissue electrical (dielectric) properties is of importance in medical diagnostic techniques (Iskander and Durney 1980), including impedance plethysmography (Anderson 1984; Wheeler and Penney 1982; Yamakoshi et al. 1980; Herscovici and Roller 1986), impedance pneumography (Pacela 1966), impedance cardiography (Penney 1986) and impedance tomography (imaging) (Barber and Brown 1984; Newell et al. 1988). It is also known that many pathological conditions change the dielectric properties of tissues and body fluids (Pfützner and Fialik 1982; Essex et al. 1977; Grant et al. 1978, Gabriel et al. 1987; Pethig and Kell 1987) and so could form the basis of diagnostic methods (Clarke et al. 1985; Kell 1987; Davey and Kell 1990).

The purely academic study of biological dielectric properties has yielded important information on cell and tissue structure and physiology. For instance the first estimate of the molecular thickness of cell plasma membranes was derived by Fricke (1925) from dielectric studies on erythrocytes, and the T-system of muscle tissue was discovered using dielectric measurements (Fatt 1964). However, two major problems beset those who would wish to measure the dielectric properties of lossy (i.e. conductive) *biological* substances at audio-to-radio frequencies (Schwan 1963).

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The first problem is that it is usually necessary to use electrodes as the interface(s) between the electronically conducting circuits of the measuring system and the ionically conducting biological milieu. The field-induced flow of charge across these interfaces manifests itself as an impedance in series with the biological impedance that one wishes to measure. Whilst electrode impedances are of great interest in their own right (e.g. Smith 1966; MacDonald and McKubre 1982; Brown and Sandifer 1986; Sluyters-Rehbach and Sluyters 1986; MacDonald 1987), the high conductivity of biological media means that the electrode polarisation impedance may in unfavourable cases dominate the biological dielectric response.

The second problem arises because of the fact that dielectric phenomena are generally dispersive rather than resonant (e.g. Debye 1929; Schwan 1957; Hasted 1973; Grant et al. 1978), and when measured at different frequencies the dielectric dispersions observed may take several decades to run to completion. This means that dielectric absorptions tend to overlap, and must be deconvoluted if one wishes to establish their true magnitudes, properties and biophysical basis.

The purpose of the present article is to describe the methods and novel spreadsheet programs we have developed to address and largely to overcome these problems, so as to improve the study of the linear dielectric properties of biological material, and in particular those of cell suspensions exhibiting the so called α- and β-dispersions, whilst an accompanying article (Ferris et al. 1990) describes a detailed study of the temperature-dependence of the β-dispersion of microorganisms. A preliminary account of the present methods has been given elsewhere (Davey and Kell 1989, 1990). We begin by considering each of the above problems in more detail.

Theory

Electrode polarisation

As mentioned above, one of the major problems with the measurement of the dielectric properties of biological materials is that at frequencies below some 500 kHz, the polarisation impedance of the electrodes used for the measurements can dominate the dielectric spectrum. When the measuring electrodes are immersed in the biological material (prior to the imposition of any applied current) there is a movement of charge as a result of the system's attempts to come to electrochemical equilibrium. This build up of charge on an electrode, and of a layer of counterions at its surface (the so called double layer), results in the formation of a potential difference across the interface. The models of the double layer are considered in detail elsewhere (e.g. Bockris and Reddy 1970; Spaarnay 1972; Bard and Faulkner 1980; Morrison 1980; Martynov and Salem 1983) but for our purposes we may consider that the surface charge of the electrodes and the corresponding layer of counter ions at the electrode-electrolyte interface are equivalent to the plates of a capacitor (C_{dl}). One can thus consider this capacitor as having been charged up by the potential difference across the interface.

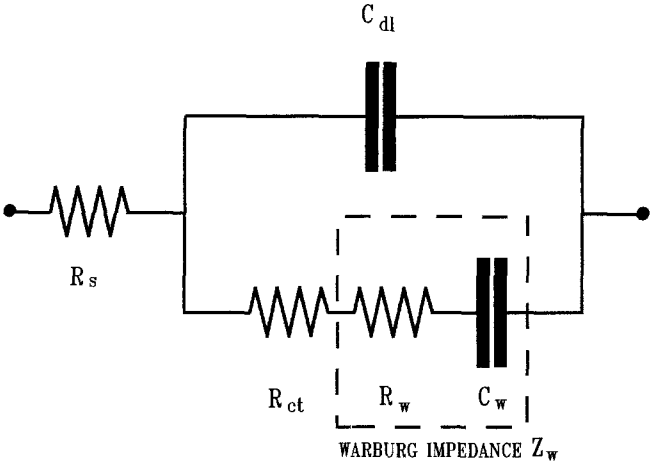

Fig. 1. The Randles equivalent circuit used to describe the frequency-dependent electrical properties of the electrode-electrolyte interface. C_{dl} represents the double-layer capacitance, R_{ct} the charge-transfer resistance, R_w and C_w the Warburg impedance and R_s the resistance of the bulk solution. It should be noted that the components used to model the Warburg impedance are strictly themselves frequency-dependent. For further details, see the text

Upon imposition of an *alternating* current (a.c) to the electrodes there is a modulation of the charge on this capacitor and hence to an alternating potential difference across it. One can thus represent this system by an impedance with a capacitive reactance. In fact the system is complicated by the presence of chemical reactions at the electrode surfaces which result in the flow of faradaic current. The generalised equivalent circuit usually used to describe such a system is that given first by Randles (1947) and is shown in Fig. 1. It can be seen that the double layer capacitance (C_{dl}) is in parallel with a resistance (R_{ct}) representing the faradaic charge transfer step. This resistance is in turn in series with the Warburg impedance (Z_w), which reflects the limitation imposed on the flow of faradaic current by the diffusion of the electroactive substances to and from the electrode surfaces. The whole arrangement is then in series with the resistance R_s (or more exactly impedance) of the bulk material between the electrodes.

When one is measuring the impedance of a biological sample it is conventional to consider it in terms of two impedances in series, one representing the electrode polarisation and the other the sample (e.g. Schwan 1963; Grant et al. 1978). At low frequencies the impedance of the electrodes will tend to be much greater than that of the biological system, but as the frequency is increased the electrodes contribution to the measured impedance rapidly approaches zero and so one tends more nearly to see the true dielectric properties of the sample. However, at frequencies above say 10 MHz, the self- and mutual-inductances of the leads between the sample and the electrodes also assume significance, and must be taken into account.

There are several numerical methods of reducing the influence of electrode polarisation on a set of previously measured biological data (see later). However, none of these methods work well if the polarisation is very serious

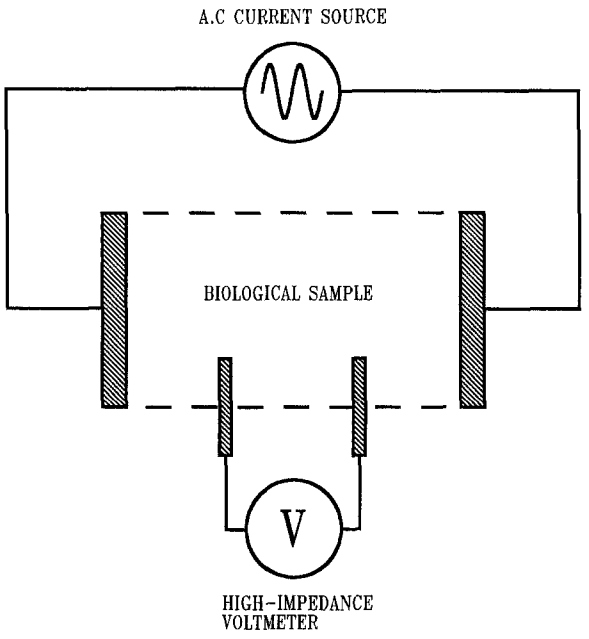

Fig. 2. The principle of the 4-terminal impedance method. Here, current is passed through the biological system of interest via 2 current electrodes, the potential drop across the biological system being measured via 2 separate voltage electrodes connected to a voltmeter of high input impedance

and it is therefore essential that steps are taken to minimize the contribution of electrode polarisation. The electrode material used should be "non-polarisable" under a.c. conditions (Schwan 1968), a suitable material being platinum, which has a very low surface impedance. A further advantage of platinum is that it is possible greatly to increase its surface area with a layer of platinum black. The procedures used to do this are outlined by Geddes (1972) and ourselves (Kell and Davey 1990), and the precautions required for the optimal performance of such electrodes are reviewed by Schwan (1963). Since the electrode impedance is inversely proportional to the electrode surface area, major reductions in electrode polarisation can easily be achieved (Schwan 1963).

Another useful technique to reduce electrode polarisation is the use of four-electrode methods (Fig. 2). In this system alternating current is forced across the material of interest by the two outer current electrodes. The resulting potential difference induced across the biological material is then measured via a very-high-impedance voltmeter connected across the inner electrodes. As the impedance is so high, virtually no current flows through the surfaces of the inner pins and so no contributions of electrode polarisation are measured by them. Thus it becomes possible to measure the impedance of the biological sample without the contribution of the electrodes (Schwan and Ferris 1968; Ferris 1974; Nakamura et al. 1981; Harris et al. 1987; Kell 1987). In the case of 4-terminal systems, it is the distance of the inner electrodes which predominantly determines the cell constant (Tamamushi and Takahashi 1974).

Finally, a general precaution that should be taken with any sample is to reduce the resistance of its suspending medium. This simple expedient results in lower electrode polarisation (see later) but this may be at the expense of physiological relevance.

Having taken electrochemical precautions to reduce the influence of the electrodes so far as is possible, there are a variety of numerical methods which can be used to subtract the electrodes' contribution to the resulting experimental data. These are reviewed in detail by Schwan (1963, 1968) and by Grant et al. (1978), but we consider only two of the most commonly used: the distance variation method and the sample substitution method.

The first method relies on changing the cell constant of the measuring electrode system by varying the distance between the two electrodes. Since one can represent the contributions of the electrodes and biological material as two impedances in series (Z_{pol} and Z_{sample} respectively) one can express the impedance actually measured at a given frequency by (1) and (2).

$$Z^1_{measured} = Z^1_{sample} + Z_{pol} \tag{1}$$

$$Z^2_{measured} = Z^2_{sample} + Z_{pol} \tag{2}$$

where the superscripts refer to the two electrode positions. Provided one ensures that the current densities through the surfaces of the electrodes are the same at the two electrode positions then Z_{pol} is a constant contribution to the measured impedances (Ferris 1974). Thus (1) and (2) can be combined resulting in the elimination of the polarisation impedance (Z_{pol}). By taking into account the change in the cell constants between the two electrode positions one can calculate the true sample impedance using (3).

$$Z^1_{sample} - Z^2_{sample} = ((d_1 - d_2)/A)/(\sigma' + j\,\omega\,\varepsilon'\,\varepsilon_0) \tag{3}$$

Here d is the inter-electrode distance, A is the area of one electrode, σ' and ε' are the conductivity and permittivity of the sample respectively, ε_0 is the permittivity of free space ($8.854 \cdot 10^{-12}$ F m^{-1}) and $j = \sqrt{-1}$.

This method requires that the electrode polarisation impedance is independent of the interelectrode distances and that the dielectric properties of the biological sample are constant over the time scale needed for the measurements. However as biological cells often leak ions or excrete metabolites, resulting in changes in the resistance of the sample (Firstenberg-Eden and Eden 1984), both the dielectric properties of the cells and the electrode polarisation can become time-dependent. This method is also unsuitable for experiments where one is interested in rapid changes in the dielectric properties of a sample, for instance when studing the interaction of toxic compounds with cells. Within these constraints the method works well. However at very low frequencies, where the electrode polarisation is large, small errors in the determination of $Z_{measured}$ can result in large errors in the estimates of Z_{sample}.

An alternative method, which is in some ways comparable, is of particular use in work with cell suspensions (Harris and Kell 1983). This involves varying the volume fraction of the suspended phase. One can then easily check for instance that the specific dielectric increment of a dispersion of interest is independent of the concentration of the suspended phase (at low volume fractions).

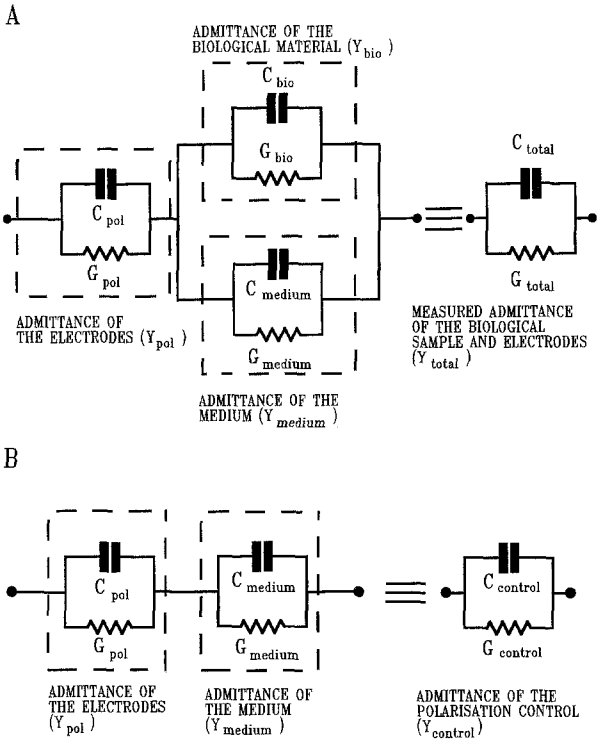

Fig. 3 A, B. The substitution method for subtracting the contributions of electrode polarisation to dielectric measurements of biological cell suspensions. **A** Equivalent circuit (*at a given frequency*) of the entire measurement system, including the electrodes, medium and biological cells. **B** Equivalent circuit (*at a given frequency*) of the electrodes plus the medium (adjusted to the appropriate conductivity) in which the cells are suspended. Under appropriate conditions (discussed in the text), the admittance due to the biological material is the difference between the admittances in **A** and **B**

Another often-used method (Schwan 1963; Grant et al. 1978), and the one we have implemented herein, is the substitution method. This has the advantage that it can be used when studying dielectric phenomena which are time-dependent over a series of experimental runs. In this technique one measures the admittance of the sample and electrodes as a function of frequency and thus the frequency-dependent values of the capacitance (C_{total}) and conductance (G_{total}) of the sample-plus-electrodes (Fig. 3 a). If the frequency scan is repeated but with the biological sample replaced by a sample of its suspending phase (suspension medium) with its conductivity adjusted to match the low-frequency conductivity of the biological sample, then one obtains the frequency-dependent values of capacitance ($C_{control}$) and conductance ($G_{control}$) of the electrodes-plus-medium (Fig. 3 b). One can then remove the polarisation contribution at each frequency by using the formulae

$$C_{bio} = C_{total} - C_{control} \quad (4)$$

$$G_{bio} = G_{total} - G_{control} \quad (5)$$

where the subscript "bio" refers to the capacitance and conductance of the biological material of interest (e.g. biological cells). For this method to work the time constants of the circuits in Fig. 3 a and b at each frequency (C_{bio}, G_{bio}, C_{pol} and G_{pol} are all frequency-dependent themselves) must be well-separated, since only under these conditions is it possible to use this subtraction method as the two circuits are not strictly in parallel with each other. Fortunately, such conditions pertain when one is measuring the dielectric properties of biological materials.

Materials and methods

Dielectric measurements were performed in a 2-terminal consisting of Pt-black pin type Pt electrodes connected to a Hewlett-Packard 4192 A Impedance analyser, under the control of an HP85 microcomputer (Harris and Kell 1983; Kell 1983; Kell 1983; Davey et al. 1988). "Zero open" corrections were performed at 1 MHz. Cell constants were obtained by measuring the capacitance of distilled water at 1 MHz and at the appropriate temperature. Frequency, conductance and capacitance data were subsequently transferred to an IBM-XT-compatible microcomputer (Opus II) via an interface conforming to the IEEE-488 standard. All analyses were subsequently performed on IBM-AT-compatible microcomputers (Viglen Vig II or Vig III).

Software

The following commercially available programs were used: VP-planner and VP-graphics (Paperback Software/NewStar Corporation), GWBASIC (Microsoft), Turbo BASIC (Borland), ANALYSER II (Number One Systems, Huntington). Other software was written in-house and is described in the Results section.

Biological material

Non-heparinised whole blood (stored at 4 °C) was obtained from the local hospital, and washed three times at room temperature (ca. 18 °C) in a 10-fold excess of a medium consisting of 1 m*M* NaCl, 10 m*M* *tris*-Cl and 290 m*M* D-sorbitol, pH 6. The buffy coat was removed after the first wash. Cells were finally resuspended to a haematocrit of approximately 50% and used almost immediately.

Chemicals were of analytical grade and were obtained from the Sigma Chemical Company or BDH Ltd, Poole, Dorset. Water was singly-distilled in an all-glass apparatus.

Results and discussion

Figure 4 shows a frequency scan of a pair of platinum-blacked platinum pin electrodes immersed in KCl solutions and measured as described (Harris and Kell 1983; Kell 1987). It is clear that the capacitance (equivalent to $C_{control}$) is still rising even when the frequency is reduced to 10 Hz, thus indicating rather long time constants (rela-

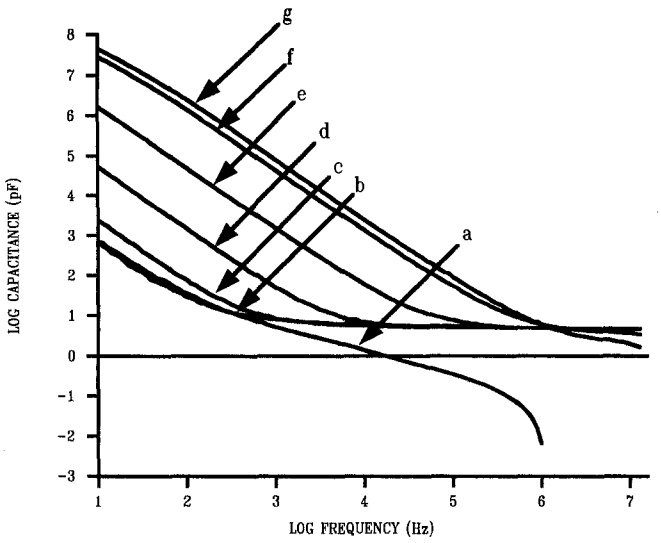

Fig. 4. The electrode polarisation admittance of Pt-black electrodes. Measurements of the equivalent parallel capacitance were made at 20 °C as described in the Methods section. The material between the electrodes consisted of (*a*) air or solutions of aqueous KCl of the millimolarities indicated: (*b*) 0, (*c*) 0.1, (*d*) 1, (*e*) 10, (*f*) 100, (*g*) 150. It may be observed that as the conductivity of the medium is increased, the polarisation capacitance is also increased, broadly in line with a thinning of the electrode double layers as expected from Debye-Huckel theory. The fall in capacitance at high frequencies in the most conducting solutions is due to the existence of inductances in the electrode leads. The cell constant was 1.405 cm^{-1}

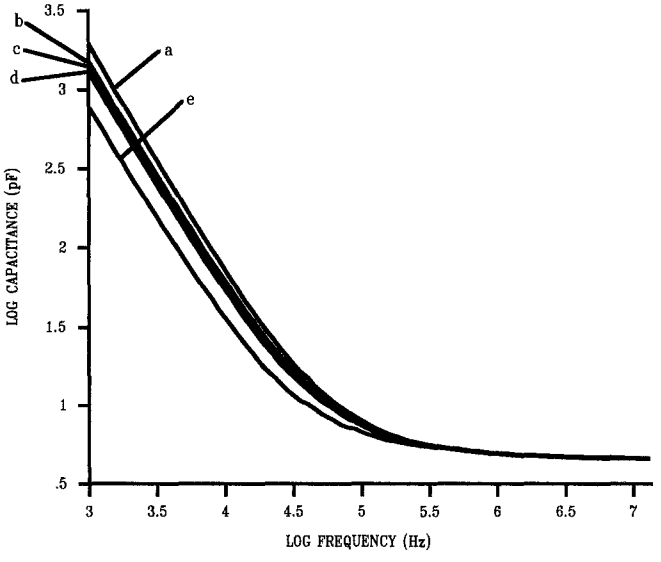

Fig. 5. The effect of ionic solutions on the electrode polarisation admittance of Pt black electrodes. Measurements were performed as described in the legend to Fig. 4 in solutions whose conductance at 1 kHz was 1.00 mS, the solutions consisting of the salts indicated: (*a*) $Mg(NO_3)_2$, (*b*) NH_4NO_3, (*c*) NaCl, (*d*) KCl, (*e*) K_2HPO_4

tive to those found in biological material) for the charge transfer and Warburg contributions to the electrode polarisation impedance, and in fact the Randles equivalent circuit implies blocked interfaces under DC conditions in the absence of electroactive material.

The suspension medium one normally uses for the polarisation control is a fresh sample of the medium the cells were originally suspended in. In principle, this ("low-frequency") conductance will be lower than that of the biological sample, since the cells, which are nonconducting at these frequencies, occupy space normally available to the ions of the suspending medium (see e.g. Harris and Kell 1983; Lovitt et al. 1986). However, the conductance of this medium (G_{medium}) will frequently differ from the conductance of the medium around the cells in the suspension because of the release of ions or metabolites by the cells. This has the effect that the conductance of the cell suspension is often higher than that of the fresh medium. In our experience both effects can cause the electrode polarisation contribution to the cell suspension data to differ from its contribution to the control data.

To allow for both effects we note the conductance (G_{total}) of the cell suspension at the lowest frequency measured and then adjust the conductance of the medium used in the polarisation control experiment so that $G_{control}$ equals G_{total} at that frequency. The conductance adjustment is done either with distilled water or KCl depending on whether the volume fraction or leakage effects dominate. Figure 5 shows that at a given $G_{control}$ the type of ions causing the polarisation do not greatly affect the magnitude of the polarisation contribution seen, provided that they are of a similar valency. In biological media, the concentrations of univalent cations and anions dominate the total conductivity.

At very high volume fractions of cells, such as found in blood, a further effect of the cells on electrode impedance becomes important. As the cells are nonconducting at low frequencies they tend to screen off parts of the electrode when present at high volume fractions (Schwan 1963). The resultant fall in effective electrode surface area means that the contribution of electrode polarisation is more prominent in the cell suspension data than in its control. It is normally possible to avoid this problem by choosing media of moderate conductivity and cell volume fractions in which the specific dielectric increment is independent of the volume fraction. If this is impossible, then it is still necessary to adjust the medium used in the control to that of the cell suspension at low frequencies, and to allow for the indeterminate influence of the screening effect by fitting to the resulting data compensated for polarisation. Even with these precautions the substitution method only works well when the electrode polarisation is not excessive and so the precautions outlined earlier to reduce it also need to be taken.

Figure 6 is a simulation of the substraction method, using the circuits in Fig. 3 with fixed (frequency-independent) values for "G_{pol}", "C_{pol}", "G_{medium}", "C_{medium}", "G_{bio}" and "C_{bio}". The two circuits were entered into a circuit analysis computer program (Analyser II) along with the fixed component values. The program then calculated the output impedances and phase angles as a function of frequency and these were then converted to their equivalent admittance capacitance and conductance values using the standard equations (see Kell 1987). It is clearly seen that the subtraction of the polarisation control data significantly reduced the contribution from the "electrodes". It is to be expected that the improvement will be even better for real data because of the increase of G_{pol} and fall in C_{pol} as the frequency is increased.

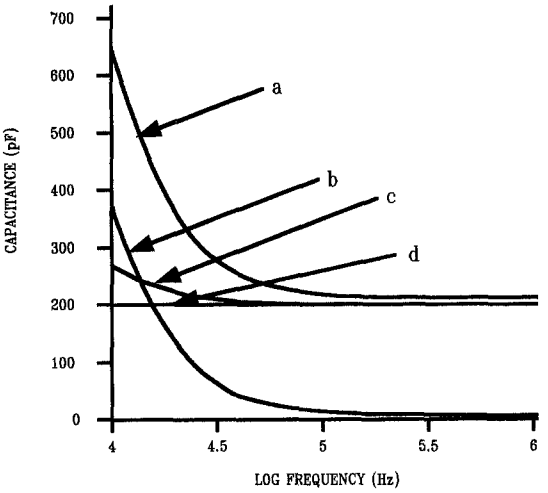

Fig. 6. A simulation of the subtraction method. The circuits of Fig. 3 were simulated using the following values: $G_{pol}=20$ mS, $C_{pol}=0.5$ μF, $G_{medium}=1$ mS, $C_{medium}=6$ pF, $G_{bio}=0.1$ mS, $C_{bio}=200$ pF. These values are similar to those observed in typical biological work. (*a*) behaviour of the circuit of Fig. 3 A (biological cells-plus-medium-plus electrodes); (*b*) behaviour of the circuit of Fig. 3 B (medium-plus-electrodes); (*c*) capacitance value of (a) *minus* capacitance value of (*b*); (*d*) frequency-independent capacitance of biological cells ("bio"). It may be observed that despite the use of frequency-independent values for the parameters, the substitution method greatly decreases the contribution of "electrode polarisation" to "biological" dielectric spectra

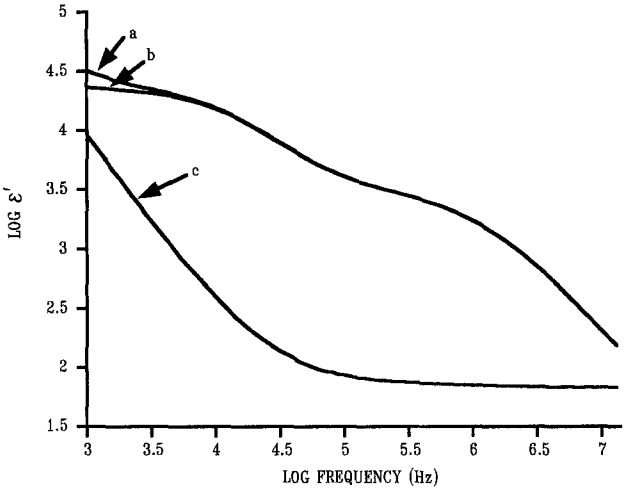

Fig. 7. Dielectric properties of human erythrocytes. Erythrocytes were prepared and resuspended to a volume fraction of 0.47. Dielectric properties were measured at 37 °C as described in the Methods section (*a*) for the cell suspension and (*c*) for the suspending medium whose conductivity had been adjusted to that (0.627(3) mS) of the suspension as measured at 1 kHz. (*b*) is the difference between the values (at each frequency) in (*a*) and (*c*) plus a term (67.8) for the permittivity of the medium at 13 MHz. It may be seen that the electrode polarisation contributes some 10 000 permittivity units at 1 kHz

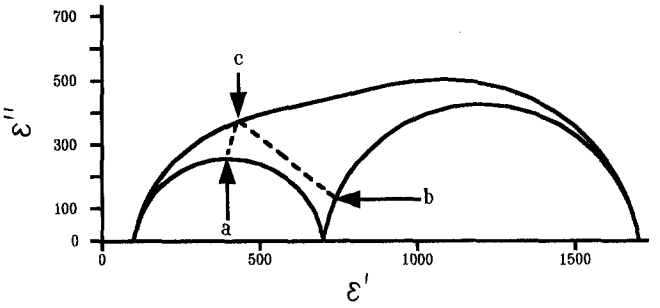

Fig. 8. Diagram to illustrate the problem of deconvoluting dielectric data. A hypothetical set of data was generated according to the double-Cole-Cole equation (6 and 7) with the values $\varepsilon'_\infty=100$, $\Delta\varepsilon'_{low}=1000$, $\Delta\varepsilon'_{high}=600$, $f_{c,low}=100$ kHz, $f_{c,high}=1$ MHz, $\alpha_{low}=0.1$, $\alpha_{high}=0.1$. The locus of these data when plotted in the complex plane is given by curve (*c*), whilst the individual dispersions are given by curves (*a*) and (*b*). The arrows show the data points at 1 MHz

Figure 7 shows the application of the substitution method to a set of data for human erythrocytes. The capacitance and conductance terms have been converted to permittivity and conductivity respectively, using the cell constant of the electrodes used. The subtraction of the polarisation control data from the cell suspension data has the effect of (negatively) offsetting the whole resulting spectrum by an extent equal to the true, frequency-independent permittivity of the suspending medium. To compensate for this one must add the high-frequency permittivity of the polarisation control data back to the cell data after the polarisation control has been subtracted, as is done in Fig. 7. It may be observed (Fig. 7) that these suspensions display substantial α- (low-frequency) and β- (high-frequency) dispersions, and that the apparent existence of a third dispersion of the erythrocytes below some 10 kHz or so, is entirely suppressed when the contribution of electrode polarisation is taken into account.

A further point to consider is that any fall in permittivity due to a dielectric dispersion must result (for a linear system) in a rise in conductivity (the Kramers-Kronig relation) and hence to a change in the extent of electrode polarisation. However at low frequencies even the large change in permittivity due to the α-dispersion results in only a very slight change in conductivity, which in our experience does not measurably affect the extent of electrode polarisation. The larger increase in conductivity due to the β-dispersion normally occurs at frequencies above those at which electrode polarisation is significant.

Deconvolution of dielectric spectra

As discussed above, dielectric spectra are by nature dispersive rather than resonant. This means that for each dispersion, the fall in permittivity (ε') and rise in conductivity (σ') with increasing frequency take several decades to run to completion. The result of this is that one typically finds that a dielectric spectrum has several overlapping dispersions which need to be separated out if one is fully to analyse the data. This problem is especially evident when one is studying the low-frequency (<30 MHz) dielectric behaviour of cell suspensions where electrode polarisation effects and the α- and β-dispersions may all occur in the same frequency range.

When two overlapping dispersions are present the traditional method of plotting the data in the complex

permittivity domain (the so-called Cole-Cole (1941) plot) results in two overlapping semicircles which are extremely difficult to separate by eye (Fig. 8). This problem has necessitated the development of procedures to deconvolute dielectric data into their component parts by fitting equations that describe the expected frequency-dependence, of say permittivity, to the permittivity values actually measured. A typical example of this is the fitting of one or more Debye dispersions to a set of data. The use of such techniques is recent as it is not practicable to do this without a moderately powerful computer.

For studying subtle phenomena in systems producing overlapping dielectric dispersions several groups have developed very sophisticated fitting routines relying on non-linear least squares fitting and statistical procedures (see e.g. Grant et al. 1978; Macdonald et al. 1982; Macdonald 1987). These programs require a substantial amount of specialist mathematical and statistical knowledge to set up (and more important to interpret) correctly and also need rather powerful computers to run them at a practicable speed. However even today the computer facilities required are out of the reach of most groups. Further, these programs are sensitive to the presence of outliers in the (unedited) data, and do not provide the user with rapid graphical output of the spectra and fits of interest.

With these points in mind we have developed a (predominantly) manual rather than automated method of deconvoluting two overlapping dispersions using a Lotus 1-2-3 type of spreadsheet (VP-Planner). This has enabled us to do the deconvolutions on standard IBM-compatible microcomputers at a practical speed, as well as exploiting the interactive nature of spreadsheets to the full. The spreadsheet program (Cole. wks) has been written so that at any time graphs of the data and the present fit can be displayed on the screen in nine different formats (including the percentage error at each frequency) using the function keys. This enables one to judge how well the fitting is progressing as well as assessing the presence of any electrode polarisation or wild points and weighting the fit appropriately. The automatic subtraction of the polarisation control data is also offered as an option and if one wants to gauge the fit against the uncompensated data this can be achieved with two key strokes. This immediacy also forces the user to be aware of any shortcommings in the data rather than to take the output of the computer as sacrosanct, as is the tendency with fully automated procedures.

Spreadsheets and the fitting programs

Spreadsheets were originally developed for business and accountancy applications but they are extremely useful within the scientific environment. Since reviews of spreadsheets are available elsewhere (e.g. LeBlond and Cobb 1983; Davey and Markx 1988; Napier and Judd 1988; Ouchi 1988), some summary remarks will suffice. A spreadsheet is basically a grid of "cells" on the computer screen and into each cell the user can enter text, numerical data or mathematical formulae. The spreadsheet has many built-in commands which enable one to do such things as graph the data, copy ranges of cells etc, and all these features are fully menu-driven, making them extremely quick and easy to use. The spreadsheets are supplied with their own programming language (macros) which enables one to evoke long sequences of commands with only a couple of keystrokes. By combining all these features one is able rapidly to build up large and sophisticated, fully- interactive and highly "user-friendly" computer applications. The fact that all the results of all the intermediate calculations are visible to the user means that debugging and maintenance of such applications is very straightforward. Unfortunately the macro languages at present available on spreadsheets are not very sophisticated and so analyses requiring complex algorithms still need to be written in normal computer languages.

The deconvolution program (Cole. wks) described in this paper is a VP-Planner spreadsheet "template" which contains the formulae required to fit the Cole-Cole equations for permittivity to a set of measured data containing one or two dispersions. Also present are the macros and formulae required for such things as the normalisation of the measured capacitance and conductance data to their equivalent permittivities (ε') and conductivities (σ'), the subtraction of the polarisation control (if required) and the generation of graphics. The Cole-Cole Eq. (Cole and Cole 1941) used for this fit is

$$\varepsilon'_\omega = \frac{\Delta\varepsilon'_L[1+(\omega\tau_L)^{1-\alpha_L}\sin(0.5\pi\alpha_L)]}{1+2(\omega\tau_L)^{1-\alpha_L}\sin(0.5\pi\alpha_L)+(\omega\tau_L)^{2-2\alpha_L}} + \frac{\Delta\varepsilon'_H[1+(\omega\tau_H)^{1-\alpha_H}\sin(0.5\pi\alpha_H)]}{1+2(\omega\tau_H)^{1-\alpha_H}\sin(0.5\pi\alpha_H)+(\omega\tau_H)^{2-2\alpha_H}} + \varepsilon'_\infty \quad (6)$$

where the subscripts L and H refer to the high and low frequency dispersions respectively (Fig. 9). ε'_ω and ε'_∞ are respectively the permittivities at the frequency of interest and at frequencies high with respect to f_c. $\Delta\varepsilon'$ is the dielectric increment of a dispersion, and the f_c (the critical frequency) is the frequency at which the fall in permittivity due to a particular dispersion is half-completed. The f_c is related to the net relaxation time of a dispersion (τ) by $\tau = 1/2\pi f_c$, where the frequency is in Hz. The Cole-Cole α value is generally taken as a measure of the distribution of relaxation times inherent within a particular dispersion and can take values between 0 and 1. When it equals the former there is a single relaxation time (Debye type dispersion) while the latter implies an infinite distribution of relaxation times.

Although there is an explicit assumption to the shape of this distribution within the Cole-Cole equations, in reality a very wide range of distributions can be made to fit a given set of data (e.g. Schwan 1957; Foster and Schwan 1989). Thus the fact that one is able to fit the Cole-Cole equations to a set of data does not imply that the underlying distribution of relaxation times is of the Cole-Cole type. In particular, the Cole-Cole analysis assumes that individual relaxations are exponential, decoupled from their environment (have no "memory function") and independent. Notwithstanding, they seem invariably to be used in biological work. More physically-based fitting procedures are available (see e.g. Dissado and Hill 1983; Jonscher 1983; Hill and Jonscher 1983) but

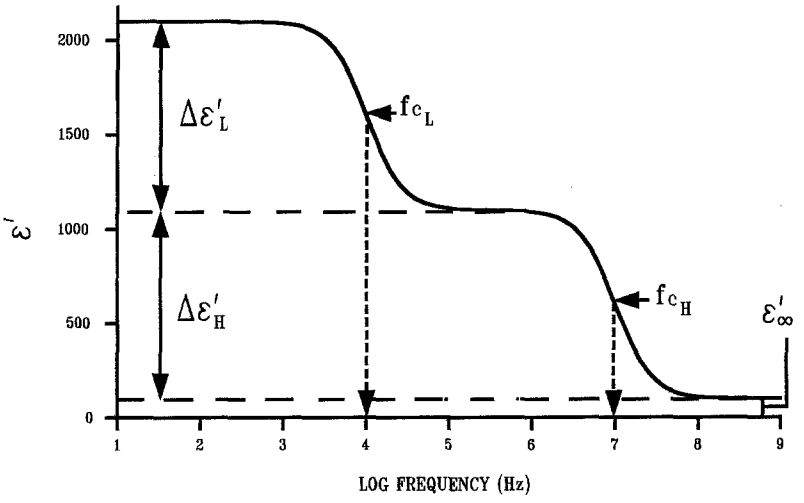

Fig. 9. The double Cole-Cole equation. The diagram shows the behaviour of the permittivity of a system conforming to the double Cole-Cole equation with the following values: $\varepsilon'_\infty = 100$, $\Delta\varepsilon'_{low} = 1000$, $\Delta\varepsilon'_{high} = 1000$, $f_{c,low} = 10$ kHz, $f_{c,high} = 10$ MHz, $\alpha_{low} = 0$, $\alpha_{high} = 0$

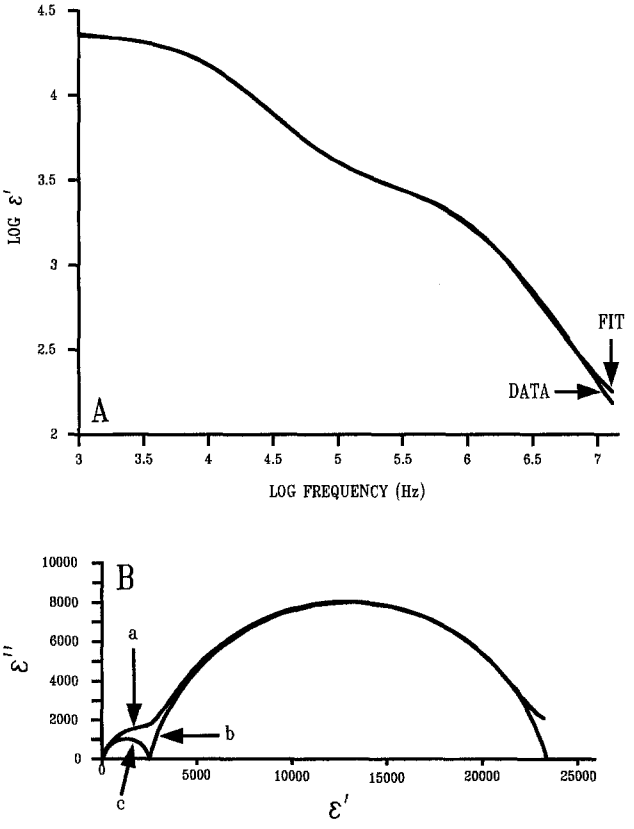

Fig. 10 A, B. Dielectric properties of human erythrocytes. Erythrocytes were prepared and resuspended as described in the legend to Fig. 7. The data, corrected for electrode polarisation as described therein are plotted, together with the best fit obtained using the Cole wks and Acole bas programs described in the text. The mean modulus percentage error was 1.62%. The time for the spreadsheet to recalculate the data (for 84 separate frequencies) following a change in a parameter was 9 s using a Vig III microcomputer containing an 80387 coprocessor. When the parameters of the "true" best fit (except ε_{inf} which was held at a constant value) were each adjusted by +1%, Acole-bas required 57 seconds to return them to a mean modulus percentage error of 1.64% (after 10 iterations). **A** Frequency-dependent permittivity. **B** Complex plane diagram. The values obtained were $\varepsilon'_\infty = 67.8$, $\Delta\varepsilon'_{low} = 20{,}900$, $\Delta\varepsilon'_{high} = 2430$, $f_{c,low} = 13.9$ kHz, $f_{c,high} = 1.41$ MHz, $\alpha_{low} = 0.165$, $\alpha_{high} = 0.105$. The cell constant was 1.208 cm^{-1}

since our aims are merely to find $\Delta\varepsilon'$ and the f_c of the dispersions, and because the measurement of the Cole α allows comparison with the published literature, we feel the use of the equation is justified. Once the permittivity (ε') has been fitted to the experimental data then the fitted values of ε' etc. can be used to calculate the imaginary component (ε'') of the complex permittivity using the Cole-Cole equation for ε''

$$\varepsilon''_\omega = \frac{\Delta\varepsilon'_L(\omega\tau_L)^{1-\alpha_L}\cos(0.5\pi\alpha_L)}{1+2(\omega\tau_L)^{1-\alpha_L}\sin(0.5\pi\alpha_L)+(\omega\tau_L)^{2-2\alpha_L}} + \frac{\Delta\varepsilon'_H(\omega\tau_H)^{1-\alpha_H}\cos(0.5\pi\alpha_H)}{1+2(\omega\tau_H)^{1-\alpha_H}\sin(0.5\pi\alpha_H)+(\omega\tau_H)^{2-2\alpha_H}}. \quad (7)$$

By the combination of (6) and (7) Cole. wks is able to generate a deconvoluted Cole-Cole plot such as those shown in Figs. 8 and 10. Interpolation routines are used to generate smooth fitted curves in those cases where the number of data points are insufficient so to do. A summary of Cole. wks and its supporting programs is shown in Fig. 11. Cole. wks reads its data from files which contain the data in the form of capacitances (in pF) and conductances (in mS) along with the equivalent frequencies (in kHz). If the subtraction of the polarisation control data is required then a given data file will contain both the cell suspension and polarisation control data. The data files (originally in ASCII) are created by any computer programs used to log data from the measuring instrumentation (or of course by manually typing the data into another spreadsheet program called Entry. wks).

Normally a good fit to the data can be achieved simply by manual adjustment of the values of each of the variables in (6). However if required the data and the fitted values can be passed to a BASIC program (Acole. bas) for fine-tuning. This program automatically adjusts the values of each of the fitted variables to achieve an "optimum" fit. The data and the optimised fit can then be passed back to Cole. wks. Both Cole. wks and Acole. bas do not use a least squares criterion for the goodness of the fit. This is because for cell suspension data showing both the α- and β-dispersions the low frequency (1 kHz) per-

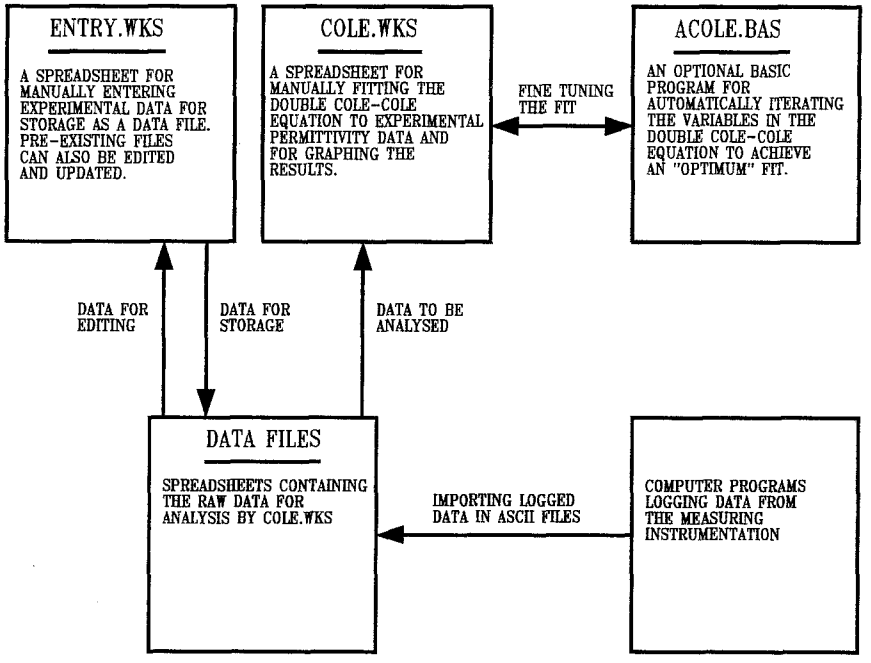

Fig. 11. A summary of the programs used herein. For further details, see the text

mittivities may be as much as 80 000, and are usually in excess of 1000, whilst the permittivities at 10 MHz will usually be close to those of the suspension medium, i.e. approximately 80. A least-squares fit will therefore *severely* weight the fit towards the low frequency data. To overcome this, the mean modulus percent error (MMPE) was calculated using

$$\text{MMPE} = \{\sum \text{modulus}[((\varepsilon'_\omega - \varepsilon' \text{ fit})/\varepsilon'_\omega) \cdot 100]\}/n \qquad (8)$$

where ε'_ω is the measured permittivity at a given angular frequency, $\varepsilon'_{\text{fit}}$ is the permittivity at the same frequency calculated using (6) and the estimates of its variables, and n is the number of data points. This method results in an equal weighting across the whole frequency range and so the user of Cole. wks must mentally weight the data according to knowledge of where the data are most reliable, even if doing this means that the "absolute minimum" of MMPE is not achieved.

Automatic iteration using Acole. bas

This program is written in Borland Turbo BASIC and is normally only used to help in the fitting when the two dispersions are very seriously overlapping (a difference in f_c of less than approximately one decade, as in Fig. 8) or for "fine-tuning" relatively error-free data. One of the variables being fitted is tested to find the largest step size in integer powers of ten (starting at its previous step size) which gives an improvement in MMPE. Once this step size is found it is used until the minimum MMPE using it is achieved, before repeating the process with the next variable. This very simple algorithm has the disadvantage that it will tend to get trapped in any local error minima present. To overcome this one should get a fairly good fit using Cole. wks and then use these estimates of the variables in equation 6 as the initial estimates for Acole. bas. When Acole. bas has completed its iterations one should take its fitted values and adjust some of them by a few percent and then rerun the program using these as the initial estimates. By doing this several times one can reduce the MMPE by overcoming any local error minima.

The program Acole. bas also allows one to put upper and lower limits on each of the variables being fitted, or to fix them at a single value. These features can be useful if a variable has a tendency to be adjusted to values well away from the correct one in the early stages of the iteration, only slowly to be iterated back later. The uniform weighting produced by the MMPE method means that weighting the data appropriately becomes a problem. However for the measuring system we typically use for cell suspensions we know that the very-low-frequency data (<10 kHz) can be affected by residual polarisation effects, while at frequencies greater than 10 MHz strays can cause distortions. To allow for this the program enables one to select upper and lower frequency limits for the fitting, and any values outside these ranges are ignored. The results from Acole. bas must however be evaluated on Cole. wks to check that a sensible fit is achieved, especially when using the frequency limits.

Concluding remarks

The approach and data described herein allow us to draw the following conclusions. The substitution method works extremely well, even when apparent (artefactual) permittivities (due to electrode polarisation) of 10^4, or nearly 30% of the biological dielectric permittivity, are observed and the spreadsheet and fitting routines allow one rapidly to obtain convincing fits to the true biological dielectric dispersions. An accompanying article (Ferris

et al. 1990) describes a further application of these techniques to the resolution of small but crucially important effects of environmental variables on biological dielectrics.

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