

THE DIELECTRIC PROPERTIES OF CELLS AND TISSUES: WHAT CAN THEY TELL US ABOUT THE MECHANISMS OF FIELD/CELL INTERACTIONS?

Christopher L. Davey and Douglas B. Kell

INTRODUCTION

"A knowledge of the passive electrical properties of biological systems must underpin any significant understanding of the nature, role and mechanisms of bioelectrical phenomena."⁴² In other words, and not least because of the possible physiological effects exerted on tissues following their absorption of non-ionising electromagnetic radiation,^{1,2,11,18,33,34,35,51,56,57,58,59,60,65,69,72,77,78,83,89,90,91,102} there is an increasing awareness that if we are to exploit electric fields in medical technology, and in other forms of diagnosis and therapy, the first thing we must do is to measure and then to understand how and why such fields actually are absorbed by the target tissues or cells. In this sense, we treat tissues as concentrated suspensions of cells, and, recognising that the magnetic susceptibility of virtually all tissues is essentially identical to that of water, we consider only the electrical component of any imposed electromagnetic field.

In the present article, therefore, we shall: 1) discuss in elementary terms how we measure the (predominantly RF) passive electrical properties of living systems; 2) describe the so-called α - and β -dispersions as examples of linear dielectric behaviour; 3) suggest some future uses that we may make of our knowledge of such properties; 4) introduce readers to the ideas and existence of the non-linear dielectric behaviour of living systems; and finally 5) suggest some novel means by which these nonlinear properties may be exploited in emerging electromagnetic devices.

MEASUREMENT OF THE DIELECTRIC PROPERTIES OF LIVING SYSTEMS

As reviewed elsewhere extensively,^{1,39,56,64,87,88,94,95,96,97,99} living systems possess dielectric (ie, passive electrical) properties very different from those generally found in inanimate matter. One of the chief characteristics of living systems, from an electrical point of view, is that they are typically rather conductive or 'lossy', ie, they contain a substantial concentration of small, mobile ions. Conductivity (σ) is a measure of the ease with which free

charges can migrate through the material under the influence of an electrical field. By contrast, if an exogenous electrical field can induce or modulate significant charge separations (ie, polarisations), the material will have a high permittivity (ϵ').

Except for unusual cases (such as nerve axons, not discussed here) in which inductive reactances and negative resistances are present,^{3,21,31,32,111} the passive electrical properties of living systems are completely characterised by their frequency-dependent conductivity and permittivity. These are related to the macroscopic conductance and capacitance of the material held between two or more electrodes by a geometric factor, the cell constant (which has units of cm^{-1}). The vector sum of the conductance and the (angular frequency times the) capacitance is known as the admittance. Thus, to determine the dielectric properties of cell suspensions at frequencies up to say 30 MHz or so, one applies a sinusoidally modulated current and measures the resulting voltage (at the frequency of excitation) and phase angle (Fig. 1 a and b).

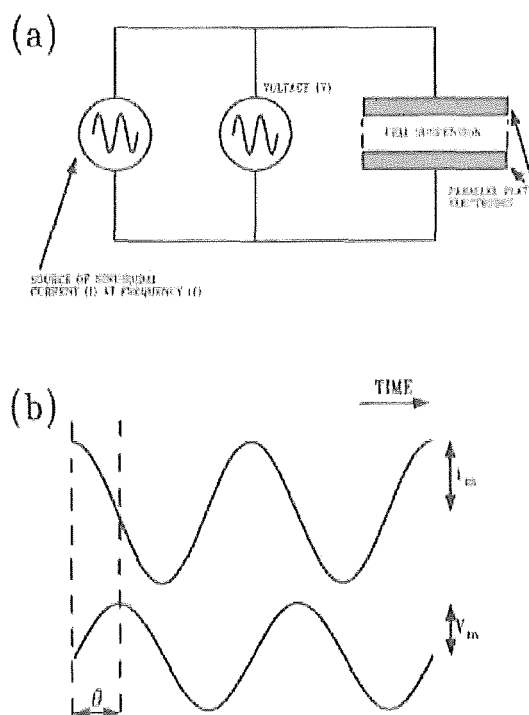


Figure 1. A dielectric measurement, in which a sinusoidally modulated AC current is applied to the system of interest, in this case a cell suspension. The result of this is that a voltage of the same frequency, phase shifted by an amount (θ radians) which reflects the macroscopic capacitance and conductance of the suspension, is generated across the system. For a pure capacitor the current leads to voltage by $\pi/2$ radians, whilst for a pure resistor the current and voltage are exactly in phase. (a) generalised measurement system. (b) waveforms of voltage and current: the overall admittance is the ratio I_m/V_m .

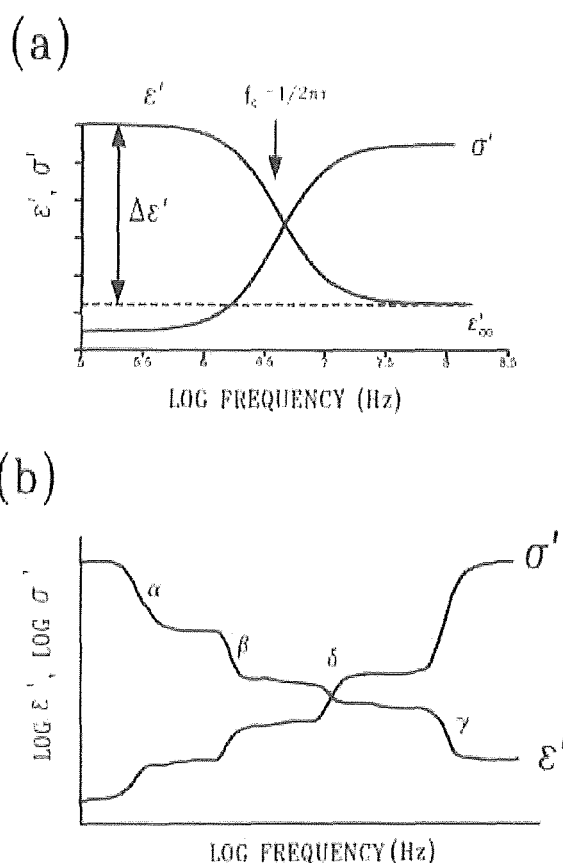
A particular disadvantage of this arrangement is that at 'low' frequencies (< 5 MHz) there is a significant contribution to the measured capacitance and conductance from reactions occurring at the electrode-solution interfaces, a phenomenon usually referred to as "electrode polarisation." To minimise this problem, one may use a 4-terminal system in which the outer electrodes are the source of current, whilst the inner pair of electrodes are used to determine the voltage drop. By connecting the inner electrodes to a voltmeter of high input impedance, one may ensure that electrode polarisation phenomena (which are caused by current flow across the electrode-solution interfaces) do not contribute to the voltage drop (and hence admittance) measured.^{56,62,98}

A. Dielectric Dispersions

When one measures the permittivity and conductivity of a cell suspension, it is found that permittivity falls and conductivity rises as the frequency is increased, in a series of steps known as dispersions (Fig. 2a). In the frequency range of interest (say DC-100 MHz), two or three main dispersions may usually be discerned, known respectively as the α -, β - and δ -dispersions (Fig. 2b).

Figure 2. Dielectric dispersions. (a) In a dielectric dispersion, the permittivity falls and the conductivity rises as the frequency of measurement is increased. The dielectric increment ($\Delta\epsilon'$) describes the magnitude of the change in permittivity, whilst the characteristic frequency (f_c) is the frequency at which the change is half-completed. Similarly one may describe a conductivity increment.

(b) The classical dispersions typically observed in living tissues. The frequency range and dielectric increments are somewhat arbitrary, but the characteristic frequencies and dielectric increments are respectively of the order of 1 kHz and 10^8 (α), 1 MHz and 10^4 (β), 100 MHz and 20 (δ) and 10 GHz and 70 (γ). The dispersions are less sharp than those of a single Debye dispersion.



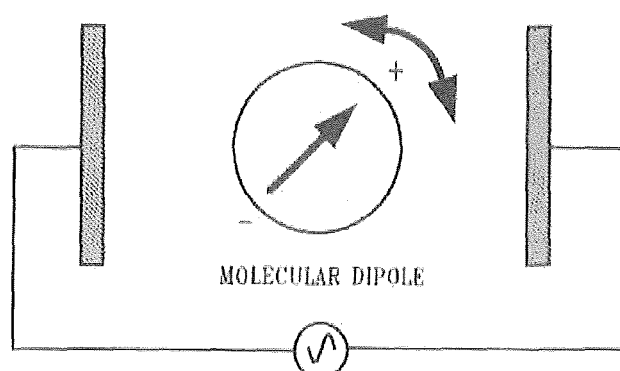
The δ -dispersion is due mainly to the rotation of the side-chains of amino acids in proteins and of bound water molecules (whilst the γ -dispersion, occurring at somewhat higher frequencies still, is dominated by the rotation of the dipoles of 'free' water molecules).³⁹ Whilst our present interest is focussed upon the α - and β -dispersions occurring at the lower audio and radio frequencies, it is convenient first to consider the simplest type of dielectric dispersion, that due to dipole rotation.²⁵

1. The Rotating Dipole

Figure 3 shows the simplest type of molecule suitable for our present purposes: the dipolar billiard ball. Such a molecule contains a permanent dipole moment due to the fact that it has two (or more) charges of opposite sign separated in space. If the (two) charges are of magnitude $+q$ and $-q$ Coulombs, and they are separated by a distance s metres, the molecule has a permanent dipole moment of $m = qs$ (in C.m). Though not an SI Unit, dipole moments are often quoted in Debye units, where $1D = 3.33 \times 10^{-30} \text{ C.m}$, and it is convenient to note that the displacement of 1 electronic charge through 10^{-10} m gives a dipole moment of $4.8D$, ie, 1 "charge-Ångström". Pure water has a dipole moment of some $1.8D$, whilst typical proteins have dipole moments of some hundreds of Debyes. As one may expect, the dielectric increments observed, ie, the change in permittivity as one passes between two plateau regions as one increases the frequency, is related both to the intrinsic dipole moment of the relaxing dipoles and to their concentration. The frequency (in Hz) at which the above transition is half-completed is known as the characteristic frequency (f_c) and is related to the relaxation time (τ) by the relation $f_c = 1/(2\pi\tau)$ (Fig. 2a). The relaxation times to be expected for the rotation of a molecular dipole are those for the spheroidal molecules whose rotation is opposed by frictional interaction with the surrounding viscous medium. Thus in this case the energy that the system absorbs by the field is transduced into heat (by virtue of the frictional interactions between the rotating dipole and the solvent). We shall find later in this paper that this type of mechanism is in contrast to certain other mechanisms by which biological systems may interact with exogenous fields.

In the situations considered here, only a miniscule fraction of the charges and dipoles in the ensemble present are effectively moving in response to the applied field, such that doubling the voltage (field) doubles the current flowing such that their vector ratio, the admittance, is voltage-independent. This is why the electrical properties referred to here are called 'passive' or 'linear'. Nonlinear properties are discussed later.

Figure 3. The rotating dipolar billiard ball. In this model system of dielectric relaxation, the system of interest is a hard sphere possessing unit charges at opposite poles. The alternating nature of the electrical field means that the dipole seeks to rotate to an orientation of minimum energy with respect to the field.



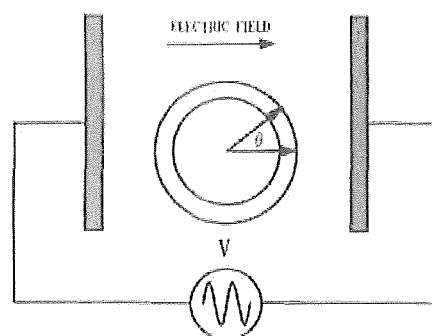
If we ask what sort of frequency ranges are characteristic of this sort of dipole rotation, we find that for water rotating in water, $f_c = (2\pi\tau)^{-1}$ is approximately 25 GHz at room temperature^{39,46,87} whilst concentrated protein solutions have an observable dispersion due to dipolar rotation centred at 1 MHz or so.^{85,109,110} In practice this is essentially invisible, since it is dominated by the β -dispersion typically occurring in this frequency range.

2. The β -Dispersion

Biological membranes have conductances of the order of perhaps $10^{-3} \text{ mS.cm}^{-2}$ and may be regarded (with respect to the extracellular and intracellular phases) 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 electric capacitor. This means that when an exciting electrical voltage is applied across a cell suspension, the membrane capacitance (C_m) is charged up by ions moving under the influence of the electrical field. Because of the essentially nonconducting nature of the membrane, the membrane has the effect (Fig. 4) of strongly amplifying the exciting field generated between the electrodes.¹¹³

However, as the frequency rises, fewer and fewer ions have time to charge up the membrane(s) before the field changes direction. Thus the electrical charge stored by the suspension for a given exciting voltage falls, and the capacitance (permittivity) of the suspension drops. At low frequencies, the admittance (i.e. the conductance to alternating current) of the cell membranes is very low, such that they behave as nonconductors suspended in a conducting medium and most of the current flowing in the suspension must flow round the cells. As the frequency increases, the membrane admittance rises and an increasing proportion

Figure 4. Amplification by a spherical shell membrane of an exogenous electrical field. In the present case, the (maximum) exogenous field strength E_0 (neglecting electrode polarisation) experienced by the spherical shell is V_{max}/d , where d is the distance between the electrodes (typically of the order of 1 cm). At the moment shown, the right-hand electrode



induced across the membrane is $\psi_m = 1.5rE_0 \cos \theta / [1 + (f/f_c)^2]^{1/2}$, where r is the sphere's radius and f and f_c respectively the excitation frequency and the characteristic frequency of the β -dispersion. This potential will superimpose vectorially upon any pre-existing transmembrane potential⁶¹. The field strength across the membrane is then ψ_m divided by the thickness of the membrane (typically 5 nm). Thus the membrane amplifies the field by an amount depending in particular upon the cell radius and the frequency of the field.

of the current can flow through the membrane and via the conductive cytoplasm of the cells. Thus the conductivity of the suspension increases. These features are illustrated in Fig. 5, whilst an electrical equivalent circuit for the shell membrane system characteristic of cell suspensions is given in Fig. 6, and a more complete overview of the β -dispersion.^{23,64}

Since only living cells have these properties (in that cells with leaky membranes are dead or moribund,⁴³ we have been able to exploit this fact to devise a biomass probe for use in laboratory and industrial fermentations, based on the 4-terminal measurement of the RF dielectric properties of fermentor broths.^{24,45,66,67} This device, the β ugmeter (Fig. 7), is now being produced commercially. Most recently, in addition to its use in fermentor broths, we and others have shown that it may be used 1) in toxicological studies to follow cell death subsequent to a challenge with toxic xenobiotics,¹⁰⁴ 2) in the control of yeast pitching in breweries,¹⁴ and 3) in solid-substrate fermentations by the mould *Rhizopus oligosporus* used in the production of Tempeh (personal communication Davey, Penaloza, Kell, Hedger).

3. The α -Dispersion

Cell surfaces are normally negatively charged, the cell surface charge density depending upon the cell type.^{9,75} The presence of this cell surface

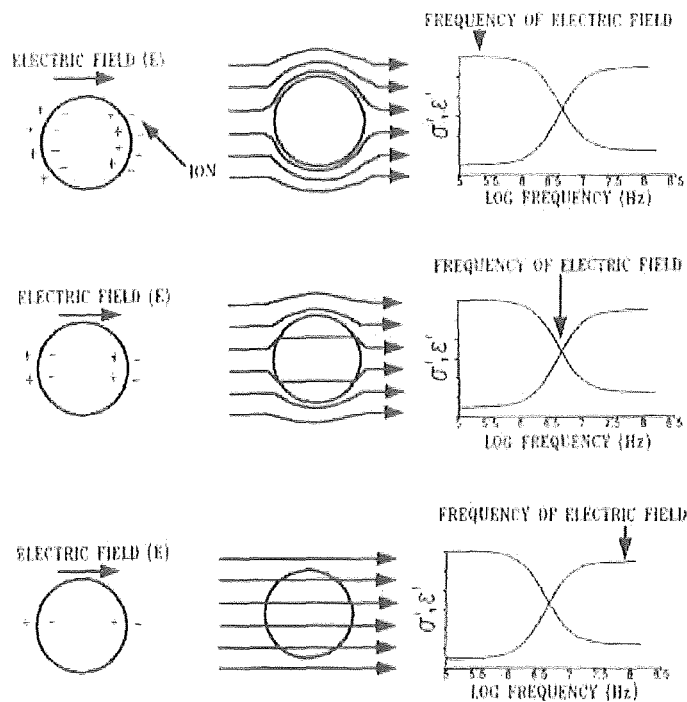
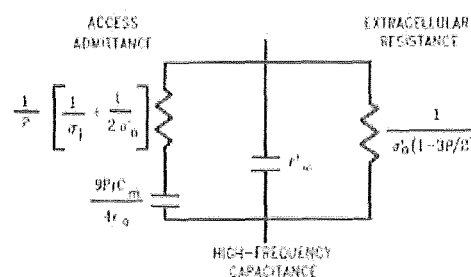


Figure 5. Effect of frequency upon the polarisation, current flow and dielectric properties of spherical shell suspensions. The frequency increases from top to bottom. It is assumed that the electrodes are to the left and right of the cells illustrated, and are observed at an instant when the right-hand electrode is negative. The left-hand portion shows the relative polarisation of the plasma membrane, the middle portion the flow of current around and/or through the cells, and the right-hand portion the approximate frequency (relative to f_c) at which the behaviour indicated would be observed. For further discussion, see the text.

Figure 6. An electrical equivalent circuit often used to describe the β -dispersion. The values of the components are dimensionally inaccurate but are used to illustrate the physical contributions of the different subsystems. In the absence of cells, the 2 right-hand components are the only ones present, whilst the membrane capacitance (lower left) is charged up via the 'access admittance' consisting of internal and external



conductivities. The symbols are for volume fraction (P), internal (σ_i) and external (σ_o) conductivities, the cell radius (r) and membrane capacitance per unit area (C_m). ϵ_o is the permittivity of free space.

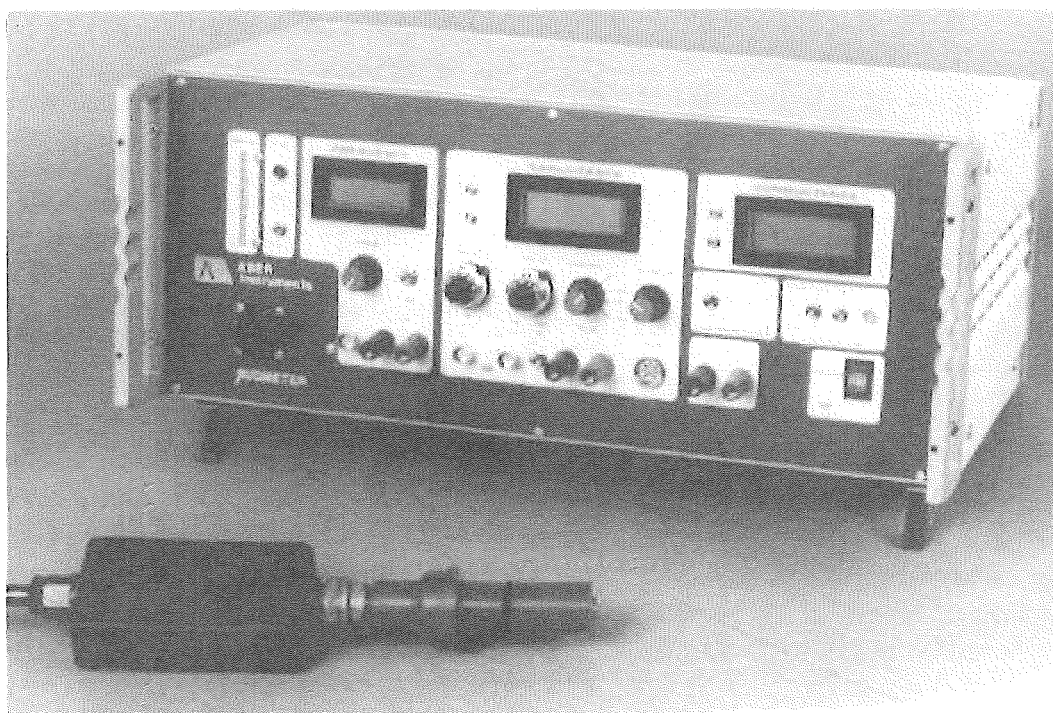
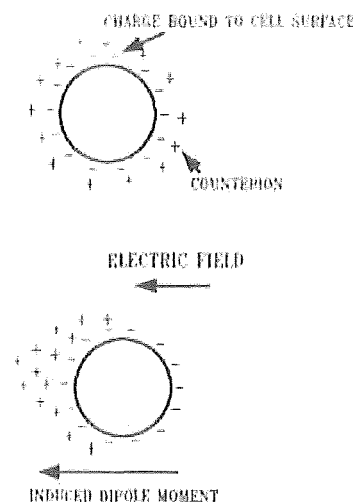


Figure 7. The bugmeter, an instrument for the real-time estimation of cellular biomass and viability, based upon the measurement of the radio frequency dielectric properties ($\Delta\epsilon'$ for the β -dispersion). The probe is a standard 25 mm fermentor probe. Figure courtesy of Aber Instruments.

charge results in a diffuse counterion layer around the cells. When an electric field is applied to this cell, the counterions move tangentially along the cell surface, so that an induced dipole is formed along the length of the cell (Fig. 8). As it takes a finite time for the counterions on the cell surfaces to reach the ends of the cell, the number doing so, and hence the measured capacitance, will again increase with decreasing frequency. As before, the conductivity rises with increasing frequency. This is because the energy in the exciting field must either be stored (as reflected in ϵ') or dissipated (as reflected in σ'), and therefore for a linear system a permittivity fall must be accompanied by a rise in conductivity (the Kronig-Kramers relationship). It is not improbable that other factors, such as the field-induced gating of transmembrane ion transfers, may also be involved in the α -dispersion, and it is certainly true to say that the classical explanations^{28,84} do not alone account for the independence of the magnitude of the α -dispersion from the number and valency of counterions in bacterial chromatophores.⁵⁵ None the less, the low-frequency dielectric properties of extended, charged macromolecules such as DNA are caused by counterion relaxation mechanisms of this type.^{76,112} Further experimental developments in this area would benefit from the adoption of simple experimental systems such as HPLC resins.⁷³

Figure 8. The production of a field-induced dipole along the length of a cell as a mechanism of dielectric dispersion. The upper half of the figure illustrates the counterion distribution in the absence of the field, whilst the lower part indicates what happens when a (low-frequency) electric field is present.



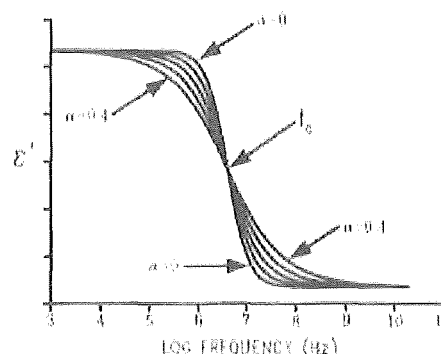
4. Other Dispersions

In essence, any field-induced relaxation or motion of a charge or dipole will result in a dielectric dispersion, those of particular interest including the passage of 'gating' charges,³ the lateral motions of charged components in the plane of biological membranes (the μ -dispersion^{44,55,63,64}) and the hydration-dependent changes in flexibility and in protonic conductivity exhibited by protein colloids (for lysozyme see^{12,13,17,30}). Space does not permit a detailed discussion of these, save to mention that they will always be present and are likely at least to contribute to some of the more classical dielectric dispersions observed in tissues.

B. 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 (and of other properties) 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 such a distribution in τ (and hence f_c) values exists then the fall of permittivity and rise in conductivity with increasing frequency will be less steep than in the case of a system exhibiting but a single relaxation time. These effects are normally discussed in terms of an empirical parameter, the Cole-Cole α^{22} (Fig. 9), which may take a value between 0 (no distribution of relaxation times) and 1 (infinite distribution), though there are many reasons to doubt that this apparent spread of relaxation times is ascribable solely to a spread in the properties of non-interacting subsystems.^{26,27,49,50,54,59,63,64,70,96,119} Notwithstanding, and as pointed out by Schwan⁹⁶ an enormous number of possible distributions of relaxation times will give behaviour that is experimentally indistinguishable from 'true' Cole-Cole

Figure 9. The effect of the magnitude of the Cole-Cole α in smoothing out a dielectric dispersion. The curves are for changes in α in steps of 0.1.



behaviour; in view of this most investigators use the Cole-Cole formalism for the analysis and condensed description of their data.

DECONVOLUTING DIELECTRIC SPECTRA

Especially given the above-mentioned spread of apparent relaxation times, 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 each other. A further problem encountered at low frequencies, especially when 2-terminal approaches are used, is that the admittance due to reactions occurring at the electrodes can dominate measurements in the sub-MHz region. To deconvolute such data one must minimise the contribution due to this electrode polarisation; in our experience²³ this can be done by subtracting the polarisation control data (obtained in a cell-free medium of identical low-frequency conductivity) from the actual cell data. Once this compensation has been carried out one fits the Cole-Cole equation for two dispersions to the resulting data points. Free parameters in the fit are the dielectric increments, the characteristic frequencies and the Cole-Cole α for the high- and low-frequency dispersions, and the permittivity at frequencies that are high relative to the frequencies of interest.

We have devised two computer programs (for use with IBM-PCs and compatibles) to deconvolute permittivity data. The first program (COLE.WKS) is written on a LOTUS 1-2-3 -type spreadsheet (VP-Planner) and allows us first to correct the data for polarisation. Estimated values for the variables of interest 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 permittivity data and the fit can then be presented on the computer screen in various ways (eg, of log permittivity vs. log frequency (f), permittivity vs. log f , permittivity and conductivity vs. log f , error vs. log f , imaginary vs. real part of the permittivity) by using the function keys. 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. The data are then fitted iteratively to (the double) Cole-Cole equation by the program, using the initial estimates. Because of the bias towards the low-frequency end, where the permittivity can become very high, the fit is not a nonlinear least-squares type of fit, as used for instance by MacDonald⁷⁴ and Grant,³⁹ but, as in the program COLE.WKS, is judged by the overall modulus of the percentage error in the permittivity domain. This program has been run using both an interpreted BASIC (GWBASIC) and a compiled BASIC (Borland Turbo BASIC), the latter running some 20-fold faster, especially in PCs possessing a maths coprocessor (80x87) chip. Together, these programs provide a convenient and accurate means of registering the linear dielectric properties of biological systems and discerning the mechanisms underlying such properties. Figure 10 gives an example of the type of fit that one may obtain, using experimental data from a suspension of erythrocytes.

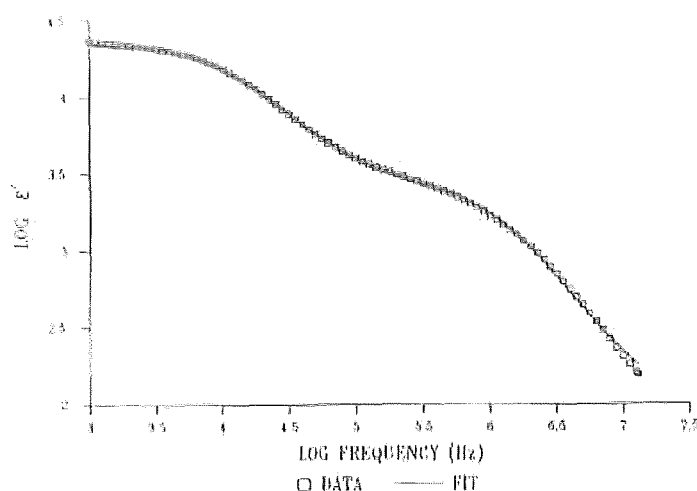


Figure 10. Fitting of dielectric data to 2 dispersions using the programs COLE.WKS and COLE.BAS. Dielectric data were obtained using an HP 4192A Impedance Analyser, as described by Harris and Kell (1983). Human erythrocytes were obtained locally, washed three times and resuspended in 1 mM NaCl, 290 mM sorbitol, 10 mM tris chloride, pH 6. The 84 data points are illustrated and the fit to the double Cole equation with the parameters $\epsilon_{inf} = 70$, $\Delta\epsilon'_{high} = 2400$, $f_c(high) = 1.41$ MHz, $\alpha_{high} = 0.11$, $\Delta\epsilon'_{low} = 20900$, $f_c(low) = 14$ kHz, $\alpha_{low} = 0.16$. The 'low' and 'high' frequency dispersions correspond to the α - and β -dispersions respectively.

EXPLOITATION OF LINEAR DIELECTRIC MEASUREMENTS IN MEDICAL TECHNOLOGY

The electronic biomass probe that we have devised for the registration of the biomass in fermentors has been alluded to above. The present instruments determine the dielectric properties of the suspension of interest in the frequency range 0.1 - 10 MHz, and use the permittivity value obtained at an appropriate frequency to estimate the amount (and in favourable cases the nature) of the biomass present. It is important to note that these properties correlate with viable biomass, and not simply with cell number.^{14,104}

Given that, as has been known for many years, the dielectric properties of living systems at both audio and radio frequencies change dramatically after death,^{96,106,107,108} it seems reasonable to propose that the measurement of such properties might provide a novel, non-invasive and useful approach to the estimation of the time of death of a subject in forensic medicine, an estimation which is still subject to many uncertainties.

The distinction between life and non-life is of course but one extreme subset of the variety of physiological states that a tissue may adopt. Diagnostic techniques that rely on dielectric measurements^{40,52} include impedance plethysmography^{4,15,48,80,86,121} and pneumography^{7,47} and electrical impedance tomography.^{5,8,29,81,82,92} Various pathological states of muscle may also be accompanied by changes in dielectric properties.⁷¹ Further, the electrical impedance of the meridians, and especially the needle points, recognised in the science of acupuncture are significantly lower than that of the surrounding tissue,^{11,53,93} a fact that seems more than coincidental. The dielectric properties at microwave frequencies of tumours and of adipose tissue are greatly different from those of other tissues,¹⁰³ such that we may expect to be able to distinguish the former in the presence of the latter. The hydration-dependencies of the microwave dielectric properties of cells are discussed from a more biological point of view by Clegg et al.^{19,20}

More generally, the interaction of electromagnetic energy with tissues is also of importance in RF and microwave hyperthermia,^{41,105} in the gentle thawing of cryogenically preserved tissue,¹⁶ in the use of pulsed electromagnetic fields to aid tissue and bone regeneration and healing.^{10,11,89} Measurement of this interaction, additional to the simple application of these electrical fields, can only improve the quality and reproducibility of these regimes. From this point of view, 'dielectric diagnosis' may (and may be expected to) be exploited in a variety of existing or projected biosensing devices.^{56,57,62}

NONLINEAR INTERACTIONS OF CELLS WITH ELECTRICAL FIELDS

We saw above that the simplest type of dipolar billiard ball rotation resulted ultimately in the simple transduction of exogenous electrical energy via frictional forces into heat. However, based on recent work of Tsong and colleagues,^{100,101} Westerhoff, Astumian and co-workers have shown that the properties required of an enzyme to 'harvest' energy from an exogenous electrical field are common to all enzymes, viz. the possession of conformational states which possess different dipole moments and which interconvert hierarchically in a fashion that is coupled to their chemical environment.^{6,114,115,118,119,120} Importantly, it was shown that such energy converters must act nonlinearly, and it was proposed that this should be visible as a conversion of the frequency of an exciting electrical field to another frequency.^{59,70,119} This would be true at field strengths in which traditional measurements (of a voltage-independent impedance) would suggest that a purely linear system was being observed.

In essence, this turns out to be a case of rose-coloured spectacles: because of the fact that impedimetric devices assume linearity, such measuring systems are normally so organised (electronically) that they reject currents at frequencies other than that of the exciting voltage. Thus a system may appear linear (in that the observable current is linear with the exciting voltage) but be nonlinear (in that the system causes what may be a constant fraction of the exciting voltage to be transformed into currents at other than the exciting frequency). We discuss one type of system explicitly.

A 4-STATE ENZYME CAPABLE OF HARVESTING ELECTRICAL ENERGY FOR THE PERFORMANCE OF USEFUL CHEMICAL WORK

We note (and would stress) that the average potential in a sinusoidally modulated field is zero, that in most cases where it interacts with an aqueous system, it merely produces heat under macroscopically isothermal conditions. However, it certainly constitutes a source of free energy, as is clear from its action as such across every electrical power point or wall socket. To provide a simple illustration of the properties of a system which does not merely turn this electric field energy into heat and which can therefore conserve this free energy as useful (electro)chemical work,^{61,116} we consider (Fig. 10) an enzyme with the following properties. We assume that the protein is a membrane-located pump (which does not therefore rotate), capable of transporting an uncharged molecule (S) which is present at a higher concentration inside the cell than that outside in a direction opposite to its (electro)chemical potential.

The protein possesses a negatively charged binding site for S which can flip-flop between the inner and outer surfaces of the membrane. (Of course it does not cross the whole membrane, merely the region where the potential drop is the greatest.) The protein possesses, and cycles between, 4 conformational states (1 to 4), which represent the combination of bound or non-bound substrate with the negatively charged binding site facing inwards or outwards. States 4 and 2 have lower basic free energies^{48,120} than states 1 and 3, and thus are more stable (ie, highly populated). Cycling of the protein in a clockwise direction would have the effect of 'pumping' S against its chemical potential, such that to effect this an exogenous source of free energy is necessary. In the absence of any field, therefore, we assume that, because of a higher affinity for its substrate than any of the other states, state 4 is the most highly populated (Fig. 11a) in the protein ensemble.

If we apply a low-frequency alternating field (whose frequency $f < f_c$ for the β -dispersion), during the first half cycle it will be oriented (say) in a direction that attracts the negatively charged binding site towards the inner face of the membrane (Fig. 11b). This effectively causes transitions from states 4 \rightarrow 1 and state 3 \rightarrow 2. Thus state 1 has become the most highly populated state and there will have been a net translocation of S from outside to inside. However, as state 2 also has its negative charge in the energetically favoured inner position, and as it has a lower free energy than state 1, there will be a re-equilibration between (the populations in) states 1 and 2 (Fig. 11c). Thus state 2 becomes more highly populated than the other states, and because the transition 1 \rightarrow 2 releases S on the inside, there will be a net release of S to the cytoplasm.

When the second half of the ac field is applied, (ie, the applied electric field is now of the opposite polarity), the favoured position of the negatively charged binding site is now at the outer face of the membrane. This causes transitions from 2 \rightarrow 3 and from 1 \rightarrow 4. The immediate result now is that state 3 becomes more populated than the other states (Fig. 11d). In other words, there has been a net movement of empty binding sites from the inside to the outside of the membrane. However, since state 4 also has its negative charge in the energetically more favoured outside position, and has a lower free energy than does state 3, there is another re-equilibration (Fig. 11e) in the direction 3 \rightarrow 4.

The net result of this is that, from the protein's point of view, we have returned to our starting position (Fig. 11a). In a sense this is to be expected, since the net potential of the field was indeed zero. Yet despite this fact the field has done work on the system, since the clockwise cycling of the protein caused it to pump S against its electrochemical gradient and under macroscopically

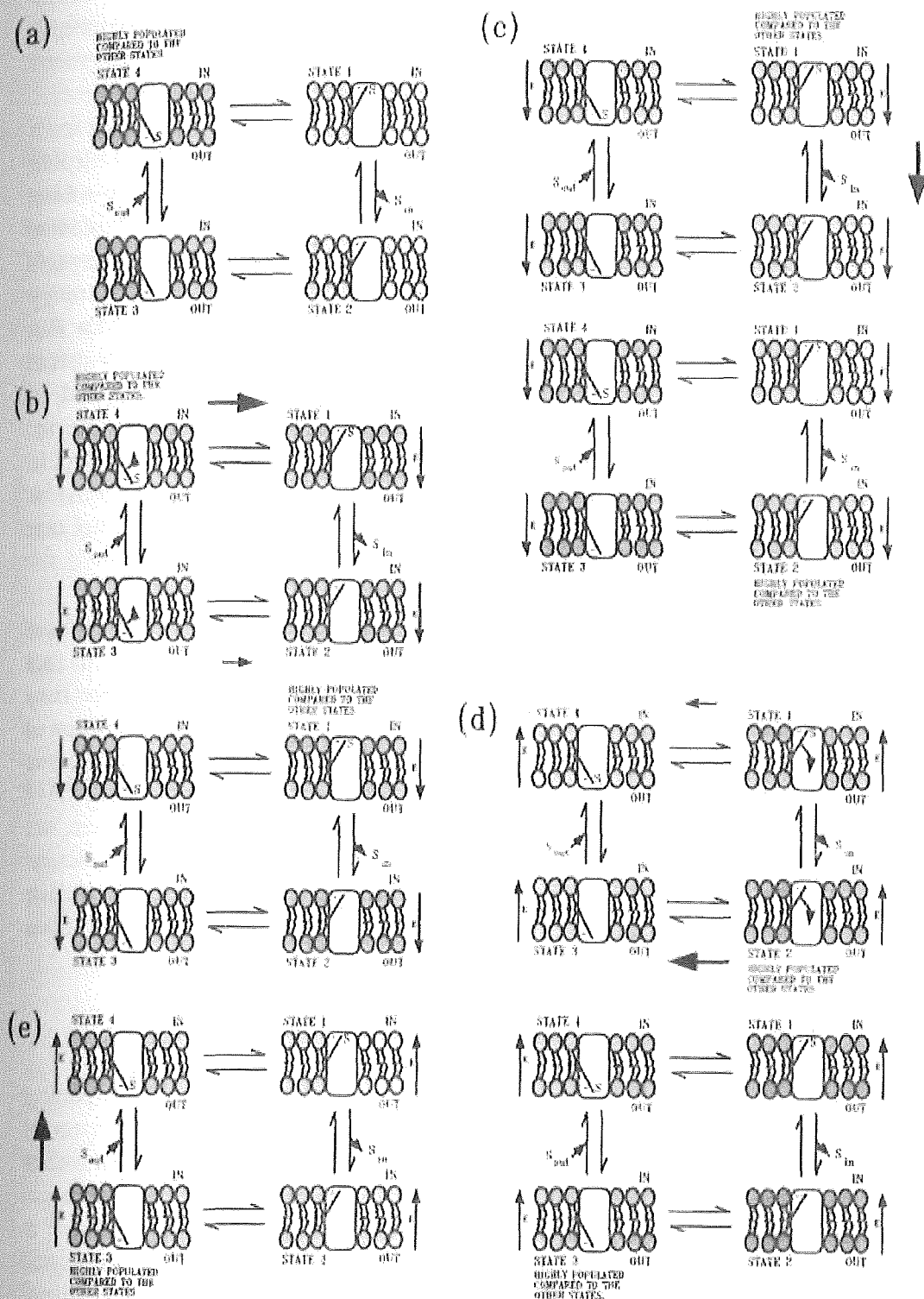


Figure 11. A 4-state enzyme capable of transducing electrical energy in the form of a non-stationary electrical field into useful (electro)chemical work. For discussion, see the text.

isothermal conditions. This does not violate the Second Law,¹¹⁶ but merely indicates that proteins are not simple dipolar billiard balls.

Clearly, the properties required of our model protein here are common to all enzymes, viz. the possession of conformational states of different dipole moments which are coupled to each other in a hierarchical fashion, which possess different free energies and the transitions between which are coupled to electrochemical reactions. Whilst this does not of itself indicate which enzymes one should seek to consider as the most likely or suitable 'targets' for low-energy exogenous electrical fields, the metabolic control analysis^{68,117} indicates that there is rarely a unique target. The amplifying effects of membranes on electrical fields suggest that initial attention might most fruitfully be directed at membranous ones. Similarly, the fact that the exogenous field causes the re-equilibration of protein conformational states which have different dipole moments indicates that one should expect to see field-induced currents at frequencies related to the (pseudo-first-order) rate constants of the normal rate constants for such transitions.

On the assumption that the fluctuation-dissipation theorem has at least some validity in this type of system,⁶¹ these frequencies would mainly in fact be the inverse of the pseudo-first-order rate constants. In this regard, we may mention that harmonics have been observed experimentally (under conditions in which the fundamental appears linear) in artificial polymers^{36,37} and in nerve axons,⁷⁹ consistent with the arguments developed herein and elsewhere.^{70,119} Whilst we can at this stage say no more about the appropriate frequencies, we may state that the design of optimal waveforms for interacting with particular targets does not in principle differ from the design of drugs aimed at selective interaction with appropriate targets or receptors.⁵⁸ What sort of considerations might we apply?

DIELECTRIC FINGERPRINTING

The answer to the previous question is to be found in the opening quotation, and requires that we jettison the idea that the dielectric properties of living systems, even at 'low' field strengths, are linear *sensu stricto*. This means that we need properly to characterise the nonlinear dielectric properties of target enzymes and tissues. The idea behind the dielectric fingerprinting approach⁵⁶ is that because of the properties of real enzymes alluded to above, and their modulation by interaction with ligands, the imposition of a high-strength field will cause time- and field-dependent changes in the dielectric properties of the target enzyme (as measured at 'low' field strengths), which may therefore be used to construct a time-, field- and frequency-dependent dielectric spectrum or

'dielectric fingerprint' of the target of interest. One way of viewing this approach, is to regard its relationship to conventional (linear) dielectric spectroscopy as in some ways analogous to that between 2-dimensional and 1-dimensional NMR spectroscopies. We are presently developing the hardware necessary for the construction of such a system. Thus, and as fore-shadowed in the opening statement (although one might well argue (and without doubt we shall soon do so) whether the word 'passive' is wholly appropriate), one should then be able to base the design of a more specific waveform on the dielectric fingerprint of the target enzyme or tissue.

AN OVERVIEW OF FUTURE USES

From the foregoing, we may anticipate future developments in this area to lie in 3 directions in particular:

- 1). **Tissue Diagnostics.** Here it is easy to envisage (linear) RF-dielectric probes for studying the physiological state of tissues, including, for instance, their adiposity, and, on a somewhat macabre note, the possibility of a means for estimating the time after death. Non-invasive 'probe' methods^{16,38} provide a particularly convenient approach.
- 2). **Biosensing.** Especially in the nonlinear regime, the possibility of studying the dynamics of proteins and of DNA by dielectric means, and how they may be modulated by interactions with ligands, provides a novel and powerful generic biosensing technology.
- 3). **Therapeutics.** If fields can affect enzymes and cells, there is no reason of principle why one should not expect to be able to tailor a waveform as a therapeutic agent in much the same way as one now modulates chemical structures to obtain pharmacological selectivity,⁵⁸ and perhaps without many of the side-effects common to pharmaceutical substances.

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