

The principles and potential of electrical admittance spectroscopy: an introduction

DOUGLAS B. KELL

24.1 Introduction and overview

In many electrochemical techniques, one applies a (clamped) DC potential to the working electrode and measures the resultant current flowing in a circuit completed by a counter electrode (e.g. Bard and Faulkner 1980; Bond 1980; Kissinger and Heineman 1984). Even in pulse voltammetric techniques, the measuring system is designed such that the potential difference between the working and reference electrodes, and the current ultimately measured, is constant for a greater or lesser period. However, the last 20 years or so have witnessed the increasing exploitation of *sinusoidal* exciting voltages in the study of electrode processes in aqueous media (e.g. Breyer and Bauer 1963; Schwan 1966; Smith 1966; Sluyters-Rehbach and Sluyters 1970; Macdonald 1977; Archer and Armstrong 1980; Bard and Faulkner 1980; Bond 1980; Gabrielli 1980; Buck 1982; Macdonald and McKubre 1982; Gabrielli *et al.* 1983), an approach which possesses two advantages in particular: (1) the sinusoid offers convenient technical and mathematical features in such systems, together with an excellent signal: noise ratio predicated upon the use of a 'steady-state' analysis (e.g. Creason *et al.* 1973; Gabrielli and Keddam 1974; Diamond and Machen 1983; Marshall 1983), and (2) the *frequency*, as well as the voltage, of the exciting wave-form may be altered, so that we may consider or use the technique as a form of spectroscopy.

To put the foregoing in another way, we may raise the idea, with which we are all familiar, that the frequency-dependent absorption of ultra-violet, visible, and infra-red light may be used in the analysis of biological (and other) materials. Yet light is only a form of electromagnetic radiation, albeit of a rather high frequency (10^{14} Hz or so), and there is thus no reason why the frequency-dependent absorption of electrical energy of *lower* frequencies might not similarly be exploited in bio-analytical devices. In such cases, at least below 30 MHz or so, one requires electrodes to act as an interface between the exciting electrical field and the sample, so that, as in the 'pure' electrochemical case above, one may study the frequency-dependent, passive electrical properties of the system consisting of the electrodes *plus* the biological sample; in other words, one may study the frequency-dependent

impedance or admittance of the system.

In the following, therefore, I shall (1) outline in very elementary terms what is meant by the concepts of electrical impedance and admittance, (2) discuss the application of such measurements in (predominantly non-faradaic) electroanalysis, and (3) introduce the cognate concept of the dielectric spectroscopy of biological substances. These considerations will pave the way for (4) a discussion of the use of AC techniques, including frequency response analysis (FRA), in biosensor applications *sensu lato*. Because of the relative magnitudes of the topic and the space available, I will make no attempt to be comprehensive; my aim will be predominantly to provide, for the general reader, an introduction to a field which I believe has been widely neglected by biologists and biophysicists (despite its many spectacular successes), yet which underlies a great many present and future biosensor applications.

24.2 Electrical impedance and admittance

Let us consider a sinusoidally modulated voltage, of the form $V = V_m \sin \omega t$, where ω is the frequency in radians s^{-1} ($\omega = 2\pi f$, where f is the frequency in Hz), V_m is the maximum (peak-to-peak) voltage, and V the voltage at any given instant. If this voltage appears across the terminals of a passive circuit, device, or 'system', which may consist of pure electrical components or of a biological or chemical sample separating a pair of electrodes, the current flowing in the circuit (after any transients have died down) may be related to the voltage both by its *magnitude* and its *phase*, and is of the form $i = i_m \sin(\omega t + \theta)$. Thus (Fig. 24.1a), although the *frequency* and *sinusoidal nature* of the wave-form are unchanged by interaction with the system, the characteristics of the system are reflected in the ratio V_m/i_m and by the value of θ .

Now, systems may exhibit resistive, capacitive, and inductive properties, properties which (by definition) may be distinguished from each other by their effects upon a sinusoidal voltage. Thus, for a pure resistor (R Ohms), the current due to our exciting waveform ($V_m \sin \omega t$) is given by:

$$i = (V_m/R) \sin \omega t. \quad (24.1)$$

For a pure capacitor (C farads):

$$i = \omega C V_m \sin \left(\omega t + \frac{\pi}{2} \right) \quad (24.2)$$

whilst for a pure (self-) inductance of L henries:

$$i = (V_m/\omega L) \sin \left(\omega t - \frac{\pi}{2} \right). \quad (24.3)$$

Thus, for a pure resistor, there is no phase difference between V and i . In

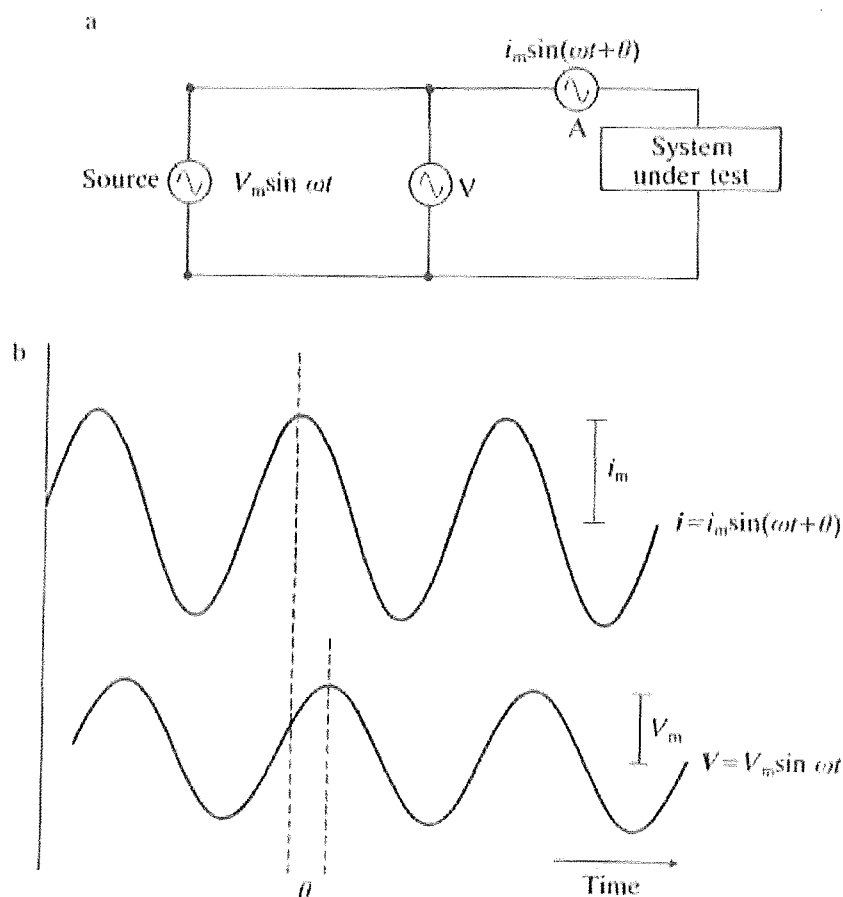


Fig. 24.1 (a), The impedimetric experiment, in which a small-amplitude perturbation, in the form of a sinusoidal voltage, is applied to the system of interest. The sinusoidal voltage across the system may be measured using a (high-impedance) AC vector voltmeter, V , whilst the sinusoidal current flowing in the circuit may be measured by means of an AC vector ammeter A . In practice (a, b), it is found that the phase of the current differs from that of the voltage by an amount θ ; in the case shown it *leads* the voltage.

contrast, for a pure capacitor, the current *leads* the voltage by $\pi/2$ radians (90°) whilst for a pure inductor the current *lags* the voltage by the same amount. Now, except in active biological systems such as nerve axons (e.g. Cole 1972; Jack *et al.* 1975; De Felice 1981), and in *certain* electrochemical systems, particularly those involving corrosion and electro-deposition (Gabrielli 1980; Macdonald and McClure 1982; Gabrielli *et al.* 1983), inductances are negligible, and we shall for the most part ignore them. We may therefore imagine intuitively (and correctly) that for a 'real' system, which possesses both resistive *and* capacitive properties (i.e. behaves as a leaky capacitor), θ takes a value between 0 and $\pi/2$, as illustrated in Fig. 24.1b.

We may then define a vector quantity Z , the *impedance*, with modulus

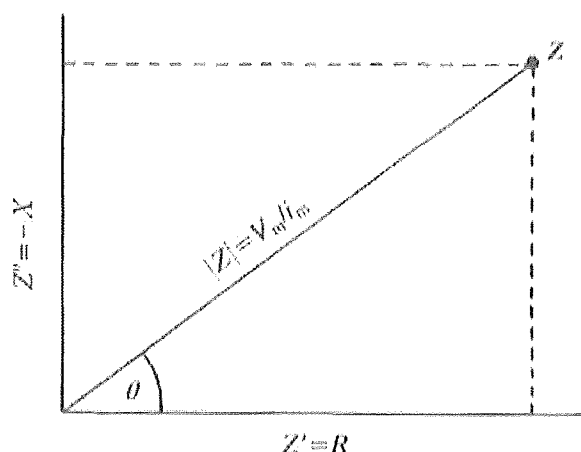


Fig. 24.2 Impedance as a complex quantity. There is a mathematical function, known as Euler's identity, which states that $Ae^{\pm j\theta} = A\cos\theta \pm Aj\sin\theta$, where $j = \sqrt{-1}$. Thus, any complex quantity may be split up into its real and imaginary part. The figure shows the manner in which this is done for the impedance function $Z = R + jX$. Simple geometrical considerations indicate (i) that $Z^2 = R^2 + X^2$, and (ii) that $R = |Z| \cos\theta$ and $X = -|Z| \sin\theta$. Thus R and X may be obtained from measurements of $|Z|$ and θ , and are known respectively as the 'in phase' and (90°) 'out-of-phase' components.

(magnitude) $|Z|$ and argument ('direction') θ , in a form analogous to that of a complex number $a + jb$ (where $j = \sqrt{-1}$) as in Fig. 24.2, where the modulus $|Z|$ of the impedance is equal to the ratio V_m/i_m . Thus, the impedance has both real and imaginary parts, and is defined as $Z = R + jX$, where the reactance $X = -1/\omega C$, and the system is treated as though it consisted of a resistance and capacitance in *series*.

We may also treat the system as consisting of an equivalent conductor (G siemens = $1/R'$ S) and capacitor (C') in *parallel*. In this case, we define an *admittance* Y , as a vector with modulus $|Y| = i_m/V_m = 1/|Z|$ and argument θ , such that $Y = 1/Z = G + jB$, where B , the susceptance, $= \omega C'$.

As succinctly stated by Falk and Fatt (1968), the distinction between the two sets of treatments is as follows: in the impedance representation, we take the impedance to represent the dependence of the voltage on the current, the terminals of the system under study (in an arrangement such as that of Fig. 24.1a) being considered as being connected to a current source of infinite resistance (i.e. open circuited). In contrast, in the admittance representation we take the admittance to represent the dependence of the current on the voltage, the terminals being considered as being connected to a voltage source of zero resistance (short-circuited).

Since the above distinctions are only distinctions in the way we *treat* the sample, it is obvious that we can move from the impedance to the admittance

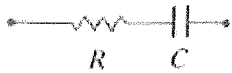
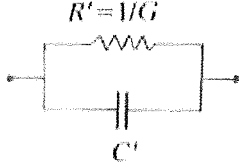
Series	Parallel
Impedance $Z = R + jX$	Admittance $Y = \frac{1}{Z} = G + j\omega C'$
Resistance $R = \frac{G}{G^2 + (\omega C')^2}$	Conductance $G = \frac{R}{R^2 + X^2}$
Reactance $X = \frac{-\omega C'}{G^2 + (\omega C')^2} = \frac{-1}{\omega C}$	Susceptance $B = \omega C' = \frac{-X}{R^2 + X^2}$
	

Fig. 24.3 The relationships between impedance and admittance, and their real and imaginary components. For discussion, see text.

domain, and *vice versa*, by the choice of appropriate values of R , C , G , and C' . For convenience, we give the relevant equations in Fig. 24.3. In other words, regardless of the *actual* complexity of (the equivalent electrical circuit of) the system between the terminals of the measuring instrument, when we make measurements at a given frequency, we merely *treat* the system as though it consists of a single resistance (conductance) in series or in parallel with a single capacitance. For real circuits, then, the impedance $Z(\omega)$ or admittance $Y(\omega)$, and their component real and imaginary parts, are *frequency-dependent quantities*, the frequency-dependence of which may be used to describe the *actual* equivalent electrical circuits. It should be noted that, by definition, the impedance and admittance are independent of the voltage across, and current flowing in, the system under study, and this 'linear property' should be taken into account when use is made of these representations.

In general, the most convenient means by which we can extract the magnitudes and topological relationship of the components constituting the equivalent circuit is by means of complex plane diagrams, a topic to which we now turn.

24.3 Impedance diagrams

If we make measurements of the frequency-dependent impedance of an electrical circuit consisting of a 2.671 k Ω resistor in parallel with a 220.4 pF

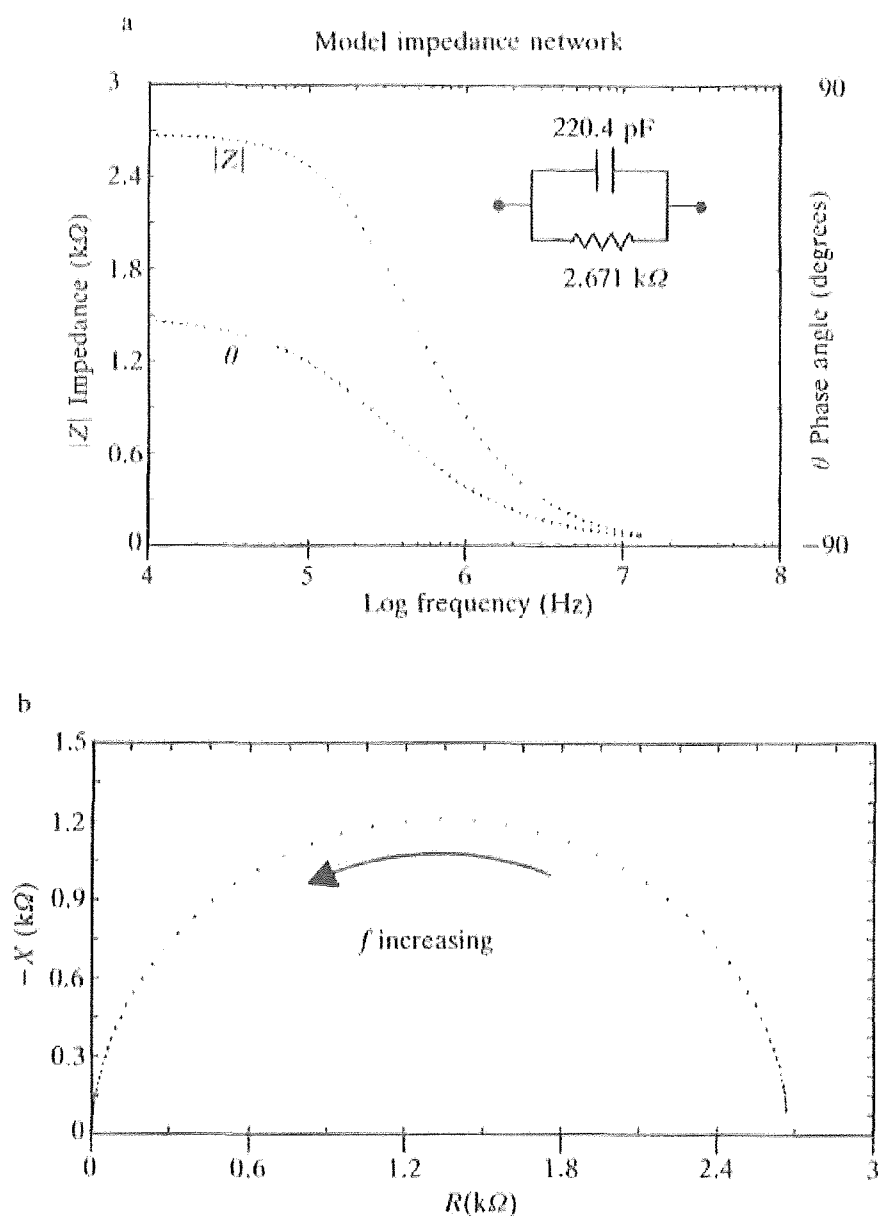


Fig. 24.4 The frequency-dependent impedance of a model electrical circuit. Measurements were made, and the data plotted, using the frequency-domain impedimetric system described by Harris and Kell (1983). (a) The impedance modulus and the phase angle as a function of the frequency. Note the existence of two plateau regions in frequency ranges that are respectively low and high relative to that of f_c . (b) A reactance/resistance plot, showing that the circuit has a single time constant. For further discussion, see text.

capacitor, the behaviour shown in Fig. 24.4 is obtained. (Remember that although the circuit is *actually* a parallel network, we *treat* it, in the impedance representation, as though components were connected in series.) Thus (Fig. 24.4a), as the measuring frequency is increased, we find (i) that the phase angle (θ , as defined in Fig. 24.1), decreases from approximately 0°

(purely resistive behaviour) to approximately -90° (purely capacitive or reactive behaviour), and (ii) the modulus of the impedance $|Z|$ decreases from roughly $2.67\text{ k}\Omega$ to roughly zero. The frequency at which the transition is half completed, the so-called critical or characteristic frequency f_c , may be seen, by inspection of Fig. 24.4a, to occur at approximately 300 kHz . Since the product of a resistance and a capacitance has the dimensions of time (seconds), and is equal by definition to the relaxation time τ ('time constant') for such a circuit, and since $\tau = 1/2\pi f_c$, we may also calculate τ ($5.89 \times 10^{-7}\text{ s}$) and f_c (270 kHz) simply from the values of the resistor and capacitor in the circuit.

Now, as shown in Fig. 24.2, we can calculate the real (R) and imaginary (X) parts of the impedance from the measured values of $|Z|$ and θ , and (since these change with frequency) plot the negative reactance against the resistance with frequency as the parameter. This is done in Fig. 24.4b, where it may be observed that the resultant plot takes the form of a semicircle, whose centre would lie on the abscissa and which has a maximal value of $-X$ which occurs (*cf.* Figs. 24.4a, 24.4b) at the characteristic frequency; further, had measurements been made over a wider frequency range, it is evident (or at least plausible) that the semicircle would have extrapolated to values of 0 and $2.67\text{ k}\Omega$. Thus, as discussed in many introductory textbooks of electrical circuit analysis (e.g. Bleaney and Bleaney 1976; Duffin 1980; Bobrow 1981; Brown *et al.* 1982; Harter and Lin 1982), these impedance diagrams reflect, and may be used to obtain, the values of the elements of an equivalent electrical circuit.

Using the equations given in Fig. 24.3, one may also derive from Fig. 24.4a the equivalent values of G and B pertinent to a representation in the admittance domain. In this case, a plot of B versus G (an admittance diagram) would also give a semicircle, with its centre on the abscissa and the maximum value of B when the exciting frequency $= f_c$. The production of such a plot is left as an exercise for the reader. As we shall also see when we come to consider complex conductivity and permittivity, although the information contained in each plot is the same, the relative *weightings* of the data can serve to enhance different frequency regions (Macdonald *et al.* 1982).

24.4 Impedance diagrams in electrochemical systems

For a variety of historical and other reasons, the impedance (R/X) representation has dominated the electrochemical literature, although J. R. Macdonald and his colleagues (e.g. Macdonald 1980; Macdonald *et al.* 1982) have stressed the utility of the three-dimensional perspective $R/X/\log f$ plot. Now, the general aim in studies of purely electrochemical and, in many cases, of solid-state (as opposed to biological), impedances is to gain information

about the mechanisms of electrode processes, i.e. of processes occurring at the electrode/electrolyte interface. Thus, since such processes are obviously dependent upon the 'mean' potential of the working electrode, one should arrange to poise this potential at a known value, either by including both pairs of a redox couple of known E_0' in the medium (faradaic impedance) or electronically. In the latter case in particular, it is usual to use a three-electrode system (Bard and Faulkner 1980; Bond 1980; Gabrielli 1980). In such two- or three-electrode measurements, of course, one should either use identical electrodes or make the impedance of the working electrode very much greater than that of the counter electrode.

The interpretation of electrochemical impedances is a vast, detailed, and complex field, and for the present purposes I shall merely give the simplest possible description of the salient ideas. These are: (i) that the electrical double layer (e.g. Mohilner 1966; Bockris and Reddy 1970; Sparnaay 1972; Martynov and Salem 1983) at the electrode/solution interface possesses, due to its molecular thickness, a significant capacitance (of some μF per cm^2 actual electrode area, under typical conditions) which must be charged up before any faradaic current can flow; (ii) that the rate of the subsequent reaction may be limited by a charge transfer step, by diffusion of electro-active reactant to the reaction layer, or by both, in which latter case one finds the superposition of a straight line and a semicircle in the R/X plot; (iii) that the residual resistance at very high frequencies represents the resistance of the bulk solution between the electrodes; (iv) the diffusional impedance is often

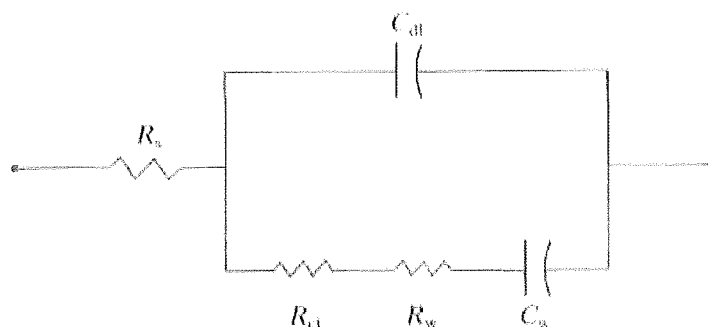


Fig. 24.5 Very general equivalent circuit for an electrochemical cell. The double layer capacitance C_{dl} is in parallel with a resistance representing the charge transfer (faradaic) step, since geometrically they occur in (essentially) the same place. This structure is in series with a 'Warburg' impedance Z_w , comprised of resistive and capacitive parts, equivalent in essence to the 'diffusion zone'. Finally, the whole arrangement is in series with the (' iR drop') bulk electrolyte solution resistance R_s . Obviously the actual magnitude of these components determines the exact frequency response of the system. The symbols used for the capacitors are to indicate the presence of some heterogeneity in the structures which they represent.

referred to as the Warburg impedance Z_w , and represented as a resistor and capacitor in series. The equivalent electrical circuit describing this behaviour, and which is usually ascribed to Randles (1947), is given in Fig. 24.5; it may be noted that we are here beginning to equate our electrical circuit components with mechanistic explanations of electrode behaviour.

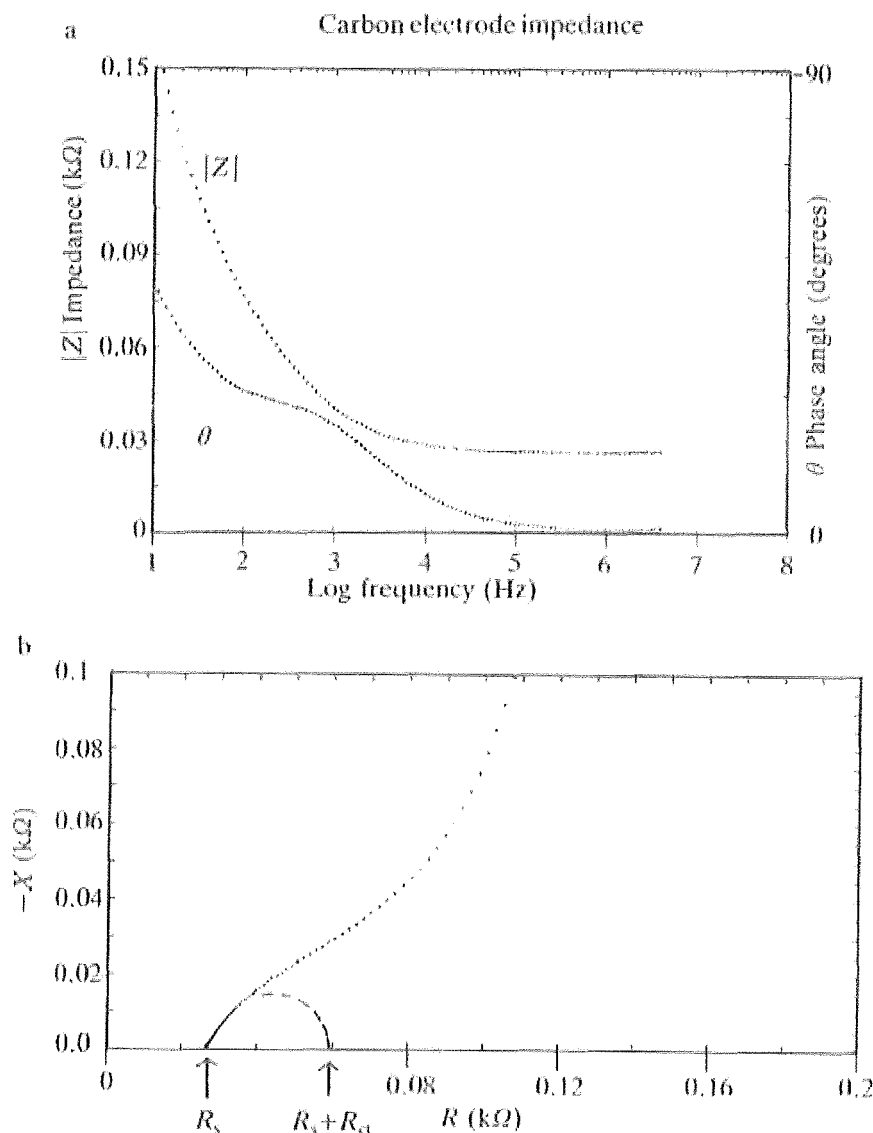


Fig. 24.6 The frequency-dependent impedance of a pair of graphite electrodes immersed in 100 mM KCl. The modulating voltage was 50 mV and measurements were performed using the apparatus described by Harris and Kell (1983). (a) Impedance modulus and phase angle versus logarithmic frequency. (b) Impedance diagram, showing how one may derive the values of R_s and R_{ct} from the semicircular portion of such a plot (Hung *et al.* 1979). In a classical Warburg-type system, the low-frequency (right hand) part of the impedance locus should make an angle of 45° with the abscissa. The characteristic frequency of the semicircular part of the plot may be used to obtain the values of C_{dl} from the relation $C_{dl} = 1/2\pi f_c R_{ct}$.

Now, it should be stressed that much more complicated behaviour than the above may be observed in practice. Nevertheless, Fig. 24.6 shows the experimentally obtained impedance diagram of a pair of cylindrical graphite electrodes (*ca.* 4 mm radius, 20 mm length, surface roughness unknown, separation 10 mm) immersed in 100 mM KCl, a diagram which, it may be observed, corresponds fairly accurately to the behaviour described above (and see Besenhard and Fritz 1983). The following points may be made with respect to this figure: (1) the semicircular locus is by no means perfect, and is poorly separated from the straight line portion, and it is not realistic to fit it such that its centre lies on the abscissa — this may be ascribed to heterogeneity in the structures underlying C_{dl} and R_{ct} ; (2) the frequency dependence of the impedance extends over an enormous range — at least seven orders of magnitude in the present case; (3) there is no frequency dependence (in this range) of the impedance of the material *between* the electrodes (which is simply an ionic solution) — all the observed frequency dependence is caused by electrochemical behaviour *at the electrodes*.

Now, it is obvious that the measured resistance and reactance of our electrochemical cell is a function of the electrode size and geometry; electrodes of larger area and closer separation will, all else being equal, appear to have a lower impedance. Since, in many cases, it is the *intensive* properties of the system which are of interest, we must needs take account of this; to do so we will make use of the admittance representation (Fig. 24.7), and introduce the notions of permittivity and conductivity.

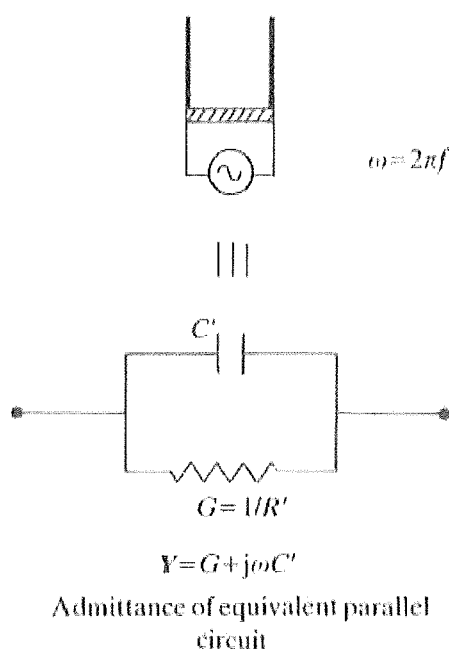


Fig. 24.7 At any given frequency, the passive electrical properties of a system may be completely described by the admittance $G + j\omega C'$ of the equivalent parallel circuit.

24.5 Permittivity, conductivity and dielectric dispersion

For a specimen held between two parallel electrodes of area A separated by a distance d , the intrinsic passive electrical properties are completely specified by the conductivity σ' and the permittivity ϵ' , which are related to the measured conductance G and capacitance C' by the equations:

$$G = \sigma' (A/d) \quad (24.4)$$

$$C' = \epsilon' \epsilon_r (A/d) \quad (24.5)$$

From eqn 24.4, we find that to obtain the conductivity, we multiply the measured conductance by d/A , a factor which has the dimensions of length⁻¹ (e.g. cm⁻¹) and is known as the cell constant. In eqn 24.5, ϵ_r (sometimes called ϵ_0 in the literature) is the capacitance of a cell of unit dimensions containing a vacuum, equal to 8.854×10^{-14} F/cm, so that any matter existing between the electrodes will have the effect of raising the capacitance by a factor ϵ' , a factor which was formerly called the dielectric constant, but (since it is not constant) is more properly referred to as the permittivity. The permittivity of water at 25°C is approximately 78.4, so that, as may be calculated from eqns 24.4 and 24.5, a cell of cell constant 1 cm⁻¹ containing water at this temperature will have a capacitance of 6.94 pF. The presence of ionic electrolytes has only a rather modest effect upon the permittivity of aqueous solutions, such that the permittivity of 1 M NaCl at 25°C is approximately 61.6 (Davies 1965).

Now, for many purposes, it is useful to make use of the complex permittivity $\epsilon^* = \epsilon' - j\epsilon''$, which, as with impedance and admittance, has both real and imaginary parts, and the imaginary part of which, the dielectric loss ϵ'' , is related to the conductivity by the equation

$$\epsilon'' = \frac{\sigma' - \sigma'_l}{2\pi f \epsilon_r} \quad (24.6)$$

where σ'_l represents any DC or 'low frequency' contribution to the conductivity.

In a given frequency range, the dielectric properties of any material between the electrodes may not be constant (i.e. the material exhibits dielectric dispersion), and, as with the impedance of the model circuit in Fig. 24.4, may change between two 'plateau' values ϵ'_l and ϵ'_∞ , according to the equation

$$\epsilon^* = \epsilon'_\infty + \frac{\epsilon'_l - \epsilon'_\infty}{1 + j\omega\tau} \quad (24.7)$$

where, as before, $\tau (= \frac{1}{2\pi f_c})$ is the relaxation time. Equation 24.7 separates into

$$\epsilon' = \epsilon'_\infty + \frac{\epsilon'_l - \epsilon'_\infty}{1 + (\omega\tau)^2}, \quad (24.8)$$

$$\epsilon'' = \frac{(\epsilon'_L - \epsilon'_\infty)\omega\tau}{1 + (\omega\tau)^2}, \quad (24.9)$$

and a plot of ϵ'' versus ϵ' gives a circle whose centre is located on the ϵ' axis. However, in practice it is often observed that semicircles result whose centre lies below the abscissa, and it was shown by Cole and Cole (1941) that this behaviour may be described by an equation of the form

$$\epsilon^* = \epsilon'_\infty + \frac{\epsilon'_L - \epsilon'_\infty}{1 + (j\omega\tau)^{1-\alpha}} \quad (25.10)$$

such that a line between the centre of the circle and the points at which the ϵ''/ϵ' locus crosses the abscissa makes an angle $\alpha\pi/2$ radians with the abscissa. Although the Cole-Cole representation is entirely empirical (it is generally taken to represent some kind of distribution of relaxation times), it is now commonplace to express data in the form of a Cole-Cole plot, such that the dispersion is characterized by the 'dielectric increment' $\Delta\epsilon' = \epsilon'_L - \epsilon'_\infty$ and by the Cole-Cole α . Many other dielectric relaxation time distributions have been suggested (reviewed by Boyd 1980 and see Marshall and Roe 1978), but they have not achieved widespread usage in biological systems, and are not discussed further here.

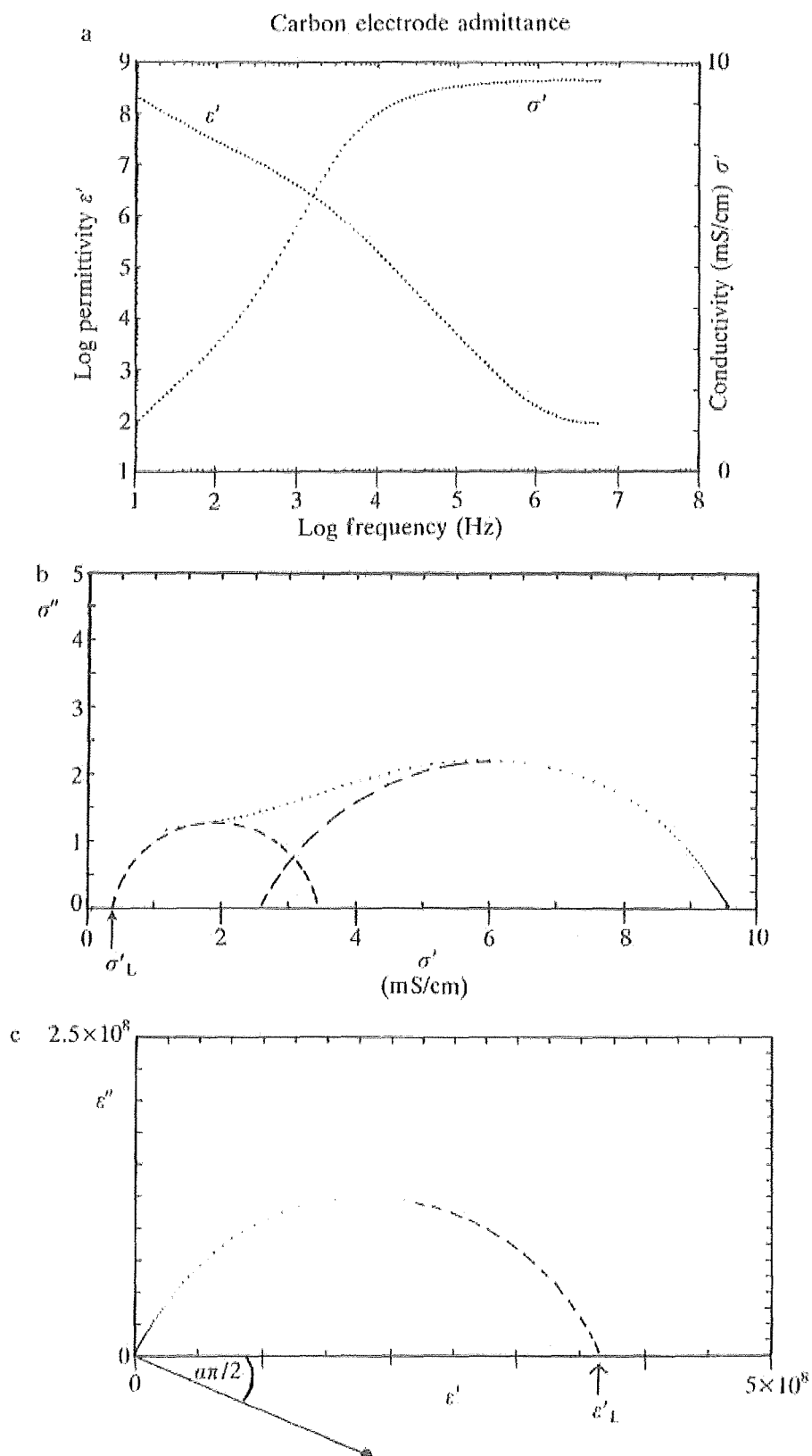
Complementarily, one may make use of the 'complex conductivity' plot of σ'' versus σ' , where

$$\sigma'' = 2\pi f\epsilon'_L(\epsilon' - \epsilon'_\infty). \quad (24.11)$$

As discussed above, the two representations have the effect of weighting the appearance of the data differently; I will illustrate this by using (in Fig. 24.8) the data (Fig. 24.6) from the carbon electrode impedance spectrum.

As Fig. 24.8a shows, the apparent permittivity of the system at low frequencies reaches truly enormous values (2×10^8 at 10 Hz), the measured capacitance at this frequency being approximately 70 μF , an effect which forms the basis of the 'electrolytic' type of capacitor used in electrical and electronic circuits. Of course, the permittivity of the electrolyte between the electrodes is only about 78, and, if we use this value for the 'high frequency'

Fig. 24.8 Admittance properties of carbon electrodes. Data were obtained as described in the legend to Fig. 24.4. (a) Conductivity and permittivity, as obtained from the measured capacitance and conductance by means of the cell constant. (b) Admittance (complex conductivity) plot, using $\epsilon'_\infty = 78.4$. The fit of the two semicircles is empirical, there being no satisfactory way (in the absence of additional knowledge) of separating overlapping dispersions. Extrapolation gives σ'_L , whence $\Delta\sigma'$ for each dispersion may be obtained. (c) Complex permittivity (Cole-Cole) plot, using the value of σ'_L obtained in b, and illustrating the estimation of the Cole-Cole α and the extrapolation (to low frequencies) by which one may obtain ϵ'_L . It may be



noted that two dispersions are *not* discernible in this diagram, illustrating how the admittance and complex permittivity plots weight the data differently. Note that, for a given dispersion in which the Cole-Cole α is not too large, τ is given by $\Delta\epsilon'\epsilon_i/\Delta\sigma'$.

permittivity, we obtain the admittance plot shown in Fig. 24.8b; as in the impedance diagram (Fig. 24.4), two separate processes may be discerned, extrapolation of the latter to low frequencies giving the value of σ_t' (0.4 mS/cm) to be used in constructing the Cole–Cole plot in Fig. 24.8c. As stressed by Macdonald *inter alia*, the Cole–Cole plot is not really suitable for use in describing *electrochemical* impedances since permittivity and conductivity are *intrinsic* properties of materials which may be held between the electrodes, and this should be borne in mind. However, the representation in Fig. 24.8 serves to illustrate the means by which we treat data of this type, and it is hoped that this elementary exposition will assist the novice or tyro who may wish to delve further into these matters. For completeness, it should be mentioned that some literature, particularly that concerned with electrical insulators, specifies the so-called ‘dissipation factor’, $D = \tan \delta = \epsilon''/\epsilon'$. For materials lacking DC conductivity, $D = G/\omega C = 1/Q$, where Q is the so-called quality factor or Q -factor.

We are now more or less in a position to consider some of the mechanistic bases for the frequency-dependent electrical behaviour of systems held between electrodes and which consist not only of ionic solutions but of biological materials. However, the dielectric (passive electrical) properties of biological and chemical (Stock 1984) substances have attracted study for a great many years (e.g. Osterhout 1922), both from a scientific and an analytical standpoint. Thus, for instance, Stewart (1899*a*) noted that the low-frequency conductivity of blood plasma exceeded that of the whole blood from which it had been derived by an amount that was a monotonic function of the haematocrit, and derived an equation wherewith to estimate the latter by means of conductivity measurements. Since this time, a vast and increasing literature on biological impedances has accumulated, an amount far too great adequately to be reviewed herein, and what I shall therefore do is: (i) draw attention to the many excellent books, review articles, and monographs on the subject of the dielectric spectroscopy of biological substances, (ii) outline the salient observable and mechanistic features of the dielectric dispersions that have been described in biological systems, and the relationships between the dielectric increment and the effective molecular dipole moments underlying the dispersions, and (iii) describe some of the analytical methods and devices that have been used or proposed, and which have as their basis the measurement of conductivity, permittivity, or their vector sum. I shall then outline some of the technical and methodological aspects which should be borne in mind when one considers making measurements of biological impedances, and draw attention to the distinctions one may make between measurements in the time and frequency domain. This will lead us to an outline of the role of time series analysis in biosensing generally. Finally, I shall seek to bring together the ideas and facts described above in suggesting some novel approaches to the design and exploitation of biosensors.

24.6 Dielectric spectroscopy of biological substances

Of the many books available on the dielectric behaviour of condensed matter, those of most biological relevance, and which are especially recommended, are by Daniel (1967), Cole (1972), Hasted (1973), Grant *et al.* (1978), Schanne and Ceretti (1978) and Pethig (1979). Schwan, the doyen of biological impedance determinations, has written several excellent reviews (e.g. Schwan 1957, 1963, 1977, 1981*a, b*, 1983*a, b*; Schwan and Foster 1980; Stoy *et al.* 1982), and overviews of these matters may also be found in the review articles of Salter (1979), Pilla (1980; Pilla *et al.* 1983), Zimmermann (1982) and Pethig (1984). The latter gives an extensive discussion of measurements on proteins, which are also discussed in the reviews by Oncley (1943), Takashima (1969; Takashima and Minakata 1975), Grant and South (1972; Grant 1982, 1983), Petersen and Cone (1975), Wada (1976), Hasted *et al.* (1983), Kell and Hitchens (1983) and Kell and Westerhoff (1985). Our own work (Harris and Kell 1983; Kell 1983; Harris *et al.* 1984; Harris and Kell 1985*a*; Kell and Harris 1985*a, b*) has concentrated on microbial membranes, the latter two articles containing a fair amount of review material on this topic. Work with natural (Pauly and Packer 1960; Pauly *et al.* 1960; Falk and Fatt 1968; Irimajiri *et al.* 1979; Asami *et al.* 1980*a*, 1984) and pure phospholipid membrane vesicles (Schwan *et al.* 1970; Redwood *et al.* 1972; Asami and Irimajiri 1984; Pottel *et al.* 1984) and planar membranes (Hanai *et al.* 1964, 1965; Tien 1974; Fettiplace *et al.* 1975; Haydon *et al.* 1977; and Laver *et al.* 1984) may also be cited, whilst an entrée to the microbial literature may also be gained from the papers of Pauly (1962), Asami *et al.* (1976, 1980*b*), Clarke *et al.* (1984, 1985), Blake-Coleman *et al.* (1984), and Harris and Kell (1985*b*). Almost all charged polyelectrolytes exhibit enormous permittivities at low frequencies (e.g. Dukhin and Shilov 1974; O'Brien 1982), whilst those displayed by DNA are discussed at some length in the articles of Vreugdenhil *et al.* (1979) and Sorriso and Surowiec (1982). Most of the papers cited in this section concern work at frequencies below 100 MHz or so; the higher frequency work, with which we have not had experience to date, is discussed by Foster and Schepps (1981), Foster *et al.* 1982, Illinger (1981), Stuchly *et al.* (1981), Clegg *et al.* (1982), Kraszewski *et al.* (1982), Gabriel *et al.* (1983), Magin and Burdette (1983) and Clegg *et al.* (1984). This extensive citation list indicates very clearly the great breadth and depth of literature on biological impedance determinations. What take-home messages may one distil from this work?

In general, it has become usual to point out that biological cells and tissues exhibit three broad and more-or-less separable dielectric dispersions, centred respectively in the audio-, radio- and UHF-frequency regions and referred to as the α -, β - and γ -dispersions. Subsidiary δ - and β_1 -dispersions, located between the β - and γ -dispersions, may also be noted, especially in protein

solutions (Essex *et al.* 1977; Grant 1982), whilst a low-frequency μ -dispersion was described by Kell (1983), Harris *et al.* (1984), and Harris and Kell (1985a). The major mechanisms thought to underlie these dispersions are as follows: relaxation of the ion cloud tangential to charged membrane surfaces (α -dispersion); Maxwell-Wagner-type relaxation at the interface between the poorly conducting cell membranes and their adjacent aqueous solutions (β -dispersion); rotation of small charged and/or dipolar molecules (γ -dispersion); relaxation of tissue-bound water (δ -dispersion); protein rotation (β_1 -dispersion) and diffusional movements of membrane-associated components (μ -dispersion). Where applicable, the superposition principle states that each of these mechanisms is independent and additive, and we would stress that *any* potential charge or dipole mobility will lead to the existence of a dielectric dispersion. In this sense, dipole rotations are electrically indistinguishable from any other motions such as the hopping of charges between different sites (Jonscher 1975; Lewis 1977; Ngai *et al.* 1979), and it is therefore obvious that a plethora of molecular mechanisms can in fact underlie the relatively broad dielectric dispersions observed in practice.

As discussed above, we can describe or characterize a dielectric dispersion by its dielectric increment, its 'mean' relaxation time and by the extent of distribution of the relaxation times as embodied in the Cole-Cole α . Now, the dielectric increment may be said to constitute the outward and visible sign of a molecular property, the dipole moment (or, for hopping of charges, etc, the *effective* dipole moment), μ . Dipole moments are traditionally measured in debyes (D), where $1\text{D} = 3.33 \times 10^{-30} \text{ C m}$; in other words, since the unit electrical charge $= 1.6 \times 10^{-19} \text{ C}$, a pair of charges of opposite sign separated by 10^{-10} m (1 \AA) have a dipole moment of 4.8 D. It is the molecular dipole moment that serves to tell us what fraction of the dipoles are *actually* responding at a given field strength, according to the Langevin function (Fig. 24.9)

$$L(x) = \coth(x) - 1/x \quad (24.12)$$

where $x = \mu E_l / kT$, E_l is the local electrical field, k is Boltzmann's constant, and T is the absolute temperature. Since, in complex biological systems especially, we are likely to know only the macroscopic field (i.e. the peak potential difference between the electrodes divided by the distance between them) rather than the local field, it is appropriate to use the former and to add an empirical constant. For the rotation of aqueous globular proteins with a permanent dipole moment, we use the factor $H = 5.8$ (obtained from a comparison between theory and experiment for the amino acid glycine) (Oncley 1943), and we have:

$$\mu = \sqrt{(9000 kT \Delta\epsilon / 4\pi NHC)} \quad (24.13)$$

where N = Avogadro's number and C is the *molar* protein concentration.

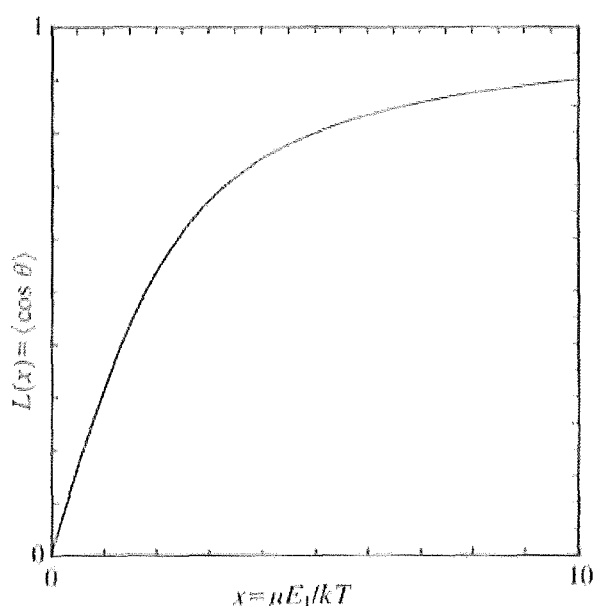


Fig. 24.9 The Langevin function. This relates the average angle between the field (at a frequency low relative to that of f_0) and the (effective) dipole of interest $\langle \cos \theta \rangle$ to the field strength and effective molecular dipole moment $\mu E_1 / kT$, where E_1 is the local field, k is Boltzmann's constant and T the absolute temperature. For $x (= \mu E_1 / kT) < 1$, the Langevin function reduces to $\langle \cos \theta \rangle = \mu E_1 / 3 kT$, and the dielectric increment is independent of the field strength (i.e. we are in the linear region), the number of particles *actually* moving in response to the field being proportional to E_1 .

The magnitudes of the dipole moments of protein solutions observed in practice are equivalent roughly to 1–15 relative permittivity units per (g/100 ml), corresponding to roughly 5–20 D per kilodalton (e.g. Gerber *et al.* 1972; Schwan 1981*a*). One may therefore calculate that, in a typical dielectric experiment in which the field strength is most unlikely to exceed 0.5 V/cm, and is likely to be as little as 1/10 of this, the Langevin function has a very small value, such that the number of proteins *actually* rotating is in fact an extremely small fraction of the total. We shall have cause to return to this point later.

To summarize the discussions in this section as they relate to our overall considerations, we may make the following remarks: (1) there is an enormous literature indicating that all types of cells, tissues, and bio-molecules possess dielectric properties different from those of simple ionic solution; (2) especially since dielectric spectroscopy is a non-invasive technique, one may exploit it to assay for the former in the presence of the latter; (3) because of the strong frequency-dependence of dielectric properties, one may assay for different substances or features by choosing different frequencies; (4) in such cases, a consideration of where the field lines go is likely to prove informative; (5) because of the relative insensitivity of the

technique, and the breadth of the spectra obtained (which reflect relaxation rather than resonance), it is likely to be most useful in 'bulk' measurements when practised conventionally. From a bioanalytical standpoint, one must also add that, especially at low frequencies, one is likely also to be measuring the electrode properties, in addition to those of the material between the electrodes, although this does not of itself impair the potential analytical utility of the method. I would also mention that a recent and otherwise excellent book *Biological Spectroscopy* (Campbell and Dwek 1984) did not even mention the concept of dielectric spectroscopy, a rather clear indication that indeed the method is ripe for exploitation.

In this vein, therefore, I turn to a discussion of some of the articles which have sought to use the principles described herein in analytical devices.

24.7 Some bioanalytical uses of conductimetry and impedimetry

Obviously this is a vast topic as well, and I shall therefore aim for some selectivity in choosing the examples with which I shall draw attention to the use of these methods. One particular use, which is attracting increasing attention (see Firstenberg-Eden and Eden 1984; Harris and Kell 1985*b*), is in the exploitation of impedimetry in assessing the numbers of micro-organisms present in sparse populations, since changes in the electrical properties of microbial culture media have been known to be associated with microbial growth since the last century (Stewart 1899*b*). Conductimetry (e.g. Richards *et al.* 1978; Mackey and Derrick 1984), impedimetry (e.g. Cady 1978) and capacitimetry (Firstenberg-Eden and Zindulis 1984) have all been used (Firstenberg-Eden and Eden 1984); in the latter case especially, the micro-organism-dependent changes are due to effects at the electrodes (Hause *et al.* 1981), since any micro-organism-dependent changes in the bulk permittivity would here be negligible.

Since the electrical conductivity (at frequencies below that of the Maxwell-Wagner type β -dispersion) of a suspension is lower than that of the fluid in which it is suspended, one may thus detect the presence of suspended matter directly by its effects upon an electrical field. Such measurements have been made both in bulk suspension (see for example Irimajiri *et al.* 1975; Harris and Kell 1983; Lovitt *et al.* 1986) and in hydrodynamically focused flowing streams in devices based upon the principle of the Coulter CounterTM (e.g. Kubitschek 1969; Dow *et al.* 1979). Clarke and his colleagues (Blake-Coleman *et al.* 1984; Clarke *et al.* 1984, 1985) have also successfully applied impedimetry to the direct assessment of microbial biomass, and our own studies and those of others (*op. cit.*) have indeed shown that the dielectric properties of cells of a given radius scale monotonically with the volume fraction of the suspended phase.

As regards the possibilities of *distinguishing or identifying* cells by their

frequency-dependent dielectric properties, it is certainly true that both the size and surface charge (density), *inter alia*, differ for different bacteria. For instance, Gram-positive and -negative bacteria have entirely different α -dispersions (e.g. Harris and Kell 1985a). However, size and surface charge depend critically on both the pH and physiological status (e.g. growth rate) of micro-organisms, and simple dielectric spectra are unlikely to contain enough information, in the absence of other tests, to be diagnostic. Similarly, in non-axenic cell suspensions, the dielectric properties of the largest cells will tend to dominate those of the suspension, so that deconvolution, already difficult, would probably be impossible in all but the most favourable cases. However, I see no reason in principle why the Coulter CounterTM method should not be extended to exploit measurements of the *frequency-dependent* electrical properties of individual cells. In particular (and see later), the magnitude of the electrical fields used would allow one to make use of the *non-linear* electrical properties of cells, properties which may be expected to be far more cell-specific than simple linear behaviour might lead one to suppose. Thus, although I do not see that the dielectric spectroscopy of microbial cell suspensions is likely to be diagnostic of the *specific* micro-organism (measurement of colonies might be more productive), the use of more advanced techniques does hold out some promise for the characterization of unknown cells. However, since published dielectric spectra of microbial cells do not cover more than ten species (of unknown physiological status) to date, much more work is required before one may make an adequate assessment of the many exciting possibilities in this area.

Other techniques exploiting the bulk permittivity, conductivity, or impedance of cells and tissues, and which have enjoyed a reasonably widespread use, include impedance plethysmography (e.g. Nyboer 1970; Wheeler and Penney 1982; Brown 1983; Anderson 1984) and pneumography (Pacula 1966; Henderson and Webster 1978), whilst measurements of the dielectric properties of excised tissue have been used in the testing of freshness (Faure *et al.* 1972; To *et al.* 1974; Kent 1975; Kent and Jason 1975), and quality (Pfutzner and Fialik 1982) of foods. As regards tissue measurements, it may also be mentioned that there are significant local decreases of skin impedance in the area of the meridian points recognized as significant in the science of acupuncture (e.g. Becker and Marino 1982; Jakoubek and Rohlicek 1982), estimations of which, it may well be argued, really constitute biosensing *sensu stricto*.

Obviously, measurements of the conductance of homogeneous solutions are widely used in environmental monitoring, and are the method of choice in estimating the salinity of the marine environment (see for example Brown 1968; Ben-Yaakov 1981; Wilson 1981). Similarly, resistivity methods have also enjoyed use in geophysical prospecting (Keller and Frischknecht 1966), although the physical and mechanistic interpretation of the data is by no

means free of difficulty (Hasted 1973; Phillips 1984). It may also be mentioned that the time resolution of solution conductivity measurements may be made extremely good by using microwave frequencies (de Haas and Warman 1982). Schugerl (1984) gives a useful discussion of an elegant conductimetric method for monitoring bubble size and velocity distribution in microbial fermenters (and see later), whilst the utility of impedimetry in the monitoring of chromatographic eluents is discussed, for instance, by Alder *et al.* (1984).

As regards conductimetry in biosensors generally, Lowe (1984, 1985) and Ballot *et al.* (1984) have recently stressed that a great many of the reactions exploited in potentiometric and amperometric enzyme electrodes, for instance the urea-dependent pH and pI change in urease-containing electrodes, might be equally or better assessed conductimetrically. Similarly, Arwin and colleagues (1982) have made use of enzyme reaction-dependent changes in the double layer capacitance of symmetrical metal electrodes as a measure of enzyme or substrate activities. Workers tend to make such measurements at a single frequency, and it goes without saying that yet more selective and informative sensors might be based upon multiple-frequency methods.

Finally, we may mention the use of conductimetry in improving the response time (Powley *et al.* 1980) and selectivity (Powley and Nieman 1983) of ion-selective electrodes that are normally used in a potentiometric mode. As one would expect from the properties of electrode impedances described above, there is an optimal time (frequency) window for these measurements, in this case a delay of 0.1 ms between the stimulus and the measurement of the response being used.

Naturally one could give many, many more examples of the above type. However, what I wish to convey is that by choosing appropriate frequencies and/or analyte matrices, a great many determinants may be monitored in real time and non-invasively by the use of impedimetry in various embodiments, and that the predominant response may be due to the behaviour of the electrode, of the bulk solution, or of the interfacial region. This concept brings us to a brief discussion of some technical aspects of this type of measurement.

24.8 The realization of impedimetric systems

I have not thus far laid much stress upon the technical and instrumental considerations underlying impedimetry, since, as far as the typical user is concerned, the methods to be employed follow directly from the underlying principles. Many reviews discuss the measurement of chemical impedances (e.g. Shedlovsky 1949; Blake 1950; Reilley 1954; Loveland 1963; Thomas and Pertel 1963; Pungor 1965; Bennett and Calderwood 1971; Hollder and Enke

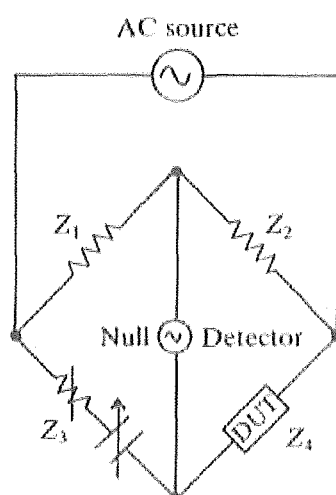


Fig. 24.10 The principle of a two-terminal impedance bridge. The device under test (DUT) forms one arm of the bridge (Z_4), which is in many ways similar to the familiar (DC) Wheatstone bridge, except that the voltage source is a sinusoidal oscillator of variable frequency, the null detector is AC sensitive and the adjustable arm of the bridge (Z_3) contains both resistive and capacitive components. When the bridge is balanced (i.e. no current flows in the part of the circuit containing the null detector), and if $Z_1 = Z_2$, then $Z_3 = Z_4$, since, generally, $Z_1 Z_4 = Z_2 Z_3$.

1984; and see electrochemical references above) and biological impedances (Schwan 1963; Hasted 1973; Grant *et al.* 1978; Pethig 1979; Marmarelis and Marmarelis 1978; de Felice 1981) in the range up to 30 MHz or so. I use this frequency criterion because it is roughly here that the wavelength of electromagnetic radiation approaches the dimensions of the measuring system, such that at frequencies greater than this, the lumped circuit description implicit in the above ceases solely to be applicable, and one should also consider a field description based on the Maxwell equations (see for example Bleaney and Bleaney 1976; Lorrain and Corson 1979; Cheng 1981). Similarly, electrode impedances are now negligible. An entrée to the recent literature on these very high frequency methodologies may be gained from the articles by Dawkins *et al.* 1979, Burdette *et al.* 1980, Stuchly and Stuchly 1980, Athey *et al.* 1982; Foster *et al.* 1982, and Steel *et al.* 1984; we do not here discuss these matters further.

In the frequency range below 10^7 to 10^8 Hz or so, bridge methods (Fig. 24.10) remain the most widely used and are appropriate. Traditionally, manually balanced bridges were used, but modern instruments are computer-controlled and auto-balancing. Frequency response analysers provide, albeit at some loss in precision, an extremely convenient means of obtaining dielectric spectra (Morse 1974; Gabrielli 1980). The system illustrated schematically in Fig. 24.11, which is that used by the present author, measures V_m , i_m , and θ (see Fig. 24.1) by means of a vector voltmeter and ammeter,

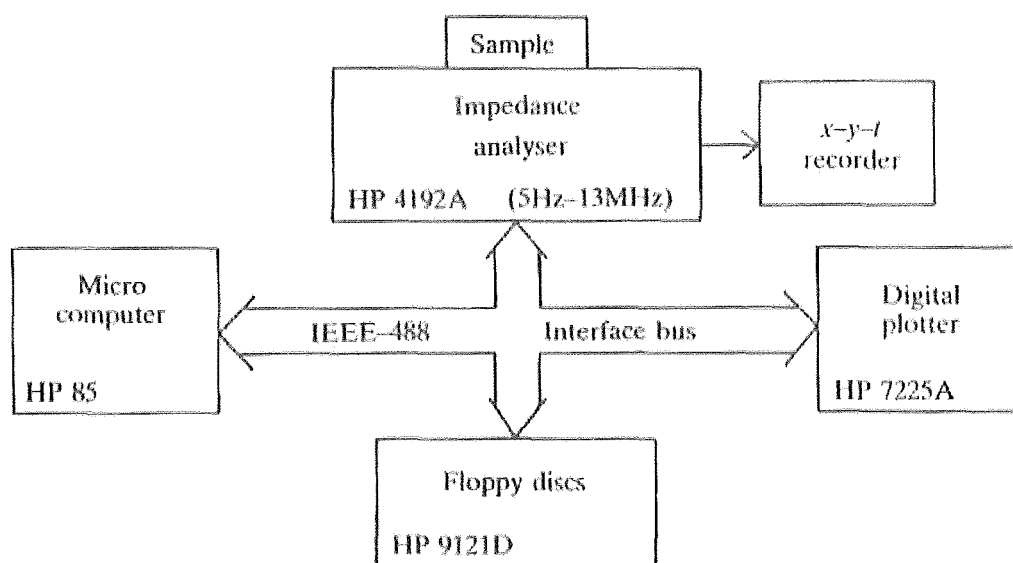


Fig. 24.11 A computer-controlled, frequency domain dielectric spectrometer, based upon commercially available components and usable in the range 5 Hz–13 MHz. The microcomputer drives the impedance analyser, stores the data obtained both in RAM and on disc, and permits the data to be plotted in a variety of forms (see Figs 24.4, 24.6, 24.8, and Harris and Kell 1983).

whence all required information may be calculated and displayed. Its implementation of the IEEE-488 standard interface makes it extremely convenient in use, and logarithmic scans may be made at a rate of 6 s (and 20 measurement frequencies) per decade. In this type of system the sinusoidal frequencies are applied one at a time, and these methods are thus called *frequency-domain* methods.

In systems of the above type, two-terminal measurements are the more common. However, this means that one is always measuring the impedance of the sample *plus* the electrodes, and, particularly at low frequencies and high conductance, the latter, which may be of no scientific or analytical interest, can dominate the measurements. In such cases, four-electrode techniques are used (Fig. 24.12), by which electrode polarization problems are in principle avoided (see for example Schwan 1963, 1966, 1968; Schwan and Ferris 1968; Nakamura *et al.* 1981), although a careful consideration of the exact location of the electrical field lines is necessary (Schwan 1955; Schwan and Ferris 1968). In such cases, the cell constant is determined by the positioning of the voltage electrodes (Tamamushi and Takahashi 1974). The minimization of electrode polarization generally, by using Pt black electrodes (e.g. Schwan 1963), and the preparation of such electrodes using electrolysis in Kohlrausch solution (Geddes 1972), are discussed elsewhere.

In recent years, time domain methods have become popular. In this type of approach, one applies a step voltage to the sample and follow, depending

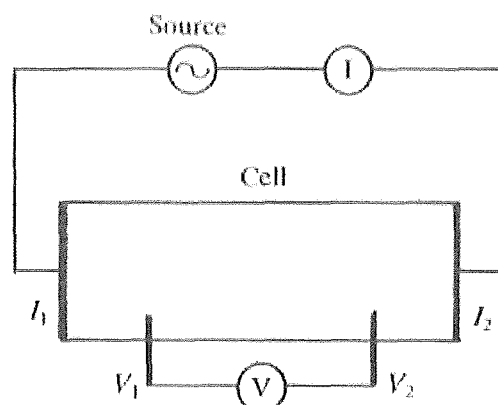


Fig. 24.12 The principle of the four-electrode technique for measuring bulk, low-frequency impedances with minimal interference from the impedance of electrode/electrolyte interfaces. Current from the AC source is measured with an ammeter and flows through the system via two current electrodes (I_1 and I_2). The voltage drop across the relevant part of the system is measured using two voltage 'pick-up' electrodes (V_1 and V_2), connected to a voltmeter of high input impedance, such that negligible current flows through them and thus no electrode polarization impedance is measured (see Schwan and Ferris 1968; Ferris 1974).

upon the frequency range, the time-dependent (dis)charging current flow, or the wave behaviour, of the equivalent RC circuit. Deconvolution of such data, usually by use of the fast Fourier transform (see later), gives the equivalent frequency-dependent dielectric properties. Such methods are of value at both high frequencies (see for example Cole 1975; Dawkins *et al.* 1979; Stuchly and Stuchly 1980; Burdette *et al.* 1980; Boned and Peyrelasse 1982; Steel *et al.* 1984) and low frequencies (e.g. Singh *et al.* 1979; Eden *et al.* 1980; Hart 1982; Schmukler and Pilla 1982; Mopsik 1984).

This concept, of the equivalence of the time- and frequency-domain behaviour of a system, leads us finally and naturally to the idea that we might broadly use an input wave-form of *any* shape in order to assess the passive electrical properties of a system, and this is in fact to a good approximation true. We will therefore include an introductory section on modern methods of signal analysis.

To summarize this section, we would stress again (i) that care must always be taken to be sure of the extent to which electrode polarization is contributing to the measured biological impedances, and (ii) that one should properly be aware of the pathways taken by the field lines between the electrodes.

24.9 Spectral analysis as an integral element of biosensing

The means most commonly used, in the general case, to analyse the frequency dependence of the response of a system to an input wave-form (e.g. Jenkins

and Watts 1968; Priestley 1981) are the same as those used in the proper characterization of any time-dependent signal or 'time series' (e.g. Bendat and Piersol 1971; Box and Jenkins 1976; Chatfield 1984). In particular, they exploit transform techniques such as the Fourier transform (e.g. Champeney 1973; Bloomfield 1976; Bracewell 1978; Marshall 1978, 1982, 1983) and, whilst yet more advanced approaches and treatments may be mentioned (e.g. Childers 1978; Kay and Marple 1981; Chen 1982*a,b*; Fu 1982; Ahmed and Natarajan 1983; Geckinli and Yavuz 1983), we shall confine our short discussion to the more standard approaches that may be applied to linear, stationary, or periodic (quasi-)ergodic systems.

Any periodic signal $x(t)$ (of period T) may be represented by a Fourier series, which may be written thus:

$$x(t) = \sum_{-\infty}^{+\infty} C_n e^{j2\pi nt} \quad (24.14),$$

where

$$C_n = \frac{1}{T} \int_{-T/2}^{T/2} x(t) e^{-j2\pi nt} dt \quad (24.15)$$

and where $j = \sqrt{-1}$ and the 'fundamental frequency' $f = 1/T$.

The Fourier series may also be written

$$\begin{aligned} S_x(f) = F[x(t)] = & X_0 + X_1(\cos 2\pi ft + j \sin 2\pi ft) \\ & + X_2(\cos 4\pi ft + j \sin 4\pi ft) + \dots \\ & + X_n(\cos 2n\pi ft + j \sin 2n\pi ft) \end{aligned} \quad (24.16)$$

For non-periodic data, a continuous spectral representation must be obtained from a Fourier integral, given by

$$X(f) = \int_{-\infty}^{+\infty} x(t) e^{-j2\pi ft} dt. \quad (24.17)$$

These equalities thus relate signals in the time domain to those in the frequency domain, and show that any signal may be represented as a sum of sinusoids of defined frequency, amplitude, and phase.

If we take an apparently 'random' signal, such as that in the top half of Fig. 24.13a, we may wish to characterize it further, and to decide, for instance, to what extent if any it may differ materially from that of another, apparently equally random, signal such as that in the top half of Fig. 24.13b. A convenient means by which this may be accomplished is by determining the autocorrelation function $R_x(\tau)$, which measures the degree to which a signal correlates with a displaced replica of itself:

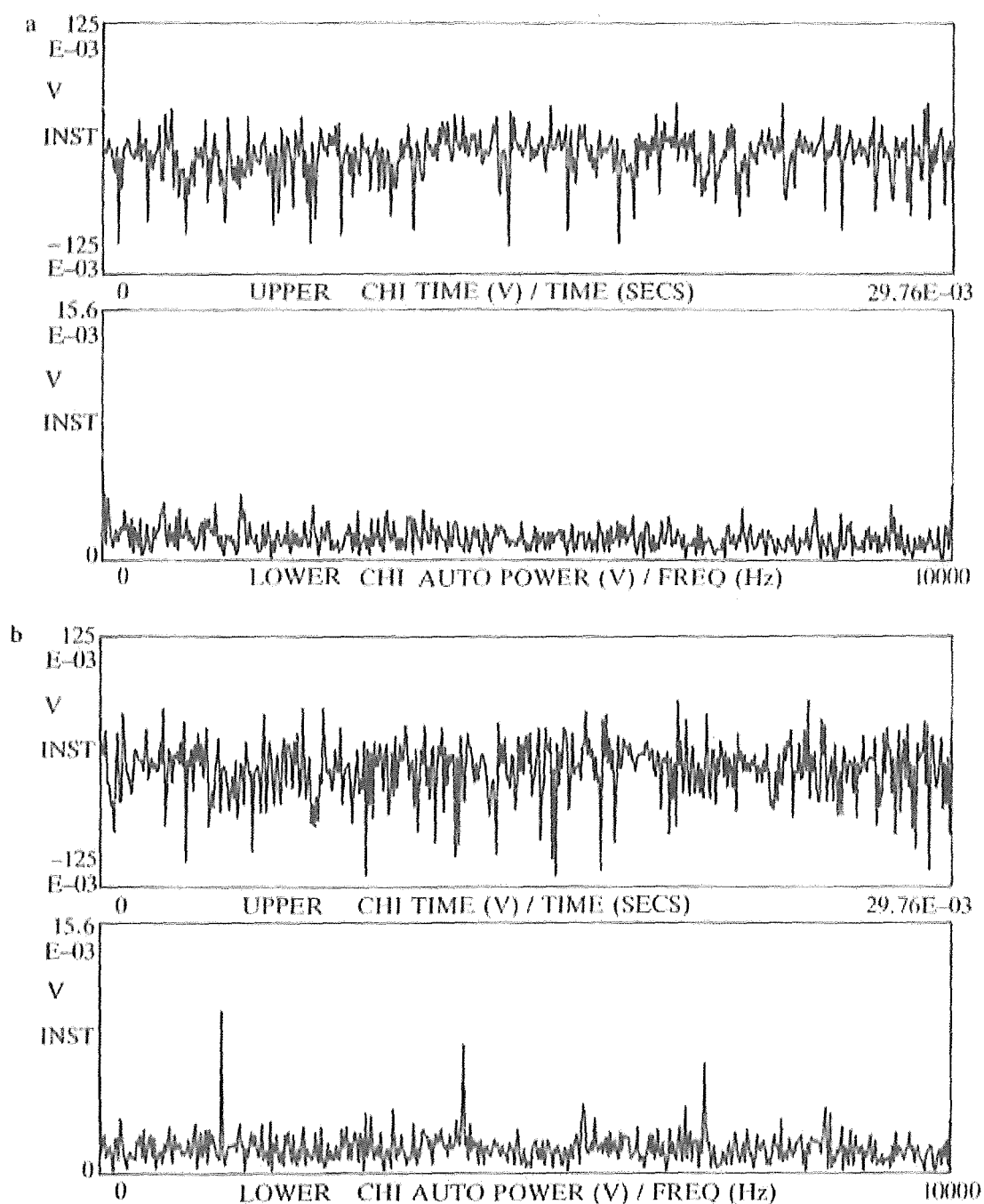


Fig. 24.13 Two 'random' signals and their autopower spectra. The upper half of each figure contains the time history of the signal, whilst the lower half is the autopower spectrum (see text) of the data. It is clear that, whilst the degree of 'randomness' in the original data is apparently similar in the two cases, the autopower spectra reveal that the signal in b has a substantial component centred at a frequency of about 1.4 kHz. In fact, the signals are constituted in each case by the output of a 'white noise' generator, that in b being mixed with the output of a sinusoidal oscillator operating at 1420 Hz. The data were analysed using a Solartron 1200 Signal Processor and plotted using a Hewlett-Packard 7470 digital plotter.

$$R_x(\tau) = \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T x(t) \cdot x(t + \tau) \cdot dt \quad (24.18)$$

At zero time displacement ($\tau = 0$), the value of $R_x(\tau)$ equals the mean square value of the signal $x(t)$. A 'purely' random signal ('white noise') has an autocorrelation function that is independent of the value of t . Such functions have found use in the on-line estimation of the time constant of electrodes (Turner and Howell 1984).

The autocorrelation function is the inverse Fourier transform of the auto-power spectrum $G_x(f)$, i.e. $R_x(\tau) = F^{-1}[G_x(f)]$, and describes the general frequency composition of a time series in terms of the spectral density of its mean square value:

$$G_x = \lim_{\Delta f \rightarrow 0} \frac{1}{(\Delta f)} \left[\lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T x^2(x, t, \Delta f) \cdot dt \right]. \quad (24.19)$$

(The autocorrelation function and autopower spectra thus ignore *phase* relations). Figure 24.13 illustrates the utility of the autopower spectrum in 'picking out' a periodic signal from a noisy set of data; whilst one would be hard pressed to perceive any analytical use for data as noisy as those in the time domain representations, the autopower spectra clearly show that signal b indeed contains a significant component with a frequency of 1.4 kHz or so (plus harmonics), and in this area of the spectrum the signal:noise is quite acceptable for analytical usage.

The above analyses have considered *single* signals alone. We may also define a cross-correlation function $R_{xy}(\tau)$ between signals $x(t)$ and $y(t)$, such that

$$R_{xy}(\tau) = \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T x(t) \cdot y(t + \tau) \cdot dt \quad (24.20)$$

This function tells us the extent to which one signal correlates with another, and is the inverse Fourier transform of the so-called cross-power spectrum $G_{xy}(f)$, i.e.

$$R_{xy}(\tau) = F^{-1}[G_{xy}(f)]. \quad (24.21)$$

Finally, we may use these concepts to define the transfer function of a system, $H(f)$, which serves to define the input/output relationship of a generalized transmission system. Thus, if in a test system such as that of Fig. 24.1a, the input signal $x(t)$ has an autopower spectrum $G_x(f)$, and the output signal $y(t)$ is so modified by the system that the cross-power spectrum is $G_{xy}(f)$, then

$$H(f) = G_{xy}(f)/G_x(f). \quad (24.22)$$

In principle, therefore, *any* input signal might therefore be used to obtain the transfer function, and hence the impedance, since in this case the transfer function may also be defined (notation as in Figs. 24.1 and 24.2) as

$$Z(f) = |Z(f)|e^{j\theta(f)}. \quad (24.23)$$

In practice, certain wave-forms are favoured, for reasons connected with the measuring time (Creason *et al.* 1973; Gabrielli *et al.* 1982); similarly, accuracy is improved by stressing frequency components related to the relaxation times of the system under study (one might here imagine the exploitation of an iterative system (see also Kell and Harris 1985a)). Nevertheless, despite the need to average, the 'pseudo-random' input remains popular in neurophysiological (e.g. Marmarelis and Marmarelis 1978; De Felice 1981; Fernandez *et al.* 1984) and dielectric (Nakamura *et al.* 1981) work, and is that exploited in Fig. 24.13.

We may therefore state that this type of analysis is already extremely important and useful, and will become increasingly cheap, widespread, and significant as digital electronic technology advances. (All the spectral functions are implemented in a hard-wired form and in real time in the system used to construct Fig. 24.13). Although I have included a discussion of these matters because they naturally complement the concept of admittance spectroscopy, I would stress that spectral analysis in general, i.e. what is often referred to as 'pattern recognition', should be considered as an *integral* design goal by all workers actively developing biosensing devices. Although these methods have been used for many years in photometric systems (e.g. Berne and Pecora 1976), and Fourier techniques are widely used in NMR and IR spectroscopy, etc. (e.g. Marshall 1983; Campbell and Dwek 1984), I do not as yet perceive their exploitation in biosensing systems on the wide scale that their potency merits. Therefore, and although the applicability of these techniques to fermentation technology constitutes our own main present direction, I will end by describing two possible general uses of fluctuation or spectral analysis.

24.10 Conductimetric correlation functions in the assessment of two-phase flows in bioreactors

Many systems, such as laboratory and industrial bioreactors and fermentors, exhibit highly complex and multiphase fluid dynamics (e.g. Bryant 1977). Leaving aside, for the present, particulate matter and biological cells, such systems may broadly be modelled as consisting of a heterogeneous suspension of non-conducting gas bubbles in an aqueous ionic solution. When stated thus, it is evident that conductivity (or impedimetry generally) can

provide a convenient approach to the measurement of the passage of gas bubbles, on a similar principle to that employed in the Coulter CounterTM (see Harris and Kell 1985*b*). In particular, the use of more than one probe in a bioreactor allows the estimation of the cross-correlation and/or coherence functions of the conductivity fluctuations between probes, a direct indication not only of bubble size and dynamics but of bubble velocity (Buchholz and Schügerl 1979*a,b*; Schügerl 1984; Sekiguchi *et al.* 1984). Spectral analysis of pressure fluctuations has also been used to gain otherwise-unobtainable, and real-time, information on the mixing dynamics in two-phase bioreactors (Gerson 1980).

Extending such ideas, we may state (accurately) that the 'problem of scale-up' (e.g. Lilly 1983) is largely ascribable to the fact that conventional measuring practice considers only the *mean*, and not the (rapid) *fluctuations about the mean*, of signals derived from probes. It should be obvious that the proper characterization of the 'state' of a culture, by means of environmental measurements, thus requires the *full* characterization of the time-dependent behaviour of such measurements, including their fluctuations. It is our view that this area in particular represents one of the most fruitful in which future progress may be expected.

Now, whilst the type of signal analysis discussed in this section relies upon the assessment of signals generated by macroscopic probes in microbial fermentors, we wish finally and speculatively to discuss a potentially novel approach to biosensing *sensu stricto*, based upon the measurement of non-linear electrical transfer functions in relatively microscopic proteinaceous systems.

24.11 Use of the multi-dimensional dielectric spectrum of intramolecular protein motions in biosensing devices

The overwhelming majority of biosensing devices proposed or realized to date rely upon the juxtaposition of an enzyme (or protein) and either a potentiometric or an amperometric electrode. What I wish to discuss here is the possibility of exploiting the specific, *non-faradaic* and *non-linear* electrical behaviour of proteins that are bound (or adjacent) to electrodes.

It is now becoming widely recognized that the atoms of even protein crystals, let alone aqueous solutions of globular proteins, exhibit many and complex fluctuations about their mean or average positions, even when at thermodynamic equilibrium (above 0°K) (reviews: Welch *et al.* 1982; Somogyi *et al.* 1984; Welch 1986; and references therein). Such intramolecular fluctuations are not wholly independent from each other (Kell and Hitchens 1983). Further, since proteins contain numerous charged and dipolar species, it is to be expected that the intramolecular mobilities of such groups will be (i) protein-specific and (ii) changed upon substrate (ligand)

binding, enzymatic activity, or energy transduction (Welch and Kell 1985), so that a non-invasive dielectric spectroscopic assessment of protein dynamics might form the basis of an entire family of novel biosensing devices (since this principle would apply to any protein-ligand (protein) interaction). However, since the (linear) dielectric dispersions exhibited by proteins are rather broad (reflecting, presumably, the numerous underlying processes contributing to the macroscopic observables), the problem reduces to that of signal handling, i.e. to *deconvoluting* the dielectric spectra. To approach this, we propose (i) to exploit the *non-linear* dielectric properties of proteins (or indeed any other macromolecule) and (ii) to exploit two- (or multi-) dimensional analysis of the electrical transfer functions of protein-ligand systems. We shall also need to consider the appropriate frequency range for maximizing the protein-specificity of the signal.

Now, as discussed above, the fraction of charges or dipoles *actually* moving in response to an electrical field of the appropriate frequency is given by the Langevin function (see Fig. 24.9), so that, in calculating the fields necessary to drive at least say 80% of a given type of dipole to its extremal position we require that $\mu E_1/kT$ exceeds 5, a value significantly outside the linear domain (see Fig. 24.9). Since many of the effective *intramolecular* dipole moments in which our interest lies probably do not exceed say 5 charge-Å (24 D), and since for $\mu E_1/kT = 5$ we require (at 298°K) a field of $6.159 \times 10^9 \text{ V m}^{-1} \text{ D}^{-1}$ (Kell and Harris 1985a), the type of field we are likely to require is of the order of $2.5 \times 10^8 \text{ V/m}$. Thus, to keep the voltages small, or at least realistic, we must use electrodes separated by as small a distance as possible, a suitable design being that of intercalated or comb electrodes (e.g. on a silicon substrate) (Fig. 24.14), as used for instance in the EumetricTM system (Micromet Instruments Inc, Cambridge, MA 02139, USA) for low-frequency permittivity measurements.

Now, because of the protein-specific intramolecular connections of the different charged and dipolar groups, the imposition of a field (such that $\mu E_1/kT$ is greater than say 5) at one frequency will measurably affect the dielectric properties measured at another frequency. Thus, by measuring the frequency-dependent dielectric properties as a function of the frequency of a *high* electric field, we may seek to deconvolute the intramolecular electrical properties, in much the same spirit as NMR spectroscopists measure the so-called J- and NOE-connectivities or cross-relaxation pathways of NMR-active nuclei by two-dimensional techniques (e.g. Kumar *et al.* 1980; Jardetsky and Roberts 1981; Winter and Kimmich 1982; Wuthrich 1982; Campbell and Dwek 1984; Markley *et al.* 1984). In other words, one would *excite* (with a high field intensity (E_1)) at *one* frequency (f_1) and *interrogate* (with a field E_{11}) at *other* frequencies (f_2), either simultaneously ($t = 0$) or subsequently ($t > 0$), with f_1 and f_2 and/or t and perhaps also E_1 and E_{11} being varied throughout. What sort of frequencies should we consider?

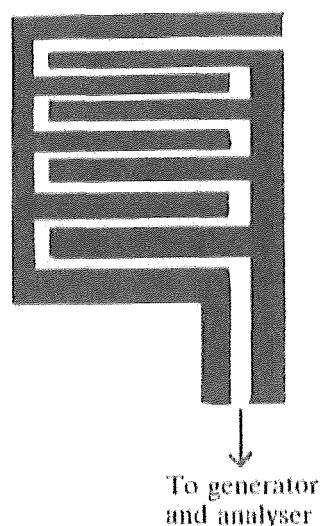


Fig. 24.14 The principle of using comb electrodes to give a high field for a reasonably low voltage, whilst covering a reasonable surface area (and thereby lowering the impedance). The alternating comb electrodes (seen here from above) are closely spaced (say $1\text{ }\mu\text{m}$ or less), and attached to the signal-generating and -analysing circuitry. The protein or biological component of interest is placed over the surface of the device, either by covalent attachment or otherwise, and the ligand-dependent change in the multi-dimensional dielectric spectrum assessed. Available variables for obtaining the multidimensional matrix include (see text) f_1 , f_2 , E_1 , E_{11} , and t .

We might expect that many of the most interesting intramolecular relaxations would lie at frequencies in the more technically difficult range above 1 MHz or so, not least because simple protein rotation is likely to dominate the measured spectra below this frequency. However, increasing the local solvent viscosity, e.g. with phospholipids, or chemical cross-linking of electrode-associated enzyme molecules, would serve to lower the appropriate frequency range. Notwithstanding, at the lower frequencies a significant contribution from double layer and faradaic electrode processes would be observed, and whilst this does not affect the pattern-recognition approach *per se*, it seems likely that the biospecific signal/noise ratio of such a device will be greater the greater the contribution from the protein dynamics.

The exact features of such multi-dimensional dielectric spectra which are likely to prove of most bioanalytical value can not easily be defined at the present time. However, it is easy to predict that a difference spectrum of (protein-plus-ligand) *minus* (protein alone) is likely to give the best type of definition of the ligand-selective signal analysis required, whether the biological response *in vivo* are a function of the occupancy or the rate of occupancy of the proteinaceous receptor in question. Similarly, whilst I do not in any way underestimate the technical difficulties involved, one should state that if *proteins* recognize (bind to) ligands and each other by means of

such frequency-dependent electrical processes, there is no *fundamental* reason why we should not do so as well.

The possibility of placing such a device, of the type alluded to herein, on an electrophoretic gel and therewith *identifying* a protein or nucleic acid in a band or a spot, seems sufficient justification alone to cause one further to explore the development of such a principle.

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