Douglas B. Kell

Department of Botany and Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, United Kingdom

Dielectric Spectroscopy of Biological Systems

Biological systems absorb electromagnetic radiation at all frequencies. Study of this absorption in the frequency range between some 0.1 kHz and 20 GHz constitutes dielectric spectroscopy. The frequency-dependent dielectric behavior is caused by the electrical field-induced rearrangement of charges and dipoles at all levels of biological organization, and may be exploited in biosensing devices.

BACKGROUND

Spectroscopy is concerned with the interaction of electromagnetic (EM) radiation with matter (1). The energy E in a quantum of EM radiation is related to the sinusoidal frequency ν of that radiation (in Hz) by $E = h\nu$ (where h = Planck's constant = 6.626×10^{-34} J·s). Microscopically, the absorption and emission of EM radiation can occur only between states whose energy difference ΔE is equal to the energy in the exciting radiation field. Thus studying the relationship between the extent of absorption of EM radiation by a system and the frequency of the radiation gives information about the structure of the system. The frequency range studied then defines the types of energies involved and the levels of organization that are observed.

UV/visible spectroscopy (where $\nu \simeq 10^{14}$ to 10^{15} Hz) probes electronic transitions, while infrared (IR) spectroscopy (where $\nu \simeq 10^{12}$ to 10^{14} Hz) detects vibrational and rotational transitions of molecules. At lower frequency we reach the millimeter and microwave bands (1 GHz to 1 THz) and then frequencies usually referred to as radio (10^5 to 10^9 Hz) and audio ($<10^5$ Hz). While there is some arbitrariness here, dielectric spectroscopy is generally taken to encompass studies up to perhaps 20 GHz. Because of the frequencies (and hence times) involved, dielectric spectroscopy gives information about electrical transitions in matter that are slower or of longer range than those observed in UV/visible/IR spectroscopy. The matter may of course consist of biological systems at any level of organization.

One finds dielectric spectroscopy turning up in many sub-disciplines, often under different names such as "impedance," "admittance," or "alternating current" spectroscopy. In these guises it has been responsible for such advances as the determination that biological membranes are of molecular thickness (2) and the discovery of the inductive behavior of nerve axons (3) (and hence the concept of voltage-gated ion channels). Despite this, however, the technique is not widely exploited, and what I shall therefore provide here is an overview of the types of information one can obtain and the ways in which one goes about doing so. Because of the somewhat different technical requirements of work above 10 MHz, I will here confine myself largely to work below this frequency. An introduction to the literature on dielectric spectroscopy may be found in a number of books and review articles (3–20).

CURRENT STATUS

The passive (i.e., voltage-independent) electrical properties of a sample of condensed matter may be completely characterized by its frequency-dependent permittivity ϵ' and conductivity σ'. These may be measured by determining respectively the electrical capacitance (C, in farads) and conductance (G, in siemens) of a sample of the material held between two or more electrodes. The conductance and capacitance reflect respectively the in-phase and 90° out-of-phase components of the current $i_m \sin(\omega t + \phi)$ flowing at the same frequency in the system when the potential has the form of a sinusoidally modulated voltage $V_m \sin \omega t$ with a frequency ω radians s^{-1} ($= 2\pi f$ Hz). G and C are extensive properties, which are related to the intensive properties of the system by $C = \epsilon'\epsilon_0/K$ and $G = \sigma'/K$. Here K is a "cell constant" that describes the electrode separation, area, and geometry and has the dimensions length^{-1}; it is usually obtained by measuring the capacitance and conductance of the electrochemical cell when it contains a solution whose properties are known. ϵ_0 is the absolute permittivity of vacuum and is numerically equal to 8.854×10^{-12} F/m. The units of σ' are thus S/m, while ϵ' (previously misnamed the dielectric constant) is dimensionless.

Traditionally, C and G were measured using manually balanced impedance bridges. In the modern era, however, devices such as digital, auto-balancing "impedance analyzers," "frequency-response analyzers," and "network analyzers" have become commercially available and are the instruments of choice, since, under micro-

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computer control, they may obtain and store the required data over a wide frequency range and at a speed considered unthinkable a few years ago. Thus for present purposes we may consider that the prime technical requirement is for such a device connected to the sample of interest via electrodes.

The major problem is that most such devices have only two terminals to which to attach the electrodes, which means that what is measured is the sample *plus* the electrodes. At lower frequencies, therefore, and especially in conducting media, the electrodes (i.e., the electrode/electrolyte interfaces) polarize, so that the sample appears to have a higher capacitance and lower conductance than it really possesses. This effect, which can dominate the measurement, may be assessed 1) by studying the dielectric behavior of ionic solutions of the same conductivity (whose true dielectric properties are frequency-independent in the range considered), 2) by varying the cell constant so that the intrinsic properties of the system remain the same, or 3) by varying intrinsic properties of the system such as the concentration of any suspended phase. These technical aspects are discussed in reviews by Schwan (4,5) and by Kell (19) and in the books by Grant and colleagues (9) and by Pethig (11).

The property of a system that underpins its dielectric behavior is its polarizability. This property reflects the ability of charges and (permanent and induced) dipoles to move in response to an imposed electrical field. Any charges separated in space constitute a dipole; the magnitude of a dipole is known as the dipole moment, μ, which has units of C·m. For historical reasons, dipole moments are often expressed in Debyes (D), where 1 D $= 3.336 \times 10^{-30}$ C·m, so that the separation of opposite unit electronic charges by 10^{-10} m constitutes a dipole moment of 4.8 D. The macroscopic permittivity is the outward and visible sign of the number and magnitude of individual molecular dipoles.

If a single species, let us say a protein with a permanent dipole moment, is present in aqueous solution, and is subjected to a sinusoidally modulated field, the magnitude of its polarizability will be frequency-dependent. This is because at frequencies low relative to the protein's rotational relaxation time it can rotate around its permanent dipole moment in concert with the field (Fig. 1), whereas at higher frequencies such motions cannot keep up with the field because of the viscosity of the aqueous phase. The capacitance of the system is therefore frequency-dependent, with the appearance of an inverted sigmoid when plotted against the logarithm of the frequency. The conductivity increases with frequency, since for any given field the energy must either be stored (as reflected in *C*) or dissipated (as reflected in *G*). The change in conductivity ($\Delta\sigma'$) and the change in permittivity ($\Delta\epsilon'$) for the relaxation of a single species are related to the time constant τ of the system by $\tau = \Delta\epsilon'\epsilon_0/\Delta\sigma'$. When the dielectric behavior of a system is frequency-dependent, the effect is known as a dielectric dispersion; it may be characterized by its dielectric increment $\Delta\epsilon'$ (the difference between ϵ' at "low" and "high" frequency) and the "critical" or "characteristic" frequency f_c (in Hz, $= 1/(2\pi\tau)$) at which it is half completed. $\Delta\epsilon'$ reflects the magnitude of the dipole moment, while f_c reflects the rate at which the polarization occurs. Measurements on a variety of proteins (9,20,21) indicate that their rather large permanent dipole moments (usually between 10^2 and 10^3 D) amount in general to the separation of some three to five electronic charges by the molecular diameter (22).

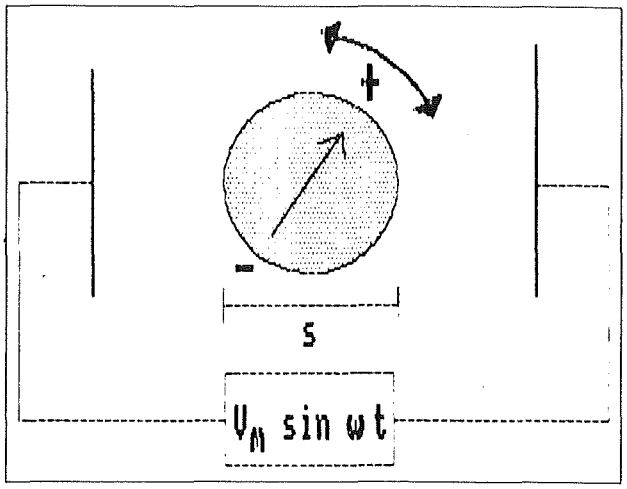

Figure 1 Rotational motions of a dipole as a mechanism of dielectric relaxation. In the case of a hard sphere representing a protein with unit charges of opposite sign at opposite poles, the dipole moment is $\mu = qs$, where s is the diameter of the sphere and q the elementary electronic charge.

The rotation of a permanent dipole is an obvious means of dielectric relaxation. It is equally obvious that *any* means by which a field can cause a charge or dipole to move will be accompanied by a dielectric disperson. A generalized "classical" dielectric spectrum of a sample of biological tissue has three major (α, β, γ) and two smaller (β_1, δ) dielectric dispersions (Fig. 2). The effects are extremely large relative to the "background" static permittivity of aqueous solutions ($\simeq$80), in that the low-frequency permittivity reaches some 10^8 permittivity units. The major mechanisms underlying these dispersions are thought to be tangential relaxation of ions adjacent to charged cell surfaces (α), charging of the capacitance(s) represented by the cellular membrane(s) (β), rotation of small dipoles, predominantly cytoplasmic water (γ), protein rotation (β_1), and the rotational and other motions of water and side-chains bound to cellular macromolecules (δ). Recent work (23,24) has also implicated the "lateral electrophoresis" of membrane proteins as a mechanism of dielectric relaxation referred to as the μ-dispersion.

The range of organisms, cells, macromolecules, and small molecules studied by the dielectric method is very extensive, as documented in the books and reviews cited. The method now constitutes a mature discipline or field of enquiry by which one may establish the overall electrical organization of any chosen biological material.

FUTURE DIRECTIONS

The technological exploitation of dielectric spectroscopy already includes the well-established techniques of impedance plethysmography (25) and pneumography (26). Progress has been made toward the development of electrical impedance tomography (27), which represents a means of deriving structural information from the dielectric behavior of tissues in situ, as do high-frequency probe methods (28). Dielectric measurements at radio frequencies permit the real-time measurement of

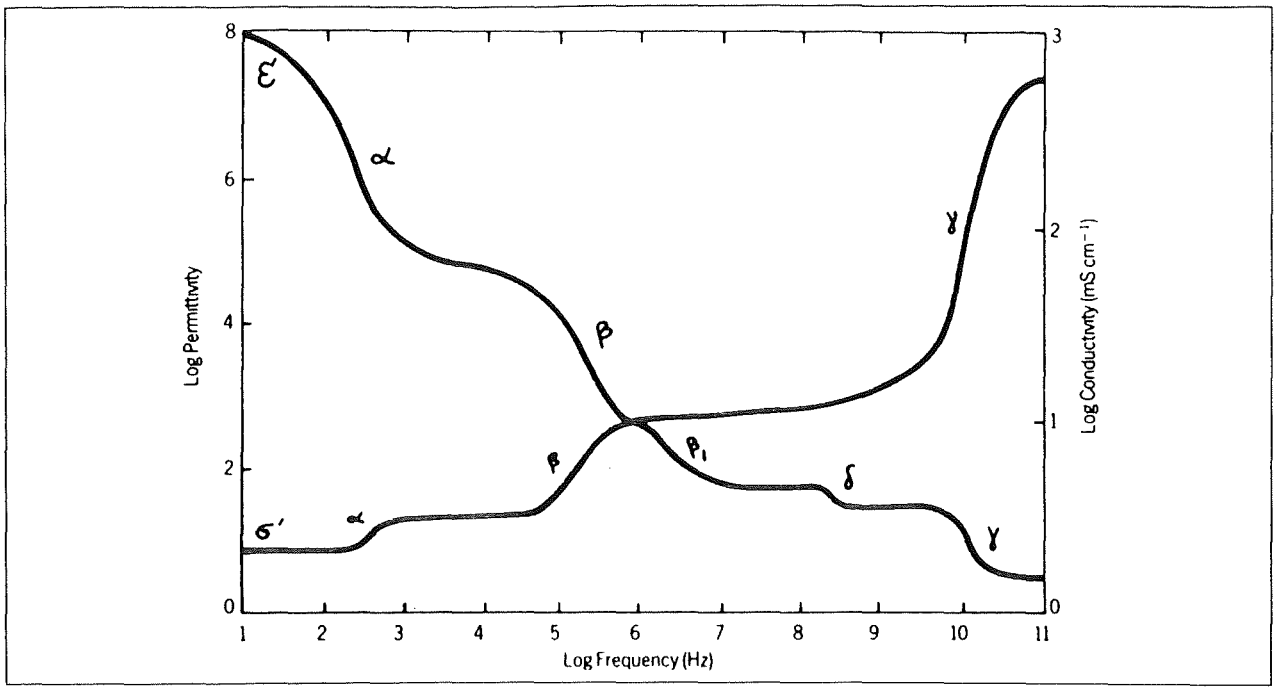

Figure 2 The frequency-dependent permittivity ϵ' and conductivity σ' of a typical biological tissue, showing the major classical dielectric dispersions that may be observed.

cellular biomass during industrial fermentations (29). Given that the technique consists only of measuring voltages, currents, and their relative phase relationships, it underpins a great many possible applications in biosensing (19).

Typically, the dielectric dispersions observed are rather broad, since they reflect relaxation rather than resonance. Similarly, the standard analysis builds in assumptions that amount to that of an equilibrium between the ground state and the field-induced ("excited") state during dielectric measurements on an ensemble, just as does the transition-state theory of chemical reactions (30). For this reason, during measurements of the linear, passive electrical properties one obtains each data point by measuring the voltage, current, and phase angle at the *same* frequencies, moving from one frequency to another in concert. Also, for a simple system we expect $\Delta\epsilon'\epsilon_0/\Delta\sigma = \tau$. In practice this latter equation is often found not to hold, especially in biological work (18); this means that even though by conventional analysis one appears to be operating in the linear region (i.e., that in which the *apparent* permittivity and conductivity are independent of the exciting voltage), the reality must be that currents are flowing at frequencies other than that of the exciting voltage (19), which implies nonlinearities of some sort within the system. These currents need not in principle be harmonics alone, since they may depend upon the native kinetic constants for secondary electrical transitions within the system. Such currents contain crucial information about the electrical organization of the system of interest, and we may expect to see the development of this type of nonlinear or multidimensional dielectric spectroscopy, in much the same way that nuclear magnetic resonance spectroscopists have developed two-dimensional nuclear magnetic resonance.

I described how the measurement of dielectric behavior at low frequencies was still bedeviled by the problems of electrode polarization. The ongoing development of accurate four-terminal devices will make possible many experiments that have previously been inaccessible. One may predict that novel molecular mechanisms of dielectric relaxation remain to be observed within biological materials and at audio frequencies.

For many workers the study of the dielectric behavior of living systems is motivated by the possibility that relatively weak exogenous electromagnetic fields may have clinical, therapeutic, or other manipulative utility. To understand this utility and to optimize the strength and waveform of the applied field, one must know how and where such fields are being absorbed by the system of interest. An entrée to this area, including the well-attested stimulation of bone regrowth, may be found in books (31,32) and in the pages of the *Journal of Bioelectricity* and *Bioelectromagnetics*. It is my opinion (33) that it is in this area in particular that one may expect some of the most important, innovative, and far-reaching developments in the bio(electro)chemistry of the future.

KEY CONTRIBUTORS

Although AC electrical measurements have been applied by many workers, the following list reflects those who have made long-term contributions to the areas covered above.

E. H. Carstensen, Department of Electrical Engineering, University of Rochester, Rochester, New York, USA.

K. Foster, Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

E. H. Grant, Department of Physics, King's College (KQC), London, United Kingdom.

T. Hanai, Institute for Chemical Research, University of Kyoto, Kyoto, Japan.

R. Pethig, Institute of Molecular and Biomolecular Electronics, University College of North Wales, Bangor, United Kingdom.

A. A. Pilla, Department of Orthopedic Surgery, Mt. Sinai School of Medicine, New York, New York, USA.

R. Pottel, Drittes Physikalisches Institut, University of Göttingen, Göttingen, Federal Republic of Germany.

H. P. Schwan, Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

M. A. Stuchly, Health and Welfare Canada, Ottawa, Ontario, Canada.

S. S. Stuchly, Department of Electrical Engineering, University of Ottawa, Ottawa, Ontario, Canada.

S. Takashima, Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

GLOSSARY

admittance: The property of a system that permits the flow of alternating current in response to an alternating voltage.

capacitance: That part of the admittance which describes the nondissipative part of the current flowing through it, i.e., that part of the total current which is 90° out-of-phase with the voltage.

conductance: That part of the admittance which describes the dissipative part of the current flowing through it; i.e., that part of the total current which is in phase with the voltage.

conductivity: The conductance normalized to take account of the electrode geometry, so as to describe an intrinsic property of a system.

dielectric dispersion(s): The change(s) in permittivity and conductivity exhibited by many systems as the frequency of measurement is altered.

dielectric spectroscopy: The study of the admittance or impedance of a system at varying frequencies, and hence the description of the permittivity and conductivity of the system as a function of frequency.

impedance: The property of a system that describes its ability to resist the flow of alternating current in response to an alternating voltage. The reciprocal of admittance.

impedance plethysmography: The study of blood flow by measuring the beat-to-beat changes in impedance of vasculated tissue.

impedance pneumography: Study of breathing patterns by measuring the time-varying impedance of the thoracic cavity.

impedance tomography: Computerized reconstruction of tissue structures based upon multiple-electrode estimation of the tissue impedance.

permittivity (previously called *dielectric constant*): The property of a system which describes its ability to store electrical charge; the capacitance normalized to take account of the electrode geometry, so as to describe an intrinsic property of a system.

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