NEW ELECTRIC FIELD METHODS IN CHEMICAL RELAXATION SPECTROMETRY

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ABSTRACT New stationary relaxation methods for the investigation of ionic and dipolar equilibria are presented. The methods are based on the measurement of nonlinearities in conductance and permittivity under high electric field conditions. The chemical contributions to the nonlinear effects are discussed in their static as well as their dynamic behavior. A sampling of experimental results shows the potential and range of possible applications of the new techniques. It is shown that these methods will become useful in the study of nonlinear responses to perturbation, in view of the general applicability of the experimental principles involved.

INTRODUCTION

Electrostatic interactions between ions or dipolar molecules are fundamental to the molecular mechanisms of some major phenomena in physiology. The theoretical description of these electrostatic effects and their experimental investigation—a brilliant chapter of classic physical chemistry—are mostly restricted to the range where current densities are linearly related to the applied electric field. However, some important processes in physiology, e.g., membrane permeability changes, are unconditionally linked to the proper function of supramolecular structures whose boundaries are the site of very intense electric fields. At such high field strengths field-dependent increments in the electric admittance appear. Their amplitude and frequency behavior are related to saturation effects and molecular mobilities, respectively. In chemically reactive systems, nonlinear field effects arise as a consequence of the coupling between chemical processes and the electric field. The physical and chemical phenomena due to electrostatic interactions can therefore be characterized completely—in their energetics as well as dynamics—from the nonlinear response of the system to which an intense electric field is applied. The understanding of the observed effects needs a theoretical description on a molecular level. The current lack of theoretical developments, in particular of the dynamic aspects, may be attributed to mathematical complexity, but even more so to the absence of relevant experimental data that would instigate the design of adequate models.

It is clear that advances in our understanding of electric field effects and of their dynamics will come from the development of new experimental techniques. In the recent past we developed methods that allow the investigation of field effects and their

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frequency dependence. Applied to chemically reactive systems, our methods yield information on rate processes that involve ionic and/or dipolar species. These stationary relaxation techniques can be applied to the kinetic investigation of nonlinear responses upon any perturbation of a reactive system, as a result of the generality of the experimental principles involved.

Chemical Systems and Electric Fields

Any system whose energy depends on the electric field density will oppose the change in energy resulting from the application of an electric field. Generally, the electric field acts upon translational and rotational properties of the species present and induces such an anisotropy in the system as will reduce the field density; these effects are linear only at the lowest field strengths. However, in chemical reactive systems the gain in electrostatic stability can be balanced by a corresponding reduction in free energy. This is revealed by a shift in chemical equilibrium composition toward the state that interacts more strongly with the electric field. The chemical effects account for the nonlinear response of a reactive system subjected to high field conditions and are well known for ionic as well as for dipolar equilibria. In this section we illustrate the theoretical approach to these effects and show their relation with chemical dynamics.

As first noted by Wien, the conductance of a solution of a weak electrolyte increases upon the application of a high electric field (1). The effect is known as the second Wien effect (the first Wien effect being the conductance increase with field in solutions of strong electrolytes) or the field-dissociation effect because it is related to increased dissociation.

A system containing free charges never reaches equilibrium in the presence of an electric field. Therefore, a thermodynamic approach to the field-dissociation effect faces many problems. A successful description was first given by Onsager (2) from an electrodiffusion treatment of the effect. Neglecting shielding by the ionic atmosphere, as well as the hydrodynamic interactions between ions, Onsager obtained for the relative increase of dissociation of an ion-pair into free ions under field conditions:

$$K(E)/K(E = 0) = 1 + 2\beta q + \tfrac{1}{3}(2\beta q)^2 + \tfrac{1}{18}(2\beta q)^3 + \dots \qquad (1)$$

The parameter $q(= -e_1 e_2/8\pi\epsilon_0\epsilon kT$, symbols with their usual meaning) represents the distance within which the mutual electrostatic interaction of the two ions with charges e_1 and e_2 becomes higher than their thermal interaction with the surrounding medium. This distance is known as the Bjerrum distance and may be regarded as the radius of the association sphere, i.e., the sphere within which the two ions are considered as bound. The parameter $2\,\beta(= |(e_1u_1 - e_2u_2)E| /(u_1 + u_2)kT$, in which E is the external field, is a reciprocal distance which becomes independent of the mechanical mobilities u for symmetrical electrolytes. In the symmetrical case $(2\beta)^{-1}$ represents the distance at which two oppositely charged ions form a dipole which gained an electrostatic stabilization energy of $kT\cos\theta$, θ being the angle between the dipole moment and the field direction.

The mathematical treatment by Onsager is very difficult in some of the crucial steps of the derivation of Eq. 1. It would therefore be worthwhile to have a simple picture of the physical process responsible for the facilitation of dissociation of an ion-pair in field conditions. Such a picture can be made for a symmetrical electrolyte where apparently no parameters of transport theory appear in Onsager's result. We consider in this case an ion-pair as any configuration of two oppositely charged ions within the Bjerrum-association sphere. The configuration with the two ions in contact—or solvent-separated—will have the highest probability. The configuration of two ions at the Bjerrum distance has a fleeting existence and may be considered as the transition state for the dissociation process; this configuration has equal probability of collapsing into the ion-pair state as of separating into free ions. It is now useful to consider the facilitated dissociation under field conditions as a consequence of the reduction in electrostatic work needed to separate the "Bjerrum dipole" into free ions. An alternative point of view would be to consider the increase in free ions under field conditions to be the result of a distortion of the Bjerrum sphere.

We can now write for the standard free energy change of dissociation with and without field, assuming a fast rotation of the Bjerrum dipole toward the most favorable orientation in the field:

$$\Delta G^\circ(E) = \Delta G^\circ(E = 0) - \mu_{\mathrm{Bj}} E N_0. \tag{2}$$

Eq. 2 may directly be transformed into an expression for the dissociation constant under field conditions:

$$K(E) = K(E = 0)\exp(\mu_{\mathrm{Bj}} E/kT). \tag{3}$$

Writing μ_{Bj} as $e_0 q$, we obtain, by expanding the exponential and retaining only the first term:

$$K(E)/K(E = 0) = 1 + 2\beta q. \tag{4}$$

In the linear approximation, this is the result of Onsager's theory. Although the picture presented may seem to be very crude—especially in neglecting the nonequilibrium aspects of the field-dissociation effect—we nevertheless gain some insight in the dynamic aspects. This picture introduces the hydrodynamic interactions within the association sphere. We may conjecture that for electric fields of short duration, or high frequency AC fields, the field-dissociation effect will disappear not as a result of the relaxation of the association-dissociation equilibrium but as a consequence of the finiteness of orientational mobility within the association sphere.

Unlike ionic equilibria, dipolar systems may reach a new equilibrium under field conditions. This situation allows a thermodynamic treatment. However, considering the dynamic features of the response of reactive dipolar systems, care should be taken of the possible coupling of orientational and chemical effects. It is, therefore, worthwhile to give a short illustration of the physical aspects of the interaction of dipoles with an electric field. Generally, orientational polarization is achieved by the electric field exerting a torque on any dipolar species present. The energy U of the polar particle decreases with the decrease of the angle θ between dipole moment and directing field F according to $U = -\mu F \cos\theta$. A consequence of chemical importance is the diffusion of polar species toward the region of highest field strength such as is encountered in systems with inherent anisotropy, e.g. membranes (3). For a mole of dipoles, one obtains for the electrostatic energy, neglecting contributions of induced polarization:

$$\bar{U} = -N_0 \mu F L(\mu F/kT), \tag{5}$$

L being the Langevin function. In the linear approximation:

$$\bar{U} = -N_0 \mu^2 F^2 / 3\,kT. \tag{6}$$

At high field strength the effect is susceptible to saturation when the polar particles become fully aligned with the field. The fact that orientational polarization becomes increasingly difficult is reflected in a decrease of the permittivity $\epsilon_0 \cdot \epsilon$ with field strength.

The dipolar contribution to polarization vanishes when the field frequency approaches the inverse of the orientational relaxation time τ_{or}, a quantity depending on particle size and solvent viscosity. Of course this is true under high field conditions as well as in the absence of an external high field; it then represents a classic case of dielectric relaxation.

Now we shall direct our attention toward the influence of high electric fields on chemical systems in dynamic equilibrium. When such a system consists of species with different polar character, the more polar ones will become stabilized most, requiring the readjustment of the true equilibrium composition under high field. Inasmuch as the stability gain is related to energy, the field-induced equilibrium shift must depend on the square of the field strength, as indicated by Eq. 6. This defines the effect as a nonlinear phenomenon. Similarly, Eq. 6 shows that the amplitude of the shift varies with $\Sigma\, \nu_i \mu_i^2$, in which ν_i are the stoichiometric coefficients of the reaction partners defined as usual. In a formal way we write a van't Hoff-type relation for the field dependence of the equilibrium constant K:

$$\mathrm{d}\ln K/\mathrm{d}E = \Delta M/RT \tag{7}$$

where ΔM is the molar change of electric moment, a quantity depending on $\Sigma \nu_i \mu_i^2$ and the electric field E.

In the foregoing we have deliberately omitted conditioning the validity of the result in terms of the ratio of chemical to orientational relaxation times. Schwarz (4) has shown that for cases where $\tau_{\mathrm{chem}}/\tau_{\mathrm{or}} \ll 1$ pseudo-orientational polarization occurs through chemical reaction, even at low field conditions, without, however, affecting the bulk equilibrium composition. Without regard to the value of the ratio $\tau_{\mathrm{chem}}/\tau_{\mathrm{or}}$ we can state that a chemical mode of incremental polarization will be induced by the action of the field, when it consists of a low-amplitude, high-frequency field superposed on a steady high field, as in the customary experimental approach (5). (It is imperative to have L significantly larger than 0 by the DC component of the field when $\tau_{\text{chem}}/\tau_{\text{or}} \ll 1$.) The polarization is revealed by a permittivity increment which will relax at frequencies dictated by the rate of the chemical process by which the polarization is effected.

The amplitude of the field-induced permittivity increments is dependent on the magnitude of the equilibrium shift ($\mathrm{d} \ln K/\mathrm{d}E$) as well as on the molar change of electric moment (ΔM), which translates the change of concentration C_i to permittivity. From this we conclude that the increment is proportional to the following quantities:

$$\Delta\epsilon_{\text{chem}}(\omega) \simeq \Gamma(\Sigma\nu_i\mu_i^2)^2 E^2 \phi(\omega). \tag{8}$$

Γ is the well-known reaction capacity ($1/\Gamma = \Sigma\nu_i^2/\bar{C}_i$) and $\phi(\omega)$ the pertinent relaxation function. The dispersion of the nonlinear effect thus carries information regarding the reaction rates of the field-perturbed equilibrium and other equilibria coupled to it. The increment has the property of any accurately measured virial coefficient which is to provide a wealth of information, in this case of structural (μ_i) and thermodynamic ($\bar{C}_i$) nature.

EXPERIMENTAL METHODS

The nonlinear contributions to the electric properties of a chemical reactive system subjected to high electric fields can in principle be measured from any electric circuit in which the system is used as a circuit element. This is the central idea for the methods we developed to measure the nonlinearities in electrolyte solutions and dipolar systems. After some investigation it became obvious that the sample solutions—subjected to changing electric fields—could be used directly as the active element in a modulator circuit whose performance is critically dependent on the nonlinear behavior of the active element.

In the past the field-dissociation effect has been measured from the conductance increase upon the application of a high field (6). To increase the sensitivity of these measurements, we developed a stationary method that uses repetitive high frequency, high amplitude pulses to modulate the conductance of the sample solution. In this way the sample becomes a circuit

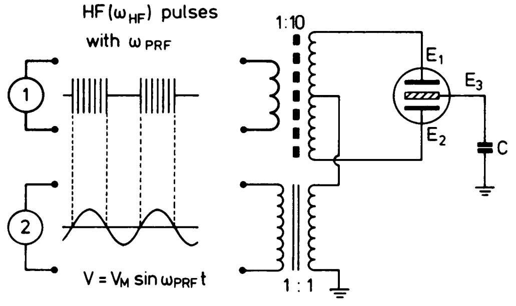

FIGURE 1 Schematic circuit for the measurement of the field-dissociation effect and its dispersion. Generator 1 is the square-wave (1 Hz-100 kHz) modulated high frequency (100 kHz-2 MHz) power oscillator (0.1–50 $\text{kV}_{\text{peak-to-peak}}$) driving the step-up (1:10) ferrite-core transformer. The ferrite core contains an adjustable air gap for tuning. Generator 2 is the low voltage sinusoidal signal generator running in synchrony with the square-wave modulating signal.

element whose nonlinear behavior can be determined from an analysis of the harmonics generated.

The principle of the method will be discussed in reference to Fig. 1 which shows a schematic of the circuit used. To the high voltage electrodes (E_1 and E_2) of the sample cell, a high-frequency voltage of high amplitude is applied as a square-wave[1] modulated signal. This high voltage is obtained from a step-up ferrite-core transformer driven from a modulated power oscillator. Depending on experimental conditions, several sample cells of a widely different design—e.g. high pressure cells—were developed. The spacing between high-voltage electrodes and the center electrode (E_2) is usually 1 mm, and electric fields of peak-to-peak amplitude of about 1–10 kV cm^{-1} are used. The center electrode of the sample cell is always short circuited for AC signals through capacitor C. The centertap terminal of the high voltage transformer is connected to a low-voltage transformer driven from a sinusoidal generator synchronously locked on the square-wave signal used to modulate the high voltage. As a consequence of the field-dissociation effect, the sample resistance will be lowered when subjected to the high-frequency high-amplitude field. At this point it should be clearly stated that this technique uses high-frequency alternating fields, whereas Onsager's theory is derived for fields of constant amplitude. In the field-on period, the capacitor C is charged through the lowered cell resistance. Because of the higher cell resistance in the field-off period, the charge on capacitor C cannot flow back completely during this period. Therefore, under stationary conditions, a net amount of charge will reside on capacitor C and can be measured as a DC voltage. It is evident that the DC signal is proportional to the amplitude of the measuring signal (V_M) and also to the relative conductance change of the sample solution. As noted in the preceeding section, an influence by the high frequency of the perturbing field pulses is also to be expected. A mathematical analysis—retaining only the square-wave envelope of the field pulses and neglecting their internal structure—of the properties of the circuit used gave for the DC voltage on capacitor C (7, 8):

$$V_{\mathrm{DC}} = (\Delta\sigma/\sigma)(V_M/\pi) = g(\Delta\sigma/\sigma)_{\mathrm{Ons}}(V_M/\pi). \tag{9}$$

g is a correction factor taking into account the deviations of the experimental value of $\Delta\sigma/\sigma$ from the value $(\Delta\sigma/\sigma)_{\mathrm{Ons}}$ calculated according to Onsager's theory.

In the foregoing discussion the response of the electrolyte solution upon perturbation was implicitly assumed to be instantaneous. This is correct as long as the pulse duration is relatively long—at low pulse repetition frequencies—and the ionic equilibrium is completely in phase with the perturbation. However, the ionic equilibrium change will progressively lag behind the perturbation as the pulse repetition frequency approaches the reciprocal relaxation time of the ionization equilibrium. This lag in response results in a smaller conductance increment lowering the modulation efficiency. The experimental consequence is a decrease of the DC voltage on capacitor C with increasing pulse repetition frequency. Ultimately, at pulse repetition frequencies much higher than the reciprocal relaxation time of the ionic equilibrium, the DC signal on capacitor C will vanish altogether. A complete analysis of the time-dependent properties of the modulator circuit yields for the DC signal as a function of pulse repetition frequency—again neglecting the high frequency structure within each pulse (7, 8):

$$V_{\mathrm{DC}} = (\Delta\sigma V_M/\sigma\pi)\, 1/(1 + \omega^2\tau^2) = V_{\mathrm{DC\,max}}/(1 + \omega^2\tau^2). \tag{10}$$

This equation, derived under the usual assumption of small perturbations, is recognized as a

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[1] In a new design sinusoidal modulation is used, which is realized more easily from an experimental point of view.

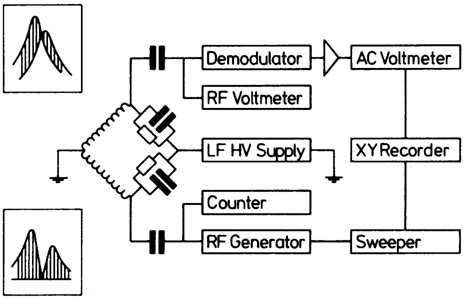

FIGURE 2 Block diagram of the apparatus. *Insets:* origin and shape of difference signals. HV, high voltage, RF, radiofrequency, and LF = 85 Hz.

classic dispersion equation. τ is the relaxation time of the ionic dissociation equilibrium, which can be expressed in the usual way as a function of the relevant kinetic constants.

A useful corollary of the technique described is the possibility of a conductance measurement from the rate of appearance or disappearance of the DC signal on the capacitor which depends, apart from the capacitance, on the cell resistance.

Nonlinear dielectric behavior appears as a change of permittivity of the system when subjected to high electric fields. Contrary to the field-dissociation effect, observable at rather low fields (~ 1 kV cm^{-1}) in low polar media, the field-induced dielectric increment is generally measurable at very high field strength (>50 kV cm^{-1}) apart from some special cases. It is also of importance to notice that as a consequence of the dependence on the field squared, the system cannot be perturbed in the limit of zero field.

Usually the field-induced dielectric increment is measured statically from the frequency shift of an oscillator, whose tank circuit includes a capacitor filled with the dielectric sample, upon the application of a constant or pulsed high electric field. We have developed a stationary perturbation technique which allows the accurate measurement of the increment of both real and imaginary parts of the permittivity in the frequency domain (9). To the dielectric losses may be added any field-induced change of conductance, either in or out of phase. A block diagram of the circuit is given in Fig. 2. As in older techniques the sample is subjected to a radio frequency (RF) low amplitude field, which is superposed on the high field. A new feature is that the sample solution is contained in a capacitor forming a parallel resonant network, which is excited externally by a RF generator. The resonant frequency of the network can be varied over a wide range (0.1–100 MHz) by choosing a coil with appropriate inductance. A second new feature is that the high field is alternating at low frequency (85 Hz).

A careful design of the sample cell—either stainless steel or gold-plated electrodes at a distance of about 0.3 mm—allows field strengths over 200 kV cm^{-1} to be sustained easily in nonconducting samples for more than 1 min.

As a consequence of nonlinear effects, the periodic high field affects the resonance characteristics of the circuit. This is reflected by the amplitude modulation of the resonant voltage at 2 × 85 Hz, illustrating the principle of harmonic generation by a nonlinear element. By sweeping the RF frequency over the resonance bandwidth, a signal proportional to the difference between resonance curves at peak high field and without field is detected. The signal is a measure of the modulation depth at each frequency. The sign of the difference signal is obtained from the phase relation between signal and perturbing high field. The amplitude and asymmetry of the recorded signal yield information on the changes of the complex permittivity. The amplitude modulation is very small, and we therefore consider the amount of modulation as the derivative of the resonant voltage $V(f)$. In general the three parameters characterizing the resonant circuit may change upon the application of the high field: the resonance voltage V_0, resonance frequency f_0, and the circuit quality Q. The amount of amplitude modulation at each frequency therefore can be written as:

$$\Delta V(f) = (\delta V/\delta f_0)\,\Delta f_0 + (\delta V/\delta V_0)\,\Delta V_0 + (\delta V/\delta Q)\,\Delta Q. \qquad (11)$$

Because ΔQ can be expressed in terms of Δf_0 and ΔV_0, it is clear that the measurement of the modulation depth over the resonance bandwidth—around frequency f_0—will yield all information on the relative changes of resonant frequency and voltage, provided the static properties of the resonance circuit are known. The changes in resonant voltage and frequency with the electric field are related to the RF frequency-dependent quantities $\Delta\epsilon'(\omega)$ and $\Delta\tan\delta(\omega)$, the incremental real part of the permittivity and the increase of dielectric loss, respectively:

$$\Delta\epsilon'(\omega)/\epsilon = 2\gamma\Delta f_0/f_0 \text{ and } \Delta\tan\delta(\omega) = (\gamma/Q)\,[(\Delta V_0/V_0) - F(\Delta f_0/f_0)]. \qquad (12)$$

γ is a calibration factor and F a correction factor. The change in the imaginary part of the permittivity, $\Delta\epsilon''$, is contained in $\Delta\tan\delta(\omega)$; that quantity may contain a contribution from the field-induced increment in conductance, $\Delta\sigma/\epsilon_0\epsilon\omega$.

For systems of ionic equilibria, changes of $\Delta\sigma$ with the frequency of the high voltage can be observed as well. The superposed RF voltage then only acts as a probing field.

The resonant circuit is arranged as a symmetrical bridge to have maximum sensitivity, a minimum of correction factors, and an exclusion of nonlinearities from the high voltage power supply.

RESULTS AND DISCUSSION

Rather than present a complete kinetic analysis of a given system, we wish to show some typical applications of the techniques.

In Fig. 3 measurements of the field-dissociation effect in solutions of tetrabu-

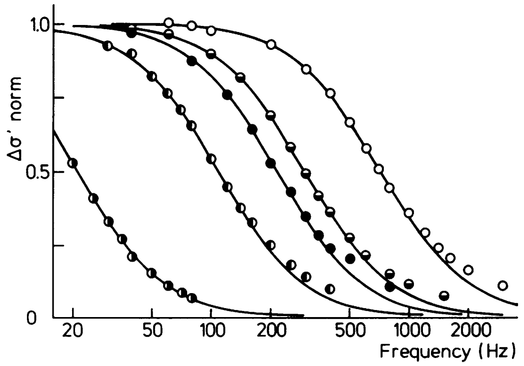

FIGURE 3 Dispersion of the field-dissociation effect in solutions of TBAP at 25°C under various conditions of pressure and solvent composition measured by the field modulation method. Solutions in benzene: ◐, 1.16 × 10^{-4} M; ◑, 3.80 × 10^{-4} M; ◓, 7.63 × 10^{-4} M; ●, 7.68 × 10^{-4} M and P = 650 atm ($\omega_{HF}/2\pi$ = 135 kHz). Solutions in chlorobenzene/benzene (30/70 by volume): ○, 1.12 × 10^{-4} M ($\omega_{HF}/2\pi$ = 80 kHz). The effective field strength is always about 3 kV cm^{-1}. The solid lines are Debye dispersions.

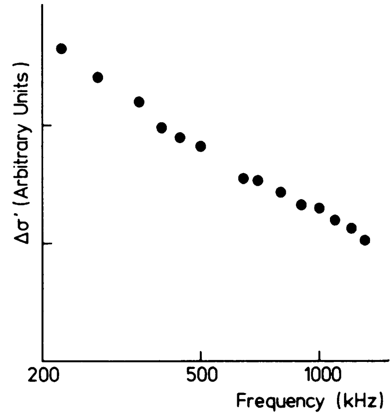

FIGURE 4 Dispersion of the field-dissociation effect in a solution of 0.95 × 10^{-4} M TBAP in chlorobenzene/benzene (20/80 by volume) at 25°C. Conductance increments are plotted as a function of the high frequency ($\omega_{HF}/2\pi$) at a modulation rate ($\omega_{PRF}/2\pi$) of 60 Hz and E = 6 kV cm^{-1}.

tylammonium picrate (TBAP) are presented. The relaxation behavior of the field effect can be described with good accuracy as a simple Debye dispersion process. Specific information on ionic dissociation rates, on processes related to ion-pairing and ionic aggregation, on solution phenomena, etc. is obtained from the dependence of relaxation time on concentration and experimental conditions. The relatively simple and noncritical design of cells for the field modulation method allows relaxation measurements over a broad range of temperature and pressure. The influence of permittivity on ionic processes, as opposed to the more specific ion-solvent interaction, is also open for a detailed investigation. The examples presented are all in relatively nonpolar media. However, the technique is also applicable to polar media provided the interference of joule heating, due to high field conditions, can be avoided or minimized.

In Fig. 4 we present a very interesting effect which remains, however, unexplained theoretically: under the experimental conditions of the field modulation method, the field-dissociation effect decreases when the high frequency (ω_{HF}) in the high field pulses is increased. However, the effect does not interfere with the chemical relaxation. As stated earlier we presume that the effect is related to hydrodynamic interactions of charged species in electrostatic contact. This unexpected high frequency dependence of the field-dissociation effect reflects omissions in the theoretical description of the dynamics of ionic processes.

The experimental results presented in Fig. 5 provide a link between the field modulation method and the resonance method. Indeed, the dispersion of the field-dissociation effect in a benzene solution of TBAP has been recorded with both techniques. Either one of the data sets fits rather well to a theoretical Debye dispersion curve. The relaxation times derived for the ionization process differ only slightly. This increases the reliability of both methods because identical results are obtained with distinct methods. We like to stress that with the field modulation method the in-phase component of the

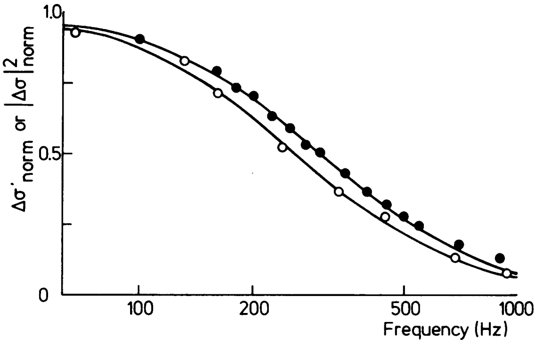

FIGURE 5 Dispersion of the field-dissociation effect in a solution of 7.83×10^{-4} M TBAP in benzene at 25°C. ●, Normalized values of the real part of the conductivity increment plotted as a function of the pulse repetition frequency ($\omega_{PRF}/2\pi$, field modulation method). E (effective) = 3 kV cm^{-1} and $\omega_{HF}/2\pi$ = 135 kHz; ○, normalized values of the square of the modulus of the conductivity increment plotted as a function of the double of the high voltage frequency (resonance method). E = 3.1 kV cm^{-1}, and resonant frequency = 123 kHz. The solid lines are Debye dispersions.

conductance increment ($\Delta\sigma'$) is measured, whereas the resonance method, being phase-insensitive, registers the modulus ($|\Delta\sigma|$) of the effect. At higher field strength and in chemical reactive systems containing dipolar species, the nonlinear parameters $\Delta\epsilon'$ and $\Delta\tan\delta$ depend most often on the square of the field, although nearly complete saturation has been observed for polypeptide solutions. Both parameters turn out to be either positive or negative depending on which contribution to the overall effect is more important: Langevin saturation and its orientational relaxation or chemical modes of permittivity increment and the related chemical relaxation.

In many instances of inter- and intramolecular processes, such effects have been observed, e.g., the self-association of *n*-butanol, of ion-pairs, and of ε-caprolactam (9), the proton transfer reaction between amines and phenols, and ring inversion in trans-1,2-dichlorocyclohexane. A typical analysis of conformational change by internal rotation is shown in Fig. 6. The frequency behavior of the measured quantities closely follows an ideal Debye-type relaxation. The *trans* $\rightleftarrows$ *gauche* equilibrium for the ethane derivative can be characterized from the ratio of chemical increment to Langevin component. The dipole moment of the polar conformer is determined. Rate and activation parameters are obtained from the relaxation times and their temperature dependence. The values agree with spectroscopic and ultrasonic absorption measurements (10). Finally, it should be noted that the conductance of the sample may not exceed such a value that heating by the high field affects the resonance parameters significantly during the RF scan.

CONCLUSION

The relaxation methods presented are suitable for the investigation of the kinetics of ionic and dipolar reactions in any medium. The frequency domain covered is very

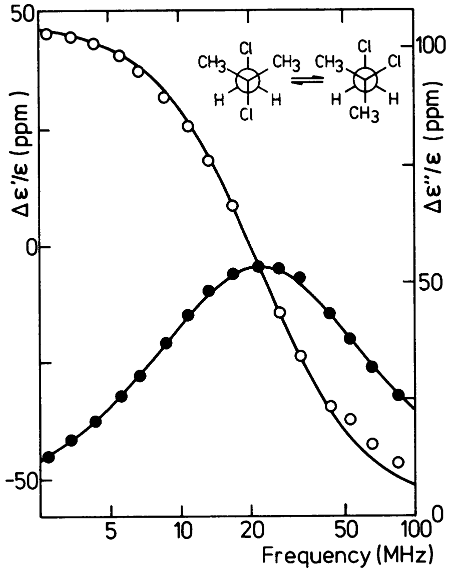

FIGURE 6 Dispersion and absorption of the nonlinear dielectric effect in a solution of 1,2-dichloro-2-methylpropane in benzene. $X = 0.112$ and $\epsilon = 2.88$ at 7.5°C and $E = 138$ kV cm^{-1}. The real ($\Delta\epsilon'$, o) and imaginary ($\Delta\epsilon''$, •) parts of the increment are plotted as a function of the RF frequency. The solid lines are Debye curves.

broad and can be increased with existing technology, e.g., with high power oscillators (radio-transmitters) or very high frequency (gigahertz-domain) generators. The stationary measurement is valuable because of high accuracy and sensitivity, but restricts the applicability of the methods. Indeed, joule heating in conducting media has always been the limitation for stationary high field methods. A combination of pulse techniques with our modulation methods, and trading off some accuracy against potential applications in conducting media, would perhaps reduce this limitation substantially. Another advantage of the electric field methods lies in the detection techniques that make them suitable for the study of nonhomogeneous systems. It is therefore apparent that these methods have a great potential for the investigation of chemical phenomena at interfaces and phase boundaries, which are abundant structures in biological systems.

An important feature of the methods described is how the relaxation behavior of a chemical system is studied. All relevant information is obtained from the intermodulation products generated from the interaction of perturbing and detecting signals. This experimental principle is easily generalized and allows the conception of a new class of techniques to investigate fast reactions. For example, a method for the study of dynamic aspects of photoconductivity is being developed. The method is based on light-induced conductance modulation. The analogy with the field modulation method is evident.

From the biophysicist's point of view the importance of these new techniques lies in the ubiquity of electric fields in biological systems. Important physiological processes invoke the intervention of phenomena induced by, or coupled to, electric field effects. Nonlinear electric behavior of membranes is often discussed in terms of the field-dissociation effect, while even a model of nervous excitation has been developed based on this effect (11). Experimental verification or refutation of such models is often impeded by the scarcity of experimental data on field effects in nonhomogeneous media. There is also a lack of theoretical work on field effects on ionic or dipolar processes in or near phase boundaries marked by a discontinuity in electric admittance.

To explain electrically induced conformational changes in polyelectrolytes and in membranes, polarization effects are often invoked. The field-dependent ionization or conformational transition of molecular groups at the membrane surface can cause a field-dependent surface charge density. These effects may be at the origin of the modulation of permeability by the electric field.

It is also important to note that structural transitions in biopolymers induced by electric fields may be the mechanism for translating electric signals in more enduring chemical states. Especially when the field-induced phenomena are cooperative, this translation mechanism may be of prime importance in biological information storage. Whatever the molecular basis of memory, this exchange mechanism between an electrical signal and a chemical state is an attractive hypothesis.

To assess the value of the hypothesis made on the intervention of electric field effects in biological processes, one of the main experimental criteria will be the time range in which the effects are operative. It is, therefore, promising that the relaxation methods described in this paper allow an accurate and sensitive measurement of the dynamics of these field effects. Moreover, our techniques can in principle with relatively minor technological changes be adapted to the direct study of biological systems.

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DISCUSSION

Scheider: This extended comment is intended to elicit an extended response from Dr. Persoons, if possible, directed to the question of the problems and possibilities of studying *dipolar* chemical coupling in proteins, and to find out what experience you have had with this. For a long time we have been looking for a system in which to see this kind of coupling. We came up against the role of the relative time constants or relative rates of the chemical and orientation events. I can't miss the opportunity to put a plug in here for the *Biophysical Journal* and myself, because in 1965 we published an article there in which we identified the importance of the ratio, $\tau_{\mathrm{chem}}/\tau_{\mathrm{orientation}}$, and of the compound relaxation rate, which is the sum of the reciprocals of the time constants for chemical and rotational relaxation.

The basic fact, in a heuristic sense, is that whether or not thermodynamics is applicable, you can't induce a chemical change by an electric field unless you have preferential orientation of the molecule in which you are trying to induce the chemistry with respect to the direction of the field. If you accept the fact that you have to orient before you can induce chemistry, then the question of the relative rates is important. In the case of small signals, if the chemistry is too fast—to be specific, if the rotation time of proteins is of the order of 1 μs; it is "slow" if it is a great deal slower than 1 μs—you may see a change but you cannot distinguish it from any other induced electronic polarization unless you use other methods, such as optical detection. If you are working strictly with the electrical signal then you can't distinguish the two processes. If the chemistry is slow compared to the rotation, then for what amounts effectively to the length of the experiment (one time constant of rotation), you have no chemical activity. Another way of saying this is that in the dispersion region for molecular rotation you don't see any of the chemistry. The system of choice therefore is one in which the ratio $\tau_{\mathrm{chem}}/\tau_{\mathrm{orient}}$ is of magnitude unity. Now the question is: To what extent can you escape these problems by using large fields? How long can you reasonably subject the protein to large enough fields when you have to use water as a solvent and cannot avoid heating due to ionic current?

Persoons: We can perhaps analyze the response of a system to an electric field as a function of the ratio of chemical relaxation time to orientational relaxation time. Each time you apply an electric field to a system, it will tend to oppose the increase of the potential energy. When the orientation is much faster than the chemical process, the system will oppose the increase in potential energy by orientation, at least at small field strength. Chemical relaxation, however, will be observed at high field strengths due to a net change in concentration of dipolar species. In conclusion, at low fields we observe no chemistry and at high fields we see chemical relaxation.