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CHEMICAL ELECTRIC FIELD EFFECTS IN BIOLOGICAL MACROMOLECULES

EBERHARD NEUMANN

Physikalische und Biophysikalische Chemie, Universität Bielefeld, Postfach 8640, D-4800 Bielefeld 1, F.R.G.

CONTENTS

I. INTRODUCTION

In order to understand the functional role of the natural electric field in bioelectric phenomena such as nerve excitation (Cole, 1968; Tasaki, 1982; Nachmansohn and Neumann, 1975; Dorogi and Neumann, 1980), electrogenic ion transport, neurostimulated secretion of hormones and transmitter substances or the photosynthesis of ATP (Witt *et al.*, 1976; Schlodder *et al.*, 1982) basic knowledge of electric field effects on simple molecules and on chemical reactions is an essential prerequisite.

External electric fields have traditionally been applied in order to probe the electric-ionic properties of atoms and molecules and to study the electronic and optical details of matter. In particular, the combination of electrical and optical techniques represents a powerful tool for the investigation of overall shape and structure and of the dynamic properties of molecules and molecular interactions. Instructive examples for the power of electro-optic analysis are the membrane-bound bacteriorhodopsin (Tsuji and Neumann, 1983) or DNA (Pörschke, 1985). In bacteriorhodopsin, electric fields cause structural transitions which involve orientation changes of the retinal, tyrosine and/or tryptophan residues and p*K*-changes of at least two types of H^+-binding sites. The conformational changes are based on a saturable induced dipole mechanism associated with an extremely large anisotropic (electric) polarizability. On a molecular level the induced polarization appears to involve a restricted electric displacement of ionic groups (ion pairs) within the protein in a highly co-operative manner. The electric field effects observed in bacteriorhodopsin membrane fragments are of functional importance for this light-driven H^+-pumping system (Tsuji and Neumann, 1981a,b).

Electro-optic data on linear polyelectrolytes like the K^+-salt of polyriboadenylate, poly(rA, K^+) demonstrate that the dissociation of counterions from the inner atmosphere and, coupled to it, the destacking of the adenine bases in high electric fields is highly anisotropic (Neumann *et al.*, 1983, 1984). The anisotropy of the counterion movement along polyionic surfaces suggests that counterion exchange as well as influx and efflux of counterionic substrates or hormones occur preferably at the border lines of the ionic atmospheres which cover polyionic regions on macromolecular enzyme and receptor proteins and membranes. Once part of the ion cloud, such substrates and activator substances may reach the active sites via surface diffusion.

In most treatments of electric field effects on chemical processes the theoretical expressions are based on the "homogeneous-field approximation" of the continuum relationship between the total polarization and the electric field strength (Maxwell field). When, however, conversion factors that account for the molecular (inhomogeneous) nature of real systems are given, they are usually only applicable for non-polar solvents and thus exclude aqueous solutions. Therefore, in the present account, particular emphasis is laid on expressions which relate experimentally observable system properties (such as optical or electrical quantities) with the applied (measured) electric field, and which include applications to aqueous solutions.

II. MATTER IN ELECTRIC FIELDS

The primary molecular-mechanical effects of electric fields are (i) the orientation of permanent dipoles (or of dipolar parts in a more complex structure) in the direction of the applied field; (ii) the deformation of polarizable systems (and also, but not necessarily, the subsequent orientation of the induced dipoles in electrically anisotropic particles) including changes in the distance between the charge centres of an ion-pair in a macromolecular structure. In general, major structural changes in electric fields require the presence of ions, or ionized groups, or permanent or induced dipolar charge configurations, preferably in macromolecular structures. Due to electrical–chemical coupling molecular conformations with large electric moments increase in concentration at the expense of those configurations with smaller moments. Secondly, the presence of electric fields increases the dissociation of weak acids and bases and promotes the separation of ion-pairs into the corresponding free ions (dissociation field effect; second Wien effect). The free ions or ionized structures then may move in the direction of the electric field (electrophoresis) and a field-dependent stationary state in the ion distribution may be established.

Thus, basically two types of electric-chemical coupling may be differentiated, (a) permanent or induced *dipolar equilibria*, and (b) *ionic* (dissociation and association) *processes* involving (macro-)ions and low molecular weight ions (of preferably opposite charge sign). Whereas dipolar equilibria in electric fields are accessible to thermodynamic analysis, ionic processes involving free ions require a kinetic approach (Eigen and DeMaeyer, 1963).

1. *Biological and Experimental Electric Fields*

In living organisms electric fields of sufficiently high intensity and of variations large enough to affect chemical processes are encountered not only within membrane phases, but also near the surfaces of membranes and protein organizations, for instance at the active sites of enzymes and receptors. The observed membrane potential differences, $\Delta\psi_m$, of up to 100 mV may correspond to average values of the electric field strength, $\bar{E}=\Delta\psi_m/d$, of about 100 kV cm^{-1} if the thickness of the dielectric membrane part $d\approx 10$ nm.

(a) *Polyionic field effects*

The electric potential $\psi(\mathbf{r})$ in the environment of polyionic macromolecules and membranes decays with increasing distance $\mathbf{r}$ from the surface of fixed ionized groups (or adsorbed ions). The corresponding electric field $\mathbf{E}=-\text{grad}\ \psi(\mathbf{r})$, however, is largely screened by counterion atmospheres at physiological ionic strengths (0.1 to 0.15 mol dm^{-3}). An effective direct interaction of these inhomogeneous fields with chemical reactions is limited to a short range of about 1 nm at 0.1 mol dm^{-3} ionic strength. The electric fields of polyionic surfaces may, however, indirectly affect chemical reactions by accumulating small ionic species in their immediate environment. In these regions of higher ionic strengths, rate and extent of chemical reactions between ionic reaction partners will be different from the behaviour in the bulk solution. This catalytic effect will be very pronounced for polyelectrolyte structures. Theoretical approaches aimed at understanding polyionic electric field effects are being advanced; for instance, partial dehydration of ionic reaction partners in the high local electric field close to a polyionic surface appears to be one of the important factors (see, e.g. Enokida *et al.*, 1980). Practically, the ionic strength dependence of rate and equilibrium constants may be used in order to establish the mechanism of polyionic field effects (Neumann *et al.*, 1980; Neumann and Nolte, 1981).

(b) *Experimental limitations*

The field intensities which are experimentally accessible are limited by dielectric breakdown. In aqueous solutions, fields up to 150 kV cm^{-1} may be controlled over distances in the mm and cm range. It is an additional limitation that in ionic solutions electric fields cannot be maintained for a long time. Due to ionic currents the field will decrease and Joule heating may cause appreciable temperature increases. These problems can be minimized by applying field pulses of limited duration to ionic solutions and suspensions.

2. *Biopolymers*

Among the early examples of the successful use of electric fields to probe ionic structures and electrical and optical anisotropies are the linear polyelectrolytes. Basic information about macromolecular dimensions, size and shape have been derived from the relaxation of field-induced changes in optical properties and in electrical parameters of the electrically and optically anisotropic systems (see, e.g. O'Konski and Haltner, 1957; Tricot and Houssier, 1976; Fredericq and Houssier, 1973). The analysis of electric conductivity measurements has demonstrated that linear polyelectrolytes are electrically anisotropic (Eigen and Schwarz, 1955, 1957, 1962). It was established that the extremely large dipole moments, which the electric field produces by displacement of the counterion atmosphere parallel to the long axis of the polyions, are responsible for their orientations in the direction of the external field. Electric fields are also capable of producing structural-conformational changes in biopolymers and membranes. Initial hints of presumably chemical contributions to field-induced changes in birefringence were reported for DNA solutions of low ionic strength (O'Konski and Stellwagen, 1965). Dielectric measurements have shown that polypeptides in viscous organic solvents may undergo intramolecular helix–coil transitions in the presence of electric fields (Schwarz and Seelig, 1968). In the meantime there are many reports on field-induced *conformational changes* in multi-stranded as well as in single-stranded polyelectrolytes (Neumann and Katchalsky, 1972; Neumann, 1973; Kikuchi and Yoshioka, 1973, 1976; Yasunaga *et al.*, 1973; Revzin and Neumann, 1974; Pörschke, 1974, 1976, 1985).

Of particular interest in the discussion of electric field effects in biological structures is the observation of threshold phenomena. It has been found that electric impulses above a certain threshold intensity are capable of triggering conformational transitions in metastable polynucleotide structures. A similar threshold effect is associated with electric field-induced permeability changes in vesicle membranes (Neumann and Rosenheck, 1972) as well as in cellular systems (Zimmermann *et al.*, 1981; Teissie and Tsong, 1981; Neumann *et al.*, 1982).

III. THERMODYNAMIC FOUNDATIONS

The dependence of biopolymer and membrane reactions on the electric field intensity are frequently very similar to that of small molecules. Therefore, it appears pertinent to introduce the analysis of field-induced macromolecular changes with relationships which are derived to describe field effects in reactions of small molecules. Chemical processes between reaction partners J

$$0 = \Sigma \nu_j J \tag{1}$$

are dependent on the intensive physical variables (z), e.g. temperature (T), pressure (P) or external electric field (**E**) and may be generally described by the z-dependence of the thermodynamic and apparent equilibrium constants, $K^{\ominus}(z)$ and $K(z)$, respectively, and by Dedonder's advancement of reaction ξ(mol) or by a degree of transition, θ. The differential change dn_j in the amount of substance n_j of the species J in a chemical process may be related to the stoichiometric coefficients ν_j (counting negative for reactants and positive for products) by:

$$d\xi = \frac{1}{\nu_j}\,dn_j = \frac{1}{\nu_j}\,V\,dc_j \tag{2}$$

where V is the volume and $c_j = n_j/V$. In integral form:

$$n_j = n_j(\text{ref}) + \nu_j\xi, \qquad c_j = c_j(\text{ref}) + \nu_j\frac{1}{V}\xi \tag{3}$$

where n_j(ref) and c_j(ref) are the reference values (for instance, at given values of P, T and **E**). As $K(z) = \Pi(\bar{c}_j/c^{\ominus})^{\nu_j}$, where $c^{\ominus} = 1$ mol dm^{-3}, is a function of z, ξ is also dependent on z. Not only are the equilibrium values $\bar{c}_j$ and thus $\bar{\xi}$ a function of z, but additionally the extent of a z-induced change, Δc_j or $\Delta\xi$, in c_j and thus in ξ depends on the actual "position" of the equilibrium. Indeed, there are optimum conditions of $\bar{c}_j$ (or $\bar{\xi}$ and $\bar{\theta}$) for major z-induced chemical transformations (see, e.g. Neumann, 1981a).

The dependence of the apparent equilibrium constant $K(z_i)$ on the intensive variable z_i (P, T, **E**) may be expressed by a generalized van't Hoff relationship according to (Eigen and DeMaeyer, 1963):

$$\left(\frac{\partial \ln K}{\partial z_i}\right)_{z \neq z_i} = \frac{\Delta Z_i}{RT} \tag{4}$$

where R is the gas constant, T is the Kelvin temperature and

$$\Delta Z_i = RT \cdot \left(\frac{\partial \ln K}{\partial z_i}\right)_{z \neq z_i} \tag{5}$$

is the extensive reaction quantity complementary to z_i. The subscript $z \neq z_i$ means all z constant except z_i.

It is recalled that when $z_i = T$, $\Delta Z_i = \Delta H/T$ where ΔH is the reaction enthalpy representing the enthalpy difference of *one* stoichiometric transition. When $z_i = P$, $\Delta Z_i = -\Delta \bar{V}$, where $\Delta \bar{V}$ is the molar partial volume change for one stoichiometric transformation. Finally, when $z_i = \mathbf{E}$, the measured electric field, then $\Delta Z_i = \Delta M$, where ΔM is the molar reaction dipole moment; ΔM refers to the components parallel to $\mathbf{E}$, of the dipole moments of the interacting dipolar molecules or macromolecular substructures.

With $\Delta = \partial/\partial\xi$ we have:

$$Z_i = \Sigma_j n_j Z_{i,j} \tag{6}$$

where

$$Z_{i,j} = \left(\frac{\partial Z_i}{\partial n_j}\right)_{n \neq n_j} \tag{7}$$

is the average value of the partial molar quantity Z_i of species j. From eqns (6) and (2) we obtain:

$$\Delta Z_i \equiv \left(\frac{\partial Z_i}{\partial \xi}\right)_z = \Sigma_j \nu_j Z_{i,j}. \tag{8}$$

The reaction quantity ΔZ_i may, in general, be dependent on the actual value of z_i and on ξ because of the dependence of the activity coefficient ratio Y on z_i and ξ.

The thermodynamic equilibrium constant K is given by

$$\left(\frac{\partial \ln K^{\ominus}}{\partial z_i}\right)_{z \neq z_i} = \frac{\Delta Z_i^{\ominus}}{RT} \tag{9}$$

where at given values of $z \neq z_i$ the reaction parameter $\Delta Z_i^{\ominus}$ is a constant, independent of ξ and of Y; $K^{\ominus} = \Pi \bar{a}_j^{\nu_j} = K \cdot \bar{Y}$, where the activity is defined by $a_j = (c_j/c^{\ominus}) y_j$, $\bar{y}_j$ is the thermodynamic activity coefficient and $\bar{Y} = \Pi \bar{y}_j^{\nu_j}$.

Transition curves. The z-dependence of the extent of reaction $\xi(z)$, of the degree of transition or the degree of dissociation $\theta(z)$ can be formulated in terms of thermodynamic quantities. By applying the chain rule of differentiation and using eqn (4) the z_i-induced change in $\xi(z_i)$ is given by

$$\left(\frac{\partial \xi}{\partial z_i}\right)_{z \neq z_i} = \left(\frac{\partial \xi}{\partial \ln K}\right)_z \left(\frac{\partial \ln K}{\partial z_i}\right)_{z \neq z_i} = \left(\frac{\partial \xi}{\partial \ln K}\right)_z \cdot \frac{\Delta Z_i}{RT}. \tag{10}$$

Since $\mathrm{d}c_j = V^{-1} \nu_j \, \mathrm{d}\xi$, the z-dependence of the reaction can be expressed in terms of the concentration change of one of the reaction partners J by

$$\mathrm{d}c_j = \nu_j \Gamma \frac{\Delta Z_i}{RT} \mathrm{d}z_i \tag{11}$$

where (Eigen and DeMaeyer, 1963):

$$\Gamma \equiv \left(\frac{\partial c_j}{\partial \ln K}\right)_z = \frac{1}{\Sigma_j \nu_j^2 / c_j}. \tag{12}$$

Integrated van't Hoff relations. When ΔZ_i is independent of z_i and ξ, it is a constant describing the respective transition at constant $z \neq z_i$. If ΔZ_i is only a function of z_i, integration of eqn (4) in the limits z_i and z_0 yields

$$\ln K(z_i) = \ln K(z_0) + \frac{1}{RT} \int \Delta Z_i \, \mathrm{d}z_i. \tag{13}$$

For further analysis it is useful to apply eqn (13) in the form of

$$K(z_i) = K(z_0) \cdot \mathrm{e}^x \tag{14}$$

where the x-quantity is defined as

$$x = \frac{1}{RT}\int \Delta Z_i \, dz_i. \tag{15}$$

1. *Chemical Affinity*

Before starting the thermodynamic analysis of electric-chemical field effects it is necessary to recall some relations from processes in the absence of external electric fields. The most general equilibrium condition for processes where the temperature and the pressure are under experimental control is that the Gibbs free energy G is at its minimum. Because of the definition

$$G \equiv U + PV - TS \tag{16}$$

where V is the volume, S is the entropy and U is the inner energy of the system, the general Gibbs equation covering "volume work" and chemical work is

$$dU = T\,dS - P\,dV + \Sigma\mu_j\,dn_j \tag{17}$$

where the chemical term refers to the changes in the n_j at given chemical potentials μ_j of the molecules. Insertion of eqn (17) in $dG = dU + P\,dV + V\,dP - T\,dS - S\,dT$ [from eqn (16)] leads to the classical Gibbs equation:

$$dG = -S\,dT + V\,dP + \Sigma_j\mu_j\,dn_j. \tag{18}$$

At constant P and T, the work function characterizing chemical systems (*in the absence of electric fields*) is given by

$$(dG)_{P,T} = \Sigma_j\mu_j\,dn_j \leqq 0. \tag{19}$$

Generally, for equilibrium processes $dG = 0$, for non-equilibrium states and irreversible processes we have $dG < 0$. From eqn (18) the chemical potential of species J is defined by

$$\mu_j = \left(\frac{\partial G}{\partial n_j}\right)_{n \neq n_j} \tag{20}$$

where all n except n_j are held constant. Introducing a standard chemical potential $\mu_j^{\ominus}$ we obtain

$$\mu_j = \mu_j^{\ominus} + RT \ln a_j \tag{21}$$

where $a_j = (c_j/c^{\ominus})y_j$; at unit activity, $a_j = 1$ and $\mu_j = \mu_j^{\ominus}$.

In a closed chemical system the amounts of substance vary during a chemical transformation. We rewrite now $\Sigma\mu_j\,dn_j = \Sigma_j\nu_j\mu_j\,dn_j/\nu_j$ and use the definition of the chemical affinity

$$A = -\Sigma_j\nu_j\mu_j. \tag{22}$$

Recalling eqn (2) the substitution of eqn (22) yields:

$$\Sigma_j\mu_j\,dn_j = -A\,d\xi. \tag{23}$$

Finally, we obtain the familiar expression

$$A = -\left(\frac{\partial G}{\partial \xi}\right)_{P,T} \geqq 0. \tag{24}$$

In line with eqn (19), the equilibrium condition is $A = 0$ and a non-equilibrium state is associated with $A > 0$.

Using eqns (21) and (22) we obtain

$$A = -\Sigma_j\nu_j\mu_j^{\ominus} - RT\cdot\Sigma_j\nu_j \ln a_j. \tag{25}$$

At equilibrium, $A = 0$ and all activities assume their equilibrium values $\bar{a}_j$. Since

$$K^{\ominus} = \Pi\bar{a}_j^{\nu_j} = \Pi(\bar{c}_j/c^{\ominus})^{\nu_j}\cdot\Pi\bar{y}_j^{\nu_j} = K\cdot\bar{Y} \tag{26}$$

where $K=\Pi(\bar{c}_j/c^{\ominus})^{\nu_j}$ and $\bar{Y}=\Pi\bar{y}_j^{\nu_j}$, we derive from eqn (25) that (at $A=0$):

$$\Sigma_j \nu_j \mu_j^{\ominus} = -RT\ln K^{\ominus}. \tag{27}$$

Substitution of this expression into eqn (25) yields

$$A=RT(\ln K^{\ominus}-\ln Q^{\ominus}) \tag{28}$$

where the notation of a non-equilibrium distribution is introduced according to

$$Q^{\ominus}=\Pi a_j^{\nu_j}=\Pi(c_j/c^{\ominus})^{\nu_j}\cdot \Pi y_j^{\nu_j}=Q\cdot Y. \tag{29}$$

From eqn (28) it may be seen that the chemical affinity represents the "thermodynamic distance" of a non-equilibrium distribution from its equilibrium distribution. On the other hand eqn (28) may be used to specify the conditions of applying the van't Hoff relations and their integrated forms to the analysis of experimental data; for instance, to relaxation kinetic amplitudes.

Step perturbations. Suppose that an external parameter z_i (P, T or **E**) can be "suddenly" changed in a practically rectangular fashion from an initial value z_0 to z_i. Immediately after this change, the previous activity ratio $K^{\ominus}(z_0)$ at z_0 becomes a non-equilibrium ratio $Q^{\ominus}(z_i)$ at z_i. Thus, in systems with inertia, initially we have $Q^{\ominus}(z_i)=K^{\ominus}(z_0)$; then the non-equilibrium will relax until the new equilibrium characterized by $K^{\ominus}(z_i)$ is obtained. Hence the sequence

$$K^{\ominus}(z_0)\rightarrow Q^{\ominus}(z_i)\rightarrow K^{\ominus}(z_i) \tag{30}$$

is a general thermodynamic representation of a chemical relaxation initiated by a stepwise variation of an intensive variable z_i.

2. *Application Limits*

There are some limitations for a straightforward application of eqn (4) and all other equations based on it. Explicitly, the reaction quantity ΔZ_i may be dependent on z_i and on ξ. We recall that the Gibbs equation (18) for the $z_i(=T, P)$-dependence of a closed chemically interacting system, to which eqn (23) applies at constant $z\neq z_i$ may be expressed as:

$$\mathrm{d}G(z_i,\xi)_{z\neq z_i}=Z_i\,\mathrm{d}z_i-A\mathrm{d}\xi, \tag{31}$$

where $Z_i=-S$, V and $z_i=T$, P, respectively. For $Z_i=M$ and $z_i=\mathbf{E}$; see Section IV. Because $\mathrm{d}G$ is a total differential the second cross differentials are equal. Hence

$$\left(\frac{\partial Z_i}{\partial\xi}\right)_z=\left(\frac{\partial A}{\partial z_i}\right)_{\xi,z\neq z_i}. \tag{32}$$

With $\Delta Z_i\equiv(\partial Z_i/\partial\xi)_z$, eqn (32) is rewritten as

$$\left(\frac{\partial(A/RT)}{\partial z_i}\right)_{\xi,z\neq z_i}=\frac{\Delta Z_i}{RT}. \tag{33}$$

As seen in eqns (4) and (5), ΔZ_i refers to the z_i-dependence of $K=K^{\ominus}/\bar{Y}$. Therefore (Schwarz, 1967):

$$\left(\frac{\partial\ln K}{\partial z_i}\right)_{\xi,z\neq z_i}=\left(\frac{\partial\ln(K^{\ominus}/\bar{Y})}{\partial z_i}\right)_{z\neq z_i}. \tag{34}$$

Since $\bar{Y}$ is the value of Y at equilibrium, i.e. at $A=0$, we may use eqn (28) in the form of

$$A/RT=\ln(K^{\ominus}/Y)-\ln Q \tag{35}$$

and specify eqn (34) as

$$\left(\frac{\partial\ln K}{\partial z_i}\right)_{z\neq z_i}=\left(\frac{\partial\ln(K^{\ominus}/Y)}{\partial z_i}\right)_{z\neq z_i,A=0}. \tag{36}$$

Equation (36) is the starting point of a derivation that finally leads to (Neumann, 1986)

$$\left(\frac{\partial \ln K}{\partial z_i}\right)_{z\neq z_i}=\frac{\Delta Z_i}{RT}\left[1-V\bar{\Gamma}^*\left(\frac{\partial \ln \bar{Y}}{\partial \ln \xi}\right)_z\right] \tag{37}$$

where $\bar{\Gamma}^*$ is the equilibrium value of Γ^*. With eqn (12) Γ^* is given by

$$\frac{1}{\Gamma^*}=\frac{1}{\Gamma}\left[1+V\Gamma\left(\frac{\partial \ln Y}{\partial \ln \xi}\right)_z\right]. \tag{38}$$

Equation (37) provides the basis to evaluate the z_i-dependence of the equilibrium concentrations $\bar{c}_j(z_i)$ or related quantities. Equation (37) reduces to the commonly applied eqn (4) provided that the dependence of the activity coefficient ratio Y on ξ is negligibly small. This condition is usually fulfilled if (i) the change δz_i only produces a small shift in K and thus in Y, or (ii) the value of Y is determined by an excess of components other than the reaction partners J; such a condition is met when ionic reactions occur in the presence of an excess of inert electrolyte.

When $z_i=T$ or P the dependence of $\Delta Z_i=\Delta H/T$ or $-\Delta\bar{V}$, respectively, on T or P may be solely expressed in terms of Y.

Since $(\partial \ln K^{\ominus}/\partial\xi)_z=0$ we have $(\partial \ln K^{\ominus}/\partial z_i)=(\partial \ln K^{\ominus}/\partial z_i)_\xi$. At $A=0$, where $Y=\bar{Y}$, eqn (9) yields

$$\frac{\Delta Z_i}{RT}=\left(\frac{\partial \ln K^{\ominus}}{\partial z_i}\right)_{\xi,z\neq z_i}-\left(\frac{\partial \ln \bar{Y}}{\partial z_i}\right)_{\xi,z\neq z_i} \tag{39}$$

and thus

$$\Delta Z_i=\Delta Z_i^{\ominus}-\left(\frac{\partial \ln \bar{Y}}{\partial z_i}\right)_{\xi,z\neq z_i} \tag{40}$$

holds (Eigen and DeMaeyer, 1963). It is instructive to recall eqns (6) and (8) and compare with eqn (40). Obviously, $\Delta Z_i=\Sigma_j\nu_jZ_{i,j}$ refers to the general non-ideal behaviour. Ideal additivity refers to $Y=1$; for this limiting case eqn (8) reads:

$$\Delta Z_i^{\ominus}=\Sigma_j\nu_jZ_{i,j}^{\ominus}\,. \tag{41}$$

Thus, as usually, non-idealities are covered by the introduction of activity coefficients. It will be shown below that a similar approach holds for the field-dipole interactions.

3. *Electrochemical Potential*

It is recalled that the electrochemical potential (Guggenheim, 1967) of a single *isolated* ion of type k is given by:

$$\tilde{\mu}_k=\mu_k+F\tilde{z}_k\psi_k^{\ominus} \tag{42}$$

where μ_k is the ordinary chemical potential, F the Faraday constant, $\tilde{z}_k$ the charge number (with sign) and $\psi_k^{\ominus}$ the ideal (Coulomb) electrostatic potential of the *isolated* ion.

In the presence of other ions it is necessary to account for the ionic screening effect of the ionic atmospheres; for instance by a Debye charging integral

$$\tilde{\mu}_k=\mu_k+F\int\bar{\psi}\,\mathrm{d}\tilde{z}_k, \tag{43}$$

where $\bar{\psi}$ is the mean electric potential.

In a solution of several ionic species the total electric work of charging from $\tilde{z}_k=0$ to $\tilde{z}_k$ is the sum over the charging integrals of all species: $F\cdot\Sigma_k(\int\bar{\psi}\,\mathrm{d}\tilde{z}_k)\,\mathrm{d}n_k$. Substitution into eqn (43) yields the chemical work

$$\Sigma_k\tilde{\mu}_k\,\mathrm{d}n_k=\Sigma_k(\mu_k+F[\int\bar{\psi}\,\mathrm{d}\tilde{z}_k])\,\mathrm{d}n_k. \tag{44}$$

The fundamental Gibbs equation (17) for ionic species thus is

$$\mathrm{d}U=T\,\mathrm{d}S-P\,\mathrm{d}V+\Sigma_k\tilde{\mu}_k\,\mathrm{d}n_k. \tag{45}$$

The Gibbs function $\mathrm{d}G$ for ionic systems is

$$\mathrm{d}G = -S\,\mathrm{d}T + V\,\mathrm{d}P + \Sigma_k \tilde{\mu}_k\,\mathrm{d}n_k \tag{46}$$

where the electrochemical potential is defined as

$$\tilde{\mu}_k = \left(\frac{\partial G}{\partial n_k}\right)_{n \neq n_k, T, P}. \tag{47}$$

(a) *Electrochemical affinity*

In line with eqn (22), the electrochemical affinity of chemically interacting ions is

$$\tilde{A} = -\Sigma_k \nu_k \tilde{\mu}_k. \tag{48}$$

Because of total charge conservation $\Sigma \nu_k \tilde{z}_k = 0$; thus $\tilde{A} = A$.

It is useful to introduce a standard value of the electrochemical potential according to

$$\tilde{\mu}_k^{\ominus} = \mu_k^{\ominus} + F \int \psi_k^{\ominus}\,\mathrm{d}\tilde{z}_k, \tag{49}$$

where $\mu_k^{\ominus}$ is the ordinary standard value for the case that the species were neutral. Analogous to eqn (21) we have

$$\tilde{\mu}_k = \tilde{\mu}_k^{\ominus} + RT \ln a_k. \tag{50}$$

(b) *Electrochemical activity coefficient*

The formalism of the eqns (43), (49) and (50) explicitly shows that the (electric) activity coefficient of ionic species accounts for deviations from the ideal (unscreened) Coulomb behaviour. It will be demonstrated below that an analogous formalism describes non-idealities in the interactions between dipolar species.

If the ionic species can be neutralized (uncharged), the chemical potential of the uncharged form is

$$\mu_k = \mu_k^{\ominus} + RT \ln a_k^{(0)} \tag{51}$$

where the superscript $^{(0)}$ is used to indicate the neutral form. Due to the ionic character, the activity, (a_k), of the ionic species is different from the activity, $a_k^{(0)}$, in the neutral form.

By this formalism the quantity $\tilde{\mu}_k$ is once expressed in terms of $a_k^{(0)}$ and $\bar{\psi}$ and, alternatively, as a function of $\psi_k^{\ominus}$ and a_k:

$$\begin{aligned} \tilde{\mu}_k &= \mu_k^{\ominus} + RT \ln a_k^{(0)} + F \cdot \int \bar{\psi}\,\mathrm{d}\tilde{z}_k \\ &= \mu_k^{\ominus} + F \int \psi_k^{\ominus}\,\mathrm{d}\tilde{z}_k + RT \ln a_k. \end{aligned} \tag{52}$$

Rearrangement leads to:

$$F \cdot \int (\bar{\psi} - \psi_k^{\ominus})\,\mathrm{d}\tilde{z}_k = RT \ln(a_k / a_k^{(0)}). \tag{53}$$

Since we refer to the same amount of species (in the uncharged form and in the ionic form), the concentrations are equal, i.e. $c_k = c_k^{(0)}$. Hence

$$(a_k / a_k^{(0)}) = y_k / y_k^{(0)}. \tag{54}$$

It is reminded that solely electrostatic interactions are covered by the terms ψ and $\psi_k^{\ominus}$. Note that

$$\psi_k^{\ominus}(\mathbf{r}) = \frac{\tilde{z}_k e_0}{4\pi\varepsilon_0 \varepsilon \cdot r} \tag{55}$$

where e_0 is the (positive) elementary charge, ε_0 the vacuum permittivity and ε the dielectric permittivity of the medium. Therefore $y_k^{(0)} = 1$ and thus $y_k / y_k^{(0)} = y_k$. From eqns (53) and (54) we obtain

$$F \cdot \int (\bar{\psi} - \psi_k^{\ominus})\,\mathrm{d}\tilde{z}_k = RT \ln y_k. \tag{56}$$

The (electrostatic) activity coefficient of ions indeed covers the *difference* between the actual and the ideal Coulomb potential of the formal charge $\tilde{z}_k e_0$.

Introducing eqn (50) into (48), we obtain

$$\tilde{A} = -\Sigma_k \nu_k \tilde{\mu}_k = -\Sigma_k \nu_k \tilde{\mu}_k^{\ominus} - RT\Sigma_k \nu_k \ln(c_k/c^{\ominus})y_k. \tag{57}$$

At equilibrium, $\tilde{A}=0$ and $c_k y_k = \bar{c}_k \bar{y}_k$; by analogy to eqn (27):

$$\Sigma \nu_k \tilde{\mu}_k^{\ominus} = -RT \ln K^{\ominus}. \tag{58}$$

Since $K^{\ominus} = K \cdot \bar{Y}$, eqn (56) leads to:

$$RT \ln \bar{Y} = F \cdot \Sigma_k \nu_k \int (\bar{\psi} - \psi_k^{\ominus})\, \mathrm{d}\tilde{z}_k. \tag{59}$$

The relationship (59) may be used to estimate charge numbers of ionic binding sites on macromolecules in the framework of the Debye–Hückel approximations (Nolte *et al.*, 1980; Neumann and Nolte, 1981).

(c) *Polyionic macromolecules*

In macromolecular biological structures ion-pairs are frequently encountered. For instance, the inner counterions surrounding the polynucleotide macroanions appear to form "diffuse ion-pairs" with the fixed polyionic matrix. Externally applied electric fields can compete with the inner fields and shift these counterions relative to the polyion thus producing large dipole moments. It is known that proteins may contain inner salt bridges, i.e. contact ion-pairs between fixed ionized side chains of the amino acid residues of the polypeptide chains and/or other ionic groups. In particular membrane proteins like the bacteriorhodopsin of the purple membranes of halobacteria appear to contain an unusually large number of charged groups within the protein structure. In such a case we may group together oppositely charged groups into ion-pairs. The total electric moment **M** of the macromolecule is then the vector sum over all individual contributions ($\mathbf{m}_j$) of the ion-pairs.

IV. THERMODYNAMICS IN ELECTRIC FIELDS

1. *The Characteristic Gibbs Function*

In order to describe ion-pair associations it is useful to extend Guggenheim's concept of an electrochemical potential of a single ion and to formulate a *dielectrochemical potential* $\check{\mu}_j$ of a (dipolar) ion-pair (Neumann, 1981, 1986). The concept of a dielectrochemical potential is already implicit in Guggenheim's treatment of dielectrics in the presence of external electric fields. In order to apply the familiar criteria for reversible (equilibrium) processes and irreversible (non-equilibrium) processes in terms of an appropriate Gibbs function, Guggenheim introduced the characteristic Gibbs function in the presence of electric fields by a transformation. Here, we may express the transformed Gibbs free energy as

$$\check{G} = G - EM \tag{60}$$

where G is the ordinary Gibbs free energy at **E** and M the absolute value of the total moment **M**.

For plate condensor geometry the measurable polarization M is the parallel component, $(\mathbf{M})_{\|}$, of the total macroscopic dipole component $\mathbf{M} = \mathbf{P} \cdot V$ where **P** is the familiar polarization per unit volume and V is the volume. Thus (Neumann *et al.*, 1984):

$$\begin{aligned} M = (\mathbf{M})_{\|} &= N_A \Sigma_j n_j \langle m_j \cos \vartheta_j \rangle \\ &= N_A \Sigma_j n_j m_j = \Sigma_j n_j M_j \end{aligned} \tag{61}$$

where N_A is the Avogadro–Loschmidt constant, n_j is the amount of substance J having the dipole moment $\mathbf{m}_j$. M is the statistical average over all orientations of the individual dipoles. M_j is the average partial molar dipole moment given by

$$M_j = (\partial M / \partial n_j)_{n \neq n_j} = N_A \langle m_j \cos \vartheta_j \rangle \tag{62}$$

and ϑ_j is the angle between the dipole vector $\mathbf{m}_j$ and the electric field $\mathbf{E}$.

With U and S denoting the inner energy and the total entropy in the field, eqn (60) is rewritten as

$$\check{G} = U - TS + PV - EM. \tag{63}$$

The general Gibbs equation for chemical changes in electric fields is:

$$\mathrm{d}U = T\,\mathrm{d}S - P\,\mathrm{d}V + \Sigma_j \check{\mu}_j\,\mathrm{d}n_j + E\,\mathrm{d}M \tag{64}$$

where $\check{\mu}_j$ denotes the chemical potential of species J in the field. Equations (63) and (64) then yield

$$\mathrm{d}\check{G} = \mathrm{d}G - \mathrm{d}(EM) = -S\,\mathrm{d}T + V\,\mathrm{d}P + \Sigma\check{\mu}_j\,\mathrm{d}n_j - M\,\mathrm{d}E. \tag{65}$$

At constant P, T eqn (65) reduces to

$$\mathrm{d}\check{G} = \Sigma\check{\mu}_j\,\mathrm{d}n_j - M\,\mathrm{d}E. \tag{66}$$

Consistent with ordinary thermodynamics we obtain from eqn (66):

$$\check{\mu}_j = (\partial\check{G}/\partial n_j)_{P,T,E,n \neq n_j} \tag{67}$$

where all n except for n_j are constant. By cross differentiation eqn (66) yields

$$(\partial\check{\mu}_j/\partial E)_{P,T} = -(\partial M/\partial n_j)_{P,T,E,n \neq n_j} = -M_j \tag{68}$$

where eqn (61) was applied to obtain M_j. Integration of eqn (68) between E and $E = 0$ yields (Kirkwood and Oppenheim, 1961):

$$\check{\mu}_j = \mu_j - \int M_j\,\mathrm{d}E \tag{69}$$

where $\check{\mu}_j(0) = \mu_j$ is the ordinary chemical potential at $E = 0$. By similarity to Guggenheim's electrochemical potential we may call $\check{\mu}_j$ the *dielectrochemical potential* of the dipolar species. Analogous to eqn (50) we introduce a standard dielectrochemical potential $\check{\mu}_j^{\ominus}$ and write

$$\check{\mu}_j = \check{\mu}_j^{\ominus} + RT \ln \check{a}_j \tag{70}$$

where $\check{a}_j = (c_j/c^{\ominus})\check{y}_j$ is the activity and $\check{y}_j$ the activity coefficient in the field. Obviously, the standard terms are connected by

$$\check{\mu}_j^{\ominus} = \mu_j^{\ominus} - \int M_j^{\ominus}\,\mathrm{d}E. \tag{71}$$

By inspection of eqns (61) and (69) it is evident that in a random distribution of dipoles or at $\mathbf{E} = 0$, the relation $\check{\mu}_j = \mu_j$ holds. In these cases the dielectrochemical potential equals the chemical potential.

2. *Dielectrochemical Affinity*

In line with eqn (48), we may now define a "dielectrochemical affinity" for chemically interacting *dipolar* species in the presence of electric fields:

$$\check{A} = -\Sigma_j \nu_j \check{\mu}_j. \tag{72}$$

Substitution of eqn (69) into eqn (72) yields:

$$\check{A} = -\Sigma_j \nu_j \check{\mu}_j = -\Sigma_j \nu_j \mu_j + \Sigma_j \nu_j \int M_j\,\mathrm{d}E. \tag{73}$$

Introducing eqn (72) and $\mathrm{d}n_j = \nu_j\,\mathrm{d}\xi$ into eqn (66) we obtain the Gibbs function of chemically reacting systems in external electric fields:

$$(\mathrm{d}\check{G})_{P,T} = -\check{A}\,\mathrm{d}\xi - M\,\mathrm{d}E. \tag{74}$$

The dielectrochemical affinity is consistently defined in terms of $\check{A}$ and ξ by

$$\check{A} = -\left(\frac{\partial\check{G}}{\partial\xi}\right)_{P,T,E}. \tag{75}$$

It is remarked that reversible and irreversible processes in external electric fields are characterized by

$$\mathrm{d}\breve{G} \leqq 0 \quad \text{and} \quad \breve{A} \geqq 0. \tag{76}$$

Thus the definitions of $\breve{G}$ and $\breve{A}$ permit the thermodynamic treatment of electric field effects in the framework of concepts which are familiar from ordinary chemical thermodynamics in the absence of electric fields.

3. *Activity Coefficients*

The standard value, $\breve{\mu}_j^{\ominus}$, of the dielectrochemical potential for the dipolar species is defined as:

$$\breve{\mu}_j = \breve{\mu}_j^{\ominus} + RT \ln \breve{a}_j \tag{77}$$

where $\breve{a}_j$ is the activity in the presence of **E**. In line with eqn (69), we have

$$\breve{\mu}_j^{\ominus} = \mu_j^{\ominus} - \int M_j^{\ominus}\, \mathrm{d}E \tag{78}$$

where $\mu_j^{\ominus}$ is the ordinary standard potential used in

$$\mu_j = \mu_j^{\ominus} + RT \ln a_j. \tag{79}$$

Note that a_j is the activity of J at $\mathbf{E} = 0$; $M_j^{\ominus}$ is the standard value of M_j associated with isolated ideal dipoles. Comparing eqns (78) and (49) the dielectrochemical potential may also be expressed in two ways.

From eqns (69), (77)–(79) we obtain

$$\begin{aligned} \breve{\mu}_j &= \mu_j^{\ominus} + RT \ln a_j - \int M_j\, \mathrm{d}E \\ &= \mu_j^{\ominus} + RT \ln \breve{a}_j - \int M_j^{\ominus}\, \mathrm{d}E. \end{aligned} \tag{80}$$

Hence, analogous to eqn (53) the relation

$$-\int (M_j - M_j^{\ominus})\, \mathrm{d}E = RT \ln(\breve{a}_j / a_j) \tag{81}$$

holds. Since $\breve{c}_j = c_j$, the activity ratio is

$$\breve{a}_j / a_j = \breve{y}_j / y_j. \tag{82}$$

If only dipolar interactions are considered, $y = 1$ and

$$-\int (M_j - M_j^{\ominus})\, \mathrm{d}E = RT \ln \breve{y}_j, \tag{83}$$

showing that for dipolar species, too, the activity coefficient covers deviations from simple ideal additivities.

In the ideal case of pure additive superposition of the formal charge-distance products $|\tilde{z}_j|\, e_0 \mathbf{r}_j = \mathbf{m}_j^{\ominus}$, where $\tilde{z}_j$ is the integer charge number, the total (standard) polarization is given by

$$M^{\ominus} = \Sigma_j n_j M_j^{\ominus} = N_A \Sigma_j n_j m_j^{\ominus} \tag{84}$$

where

$$m_j^{\ominus} = \langle \mathbf{m}_j^{\ominus} \cos \vartheta_j \rangle = m_j^{\ominus} \langle \cos \vartheta_j \rangle$$

and

$$M_j^{\ominus} = N_A \cdot m_j^{\ominus}. \tag{85}$$

In all real cases of finite concentrations the eqns (61) and (62) have to be applied.

4. *Van't Hoff Relationship for* K(E)

From eqns (72) and (77) we obtain

$$\breve{A} = -\Sigma_j \nu_j \breve{\mu}_j^{\ominus} - RT\, \Sigma_j \nu_j \ln \breve{a}_j. \tag{86}$$

At equilibrium, $\breve{A}=0$ and all $\breve{a}_j$ are equilibrium values. In this case $K^{\ominus}(E)=\Pi\breve{a}_j^{\nu_j}$ and eqn (86) yields

$$RT\ln K^{\ominus}(E)=-\Sigma_j\nu_j\breve{\mu}_j^{\ominus}. \tag{87}$$

Substitution of eqn (78) results in:

$$\Sigma_j\nu_j\breve{\mu}_j^{\ominus}=\Sigma_j\nu_j\mu_j^{\ominus}-\Sigma_j\nu_j\int M_j^{\ominus}\,\mathrm{d}E. \tag{88}$$

When we now differentiate eqn (84) with respect to ξ and use $\mathrm{d}n_j/\mathrm{d}\xi=\nu_j$, we have

$$\left(\frac{\partial M^{\ominus}}{\partial\xi}\right)_{E,T,P}=\Sigma\nu_jM_j^{\ominus}=\Delta M^{\ominus}. \tag{89}$$

Introduction of the eqns (88) and (27) in the form of $RT\ln K^{\ominus}(0)=-\Sigma\nu_j\breve{\mu}_j^{\ominus}$, at $\mathbf{E}=0$, into eqn (87) finally leads to

$$\ln K^{\ominus}(E)=\ln K^{\ominus}(0)+\frac{1}{RT}\int\Delta M^{\ominus}\,\mathrm{d}E \tag{90}$$

as the integrated van't Hoff relationship for a dipolar equilibrium in an external electric field.

From eqn (74) we obtain by cross differentiation

$$\left(\frac{\partial\breve{A}}{\partial E}\right)_{\xi,T,P}=\left(\frac{\partial M}{\partial\xi}\right)_{E,T,P}=\Delta M=\Sigma_j\nu_jM_j. \tag{91}$$

Note that the relationship between the affinity and the M_j values refers to constant ξ and thus to given values of c_j. For chemically interacting species eqn (83) is rewritten as

$$-\Sigma_j\nu_j\int(M_j-M_j^{\ominus})\,\mathrm{d}E=RT\Sigma\nu_j\ln\breve{y}_j \tag{92}$$

Substitution of eqns (89) and (90) and of the definition $\breve{Y}=\Pi\breve{y}^{\nu_j}$ yields:

$$\Delta M=\Delta M^{\ominus}-RT\left(\frac{\partial\ln\breve{Y}}{\partial E}\right)_{\xi}. \tag{93}$$

Since $K^{\ominus}=K\cdot\breve{Y}$, the comparison of

$$\left(\frac{\partial\ln K^{\ominus}}{\partial E}\right)_{P,T}=\frac{\Delta M^{\ominus}}{RT} \tag{94}$$

$$\left(\frac{\partial\ln K}{\partial E}\right)_{P,T}=\frac{\Delta M}{RT}=\frac{\Delta M^{\ominus}}{RT}-\left(\frac{\partial\ln Y}{\partial E}\right)_{\xi,P,T} \tag{95}$$

with eqn (83) shows that the activity coefficient product is given by

$$RT\ln\left(\frac{\breve{Y}(E)}{\breve{Y}(0)}\right)_{\xi}=-\int(\Delta M-\Delta M^{\ominus})\,\mathrm{d}E. \tag{96}$$

If $\breve{Y}$ is independent of E, then $\Delta M=\Delta M^{\ominus}$ is independent of ξ (or of the concentrations of the reaction partners). The relationship (95) provides the basis for the analysis of electric field induced concentration shifts in dipolar equilibria.

V. RATE CONSTANTS IN ELECTRIC FIELDS

1. *Dipolar Equilibria*

Since the equilibrium constant, K, of an elementary chemical reaction is a function of $z_i(=P, T, \mathbf{E})$, the rate constants must depend differently on the z_i-values. Consider a chemical reaction $0=\Sigma\nu_j J$, rewritten as

$$\Sigma\nu_rJ_r \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \Sigma\nu_pJ_p \tag{97}$$

where the subscripts r and p refer to reactants and products, respectively. In this notation the stoichiometric coefficients $v_j = v_r$ and $|v_j| = v_p$ are definite positive. Obviously,

$$K = \Pi(\bar{c}_p/c^{\ominus})^{v_p}/\Pi(\bar{c}_r/c^{\ominus})^{v_r} = |k_f|/|k_r| \tag{98}$$

where k_f and k_r are the rate constants of the forward and reverse direction, respectively; the number value $|k|$ is equal to k divided by the respective dimension. $K^{\ominus}$ and the rate constants $k_f^{\ominus}$ and $k_r^{\ominus}$ are related by:

$$K^{\ominus} = \Pi\bar{a}_p^{v_p}/\Pi\bar{a}_r^{v_r} = |k_f^{\ominus}|/|k_r^{\ominus}|. \tag{99}$$

At equilibrium we have:

$$k_f\Pi\bar{c}_r^{v_r} = k_r\Pi\bar{c}_p^{v_p} \tag{100}$$

and

$$k_f^{\ominus}\Pi y_r^{v_r}\cdot\Pi\bar{c}_r^{v_r} = k_r^{\ominus}\Pi y_p^{v_p}\cdot\Pi\bar{c}_p^{v_p}. \tag{101}$$

We now recall eqn (13):

$$\ln K(z_i) = \ln K(z_0) + \frac{1}{RT}\int\Delta Z_i\,\mathrm{d}z_i \tag{102}$$

where z_0 is a reference value. In line with eqn (98) the relation (8) is rewritten as

$$\Delta Z_i = \Sigma_j v_j Z_{i,j} = \Sigma_p v_p Z_{i,p} - \Sigma_r v_r Z_{i,r}. \tag{103}$$

By the definitions:

$$\Delta Z_{i,p} = \Sigma_r v_r Z_{i,p} \tag{104}$$

$$\Delta Z_{i,r} = \Sigma_r v_r Z_{i,r} \tag{105}$$

the contributions of the products and those of the reactants are explicitly separated. With these expressions and with eqn (98) we may express eqn (102) in terms of the rate coefficients as

$$\ln\frac{k_f(z_i)}{k_r(z_i)} = \ln\frac{k_f(z_0)}{k_r(z_0)} + \frac{\int(\Delta Z_{i,p} - \Delta Z_{i,r})\,\mathrm{d}z_i}{RT}. \tag{106}$$

After term separation we obtain

$$k_f(z_i) = k_f(z_0)\exp[\textstyle\int\Delta Z_{i,p}\,\mathrm{d}z_i/RT],$$

$$k_r(z_i) = k_r(z_0)\exp[\textstyle\int\Delta Z_{i,r}\,\mathrm{d}z_i/RT]. \tag{107}$$

The relations between $k^{\ominus}(z_i)$ and $\Delta Z_i^{\ominus}$ are analogous to the eqns (107).

The general formalism developed here, is particularly useful for the description of electric field effects on the rate constants of dipolar equilibria. We may choose $z_0 = E = 0$ as a suitable reference and specify the eqns (107) as

$$k_f(E) = k_f(0)\exp[\textstyle\int\Delta M_p\,\mathrm{d}E/RT]$$

$$k_r(E) = k_r(0)\exp[\textstyle\int\Delta M_r\,\mathrm{d}E/RT] \tag{108}$$

Thus the rate constants of the product formation are dependent on the dipole moment contributions of the products and the rate constants of the reactant formation are a function of the dipole moment contributions of the reactants. *Whereas the field dependence of the equilibrium constant only yields the difference* ΔM *of the reaction partners, the rate constants provide a means to determine the dipole moments of the reactants and, separately, those of the products.*

Equations (108) were used to discuss the rate aspects of electric field-induced permeability changes in membranes (Neumann and Rosenheck, 1972) in the context of electric membrane fusion (Neumann *et al.*, 1980) and electric gene transfer (Neumann *et al.*, 1982) by electroporation (Sugar and Neumann, 1984).

2. *Ionic Equilibria*

According to Onsager it is the dissociation rate constant, k_d, of the separation of an ion-pair that is mainly affected by the electric field; the association rate constant, k_a, remaining practically unchanged (Onsager, 1934). Consider the ion-pairing equilibrium:

$$(\mathrm{L}^+\cdot\mathrm{B}^-) \underset{k_a}{\overset{k_d}{\rightleftharpoons}} \mathrm{L}^+ + \mathrm{B}^-. \tag{109}$$

The Onsager treatment provides an expression for the electric field dependence of $k_d^{\ominus}$. Note that $k_d^{\ominus} = k_d y_{(\mathrm{L}\cdot\mathrm{B})}$. The electric field induced increase in the conductivity of electrolytes usually starts non-linear, followed by a range where the relative conductivity change, $\Delta\kappa/\kappa(0)$, is linearly dependent on the electric field strength and finally approaches a field-independent saturation value. For the linear range Onsager's theory of diluted weak electrolytes yields

$$\left(\frac{\partial \ln |k_d^{\ominus}|}{|\partial \mathbf{E}|}\right)_{P,T} = \frac{\tilde{z}_L u_L - \tilde{z}_B u_B}{u_L + u_B}\,\frac{|\tilde{z}_L \tilde{z}_B|\, e_0^3}{8\pi\varepsilon_0\varepsilon (kT)^2} = \gamma \tag{110}$$

where u is the ionic mobility of the free ions and where $|k_d^{\ominus}| = k_d^{\ominus}/s^{-1}$.

For symmetric electrolytes $\tilde{z}_L = -\tilde{z}_B = |\tilde{z}|$, eqn (110) is reduced to

$$\left(\frac{\partial \ln |k_d^{\ominus}|}{\partial |\mathbf{E}|}\right)_{P,T} = \frac{|\tilde{z}^3|\, e_0^3}{8\pi\varepsilon_0\varepsilon (kT)^2} = \gamma. \tag{111}$$

Note the strong dependence on the charge number. The equilibrium constant of a 1:1 weak electrolyte like acetic acid is increased by an electric field of 100 kV cm^{-1} to about 14%, that for a 2:2 electrolyte like $MgSO_4$ to about 110% (Neumann, 1981a, 1986). Compared to simple dipolar equilibria of small molecules where electric field induced changes in K are very small, we see that the dissociation step of simple ion-pairs is associated with a relatively large electric field effect. If $y_{LB} \approx 1$, then $k^{\ominus} = k$ and eqns (110) and (111) apply to k.

Following the analysis in the previous section the association step in eqn (109) must also be field dependent. According to eqns (108) we have now [with $|\mathbf{E}| = E$ and $|k_a^{\ominus}| = k_a^{\ominus}/(\mathrm{dm^3\,mol^{-1}\,s^{-1}})$]:

$$\left(\frac{\partial \ln |k_a^{\ominus}|}{\partial E}\right)_{P,T} = \frac{\Delta M_a^{\ominus}}{RT} \tag{112}$$

where $\Delta M_a^{\ominus}$ is given by

$$\Delta M_a^{\ominus} = M_{\mathrm{L}\cdot\mathrm{B}}^{\ominus} = N_A m_{\mathrm{L}\cdot\mathrm{B}}^{\ominus}. \tag{113}$$

Note that in line with eqn (84) we have

$$m_{\mathrm{L}\cdot\mathrm{B}}^{\ominus} = \mathbf{m}_{\mathrm{L}\cdot\mathrm{B}}^{\ominus} \langle \cos \vartheta_{\mathrm{L}\cdot\mathrm{B}} \rangle. \tag{114}$$

Therefore the field dissociation effect (or second Wien effect) as a whole is also determined by the dipole moment of the ion-pair which can dissociate into the free ions.

At finite electrolyte concentration the activity coefficients have to be considered. For eqn (109), $k_a^{\ominus} = k_a \cdot y_L \cdot y_B$; thus

$$\left(\frac{\partial \ln |k_a^{\ominus}|}{\partial E}\right)_{P,T} = \left(\frac{\partial \ln |k_a|}{\partial E}\right)_{P,T} + \left(\frac{\partial \ln(y_L \cdot y_B)}{\partial E}\right)_{P,T} \tag{115}$$

and from eqn (93):

$$\Delta M_a^{\ominus} = \Delta M_a + RT\left(\frac{\partial \ln(y_L \cdot y_B)}{\partial E}\right)_{\xi,P,T}. \tag{116}$$

Usually the effect of an electric field on the activity coefficient of free ions is apparent from the first Wien effect, i.e. from the perturbation (and finally the destruction) of the ionic atmosphere by an external electric field. For example, in the case of the conductivity data on $MgSO_4$, the contribution of the first Wien effect can be quantitatively covered in terms of the Wilson theory (Schallreuter, 1982).

Since $K = |k_d|/|k_a|$, ionic equilibria of the type (109) are characterized by

$$\left(\frac{\partial \ln K}{\partial E}\right)_{P,T} = \gamma - \frac{\Delta M_a^{\ominus}}{RT} - \left(\frac{\partial \ln \tilde{Y}}{\partial E}\right)_{\xi,P,T} \tag{117}$$

where $\tilde{Y}$ is given by $\tilde{Y} = \tilde{y}_L \cdot \tilde{y}_B / \check{y}_{L \cdot B}$ at a field strength $\mathbf{E}$. At very high field strengths the ionic atmosphere screening may be reduced to a large extent such that $\tilde{Y} = 1$; at very diluted solutions we may set $\tilde{Y} = 1$. In both cases the approximation $(\partial \ln \tilde{Y}/\partial E)_{\xi,P,T} = 0$ may be used.

3. *Polyelectrolytes*

The second Wien effect is particularly large in linear polyelectrolytes (e.g. Schödel *et al.*, 1958). Compared to simple electrolytes the linear range of the conductivity increase with increasing electric fields starts already at relatively low field strengths (5–10 kV cm^{-1}). Because of the rather extended linear region Onsager's equation for the dissociation field effect is frequently used as the basis for a semi-quantitative discussion of the second Wien effect in polyelectrolytes.

Denoting by B_n the polyion and by L the counterions, the large mass difference justifies the approximation $u_B \ll u_L$ for small observation times, n being the degree of polymerization. The counterions which interact in multiple ion pair equilibria with the fixed charges of the polyion experience a larger attraction potential than that arising from a single charged residue. On the other hand, the accumulation of the counterions lowers the overall attraction potential because repulsive contributions are superimposed. It should also be mentioned that counterion accumulation creates a lower (local) dielectric constant as compared to that of the bulk aqueous solution.

If now the diffuse counterion binding is viewed in terms of an effective charge $\tilde{z}_B^{\mathrm{eff}} e_0$ we may apply eqn (117) in the suggestive form

$$\left(\frac{\partial \ln |k_d^{\ominus}|}{\partial E}\right)_{P,T} = \frac{(\tilde{z}_L e_0)^2 \, |\tilde{z}_B^{\mathrm{eff}}| \, e_0}{8\pi\varepsilon_0\varepsilon(kT)^2}. \tag{118}$$

The analysis of electric conductivity relaxations of the linear polyelectrolyte poly(riboadenylate, K^+) according to eqn (118) at 293 K yields an effective charge of about -6, suggesting an end effect for the dissociation of K^+-ions from the inner counterion atmosphere (Schallreuter, 1982). The dissociation field effect in polyelectrolytes is generally larger by a factor of about 10 to 100 as compared to simple electrolytes. Thus the values for the relative displacements of the distribution constant, $\delta K/K(0) = \partial \ln K/\partial E$, in polyelectrolytes for the same conditions that have been used for simple 1:1 electrolytes (see above), are in the range of 1.4 for $E = 10^4$ V cm^{-1} and 14 for $E = 10^5$ V cm^{-1}. We conclude from these estimates, that already for moderately large changes in the field intensity the degree of counterion binding may change to a large extent. Since usually the conformation and the degree of stretching of flexible polyelectrolytes depends on counterion binding, external electric field changes may readily affect structural changes. Due to the efficiency of dissociation field effects in polyelectrolytes, major structural changes are induced by already moderately high electric fields. As an additional remark, the decrease in the number of counterions near the polyion in the presence of high electric fields, will also decrease the counterion polarization and thus the magnitude of the induced dipole moment. This, in turn, will change the reaction moment of chemical transformations involving induced dipoles. In any case, macromolecular complexes in which polyelectrolytic subunits are associated decrease in stability with increasing electric fields.

VI. REACTION MOMENT AND ELECTRIC-CHEMICAL MECHANISM

The reaction dipole moment ΔM of a dipolar equilibrium may be obtained from the measurement of continuum properties such as the dielectric permittivity as well as from direct monitoring of concentration shifts produced by an externally applied electric field. In both approaches it is primarily the chemical part of the total polarization that is aimed at. However, the *chemical* processes are intimately connected with the *physical* processes of polarization and dipole rotation. In the case of small molecules the orientational relaxations are usually rapid compared to the diffusion limited chemical reactions. When, however, macromolecular structures are involved, the rotational processes of the macromolecular dipoles may control a major part of the chemical relaxations (Schallreuter, 1982; Neumann *et al.*, 1983, 1984).

It is known that in a random distribution of permanent dipolar or induced dipolar reaction partners the (local) extent of the electric field effect depends on the orientation of the individual dipoles relative to the field direction (Schwarz, 1967; DeMaeyer, 1969; DeMaeyer and Persoons, 1974). Therefore the measured bulk effects always represent orientational averages. In this context it is stressed that the total macroscopic polarization, **M**, caused by an electric field in a random distribution of particles, is a statistical average which results from the polarizing and orienting action of the field vector against the randomizing thermal agitations (Böttcher, 1973).

It is recalled from eqn (61) that

$$M=\langle \mathbf{M}\rangle = N_{\mathrm{A}}\Sigma_j n_j\langle \mathbf{m}_j \cos \vartheta_j\rangle = N_{\mathrm{A}}\Sigma_j n_j m_j. \tag{119}$$

Whereas eqn (119) expresses **M** in terms of the average contributions of the individual molecular moments $\mathbf{m}_j$, the continuum approach to **M** represents the total moment in terms of an overall macroscopic dielectric permittivity ε. The fundamental relationship between the absolute amounts M and E is:

$$M=\varepsilon_0(\varepsilon-1)VE. \tag{120}$$

(The vector **M** is in the same direction as the field vector **E**.) When eqn (120) is applied as a general expression for homogeneous dielectrics in electric fields, the permittivity tensor is an overall quantity that depends on the intensive variables z $(=T, P, \mathbf{E})$. If, in addition, chemical transformations are caused by changes in z, then ε also depends on the extent of reaction ξ. In this manner "non-linearities" are hidden in ε (T, P, E, ξ).

1. *Reaction Moments from Dielectric Data*

The chemical reaction moment ΔM refers to that part of the total moment **M** which changes in the course of a chemical-conformational transition. Thus from eqn (120) we have

$$\Delta M=\left(\frac{\partial M}{\partial \xi}\right)_{V,E}=\varepsilon_0 VE\left(\frac{\partial \varepsilon}{\partial \xi}\right)_{V,E} \tag{121}$$

which is the continuum expression for ΔM at constant total volume between the capacitor plates; a condition usually realized at low field intensities and in dilute solutions. When volume changes occur, electrostriction terms must be explicitly considered (Eigen and DeMaeyer, 1963).

Under isothermal-isobaric-isochoric conditions, **M** is solely a function of ξ and **E**. The dependence on **E** at constant ξ defines a (normal) physical part whereas the dependence of **M** on ξ at constant **E** may be referred to as the chemical contribution to a change in **M**. The field dependence of the total moment may then be expressed as (Bergmann *et al.*, 1963):

$$\left(\frac{\partial M}{\partial E}\right)_V=\left(\frac{\partial M}{\partial E}\right)_{V,\xi}+\left(\frac{\partial M}{\partial \xi}\right)_{V,E}\cdot\left(\frac{\partial \xi}{\partial E}\right)_V. \tag{122}$$

At equilibrium in the presence of **E**, the characteristic (dielectro-) chemical affinity $\check{A}$ of dipolar systems is zero and $\mathrm{d}\check{A}=0$. Hence the term $(\partial\xi/\partial E)_{z,\check{A}=0}$ can be calculated. We finally obtain

$$\left(\frac{\partial \xi}{\partial E}\right)_{V,\check{A}=0}=\frac{V\Gamma^*\Delta M}{RT}. \tag{123}$$

At equilibrium there is no further change of **M** in the field **E**. Therefore $(\partial M/\partial E)_{V,\check{A}=0}$ is a constant at a given field strength.

From eqn (120) we obtain the physical term:

$$\Delta\varepsilon^{(\mathrm{ph})}=\frac{1}{\varepsilon_0 V}\left(\frac{\partial M}{\partial E}\right)_{V,\xi}=\left(\frac{\partial\varepsilon}{\partial E}\right)_{V,\xi}. \tag{124}$$

The chemical contribution is defined by

$$\Delta\varepsilon^{(\mathrm{ch})}=\left(\frac{\partial\varepsilon}{\partial\xi}\right)_{V,E}=\frac{1}{\varepsilon_0 V}\left(\frac{\partial M}{\partial\xi}\right)_{V,E}\cdot\left(\frac{\partial\xi}{\partial E}\right)_{V,\check{A}=0}. \tag{125}$$

Substitution of eqns (121) and (123) into (125) leads to (Bergmann *et al.*, 1963):

$$\Delta\varepsilon^{(\mathrm{ch})}=\frac{\Gamma^{*}(\Delta M)^{2}}{\varepsilon_0 RT}. \tag{126}$$

Thus the chemical part of a change in **M** by **E** may be derived from the electric field dependence of dielectric relaxation curves.

2. *Permanent and Induced Dipole Moments*

Two types of polarization processes contribute to the total macroscopic polarization.

$$\mathbf{M}=\mathbf{M}_{(\alpha)}(\mathbf{E}_{\mathrm{int}})+\mathbf{M}_{(\mathrm{p})}(\mathbf{E}_{\mathrm{dir}}). \tag{127}$$

According to Onsager the induced moment term $\mathbf{M}_{(\alpha)}$ is determined by the internal or local field $\mathbf{E}_{\mathrm{int}}$ whereas the permanent dipole term $\mathbf{M}_{(\mathrm{p})}$ is related to the directing field $\mathbf{E}_{\mathrm{dir}}$, orienting the permanent dipoles **p**. The combination of eqns (127) and (120) requires that the two different field vectors must be expressed in terms of the measured (Maxwell) field. The calculations of the terms $\mathbf{M}_{(\alpha)}$ and $\mathbf{M}_{(\mathrm{p})}$ as functions of $\mathbf{E}_{\mathrm{int}}$ and $\mathbf{E}_{\mathrm{dir}}$ usually are approximations. The final expressions may be written in terms of conversion factors, e.g. g-factors (Peterlin and Stuart, 1939). The factors are a function of particle anisotropies as well as of the properties of the medium in which the particles are embedded (polar, non-polar, gas phase or fluid phase).

(a) *Individual dipole moments*

In line with eqn (127) the molecular dipole moments **m** may generally be expressed as

$$\mathbf{m}=\mathbf{m}_{(\alpha)}+\mathbf{p} \tag{128}$$

where $\mathbf{m}_{(\alpha)}$ represents the induced moment and **p** is the permanent dipole moment.

In anisotropic molecules **m** is the vector sum of all dipolar contributions. The calculations are readily performed for ellipsoidal molecules and for simple geometries like a sphere, long cylinders or flat discs (Böttcher, 1973). The total moment of an ellipsoid where the main polarization axes are the (half-)axes $q=a$, b, c, is given by the vector sum: $\mathbf{m}=\mathbf{m}_a+\mathbf{m}_b+\mathbf{m}_c$.

The dipole moment component along the axis q is expressed analogous to eqn (128) as

$$\mathbf{m}_q=\mathbf{m}_{(\alpha)q}+\mathbf{p}_q. \tag{129}$$

The induced dipole moment is given by

$$\mathbf{m}_{(\alpha)q}=\alpha_q(\mathbf{E}_{\mathrm{int}})_q \tag{130}$$

where α_q is the component of the polarizability tensor α in the direction of the q-axis and $(\mathbf{E}_{\mathrm{int}})_q$ is the internal field in q-direction. When α is independent of **E**, then $\mathbf{m}_{(\alpha)}$ is obviously linearly dependent on **E**. The polarizability tensor reflecting charge displaceability, may depend on **E**. Thus a more general definition of the polarizability is given by:

$$\alpha=\left(\frac{\partial\mathbf{m}_{(\alpha)}}{\partial\mathbf{E}_{\mathrm{int}}}\right)_{\mathbf{E}\to 0}. \tag{131}$$

As outlined previously only that component $(\mathbf{m})_{\parallel}$ of $\mathbf{m}$ which is parallel to the field contributes to the total polarization. Thus for the q-axis we have

$$(\mathbf{m}_q)_{\parallel} = \mathbf{m}_q \cos \vartheta_q \tag{132}$$

where ϑ_q is the angle between the q-axis and the electric field.

The actual field-parallel contribution of $\mathbf{m}$ can be calculated from Onsager's concepts of the cavity field and of the reaction field (Onsager, 1936; Böttcher, 1973). At first, eqn (132) is specified as

$$(\mathbf{m}_q)_{\parallel} = \alpha \cos \vartheta_q (\mathbf{E}_{\text{int}})_q + \mathbf{p}_q \cos \vartheta_q \tag{133}$$

where $\alpha_q = \alpha \cos \vartheta_q$. The internal field is given by

$$(\mathbf{E}_{\text{int}})_q = \breve{g}_q g_q \mathbf{E} \cos \vartheta_q. \tag{134}$$

For isotropic particles where the polarizability is equal in all directions the internal field is simply

$$\mathbf{E}_{\text{int}} = \breve{g} g \mathbf{E}. \tag{135}$$

The factor $\breve{g}_q$ represents the reaction field contribution of permanent dipoles according to

$$\breve{g}_q = [1 + f_q(1 - \alpha_q f_q)^{-1} \langle \mathbf{p}_q \rangle] \tag{136}$$

where f_q is the reaction field factor and $\langle \mathbf{p}_q \rangle$ is the average contribution of the permanent dipoles. For non-polar particles (where $p_q = 0$), $\breve{g}_q = 1$ and $(\mathbf{E}_{\text{int}})_q = g_q \mathbf{E} \cos \vartheta_q$. Obviously, the total value of the field-parallel components is $(\mathbf{m})_{\parallel} = \Sigma_q (\mathbf{m}_q)_{\parallel}$. In a system of statistically distributed, mobile dipolar species, the total field-parallel contribution to the polarization is the statistical average over the cos ϑ-projections on the field vector.

For the sake of transparentness we shall confine the further analysis to uniaxial anisotropic particles J, i.e. to uniaxial dipole moments $\mathbf{m}_j$. For this case eqns (129) and (133) read, respectively,

$$\mathbf{m}_j = \alpha_j (\mathbf{E}_{\text{int}})_j + \mathbf{p}_j \tag{137}$$

and

$$(\mathbf{m}_j)_{\parallel} = \alpha_j \breve{g}_j g_j \mathbf{E} \cos^2 \vartheta_j + \mathbf{p}_j \cos \vartheta_j. \tag{138}$$

The effective average contribution to M is finally given by

$$m_j = \langle (\mathbf{m}_j)_{\parallel} \rangle = \alpha_j \breve{g}_j g_j E \langle \cos^2 \vartheta_j \rangle + p_j \langle \cos \vartheta_j \rangle. \tag{139}$$

(b) *Total polarization*

In a mixture of N_j molecules of type J, the total moment is:

$$\begin{aligned} M &= \Sigma_j N_j m_j = N_{\text{A}} \Sigma_j n_j m_j \\ &= \Sigma_j N_j \langle \alpha_j (\mathbf{E}_{\text{int}})_j \rangle + \Sigma_j N_j \langle \mathbf{p}_j \rangle. \end{aligned} \tag{140}$$

In order to reduce the complexity the two contributions to M, the induced moment $M_{(\alpha)}$ and the permanent moment $M_{(\text{p})}$ will be treated separately. Applying eqn (139) to (140) we obtain

$$M_{(\alpha)} = \Sigma_j N_j \alpha_j \breve{g}_j g_j E \langle \cos^2 \vartheta_j \rangle \tag{141}$$

and

$$M_{(\text{p})} = \Sigma_j N_j p_j \langle \cos \vartheta_j \rangle \tag{142}$$

where the summation is over all particle types. The average values $\langle \cos^2 \vartheta_j \rangle$ and $\langle \cos \vartheta_j \rangle$ are thermal averages under the polarizing and orienting action of the electric field; they are therefore dependent on $\mathbf{E}$, on the molecular shape and size and on temperature.

(i) *Induced moment*. In the absence of permanent dipoles (i.e. $\breve{g}=1$) the absolute value $M_{(\alpha)}$ of the total induced moment $\mathbf{M}_{(\alpha)}$ is derived from eqn (141):

$$M_{(\alpha)}=\Sigma_j N_j \alpha_j g_j \langle \cos^2 \vartheta_j \rangle E. \tag{143}$$

In isotropic particles the total induced moment is given by:

$$M_{(\alpha)}=\Sigma_j N_j \alpha_j g_j E. \tag{144}$$

In the case of uniaxial anisotropic molecules the thermal average $\langle \cos^2 \vartheta_j \rangle$ may be expressed as a function of the orientation factor

$$\phi_j=\tfrac{1}{2}[3\langle \cos^2 \vartheta_j \rangle-1]. \tag{145}$$

This factor can be directly obtained from electro-optic data, for instance from linear dichroism (Kuhn *et al.*, 1939) and birefringence (Peterlin and Stuart, 1939; see also O'Konski *et al.*, 1959). The orientation can also be expressed in terms of the dipole moments involved. Since from eqn (145), $\langle \cos^2 \vartheta_j \rangle=\frac{1}{3}(1+2\phi_j)$, substitution into eqn (143) yields:

$$M_{(\alpha)}=\tfrac{1}{3}\Sigma_j N_j \alpha_j g_j (1+2\phi_j) E. \tag{146}$$

Two limiting cases are of practical importance. The low-field condition $m_j E \ll kT$ means negligible orientation in the field direction, i.e. $\phi \ll 1$. Hence eqn (146) reduces to

$$M_{(\alpha)}=\tfrac{1}{3}\Sigma_j N_j \alpha_j g_j E. \tag{147}$$

At high fields when $\phi \rightarrow 1$, corresponding to total alignment of the induced moments in the field direction, we obtain:

$$M_{(\alpha)}=\Sigma_j N_j \alpha_j g_j E. \tag{148}$$

Should the induced moment be saturated at high fields, i.e. $m_{(\alpha)}=m_s$, then for $\phi \rightarrow 1$,

$$M_{(\alpha),s}=\Sigma_j N_j (m_s)_j. \tag{149}$$

In this case the induced moment is independent of the field strength: *dielectric saturation*.

(ii) *Permanent moment*. For freely mobile permanent dipoles p_j the thermal average of $\cos \vartheta_j$ in

$$M_{(p)}=\Sigma_j N_j p_j \langle \cos \vartheta_j \rangle \tag{150}$$

is given by the Langevin function $L[r_j]$ of the directing field $(\mathbf{E}_{\text{dir}})_j$, where

$$(\mathbf{E}_{\text{dir}})_j=g_j \mathbf{E}. \tag{151}$$

Since $r_j=p_j g_j E/(kT)$ we have

$$\langle \cos \vartheta_j \rangle = L\left[\frac{p_j g_j E}{kT}\right]. \tag{152}$$

Note that $L[r_j]=\coth r_j - r_j^{-1}$. Introducing eqn (152) into (142) leads to

$$M_{(p)}=\Sigma_j N_j p_j L[p_j g_j E/kT]. \tag{153}$$

At low field strengths ($r_j \ll 1$), $L[r_j]=g_j p_j E/(3kT)$ and

$$M_{(p)}=\frac{1}{3kT}\Sigma_j N_j p_j^2 g_j E. \tag{154}$$

At high fields ($r_j \gg 1$), $L[r_j]=1$ and eqn (153) reduces to

$$M_{(p)}=\Sigma_j N_j p_j. \tag{155}$$

From eqns (147) and (154) it is seen that at low field strengths both the contributions of the induced moment $M_{(\alpha)}$ and the permanent dipole moment $M_{(p)}$ are linear in E; compare the continuum expression for M in eqn (120).

(c) *Form factors and g-factors*

The conversion factors g and $\check{g}$ contain the so-called form factors which account for shape-anisotropies. In an ellipsoidal molecule the form factors (sometimes called depolarizing factors which are the components of the depolarizing tensor) of the main polarization axes are $A_q = A_a, A_b, A_c$. In line with the vectorial character of the internal and directing fields the g-factors of anisotropic molecules are tensors (Böttcher, 1973). If the environment of the molecules (which are characterized by the polarizability tensor α and the permanent dipole moment $\mathbf{p}$), can be considered as *non-polar* and the overall dielectric permittivity is ε, the g-factor of the q-axis and the polarizability, respectively, are:

$$g_q = \frac{\varepsilon\{1 + [(\varepsilon_\infty)_q - 1]A_q\}}{\varepsilon + [(\varepsilon_\infty)_q - \varepsilon]A_q}, \tag{156}$$

$$\alpha_q = \varepsilon_0 V_j \frac{(\varepsilon_\infty)_q - 1}{1 + [(\varepsilon_\infty)_q - 1]A_q}, \tag{157}$$

where $V_j = (4/3)\pi \cdot abc$ is the molecular volume of the ellipsoidal molecules J with the half-axes $q = a, b, c$. It is stressed that the value of ε in the eqns (156) and (157) is the effective dielectric permittivity of the total system. The quantity ε_∞ may be considered as the "molecular permittivity" at frequencies of the polarizing field, where the permanent dipoles do not contribute any more to the total polarization; $(\varepsilon_\infty)_q$ is the q-component of the permittivity tensor ε_∞. To facilitate comparison with familiar representations of dielectrics note that the reaction field factor of the q-axis is given by

$$f_q = \frac{1}{\varepsilon_0 V_j} \frac{A_q(1 - A_q)(\varepsilon - 1)}{\varepsilon + (1 - \varepsilon)A_q}. \tag{158}$$

For particular geometries the form factors are analytically expressed in a simple form. For very long cylinders the depolarizing factor along the long axis is zero; thus $g = 1$. Therefore the local field which affects counterion polarization in linear polyelectrolytes is equal to the externally applied electric field. Another type of shape which is relevant for biological systems is the flat disc; flat patches of biological membranes may be described in terms of the disc geometry. The depolarizing factor for the polarization direction along the disc-normal, i.e. perpendicular to the disc plane is $A_\perp = 1$; therefore $g = 1/\varepsilon$.

The form factor of spherical isotropic systems is $A_q = 1/3$. Thus for polarizable dipolar spheres (with $(\varepsilon_\infty)_j$ and $\mathbf{p}_j$) in a non-polar medium of the effective bulk permittivity ε, we obtain from eqns (156) and (157):

$$g_j = \frac{\varepsilon[(\varepsilon_\infty)_j + 2]}{2\varepsilon + (\varepsilon_\infty)_j}. \tag{159}$$

Substituting $A_q = 1/3$ into eqn (157), we obtain the familiar Clausius–Mosotti equation:

$$\alpha_j = 3\varepsilon_0 V_j \frac{(\varepsilon_\infty)_j - 1}{(\varepsilon_\infty)_j + 2} \tag{160}$$

generally valid at high frequencies of the polarizing electric field where permanent dipoles do not contribute to the polarization; V_j is the "volume of the particle J".

For spherical molecules at the same conditions

$$f_j = \frac{1}{3\varepsilon_0 V_j} \frac{2(\varepsilon - 1)}{2\varepsilon + 1}. \tag{161}$$

In a pure condensed medium of non-polar molecules we have $\varepsilon_\infty = \varepsilon$. Isotropic spheres are characterized by $g = (\varepsilon + 2)/3$, and

$$\alpha = 3\varepsilon_0 V_j(\varepsilon - 1)/(\varepsilon + 2). \tag{162}$$

In the case of a pure liquid of non-polarizable dipoles (**p**) we have $\varepsilon_{\infty}=1$; hence for spherical permanent point dipoles:

$$g=3\varepsilon/(2\varepsilon+1). \tag{163}$$

For spherical molecules in the gas phase:

$$g=(\varepsilon+2)/3. \tag{164}$$

The g-factors for the various specific cases are compiled in Table 1.

TABLE 1. CONVERSION FACTORS (g, g_j) FOR SPHERICAL MOLECULES, RELATING INTERNAL FIELD ($\mathbf{E}_{\mathrm{int}}$), DIRECTING FIELD ($\mathbf{E}_{\mathrm{dir}}$) AND FRÖHLICH FIELD ($\mathbf{E}_{\mathrm{F}}=\mathbf{E}_{\mathrm{dir}}$) TO THE (MEASURED) MAXWELL FIELD ($\mathbf{E}$)

I. Pure liquids (ε)	g	$\mathbf{E}_{\mathrm{int}}$	$\mathbf{E}_{\mathrm{dir}}$
(a) polarizable polar spheres [Onsager] (p, ε_{∞}, ε)	$\frac{\varepsilon(\varepsilon_{\infty}+2)}{2\varepsilon+\varepsilon_{\infty}}$	$\breve{g}g\mathbf{E}_{\|}$	$g\mathbf{E}$
(b) polarizable non-polar spheres ($p=0$, $\varepsilon_{\infty}=\varepsilon$)	$\frac{\varepsilon+2}{3}$	$g\mathbf{E}_{\parallel}$	
(c) polar non-polarizable spheres (p, $\varepsilon_{\infty}=1$, ε)	$\frac{3\varepsilon}{2\varepsilon+1}$		$g\mathbf{E}$
(d) polarizable permanent dipoles (Fröhlich, $p=p_{\mathrm{G}}(\varepsilon_{\infty}+2)/3$; ε)	$\frac{3\varepsilon}{2\varepsilon+\varepsilon_{\infty}}$		$g\mathbf{E}$
II. Molecules [p_j, $(\varepsilon_{\infty})_j$] diluted	g_j	$(E_{\mathrm{int}})_j$	$(E_{\mathrm{dir}})_j$
(1) in non-polar fluid medium (ε)			
(a) polarizable polar spheres [p_j, $(\varepsilon_{\infty})_j$]	$\frac{\varepsilon[(\varepsilon_{\infty})_j+2]}{2\varepsilon+(\varepsilon_{\infty})_j}$	$\breve{g}_j g_j\mathbf{E}_{\parallel}$	$g_j\mathbf{E}$
(b) polarizable non-polar spheres [$p_j=0$, $(\varepsilon_{\infty})_j$, ε]		$g_j\mathbf{E}_{\parallel}$	
(c) polar non-polarizable spheres [p_j, $(\varepsilon_{\infty})_j=1$; ε]	$\frac{3\varepsilon}{2\varepsilon+1}$		$g_j\mathbf{E}$
(2) in polar fluid medium (ε) (Fröhlich, Kirkwood, g_{K}-factor), $p_j=(p_j)_G[(\varepsilon_{\infty})_j+2]/3$	$\frac{3\varepsilon}{2\varepsilon+(\varepsilon_{\infty})_j}$		$g_j\mathbf{E}$
(3) in gas phase (ε), polar polarizable spheres [$(p_j)_G$, $(\varepsilon_{\infty})_j$, ε]	$\frac{(\varepsilon_{\infty})_j+2}{3}$	$g_j\mathbf{E}_{\parallel}$	$g_j\mathbf{E}$

In the case of isotropic polarization, $\mathbf{E}_{\parallel}=E$; for uniaxial anisotropic polarizability $\mathbf{E}_{|}=\mathbf{E}\cos\vartheta_j$ where ϑ_j is the angle between the dipole axis and **E**. ε, total dielectric permittivity; ε_{∞}, dielectric permittivity of the induced (high frequency) polarization. The factor $\breve{g}$ refers to the permanent dipoles' contribution of $\mathbf{E}_{\mathrm{int}}$; $\breve{g}_j=1+f_j(1-\alpha_j f_j)^{-1}\langle p_j\rangle$ where f_j is the reaction field factor and α_j the polarizability tensor of the molecule j [if $p_j=0$ or $\langle p_j\rangle=0$, $\breve{g}_j=1$].

3. *Reaction Moment and Equilibrium Constant*

When changes in the concentration of the reaction partners can be measured directly, say by an optical method, the analysis of equilibrium properties is based on the eqns (14) and (93). The electric field dependence of the apparent equilibrium constant (concentration ratio) is given by

$$K(E)=K(0)e^{x} \tag{165}$$

where

$$x=\frac{1}{RT}\int_0^E \Delta M\,\mathrm{d}E. \tag{166}$$

It is frequently observed that the reaction moment ΔM is dominated either by the induced dipole term $M_{(\alpha)}$ or by the permanent dipole term $M_{(\mathrm{p})}$. It is therefore useful to follow eqn (127) and to write ΔM also in two terms:

$$\Delta M=\Delta M_{(\alpha)}+\Delta M_{(\mathrm{p})}, \tag{167}$$

corresponding to

$$x = x_{(\alpha)} + x_{(\mathrm{p})} \tag{168}$$

where

$$x_{(\alpha)} = \frac{1}{RT} \int \Delta M_{(\alpha)} \, \mathrm{d}E \tag{169}$$

$$x_{(\mathrm{p})} = \frac{1}{RT} \int \Delta M_{(\mathrm{p})} \, \mathrm{d}E. \tag{170}$$

The investigation of chemical processes in solutions is preferably performed under the condition of higher dilution such that the individual reaction partners can be considered independent and the thermodynamic activity coefficients are either constant or equal to one. For chemical and physical processes in the presence of an applied electric field the solvent may then be treated as an infinite fluid dielectric in which the molecules or particles are immersed. On a microscopic scale, however, the solvent molecules are more or less densely packed, probably forming dynamic fluctuating clusters. The space in between the molecules and clusters certainly is vacuum. Even if the internal and directing fields which actually work on the molecules are homogeneous, the local Maxwell field in the vicinity of the molecules is inhomogeneous. Therefore, the classical relations between $\mathbf{E}_{\mathrm{int}}$ and $\mathbf{E}_{\mathrm{dir}}$ and the macroscopic average field (Maxwell field) of real molecular dielectrics are only approximations, covering the homogeneous part of the Maxwell field, $\mathbf{E}$. The approximations involve the assumption that the bulk of the dielectric can be represented by an effective average permittivity (ε), being a constant over the dielectric.

(a) *Non-polar polarizable spheres*

In the case of pure induced polarization the solute molecules may be approximated as polarizable spheres associated with a "molecular" permittivity $(\varepsilon_\infty)_j$ and $\mathbf{p}_j = 0$. For diluted solutions the total permittivity of the solution ε is practically that of the non-polar solvent.

For particles of uniaxial anisotropic polarizability α_j, eqns (120) and (141) for $M = M_{(\alpha)}$ are combined with eqn (146). Since $N_j = N_A \cdot n_j$, the resulting expression reads:

$$\varepsilon_0(\varepsilon - 1)V = \tfrac{1}{3} N_{\mathrm{A}} \Sigma_j n_j \alpha_j (1 + 2\phi_j) g_j. \tag{171}$$

The appropriate expressions of g_j and α_j are obtained from eqns (161) and (162), respectively. Assuming now that all $(\varepsilon_\infty)_j$ are equal such that for all J we have $(\varepsilon_\infty)_j = \varepsilon_\infty$, eqn (171) may be formulated for the low-field limiting case $\phi_j \to 0$ as:

$$\varepsilon_0(\varepsilon - 1)\frac{2\varepsilon + \varepsilon_\infty}{\varepsilon} = \frac{N_{\mathrm{A}}}{3V} \Sigma_j n_j \alpha_j (\varepsilon_\infty + 2). \tag{172}$$

In this way the quantities which change upon a chemical transformation, the total permittivity ε and the molar quantities n_j, are separated. Hence the differentiation with respect to the reaction variable ξ is readily performed separately at both sides of the equation. Using $\mathrm{d}n_j/\mathrm{d}\xi = \nu_j$ we finally obtain:

$$\left(\frac{\partial \varepsilon}{\partial \xi}\right)_{E,V} = \frac{N_{\mathrm{A}}}{3V\varepsilon_0} \frac{\varepsilon^2}{2\varepsilon^2 + \varepsilon_\infty} \Sigma_j \nu_j \alpha_j (\varepsilon_\infty + 2). \tag{173}$$

Substitution of eqn (173) into (121) leads to:

$$\Delta M_{(\alpha)} = \frac{N_{\mathrm{A}}}{3} \frac{3\varepsilon^2}{2\varepsilon^2 + \varepsilon_\infty} \Sigma_j \nu_j \alpha_j \frac{\varepsilon_\infty + 2}{3} E. \tag{174}$$

Insertion into (169) and integration yields

$$x_{(\alpha)} = \frac{3\varepsilon^2}{2\varepsilon^2 + \varepsilon_\infty} \frac{\Sigma_j \nu_j \alpha_j}{6kT} \left(\frac{\varepsilon_\infty + 2}{3}\right) E^2. \tag{175}$$

For isotropic polarizable spheres eqn (144) applies and:

$$x_{(\alpha)}=\frac{3\varepsilon^2}{2\varepsilon^2+\varepsilon_\infty}\frac{\Sigma_j\nu_j\alpha_j}{2kT}\left(\frac{\varepsilon_\infty+2}{3}\right)E^2. \tag{176}$$

In the limiting case of saturated induced dipole moments, eqn (149) is used to obtain the reaction moment:

$$\Delta M_s=N_A\Sigma_j\nu_j(m_s)_j \tag{177}$$

and

$$x_s=\frac{1}{kT}\Sigma_j\nu_j(m_s)_jE. \tag{178}$$

Inspecting the eqns (175) to (178) we realize that an induced dipole mechanism is associated with a quadratic dependence of the x-quantity on the field strength. The temperature dependence is linear in $1/T$. At high field strengths saturation may occur and a transition to a linear dependence on E/T will be observed.

(b) *Polar non-polarizable spheres*

The special case of uniaxial non-polarizable point dipoles refers to $(\varepsilon_\infty)_j=1$ and $\mathbf{p}_j\neq 1$ in the general expressions. The solvent is non-polar; the total permittivity is ε.

The combination of eqns (149) and (121) only leads to general analytical forms of $(\partial\varepsilon/\partial\xi)_{V,E}$ and of the $x_{(p)}$-factor if the g_j-factors were independent of ξ. Simple analytical expressions can only be derived for the limiting cases of small and large field strengths, respectively.

At low field strengths where eqn (154) applies we derive from the general eqn (120) that:

$$\varepsilon_0(\varepsilon-1)V=\frac{N_A}{3kT}\Sigma_jn_jp_j^2g_j. \tag{179}$$

From eqn (163) we have:

$$g_j=g=3\varepsilon/(2\varepsilon+1).$$

Term separation in eqn (179) then leads to:

$$\varepsilon_0(\varepsilon-1)\frac{2\varepsilon+1}{3\varepsilon}=\frac{N_A}{V}\frac{1}{3kT}\Sigma_jn_jp_j^2. \tag{180}$$

Differentiation with respect to ξ results in

$$\left(\frac{\partial\varepsilon}{\partial\xi}\right)_{V,E}=\frac{N_A}{\varepsilon_0V}\frac{3\varepsilon^2}{2\varepsilon^2+1}\frac{\Sigma_j\nu_jp_j^2}{3kT}. \tag{181}$$

Substitution into (6.3) yields

$$\Delta M_{(p)}=N_A\frac{3\varepsilon^2}{2\varepsilon^2+1}\frac{\Sigma_j\nu_jp_j^2}{3kT}E. \tag{182}$$

Substitution into eqn (170) and integration leads to

$$x_{(p)}=\frac{3\varepsilon^2}{2\varepsilon^2+1}\frac{\Sigma_j\nu_jp_j^2}{6(kT)^2}E^2. \tag{183}$$

For the high field strength range where eqn (155) applies we obtain for the orientational saturation:

$$\Delta M_s=N_A\Sigma_j\nu_jp_j \tag{184}$$

$$x_s=\frac{1}{kT}\Sigma_j\nu_jp_jE. \tag{185}$$

Thus, also a permanent dipole mechanism may be characterized by a transition of the x-quantity from a quadratic field strength dependence to a linear one; concomitant with the E-dependence the temperature variation changes from T^{-2} to T^{-1}. Therefore the temperature dependence of equilibrium and rate constants may be used to differentiate between permanent and induced moments. The assumption of constant ε is inherent in all integrations according to eqns (169) and (170). The ε-values refer to ε in the presence of **E**.

(c) *Polar polarizable spheres* (*Onsager*)

Real molecules are always polarizable. When these particles have a permanent dipole moment ($\mathbf{p}_j$) they are characterized by the set (p_j, α_j); α_j may be expressed in terms of the dielectric permittivity $(\varepsilon_\infty)_j$ of the induced high frequency polarization (by the Clausius–Mosotti equation).

In the case of isotropic polarizabilities and uniaxial permanent dipoles, the fields which actually work on the molecules are given by eqns (135) and (151); thus

$$\begin{aligned}(\mathbf{E}_{\text{int}})_j &= \check{g}_j g_j \mathbf{E}\\ &= [1 + f_j(1-\alpha_j f_j)^{-1}\langle \mathbf{p}_j\rangle] g_j \mathbf{E}.\end{aligned} \tag{186}$$

For spherical particle geometry the factors g_j, α_j and f_j are obtained from eqns (159)–(161), respectively. The low-field approximation of $\langle \mathbf{p}_j\rangle$ is given by

$$\langle \mathbf{p}_j\rangle = p_j\langle \cos \vartheta_j\rangle = p_j^2 g_j E/(3kT). \tag{187}$$

Equation (140) may now be written as:

$$M = N_\text{A}\{\Sigma_j n_j \alpha_j (\mathbf{E}_{\text{int}})_j + \Sigma_j n_j \langle \mathbf{p}_j\rangle\}. \tag{188}$$

For the low-field range we substitute eqns (186) and (187) into (188). Using now eqns (159) to (161) for g_j, α_j and f_j, respectively, together with the approximation that all $(\varepsilon_\infty)_j$ are equal, i.e. $(\varepsilon_\infty)_j = \varepsilon_\infty$, we obtain the familiar Onsager (1936) equation for pure dipole liquids in the suggestive form

$$\frac{\varepsilon_0(\varepsilon-\varepsilon_\infty)(2\varepsilon+\varepsilon_\infty)}{3\varepsilon} = \frac{N_\text{A}}{V}\frac{\Sigma_j n_j p_j^2}{3kT}\left(\frac{\varepsilon_\infty+2}{3}\right)^2. \tag{189}$$

Differentiation with respect to ξ yields

$$\left(\frac{\partial \varepsilon}{\partial \xi}\right)_{V,E} = \frac{N_\text{A}}{\varepsilon_0 V}\frac{3\varepsilon^2}{2\varepsilon^2+\varepsilon_\infty^2}\frac{\Sigma_j \nu_j p_j^2}{3kT}\left(\frac{\varepsilon_\infty+2}{3}\right)^2 \tag{190}$$

and applying eqn (121) results in

$$\Delta M = N_\text{A}\frac{3\varepsilon^2}{2\varepsilon^2+\varepsilon_\infty^2}\frac{\Sigma_j \nu_j p_j^2}{3kT}\left(\frac{\varepsilon_\infty+2}{3}\right)^2 E. \tag{191}$$

With eqn (166) the equilibrium shift by small fields is described by

$$x = \frac{3\varepsilon^2}{2\varepsilon^2+\varepsilon_\infty^2}\frac{\Sigma_j \nu_j p_j^2}{6(kT)^2}\left(\frac{\varepsilon_\infty+2}{3}\right)^2 E^2. \tag{192}$$

In a similar manner we may derive the expressions of ΔM and x for anisotropic polarizable permanent dipoles, interacting in non-polar media. Here again, general expressions in terms of the Langevin function cannot be derived in closed analytical form.

The Onsager approach is adequate for *non-polar* media. For polar liquids like water which is of particular interest for biochemical processes, a modified model developed by Fröhlich (1958) appears to be more adequate (Grant *et al.*, 1978).

4. *Reactions in Polar Media*

According to Fröhlich, a pure condensed dielectric consisting of polarizable molecules with a permanent dipole moment (**p**) may be formally represented by a continuum permittivity ε_∞ (accounting for the "molecular" polarizability, "embedded" in the bulk continuum with the effective permittivity ε). Thus:

$$M = \varepsilon_0(\varepsilon_\infty - 1)VE + N_A\Sigma_j n_j\langle \mathbf{p}_j\rangle \tag{193}$$

where the induced part is formally separated from the permanent dipole moment contribution. In Fröhlich's version of the Onsager model the spherical dipoles have the effective dipole moment

$$\mathbf{p}_j = (\mathbf{p}_j)_G \frac{(\varepsilon_\infty)_j + 2}{3} \tag{194}$$

where $(\mathbf{p}_j)_G$ is the dipole moment of species J in the gas phase. The actual field working as the directing field is the Fröhlich field $\mathbf{E}_F$ which is given by

$$(\mathbf{E}_F)_j = g_j\mathbf{E} \tag{195}$$

where

$$g_j = 3\varepsilon/(2\varepsilon + (\varepsilon_\infty)_j). \tag{196}$$

In a medium of polar molecules, specific intermolecular interactions such as, for instance, H-bridges in water may occur; they are accounted for by the Kirkwood correlation factor g_K.

The average contribution of the "Fröhlich-dipoles" is analogous to eqn (187):

$$\langle p_j\rangle = p_j(g_K)_j L[p_j g_j E/kT]. \tag{197}$$

The low-field approximation reads

$$\langle p_j\rangle = p_j^2(g_K)_j g_j E/(3kT) \tag{198}$$

and the limiting case of orientational saturation has an average moment contribution of

$$\langle p\rangle_s = p_j(g_K)_j. \tag{199}$$

(a) *Kirkwood–Fröhlich equation*

For pure liquids where all $(\varepsilon_\infty)_j = \varepsilon_\infty$, the combination of the eqns (120), (193), (196) and (198) leads to the familiar Kirkwood–Fröhlich equation, written here in the suggestive form of the separated variables ε and n_j

$$\frac{\varepsilon_0(\varepsilon - \varepsilon_\infty)(2\varepsilon + \varepsilon_\infty)}{3\varepsilon} = \frac{N_A}{V}\frac{\Sigma_j n_j(g_K)_j(p_j)_G^2}{3kT}\left(\frac{\varepsilon_\infty + 2}{3}\right)^2. \tag{200}$$

Since for a pure liquid $\Sigma_j n_j(g_K)_j(p_j)_G^2 = n g_K p_G^2$, eqn (200) is useful for the determination of dipole moments of polar liquids by dielectric measurements. The g_K-factor must be calculated.

(b) *Ion-pair equilibria in aqueous solution*

Chemical equilibria such as ion-pair formation of electrolytes in aqueous solution where the hydrated ion-pairs behave as polarizable dipoles, may be treated in terms of the Fröhlich formalism.

If the $(\varepsilon_\infty)_j$-values of the individual ions and ion-pairs are basically determined by the hydration shells we may use the approximation $(\varepsilon_\infty)_j = \varepsilon_\infty$. In water $\varepsilon_\infty = 5(\pm 1)$ at 20°C (Grant *et al.*, 1978).

Since the H-bond coordination number of a hydrated ion or ion-pair is not known, plausibility arguments of a symmetric interaction pattern with the bulk water may justify the approximation $(g_K)_j = 1$.

The low-field limiting case for ion-pair equilibria in water may be derived from eqn (200):

$$\frac{\varepsilon_0(\varepsilon - \varepsilon_\infty)(2\varepsilon + \varepsilon_\infty)}{3\varepsilon} = \frac{N_A}{V}\frac{\Sigma_j n_j p_j^2}{3kT} \tag{201}$$

where the effective dipole moment p_j is given by eqn (194). Substitution of the expression

$$\left(\frac{\partial \varepsilon}{\partial \xi}\right)_{V,E} = \frac{N_A}{\varepsilon_0 V} \frac{3\varepsilon^2}{2\varepsilon^2 + \varepsilon_\infty^2} \frac{\Sigma_j \nu_j p_j^2}{3kT} \tag{202}$$

into (121) yields the reaction moment

$$\Delta M = N_A \frac{3\varepsilon^2}{2\varepsilon^2 + \varepsilon_\infty^2} \frac{\Sigma \nu_j p_j}{3kT} E. \tag{203}$$

The use of eqn (166) and integration result in the x-factor of ion-pair equilibrium displacements at low field strengths:

$$x = \frac{3\varepsilon^2}{2\varepsilon^2 + \varepsilon_\infty^2} \frac{\Sigma_j \nu_j p_j^2}{6(kT)^2} E^2. \tag{204}$$

At high field strengths that lead to orientational saturation, $\langle p_j \rangle = p_j$; the combination of the eqns (120) and (193) yield:

$$\varepsilon_0(\varepsilon - \varepsilon_\infty) V E = N_A \Sigma_j n_j p_j \tag{205}$$

$$\Delta M = \varepsilon_0 V E \left(\frac{\partial \varepsilon}{\partial \xi}\right)_{V,E} = N_A \Sigma_j \nu_j p_j \tag{206}$$

$$x = \Sigma_j \nu_j p_j / (kT). \tag{207}$$

Due to eqn (194), eqns (200) and (201) are identical to (189) and eqns (202) and (203) are identical to eqns (190) to (192), respectively. On the basis of eqn (204) the dipole moments of ion-pairs formed by Mg^{2+} and SO_4^{2-} in aqueous solution have been estimated (Schallreuter, 1982; Neumann *et al.*, 1983, 1984).

5. *Induced Dipole Moments in Polyionic Macromolecules*

Large induced dipole moments may result from atomic polarization. When the polarization is caused by displacements of ionic groups within macromolecules the conformational folding of the polymer structure may restrict the local mobility of the charged groups and dipolar ion-pairs. Restricted conformational displaceability may then lead to a saturatable induced dipole moment (Tsuji and Neumann, 1983). Consider a dipolar ion-pair between a positively charged (lysine) group and a negatively charged (glutamic acid) group in a protein. An external electric field may induce an increase, $\delta\mathbf{r}$, of the distance vector $\mathbf{r}$ between the charge centres of the ion-pair. When this protein is part of a membrane structure, then the field-induced distance change $\delta\mathbf{r}$ may not only lead to an increase in the scalar amount of the dipole moment, but may also be accompanied by a rotation of the dipole vector.

According to this model an electric field $\mathbf{E}$ increases the dipole moment of an ion-pair from a value $\mathbf{m}(0) = |\tilde{z}|\, \mathrm{e}_0 \mathbf{r}_0$ at $\mathbf{E} = 0$ to a value $\mathbf{m}(E) = |\tilde{z}|\, e_0 \mathbf{r}$, with $|\mathbf{r}| > |\mathbf{r}_0|$. The distance increase corresponds to an induced dipole moment

$$\mathbf{m}_{(\alpha)} = \mathbf{m}(r) - \mathbf{m}(r_0) = |\tilde{z}|\, e_0\, \delta\mathbf{r} = \alpha \mathbf{E}_{\mathrm{int}}. \tag{208}$$

Due to conformational restrictions of (membrane) proteins, it is likely that a major unidirectional charge displacement may only occur along the membrane normal and the induced dipole moment $\mathbf{m}_{(\alpha)}$ will reach a saturation value $\mathbf{m}_s$.

VII. MEASUREMENTS OF ELECTRIC FIELD EFFECTS

The majority of biological processes involves ionic species in aqueous environments. Furthermore, the structural stability of many biopolymer systems requires a finite ionic strength. Thus, electric field effects in these systems have to be studied in conducting solutions and suspension. Traditionally, dielectric measurements, conductivity relaxations and electro-optic techniques were used to study electric field effects on chemical reactions

(Eigen and DeMaeyer, 1963; Bergmann *et al.*, 1963). The analysis of field induced changes is particularly straightforward for rectangular pulses. However, if the system is simple and only one process occurs or if the field-induced changes are long-lived and relax with time constants large compared to the field duration, Joule heating temperature jump spectrometers may be used (Neumann and Katchalsky, 1972).

In detail, the relaxation kinetic methods provide the most powerful approach to chemical field effects. The quantitative analysis of relaxation kinetic data is appreciably simplified when the z-induced changes are small, such that

$$A \ll RT. \tag{209}$$

In terms of eqn (97) the reaction rate v is

$$v = v_{\mathrm{f}} - v_{\mathrm{r}} \tag{210}$$

where the rates of product and reactant formation, respectively, are given by

$$v_{\mathrm{f}} = |k_{\mathrm{r}}^{\ominus}| \Pi a_{\mathrm{r}}^{\nu_{\mathrm{r}}}, \qquad v_{\mathrm{r}} = |k_{\mathrm{r}}^{\ominus}| \Pi a_{\mathrm{p}}^{\nu_{\mathrm{p}}}. \tag{211}$$

(At equilibrium, $\bar{v} = 0$, thus $\bar{v}_{\mathrm{p}} = \bar{v}_{\mathrm{r}}$ and $a_j = \bar{a}_j$.) Equation (210) is now rewritten as:

$$v = v_{\mathrm{f}}[1 - v_{\mathrm{r}}/v_{\mathrm{f}}] = v_{\mathrm{r}}[(v_{\mathrm{f}}/v_{\mathrm{r}}) - 1]. \tag{212}$$

With $Q^{\ominus} = \Pi a_{\mathrm{p}}^{\nu_{\mathrm{p}}}/\Pi a_{\mathrm{r}}^{\nu_{\mathrm{r}}}$, $K^{\ominus} = \Pi \bar{a}_{\mathrm{p}}^{\nu_{\mathrm{p}}}/\Pi \bar{a}_{\mathrm{r}}^{\nu_{\mathrm{r}}}$ and [from eqn (28)] $A = RT \ln(K^{\ominus}/Q^{\ominus})$ we obtain:

$$v = v_{\mathrm{f}}(1 - \mathrm{e}^{-(A/RT)}) = v_{\mathrm{r}}(\mathrm{e}^{A/RT} - 1). \tag{213}$$

Near equilibrium where $A \ll RT$, we may set $v_{\mathrm{f}} = \bar{v}_{\mathrm{f}}$ and $v_{\mathrm{r}} = \bar{v}_{\mathrm{r}}$. Series expansion of the exponentials in eqn (213) and neglect of higher order terms result in an expression for the near-equilibrium chemical rate:

$$v_{\mathrm{eq}} = \bar{v}_{\mathrm{r}} \frac{A}{RT} = \bar{v}_{\mathrm{f}} \frac{A}{RT}. \tag{214}$$

1. *Chemical Relaxations*

As seen in eqn (214) the chemical reaction rate is linearly dependent on the affinity. Close to equilibrium the z-induced changes in the extent of the reaction, ξ, also depend linearly on z. Thus, for small perturbations:

$$\delta\xi = \left(\frac{\partial \xi}{\partial z_i}\right)_{z \neq z_i} \delta z_i = \xi(z_i) - \xi_{\mathrm{ref}} \ll \xi_{\mathrm{ref}}. \tag{215}$$

With eqn (11) the chemical relaxation condition in electric fields in terms of the concentration of the reaction partner J is:

$$\delta c_j = \nu_j \Gamma \frac{\Delta M}{RT} E \tag{216}$$

where $\delta c_j \ll c_j(0)$ must hold. The definition (12) for the equilibrium at E is rewritten as

$$\Gamma = \frac{1}{\Sigma_j \nu_j^2/\bar{c}_j}. \tag{217}$$

If the thermodynamic activity coefficients depend on the extent of reaction, then the quantity Γ must be replaced by the term Γ^*; see eqn (38). Therefore the amplitude of the E-induced concentration shift is:

$$(\delta c_j)_{\infty} = \nu_j \Gamma^* \frac{\Delta M}{RT} E. \tag{218}$$

The response to a step perturbation is a relaxation spectrum containing exponentials of time, t:

$$\delta\xi(t) = \Sigma_q (\delta\xi)_{\infty, q} \mathrm{e}^{-t/\tau_q} \tag{219}$$

where $(\delta\xi)_{\infty,q}$ is the amplitude and τ_q is the relaxation time, respectively, of the relaxation mode q.

An elementary step is always described by:

$$\delta c_j(t) = (\delta c_j)_\infty e^{-t/\tau}. \tag{220}$$

This expression is generally applicable for intramolecular elementary steps; for bimolecular steps provided that the perturbation is small $[\delta\xi \ll \xi_{\text{ref}}$ or $\delta c_j \ll (c_j)_{\text{ref}}]$. For practical details consult references (Eigen and DeMaeyer, 1963; Yapel and Lumry, 1971; Jovin, 1976).

Recent theoretical developments offer a simple formalism to evaluate time constants and amplitudes in terms of total concentrations, c_j^0, rather than equilibrium concentrations, $\bar{c}_j$ (Winkler-Oswatitsch and Eigen, 1979; Neumann and Chang, 1976; Nolte and Neumann, 1979). In a kinetic titration according to $L+B=LB$ where for instance c_B^0 is kept constant and c_L^0 is varied, the amplitude factor Γ is zero at $c_L^0=0$, passes then through a maximum at $(c_L^0)\Gamma_{\max} = c_B^0 + K$ and finally approaches zero again. Provided the conditions can be chosen such that $K < c_B^0$, also the relaxation time passes through a maximum; at $(c_L^0)\tau_{\max} = c_B^0 - K$. We may therefore use two maxima and determine the value of K as well as that of the total number of binding sites participating in the relaxation process by:

$$K = [(c_L^0)_{\Gamma_{\max}} - (c_L^0)_{\tau_{\max}}]/2 \tag{221}$$

$$c_B^0 = [(c_L^0)_{\Gamma_{\max}} + (c_L^0)_{\tau_{\max}}]/2. \tag{222}$$

In Table 2 the key relations for the elementary chemical reactions are summarized.

TABLE 2. RELAXATION PARAMETERS OF ELEMENTARY CHEMICAL REACTIONS (KINETIC TITRATION)

Reaction	Relaxation time	Amplitude factor
$L+B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LB$	$\tau = \dfrac{1}{k_1\sqrt{(c_L^0+c_B^0+K)^2-4c_L^0c_B^0}}$	$\Gamma = \dfrac{K}{2}\left\{\dfrac{1}{\left[1-\dfrac{4c_L^0c_B^0}{(c_L^0+c_B^0+K)^2}\right]^{1/2}} - \right\}$
$c_B^0=\text{const.},\ c_L^0=0$	$\tau_0 = \dfrac{1}{k_1(c_B^0+K)}$	$\Gamma_0=0$
$c_B^0=\text{const.},\ (c_L^0)_{\text{m}}$	$\tau_{\text{m}} = \dfrac{1}{2k_1\sqrt{Kc_B^0}},\ c_B^0>K$	$\Gamma_{\text{m}} = \dfrac{K}{2}\left[\dfrac{c_B^0+K}{K}-1\right]^{1/2}$
	at $(c_L^0)\tau_{\text{m}} = c_B^0-K$	at $(c_L^0)\Gamma_{\text{m}} = c_B^0+K$
$2b \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BB$	$\tau = \dfrac{1}{k_1[K(K+8c_B^0)]^{1/2}}$	$\Gamma = \dfrac{K}{8}\left\{\dfrac{K+4c_B^0}{\sqrt{K(K+8c_B^0)}}-1\right\}$
$c_B^0=0$	$\tau_0=(k_1K^2)^{-1}=(k_{-1}K)^{-1}$	$\Gamma_0=0$
$B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$	$\tau = \dfrac{1}{k_1+k_{-1}} = \dfrac{1}{k_1(1+K)}$	$\Gamma = \dfrac{c_B^0\cdot K}{(1+K)^2}$

Superscript ^{0} refers to total (analytical) concentration; the amplitude factor Γ is defined by eqn (68) of the text.

2. *Indication of Concentration Changes*

For the measurement of concentration changes and for the recording of orientational changes in solutions of optically anisotropic molecules, optical techniques have proven to be widely applicable. If ionic species are involved, conductivity measurements are suitable to monitor concentration as well as orientation changes in electrically anisotropic molecules. The Wien effects are directly accessible from the conductivity change $\delta\kappa/\kappa(0)$, relative to the κ-value at $E=0$. For a 1:1 ionic equilibrium like that in eqn (109) the degree of dissociation is $\theta = c_L/c_B^0$. Thus

$$\delta c_L/c_L(0) = \delta\kappa/\kappa(0) = \delta\theta/\theta^0. \tag{223}$$

From eqns (223) and (216) the conductivity relaxation amplitude is (see, e.g. Neumann, 1986):

$$(\delta\kappa)_\infty = \kappa(0)\,\frac{1-\theta^0}{2-\theta^0}\left(\frac{\partial \ln K}{\partial E}\right)_{P,T} E. \tag{224}$$

In the linear range of the E-dependence the substitution of eqn (117) into (224) leads to

$$\frac{(\delta\kappa)_\infty}{\kappa(0)} = \frac{1-\theta^0}{2-\theta^0}\left\{\gamma - \frac{\Delta M^{\ominus}_{\mathrm{ass}}}{RT} - \left(\frac{\partial \ln \tilde{Y}}{\partial E}\right)_{\xi}\right\}E. \tag{225}$$

The relaxation time of an ionic process derived from the conductivity relaxation

$$\delta\kappa(t) = (\delta\kappa)_\infty \mathrm{e}^{-t/\tau}. \tag{226}$$

It is, however, remarked that in electrically anisotropic systems like the linear polyelectrolytes the measured conductivity relaxation may not be determined by the rate of the chemical reaction ($\tau = \tau^{(\mathrm{ch})}$), but may rather be rate-controlled by orientational processes, i.e. $\tau = \tau^{(\mathrm{rot})}$ (e.g. Schallreuter, 1982).

When optical changes are induced by the electric fields, light transmission and fluorescence emission appear to cover, in general, both concentration changes and rotational contributions in anisotropic systems. The linear dichroism seems to yield maximum information on molecular shape or chromophor position relative to rotation axis (e.g. Fredericq and Houssier, 1973).

The absorbance A of polarized light is correlated to concentration and to absorption anisotropy of the molecules through Lambert–Beer's law:

$$A_\lambda = \Sigma_j (A_\lambda)_j = l\Sigma_j \varepsilon_j c_j \tag{227}$$

where ε_j is the (decadic) absorption coefficient of component J in a composite system, l is the optical pathway, and λ is the wavelength of the light. When the absorbance is measured with normal, unpolarized light, then from eqn (227) the absorbance change per cm:

$$\delta A^1_\lambda = \Sigma_j \varepsilon_j\, \delta c_j \tag{228}$$

is a function of the concentration changes δc_j. On the same line, absorbance relaxations directly reflect concentration relaxations.

Experimentally, recent progress in instrumentation has opened the way for measuring field-induced, rotational and chemical relaxations in parallel, both optically and electrically (Schallreuter, 1982).

3. *Component Contributions to Absorbance*

In solutions and suspensions of anisotropic molecules directing external forces such as a hydrodynamic flow or electric field forces may cause large signal changes originating from molecule rotations, leading to large values of the electric dichroism and electric birefringence.

There are numerous reviews on dichroism and birefringence as well as on the use of optical signals (light transmission, fluorescence emission, light scattering, optical rotation, circular dichroism) to indicate concentration changes. Less frequent, however, are accounts where it is emphasized that both chemical and orientational changes may contribute to the measured optical signals (Revzin and Neumann, 1974; Dourlent *et al.*, 1974). We now recall that the absorbance for a multi-component system per cm light path is:

$$A^1_\lambda = \Sigma_j \varepsilon_j c_j. \tag{229}$$

It is of great practical relevance to use linearly polarized light and to choose the direction of the electric field (of a parallel-plate measuring cell) as a reference of the light polarization plane.

If an electric field is applied to a chemical system which exhibits both electrical and optical anisotropy, both the ε_j and the c_j-terms in eqn (22a) may be field dependent. Note that the usual extinction coefficient of optically anisotropic molecules reflect random average values $\bar{\varepsilon}_j$ of all chromophor orientations of the system when measured with polarized light.

In order to cover field effects on ε_j and c_j the field induced absorbance change is analysed in terms of the two types of components:

$$\mathrm{d}A_\lambda^1(E)=\left\{\Sigma_j c_j\left(\frac{\partial\varepsilon_j}{\partial E}\right)_{c_j}+\Sigma_j\varepsilon_j\left(\frac{\partial c_j}{\partial E}\right)_{\varepsilon_j}\right\}\mathrm{d}E \tag{230}$$

one at constant c_j (orientational) and one at constant ε_j (chemical concentration shifts). Therefore the field induced absorbance change

$$\delta A_\sigma=A_\sigma(E)-A(0) \tag{231}$$

measured with light polarized at the angle σ relative to the field vector, where $A_\sigma(E)$ is the absorbance in the presence of the field and $A(0)$ that at $E=0$, covers both orientational changes $\delta(\varepsilon_j)_\sigma$ and concentration changes δc_j.

Whereas in the absence of E the absorbance is independent of σ,

$$A^1(0)=\Sigma_j\bar{\varepsilon}_j c_j(0) \tag{232}$$

the absorbance in the field,

$$A_\sigma^1(E)=\Sigma_j(\varepsilon_j)_\sigma c_j(E) \tag{233}$$

is dependent on σ because of $(\varepsilon_j)_\sigma$ (Tsuji and Neumann, 1981).

Denoting the field induced changes in ε_j and c_j relative to the zero-field values by

$$\delta(\varepsilon_j)_\sigma=(\varepsilon_j)_\sigma-\bar{\varepsilon}_j \tag{234}$$

and

$$\delta c_j=c_j(E)-c_j(0) \tag{235}$$

respectively, eqn (231) is rewritten in terms of eqns (232) and (233):

$$\begin{aligned}\delta A_\sigma^1&=\Sigma_j\{(\varepsilon_j)_\sigma c_j(E)-\bar{\varepsilon}_j c_j(0)\}\\&=\Sigma_j\{\delta(\varepsilon_j)_\sigma[c_j(0)+\delta c_j]+\bar{\varepsilon}_j\,\delta c_j\}\end{aligned} \tag{236}$$

where the separation of the terms depending on σ from those independent of σ is evident.

Introducing the definitions

$$\delta A_\sigma^{(\mathrm{rot})}=\Sigma_j\,\delta(\varepsilon_j)_\sigma[c_j(0)+\delta c_j] \tag{237}$$

$$\delta A^{(\mathrm{ch})}=\Sigma_j\bar{\varepsilon}_j\,\delta c_j \tag{238}$$

eqn (236) may be generally written in terms of a rotational and a chemical contribution (Revzin and Neumann, 1974):

$$\delta A_\sigma^1=\delta A_\sigma^{(\mathrm{rot})}+\delta A^{(\mathrm{ch})}. \tag{239}$$

For axially symmetric measuring-geometry like that of a parallel-plate capacitor cell, $\delta A^{(\mathrm{ch})}$ can be experimentally obtained in *two independent ways*, using the three light polarization modes $\sigma=0$, $\sigma=\pi/2$ and $\sigma=\sigma^*$. Provided that $\delta A\ll A(0)$ we have $\sigma^*=0.955$ (54.7°). Axial symmetry provides the relationship

$$\delta A_\parallel^{(\mathrm{rot})}+2\,\delta A_\perp^{(\mathrm{rot})}=0 \tag{240}$$

where the subscript $_\parallel$ refers to $\sigma=0$ (parallel mode) and $_\perp$ denotes $\sigma=\pi/2$ (perpendicular mode). At $\sigma=\sigma^*$, $\delta A_{\sigma^*}^{(\mathrm{rot})}=0$. If $\sigma^*=0.955$, then

$$\delta A^{(\mathrm{ch})}=\delta A_{0.955}^1. \tag{241}$$

On the other hand eqns (239) and (240) can be combined to (Revzin and Neumann, 1974):

$$\delta A^{(\mathrm{ch})}=\tfrac{1}{3}(\delta A_\parallel^1+2\,\delta A_\perp^1). \tag{242}$$

In the framework of this formalism the nature of the absorbance change, either chemical or purely rotational, may be derived from the amplitudes. More detailed information can, of course, be only obtained from the analysis of the time course of the relaxations. Due to coupling between chemical-conformational transitions to the orientations of the molecules relative to the electric field vector, orientational and chemical relaxations can be coupled, and the rate-limiting process may determine both chemical and rotational contributions. The analysis is straightforward when the time scales of chemical and rotational processes are different. In any case, from a practical point of view, the analysis of the time course of the measured signal is indispensible to determine the various components and their amplitudes.

4. *Linear Dichroism*

The time course of orientational changes induced by electric fields contains information on the orientation mechanism, and on the electrical and geometrical properties (main dipole axis, length) of the aligning and deorienting molecules. For instance, permanent dipole orientation of a given particle type in the presence of a constant electric field builds up with zero slope and has two modes, whereas the build-up of induced dipole orientation starts with maximum slope and is characterized by only one time constant. The deorientational relaxation of a system of identical particles, after termination of the step pulse is monophasic, independently of the presence of permanent or induced dipoles. For details see references (O'Konski and Haltner, 1957; Tinoco and Yamaoka, 1959; Tricot and Houssier, 1976; Neumann, 1986).

The analysis of orientational changes faces problems when non-rigid molecules or molecules of non-homogeneous length distribution are present. The quantitative treatment of field-induced changes in molecule shape is still very difficult. Chain bending or stretching, structural changes, dimer formation, or multimeric aggregation will change the anisotropy components arising from long-range optical interactions as well as short-range interactions with the solvent having, in general, a refractive index different from that of the absorbing molecules considered (form anisotropy). Such contributions, however, are small for solutions of low turbidity, i.e. if the size of the aggregates remains small compared to the wavelength of the monochromatic light used (Tricot and Houssier, 1976).

The linear dichroism ΔA has been originally defined for absorbance changes of purely rotational origin. In the notation used here, we have

$$\Delta A = A_{\parallel}^{(\mathrm{rot})} - A_{\perp}^{(\mathrm{rot})} = \delta A_{\parallel}^{(\mathrm{rot})} - \delta A_{\perp}^{(\mathrm{rot})}. \tag{243}$$

It can, however, be shown that a more general definition of ΔA (as the difference between the absorbances at $\sigma = 0$ and at $\sigma = \pi/2$), holds, independently of whether there are chemical contributions present or not. Because $\delta A^{(\mathrm{ch})}$ is independent of σ, i.e. $\delta A_{\parallel}^{(\mathrm{ch})} = \delta A_{\perp}^{(\mathrm{ch})}$, the application of eqn (239) leads to the general form

$$\Delta A = A_{\parallel} - A_{\perp} = \delta A_{\parallel} - \delta A_{\perp}. \tag{244}$$

Note that the linear dichroism is given by the measured absorbance changes in the electric field.

At high field strengths orientational changes may reach saturation ($E \to \infty$, $\Delta A = \Delta A_s$). The degree of orientation is given by the orientation factor (eqn 145). Thus (Stoylov, 1971):

$$\phi = \Delta A / \Delta A_s = (\delta A^{(\mathrm{rot})} / \delta A_s^{(\mathrm{rot})})_{\sigma}. \tag{245}$$

The dependence of ϕ on the electric field strength contains information on the electrical properties of the molecules: permanent dipole moment $\mathbf{p}$ and/or polarizability tensor α. General equations for the field dependence of ϕ are given by O'Konski *et al.* (1959).

Equation (245) provides the basis for the rigorous analysis of chemical contributions of the induced or permanent dipole moments of the reaction partners, according to eqns (147) and (181). Note that the Langevin function can also be expressed in terms of ϕ: $L[r] = (r/3)(1 - \phi)$, valid for $\phi \ll 1$.

5. *Chemical Transition Factor*

We recall eqn (166): $K(E) = K(0)e^x$, where $x = \int \Delta M \, dE/RT$. If the field induced concentration shifts can be measured by absorbance changes, then eqn (238) can be used. Applied to the intramolecular transition $B_1 \rightleftharpoons B_2$ we have:

$$\delta A^{(\mathrm{ch})} = \bar{\varepsilon}_2\,\delta c_2 + \bar{\varepsilon}_1\,\delta c_1 \tag{246}$$

where $\bar{\varepsilon}_2$ and $\bar{\varepsilon}_1$ are the (random) average values of the extinction coefficients of the conformations B_2 and B_1, respectively. Mass conservation dictates that $\delta c_2 + \delta c_1 = 0$. By definition, $\theta = c_2/(c_1 + c_2) = c_2/c^0$. With $\delta c_2 = c^0\,\delta\theta$ eqn (246) is rewritten as

$$\delta A^{(\mathrm{ch})} = (\bar{\varepsilon}_2 - \bar{\varepsilon}_1)c^0\,\delta\theta \tag{247}$$

where

$$\delta\theta = \theta(E) - \theta^0 \tag{248}$$

θ^0 being the θ-value at $\mathbf{E} = 0$.

Because in a two-state transition $K = \theta/(1-\theta)$ holds we have $K(E) = \theta(E)/[1-\theta(E)]$ and at $\mathbf{E} = 0$, $K(0) = \theta^0/(1-\theta^0)$. Hence,

$$\theta(E) = K(0)\mathrm{e}^x/[1 + K(0)\mathrm{e}^x]. \tag{249}$$

Substitution of eqns (248) and (249) into (247) yields

$$\delta A^{(\mathrm{ch})} = (\bar{\varepsilon}_2 - \bar{\varepsilon}_1)c^0\,\frac{(\mathrm{e}^x - 1)(1-\theta^0)}{\mathrm{e}^x + (1-\theta^0)/\theta^0}. \tag{250}$$

Analogous to the orientation factor ϕ of the linear dichroism and the birefringence, we may define a chemical transition factor according to

$$\phi^{(\mathrm{ch})} = \delta A^{(\mathrm{ch})}/\delta A_s^{(\mathrm{ch})} \tag{251}$$

where $\delta A_s^{(\mathrm{ch})}$ is the saturation value of the chemical absorbance amplitude at high field strengths. Equations (247) and (248) yield:

$$\phi^{(\mathrm{ch})} = \frac{\theta(E_0) - \theta^0}{1-\theta^0}. \tag{252}$$

It is evident that in the limiting case $\theta^0 = 0$ at $\mathbf{E} = 0$, $\phi^{(\mathrm{ch})} = \theta$ holds.

Finally, the combination of eqns (249) and (252) leads to

$$\phi^{(\mathrm{ch})} = \frac{\mathrm{e}^x - 1}{e^x + (1-\theta^0)/\theta^0}. \tag{253}$$

Equation (253) is very useful for the description of chemical transitions as a function of the externally applied electric field (Tsuji and Neumann, 1983).

6. *Differentiation Between Component Contributions*

Among the extreme cases, the analysis of chemical electric field effects is simplest when the rotational equilibria are established faster than the diffusion-limited chemical processes. The other extreme is the complete control of the chemical process by the rate of the orientational relaxations. As seen in Table 2, bimolecular chemical reactions exhibit a characteristic dependence of time constant and amplitude on concentration.

Independent of the time course of the absorbance changes there are a number of ways to differentiate between chemical and orientational contributions of anisotropic molecules. If chemical transformations are associated with isobestic or isochromic wavelengths, then at these wavelengths chemical contributions are zero, i.e. $\delta A^{(\mathrm{ch})} = 0$. When plane-polarized light is used at the light polarization angle σ^*, the rotational contributions cancel, i.e.: $\delta A_{\sigma^*} = 0$. A chemical concentration shift of randomly distributed reaction partners is associated with an absorbance change which is independent of σ. The pure rotational contributions always obey eqn (240). It is obvious that the time constants of the chemical and rotational parts of the relaxations must also be independent of σ.

It should be remarked that chemical contributions of interacting anisotropic molecules are usually negligibly small if simple dipolar equilibria are concerned. Appreciable field effects are encountered only in macromolecular dipolar systems. The second Wien effect and structural changes coupled to ionic dissociation-association processes may occur at already low field intensities.

In any case, large reaction dipole moments (ΔM) are required to produce major displacements of dipolar equilibria; high ionic valences are necessary for larger dissociation field effects in ionic association–dissociation reactions.

These conditions generally require that the reaction partners themselves have either large dipole moments or large polarizabilities or a high density of fixed ionic groups. The structures which fulfill these conditions are macromolecules and macromolecular organizations such as polyionic biopolymers, biopolymer complexes, or the proteins of biomembranes.

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