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Elementary Analysis of Chemical Electric Field Effects in Biological Macromolecules

II. Kinetic Aspects of Electro-Optic and Conductometric Relaxations

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ABSTRACT: Electric field effects in macromolecular organizations such as proteins, nucleic acids, and membranes frequently involve both chemical-conformational changes and physical-orientational displacements of molecular subgroups. Electro-optic techniques in conjunction with relaxation kinetics in high electric fields provide a tool for the investigation of the complex processes encountered in bioelectric phenomena on the level of macromolecules, membrane fragments, and other cellular units. Whereas Part I covers the thermodynamics of electric field effects, this part deals with practical and theoretical aspects of kinetics and mechanisms in aqueous solutions of macromolecules and membranes.

1. Introduction

The electrophysiological voltage clamp technique is a widely used method to approach mechanisms of ion transport across cell membranes. Basically, the voltage clamp is the application of a rectangular electric field and the measurement of relaxations of electric currents which are frequently rate-controlled by structural changes in the ion transport gating proteins. In a similar manner chemical relaxtion kinetics appears to be the method of

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choice to investigate electric field effects of biological systems in solution or suspension in the presence of electric fields which are as high as those in natural cell membranes. Since, in principle, dynamic details of chemical-conformational transitions as well as rotational processes are accessible from relaxation kinetic measurements, the kinetic analysis of chemical electric field effects and electrical chemical mechanisms are outlined in some detail.

2. *Rate Constants in Electric Fields*

2.1. *Dipolar Equilibria*

The equilibrium constant of an elementary chemical reaction is a function of the physical state variables $z_i(=P, T, E)$. Therefore the rate constants must depend differently on the z_i values. Consider a chemical reaction

$$\sum_r \nu_r B_r \underset{k_r}{\overset{k_p}{\rightleftharpoons}} \sum_p \nu_p B_p \tag{2.1}$$

where the subscripts r and p refer to reactants and products, respectively. In this notation the stoichiometric coefficients ν_r and ν_p are positive definite and the apparent equilibrium constant K is expressed as

$$K = \prod \bar{c}_p^{\nu_p} \Big/ \prod \bar{c}_r^{\nu_r} = k_p/k_r \tag{2.2}$$

where k_p and k_r are the rate constants of the forward and reverse direction, respectively. The thermodynamic equilibrium constant K and the rate constants for unit activity $k_p^\ominus$ and $k_r^\ominus$ are given by

$$K^\ominus = \prod \bar{a}_p^{\nu_p} \Big/ \prod \bar{a}_r^{\nu_r} = k_p^\ominus / k_r^\ominus \tag{2.3}$$

At equilibrium we have

$$k_p \prod \bar{c}_r^{\nu_r} = k_r \prod \bar{c}_p^{\nu_p} \tag{2.4}$$

$$k_p^\ominus \prod \bar{y}_r^{\nu_r} \cdot \prod \bar{c}_r^{\nu_r} = k_r^\ominus \prod \bar{y}_p^{\nu_p} \cdot \prod \bar{c}_p^{\nu_p} \tag{2.5}$$

Recalling Eq. (3.24) of part I,(1) the z_i dependence of K is

$$\ln K(z_i) = \ln K(z_i^{(0)}) + \int \Delta Z_i \, dz_i/(RT) \tag{2.6}$$

where $z_i^{(0)}$ is a reference value. In line with Eq. (2.2), the relation (3.10) of part I is rewritten as

$$\Delta Z_i = \sum_j \nu_j Z_{i,j} = \sum_p \nu_p Z_{i,p} - \sum_r \nu_r Z_{i,r} \tag{2.7}$$

where

$$\Delta Z_{i,p} = \sum_p \nu_p Z_{i,p} \tag{2.8}$$

and

$$\Delta Z_{i,r} = \sum_r \nu_r Z_{i,r} \tag{2.9}$$

the contributions of the products and those of the reactants are explicitly separated. With these expressions and with Eq. (2.2) we may express Eq. (2.6) in terms of the rate coefficients as

$$\begin{aligned}\ln[k_p(z_i)/k_r(z_i)] &= \ln[k_p(z_i^{(0)})/k_r(z_i^{(0)})] \\ &\quad + \int (\Delta Z_{i,p} - \Delta Z_{i,r})\, dz_i/RT\end{aligned} \tag{2.10}$$

After term separation we obtain

$$\begin{aligned}k_p(z_i) &= k_p(z_i^{(0)}) \exp\left[\int \Delta Z_{i,p}\, dz_i/RT\right] \\ k_r(z_i) &= k_r(z_i^{(0)}) \exp\left[\int \Delta Z_{i,r}\, dz_i/RT\right]\end{aligned} \tag{2.11}$$

The relations between the unit-activity quantities $k^{\ominus}(z_i)$ and $\Delta Z_i^{\ominus}$ are analogous to Eqs. (2.11).

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 $z_i^{(0)} = E = 0$ as a suitable reference and specify Eqs. (2.11) as

$$\begin{aligned}k_p(E) &= k_p(0) \exp\left[\int \Delta M_p\, dE/RT\right] \\ k_r(E) &= k_r(0) \exp\left[\int \Delta M_r\, dE/RT\right]\end{aligned} \tag{2.12}$$

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 (2.12) were used to discuss the rate aspects of electric-field-induced permeability changes in membranes(2) in the context of electric membrane fusion(3) and electric gene transfer(4) by electroporation.(5)

2.2. *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.(6) Consider an ion-pairing equilibrium:

$$\mathrm{L^+ \cdot B^-} \underset{k_a}{\overset{k_d}{\rightleftharpoons}} \mathrm{L^+ + B^-} \tag{2.13}$$

The Onsager treatment provides an expression for the electric field dependence of $k_d^\ominus$. Note that $k_d^\ominus = k_d Y_{L \cdot B}$. The electric-field-induced increase in the conductivity of electrolytes usually starts nonlinear, 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. In the linear range Onsager's theory of diluted weak electrolytes yields

$$\left(\frac{\partial \ln k_d^\ominus}{\partial |\mathbf{E}|}\right)_{P,T} = \frac{(\tilde{z}_\mathrm{L} u_\mathrm{L} - \tilde{z}_\mathrm{B} u_\mathrm{B})|\tilde{z}_\mathrm{L}\tilde{z}_\mathrm{B}|e_0^3}{(u_\mathrm{L} + u_\mathrm{B})\, 8\pi\varepsilon_0\varepsilon(kT)^2} \tag{2.14}$$

where u is the ionic mobility of the free ions ($u \approx 10^{-4}\ \mathrm{cm^2\,V^{-1}\,s^{-1}}$).

For symmetric electrolytes where $\tilde{z}_\mathrm{L} = -\tilde{z}_\mathrm{B} = |\tilde{z}|$, Eq. (2.14) is reduced to

$$\left(\frac{\partial \ln k_d^\ominus}{\partial |\mathbf{E}|}\right)_{P,T} = |z^3|\, e_0^3/[8\pi\varepsilon_0\varepsilon(kT)^2] = \gamma \tag{2.15}$$

Note the strong dependence on the charge number. As shown elsewhere, 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 $\mathrm{MgSO_4}$ to about 110%.(9,10) 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 the activity coefficient of the ion pair can be taken equal to unity, we have $k^{\overset{\leftrightarrow}{\ominus}} = k$, and Eqs. (2.14) and (2.15) apply to k.

Following the analysis in the previous section the association step in Eq. (2.13) must also be field dependent. According to Eqs. (2.12) we readily see that (with $|\mathbf{E}| = E$)

$$\left(\frac{\partial \ln k_a^{\leftrightarrow}}{\partial E}\right)_{P,T} = \Delta M_a^{\overset{\leftrightarrow}{\ominus}}/RT \tag{2.16}$$

where ΔM_a is given by

$$\Delta M_a^{\overset{\leftrightarrow}{\ominus}} = M_{\mathrm{L \cdot B}}^{\overset{\leftrightarrow}{\ominus}} = N_A m_{\mathrm{L \cdot B}}^{\overset{\leftrightarrow}{\ominus}} \tag{2.17}$$

Note that in line with Eq. (4.31) of part I(1) we have

$$m_{\mathrm{LB}}^{\leftrightarrow} = \mathbf{m}_{\mathrm{L \cdot B}}^{\overset{\leftrightarrow}{\ominus}} \langle \cos \vartheta_{\mathrm{LB}} \rangle \tag{2.18}$$

Therefore the actual 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. Since for Eq. (2.13), $k_a^{\overset{\leftrightarrow}{\ominus}} = k_a \cdot y_{\mathrm{L}} \cdot y_{\mathrm{B}}$,

$$(\partial \ln k_a^{\overset{\leftrightarrow}{\ominus}}/\partial E)_{P,T} = (\partial \ln k_a/\partial E)_{P,T} + (\partial \ln[y_{\mathrm{L}} \cdot y_{\mathrm{B}}]/\partial E)_{P,T} \tag{2.19}$$

and from Eq. (4.40) of part I:

$$\Delta M_a^{\overset{\leftrightarrow}{\ominus}} = \Delta M_a + RT(\partial \ln[y_{\mathrm{L}}\, y_{\mathrm{B}}]/\partial E)_{\xi,P,T} \tag{2.20}$$

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.

As shown for conductivity data on $MgSO_4$, the contribution of the first Wien effect can be quantitatively covered in terms of the Wilson theory.(8)

We recall that the (practical) equilibrium constant (concentration ratio) is given by $K = k_d/k_a$. Thus we may formally express K of ionic (-dipolar) equilibria of the type (2.13) by

$$(\partial \ln K)/\partial E)_{P,T} = \gamma - (\Delta M_a^{\overset{\leftrightarrow}{\ominus}}/RT) - (\partial \ln \tilde{Y}/\partial E)_{\xi,P,T} \tag{2.21}$$

where $\tilde{Y}$ is given by $\tilde{Y} = \tilde{y}_{\mathrm{L}}\, \tilde{y}_{\mathrm{B}}/\tilde{y}_{\mathrm{LB}}$ at the field strength $\mathbf{E}$. At very high field strengths the ionic atmosphere screening may be reduced to a large extent such that $\tilde{Y} = 1$; also, 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.

2.2.1. Polyelectrolytes

It is well established that the second Wien effect is particularly large in linear polyelectrolytes.(11,12) 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 has been used as the basis for a qualitative discussion of the second Wien effect in polyelectrolytes.

Denoting by B_n the polyion and by L the counterion, 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. Owing to the neighboring fixed charges the effective charge, $z_B^{\mathrm{eff}} e_0$ per residue, B, is larger than $z_B e_0$ itself. 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 one residue of the polyion, we may apply Eq. (2.21) in the suggestive form

$$(\partial \ln k_d/\partial E)_{P,T} = (\tilde{z}_L e_0)^2 |\tilde{z}_B^{\mathrm{eff}}| e_0/[8\pi\varepsilon_0\varepsilon(kT)^2] \qquad (2.22)$$

and thus quantify the influences of counterion valency and effective polyionic charge per residue.

The analysis of electric conductivity relaxations of the linear polyelectrolyte poly(riboadenylate, K$^+$) according to Eq. (2.22) at 293 K yields a formal effective charge of about -6 for the interaction of a K$^+$ ion with the inner counterion atmosphere of this polyanion.(7,8)

It has been found that the dissociation field effect in polyelectrolytes is generally larger by a factor of about 10–100 as compared to simple electrolytes. Thus the values of 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. Owing to the efficiency of dissociation field effects in polyelectrolytes, it can be expected that appreciably large 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.

A theoretical approach for the second Wien effect of polyelectrolytes has been initiated in terms of the counterion condensation model by Manning.(13) According to this theory the degree of counterion dissociation from the condensed layer is linearly dependent on the field strength.

3. *Reaction Moment and Electric–Chemical Mechanism*

One of the main aims of investigating electric field effects on chemical transformations is to determine the reaction mechanism and, in simple cases, the dipole moments of the reaction partners. As outlined above, the rate constants provide the key information.

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 to reaction properties 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.(7,8) Two types of processes may be involved if a vectorial perturbation like an external electric field is applied: a chemical concentration change and a change in the orientation of the reaction partners.

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.(14–16) 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 that results from the polarizing and orienting action of the field vector against the randomizing thermal agitations.[17]

For plane-plate capacitor geometry, which is experimentally most adequate, the field-parallel component M of $\mathbf{M}$ is the sum over all field-parallel components m_j of the individual moments $\mathbf{m}_j$. We recall that[1]

$$M = \langle \mathbf{M} \rangle = N_A \sum_j n_j \langle \mathbf{m}_j \cos \vartheta_j \rangle = N_A \sum_j n_j m_j \tag{3.1}$$

Whereas Eq. (3.1) expresses $\mathbf{M}$ in terms of the average contributions of the individual molecular moments $\mathbf{m}_j$, the continuum approach to $\mathbf{M}$ represents the total moment in terms of an overall macroscopic dielectric permittivity ε.

The fundamental relationship between the total moment $\mathbf{M}$ and the measured electric field $\mathbf{E}$ may be written in terms of the absolute amounts (M and E) as

$$M = \varepsilon_0(\varepsilon - 1)\, VE \tag{3.2}$$

where the vector $\mathbf{M}$ is in the same direction as the field vector $\mathbf{E}$.

In classical electrostatics the polarization is given by the macroscopic dipole moment per unit volume, $\mathbf{P}$. Hence $\mathbf{M} = V \cdot \mathbf{P}$. Further on, $\mathbf{P}$ is represented in terms of linear and nonlinear contributions:

$$\mathbf{P} = \chi \mathbf{E} + \chi' E^2 \mathbf{E} + \cdots \tag{3.3}$$

where the susceptibility tensor is given by $\chi = \varepsilon_0(\varepsilon - 1)$. In this form the dielectric permittivity tensor ε is considered as a constant and is independent of $\mathbf{E}$. Chemical contributions to $\mathbf{P}$ only appear as odd powers of $\mathbf{E}$ and are qualified as nonlinear terms.

When Eq. (3.2) 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 $z(=T, P, E)$. If, in addition, chemical transformations are caused by changes in z, then ε also depends on the extent of reaction ξ. In this manner "nonlinearities" are hidden in ε (T, P, E, ξ).

3.1. *Reaction Moments from Dielectric Data*

The chemical reaction moment ΔM refers to that part of the total moment $\mathbf{M}$ which changes in the course of a chemical-conformational transition. Differentiation of Eq. (3.2) with respect to the reaction variable ξ results in

$$\Delta M = (\partial M / \partial \xi)_{V,E} = \varepsilon_0 V E (\partial \varepsilon / \partial \xi)_{V,E} \tag{3.4}$$

Equation (3.4) may be called the continuum expression for the chemical reaction moment at constant total volume between the capacitor plates, usually realized at low field intensities and diluted solutions of the reaction partners. When volume changes occur electrostriction terms must be explicitly considered.(18,19)

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

$$(\partial M/\partial E)_V = (\partial M/\partial E)_{V,\xi} + (\partial M/\partial \xi)_{V,E}(\partial \xi/\partial E)_V \tag{3.5}$$

At equilibrium in the presence of **E** the characteristic (dielectro-)chemical affinity $\tilde{A}$ of dipole systems is zero and $d\tilde{A} = 0$. Hence the term $(\partial \xi/\partial E)_{z,\tilde{A}=0}$ can be calculated; from Eq. (3.57) of part I(1) we obtain

$$(\partial \xi/\partial E)_{V,\tilde{A}=0} = V\bar{\Gamma}^* \Delta M/RT \tag{3.6}$$

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

We may now call the term $(\partial M/\partial E)_{V,\xi}$ the (normal) physical term, because it refers to a fixed value of ξ. From Eq. (3.2) we obtain

$$\Delta\varepsilon^{(\mathrm{ph})} = (\partial M/\partial E)_{V,\xi}/(\varepsilon_0 V) = (\partial \varepsilon/\partial E)_{V,\xi} \tag{3.7}$$

In a similar way we may define the chemical contribution of changes in **M** by

$$\Delta\varepsilon^{(\mathrm{ch})} = (\partial \varepsilon/\partial \xi)_{V,E} = (\partial M/\partial \xi)_{V,E}(\partial \xi/\partial E)_{V,\tilde{A}=0}/(\varepsilon_0 V) \tag{3.8}$$

Substitution of Eqs. (3.4) and (3.6) into (3.8) leads to the well-known relation

$$\Delta\varepsilon^{(\mathrm{ch})} = \Gamma^*(\Delta M)^2/(\varepsilon_0 RT) \tag{3.9}$$

Thus the chemical part of a change in **M** by **E** may be derived from the electric field dependence of dielectric relaxation curves.(14,19)

3.2. *Permanent and Induced Dipole Moments*

Two types of polarization processes may contribute to the total macroscopic polarization:

$$\mathbf{M} = \mathbf{M}_{(\alpha)}(\mathbf{E}_{\mathrm{int}}) + \mathbf{M}_{(p)}(\mathbf{E}_{\mathrm{dir}}) \tag{3.10}$$

According to Onsager the induced moment term $\mathbf{M}_{(\alpha)}$ is determined by the internal or local field $\mathbf{E}_{\text{int}}$, whereas the permanent dipole term $\mathbf{M}_{(p)}$ is related to the directing field $\mathbf{E}_{\text{dir}}$ orienting the permanent dipoles $\mathbf{p}$. The combination of Eqs. (3.10) and (3.2) requires that the two different field vectors must be expressed in terms of the measured Maxwell field. The calculations of the terms $M_{(\alpha)}$ and $M_{(p)}$ as functions of $\mathbf{E}_{\text{int}}$ and $\mathbf{E}_{\text{dir}}$ usually are approximations. The final expressions may be written in terms of conversion factors (g factors(20)) which are a function of particle anisotropies as well as of the properties of the medium in which the particles are embedded (polar, nonpolar, gas phase, or fluid phase).

3.2.1. Individual Dipole Moments

In line with Eq. (3.10) the molecular dipole moments $\mathbf{m}$ may generally be expressed as

$$\mathbf{m} = \mathbf{m}_{(\alpha)} + \mathbf{p} \tag{3.11}$$

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

In anisotropic molecules $\mathbf{m}$ represents 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 disks.(17) 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 analogously to Eq. (3.11) as

$$\mathbf{m}_q = \mathbf{m}_{(\alpha)q} + \mathbf{p}_q \tag{3.12}$$

The induced dipole moment is given by

$$\mathbf{m}_{(\alpha)} = \alpha_q (\mathbf{E}_{\text{int}})_q \tag{3.13}$$

where α_q is the component of the polarizability tensor α in the direction of the q axis and $(\mathbf{E}_{\text{int}})_q$ the internal field in the q direction.

When α is independent of $\mathbf{E}$, then $\mathbf{m}_{(\alpha)}$ is obviously linearly dependent on $\mathbf{E}$. Generally, however, the polarizability tensor reflecting charge displaceability may depend on $\mathbf{E}$. Thus a more general definition of the polarizability is given by

$$\alpha = (\partial \mathbf{m}_{(\alpha)} / \partial \mathbf{E}_{\text{int}})_{E \to 0} \tag{3.14}$$

As outlined previously the parallel component $(\mathbf{m})_{\|}$ of $\mathbf{m}$ contributes to the total polarization. Thus for the q axis we have

$$(\mathbf{m}_q)_{\|} = \mathbf{m}_q \cos \vartheta_q \tag{3.15}$$

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.(17,21) At first, Eq. (3.15) is specified as

$$(\mathbf{m}_q)_{\|} = \alpha \cos \vartheta_q (\mathbf{E}_{\mathrm{int}})_q + p_q \cos \vartheta_q \tag{3.16}$$

where $\alpha_q = \alpha \cos \vartheta_q$. The internal field in the q direction is given by

$$(\mathbf{E}_{\mathrm{int}})_q = \tilde{g}_q g_q \mathbf{E} \cos \vartheta_q \tag{3.17}$$

It is obvious that in isotropic particles where the polarizability is equal in all directions the internal field is simply

$$\mathbf{E}_{\mathrm{int}} = \tilde{g} g \mathbf{E} \tag{3.18}$$

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

$$\tilde{g}_q = [1 + f_q(1 - \alpha_q f_q)^{-1} \langle \mathbf{p}_q \rangle] \tag{3.19}$$

where f_q is the reaction field factor(17,21) and $\langle \mathbf{p}_q \rangle$ is the average contribution of the permanent dipoles. For nonpolar particles (where $p_q = 0$), $\tilde{g}_q = 1$ and $(\mathbf{E}_{\mathrm{int}}) = g_q \mathbf{E} \cos \vartheta_q$.

Obviously, the total value of the field-parallel components is $(\mathbf{m})_{\|} = \sum_q (\mathbf{m}_q)_{\|}$.

In a collection of statistically distributed, mobile dipolar species, the total field-parallel contribution to the polarization is the statistical average over the $\cos \vartheta$ projections on the field vector. For the sake of transparentness we shall confine the further analysis to *uniaxial anisotropic particles* B_j, i.e., to uniaxial dipole moments $\mathbf{m}_j$. In this case Eqs. (3.12) and (3.16) read, respectively,

$$\mathbf{m}_j = \alpha_j (\mathbf{E}_{\mathrm{int}})_j + \mathbf{p}_j \tag{3.20}$$

$$(\mathbf{m}_j)_{\|} = \alpha_j \tilde{g}_j g_j \mathbf{E} \cos^2 \vartheta_j + \mathbf{p}_j \cos \vartheta_j \tag{3.21}$$

The effective average contribution to $\mathbf{M}$ is finally given by

$$m_j = \langle (\mathbf{m}_j)_{\|} \rangle = \alpha_j \tilde{g}_j g_j E \langle \cos^2 \vartheta_j \rangle + p_j \langle \cos \vartheta_j \rangle \tag{3.22}$$

3.2.2. Total Polarization

In a mixture of N_j molecules to type B_j, the total polarization moment can be generally expressed as

$$M = \sum_j N_j m_j = N_A \sum_j n_j m_j$$

$$= \sum_j N_j \langle \alpha_j (E_{\mathrm{int}})_j \rangle + \sum_j \mathrm{N}_j \langle \mathbf{p}_j \rangle \tag{3.23}$$

In order to reduce the complexity the two contributions to M, the induced moment $M_{(\alpha)}$ and the permanent moment $M_{(p)}$ will be treated separately. Applying Eq. (3.22) to (3.23) we obtain

$$M_{(\alpha)} = \sum_j N_j \alpha_j \tilde{g}_j g_j E \langle \cos^2 \vartheta_j \rangle \tag{3.24}$$

$$M_{(p)} = \sum_j N_j p_j \langle \cos \vartheta_j \rangle \tag{3.25}$$

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

3.2.3. Induced Moment

In the absence of permanent dipoles (i.e., $\tilde{g} = 1$), the absolute value $M_{(\alpha)}$ of the total induced moment $\mathbf{M}_{(\alpha)}$ is derived from Eq. (3.24):

$$M_{(\alpha)} = \sum_j N_j \alpha_j g_j \langle \cos^2 \vartheta_j \rangle \, E \tag{3.26}$$

In isotropic particles the total induced moment is given by

$$M_{(\alpha)} = \sum_j N_j \alpha_j g_j E \tag{3.27}$$

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

$$\phi_j = [3 \langle \cos^2 \vartheta_j \rangle - 1]/2 \tag{3.28}$$

This factor can be directly obtained from electro-optic data, for instance, linear dichroism(22,24) and birefringence.(20,23,24) The orientation can also be expressed in terms of the dipole moments involved.(22–24)

Since from Eq. (3.28), $\langle\cos^2\vartheta_j\rangle=\frac{1}{3}(1+2\phi_j)$, substitution into Eq. (3.26) yields

$$M_{(\alpha)}=\left[\sum_j N_j\alpha_j g_j(1+2\phi_j)E\right]\Big/3 \tag{3.29}$$

Two limiting cases are of practical importance. The low-field condition $m_jE\ll kT$ means negligible orientation in the field direction, i.e., $\phi\ll 1$. Hence Eq. (3.29) reduces to

$$M_{(\alpha)}=\left(\sum_j N_j\alpha_j g_j E\right)\Big/3 \tag{3.30}$$

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

$$M_{(\alpha)}=\sum_j N_j\alpha_j g_j E \tag{3.31}$$

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

$$M_{(\alpha),s}=\sum_j N_j(m_s)_j \tag{3.32}$$

In this case the induced moment is independent of the field strength (dielectric saturation).

3.2.4. Permanent Moment

When the molecules B_j have a permanent dipole moment $\mathbf{p}_j$ and are freely mobile, the thermal average of $\cos\vartheta_j$ in the total permanent moment

$$M_{(p)}=\sum_j N_j\, p_j\langle\cos\vartheta_j\rangle \tag{3.33}$$

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

$$(\mathbf{E}_{\text{dir}})_j=g_j\mathbf{E} \tag{3.34}$$

Since $r_j=p_jq_jE/(kT)$, we have

$$\langle\cos\vartheta_j\rangle=L[p_j g_j E/(kT)] \tag{3.35}$$

Note that $L[r_j] = \coth r_j - r_j^{-1}$. Introducing Eq. (3.35) into (3.25) leads to

$$M_{(p)} = \sum_j N_j p_j L[p_j g_j E/(kT)] \tag{3.36}$$

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

$$M_p = \sum_j N_j p_j^2 g_j E/(3kT) \tag{3.37}$$

At high fields ($r_j \gg 1$), $L[r_j] \to 1$ and Eq. (3.36) reduces to

$$M_{(p)} = \sum_j N_j p_j \tag{3.38}$$

From Eqs. (3.30) and (3.37) it is seen that at low field strengths both the contributions of the induced moment $M_{(\alpha)}$ and of the permanent dipole moment $M_{(p)}$ are linear in E. This correlates well with the continuum expression for M as discussed in the context of Eq. (3.2).

3.2.5. *Form Factors and g Factors*

The conversion factors g and $\tilde{g}$ 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.(17,20) 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 *nonpolar* and the overall dielectric permittivity is ε, the g factor of the q axis is given by

$$g_q = \varepsilon\{1 + [(\varepsilon_\infty)_q - 1]A_q\}/\{\varepsilon + [(\varepsilon_\infty)_q - \varepsilon]A_q\} \tag{3.39}$$

The polarizability along the q axis is

$$\alpha_q = \varepsilon_0 V_j[(\varepsilon_\infty)_q - 1]/\{1 + [(\varepsilon_\infty)_q - 1]A_q\} \tag{3.40}$$

where $V_j = (4/3)\pi abc$ is the molecular volume of the ellipsoidal molecules B_j with the half-axes $q = a, b, c$. It is stressed that the value of ε in Eqs. (3.39) and (3.40) 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(17) note that the reaction field factor of the q axis is given by

$$f_q = A_q(1 - A_q)(\varepsilon - 1)/\{\varepsilon_0 V_j[\varepsilon + (1-\varepsilon)A_q]\} \tag{3.41}$$

At particular geometries the form factors are analytically expressed in a simple form. For very long cylinders the depolarizing factors 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 disk; flat patches of biological membranes may be described in terms of the disk geometry. The depolarizing factor for the polarization direction along the disk-normal, i.e., perpendicular to the disk plane, is $A_\perp = 1$; therefore, $g = 1/\varepsilon$. The form factor of spherical isotropic systems is $A_q = 1/3$. In the case of polarizable dipolar spheres B_j [with $(\varepsilon_\infty)_j$ and $\mathbf{p}_j$] immersed in (a large excess of) a nonpolar medium of the effective bulk permittivity ε, Eqs. (3.39) and (3.40) yield

$$g_j = \varepsilon[(\varepsilon_\infty)_j + 2]/[2\varepsilon + (\varepsilon_\infty)_j] \tag{3.42}$$

Substituting $A_q = 1/3$ into Eq. (3.40), we obtain the familiar Clausius–Mosotti equation:

$$\alpha_j = 3\varepsilon_0 V_j[(\varepsilon_\infty)_j - 1]/[(\varepsilon_\infty)_j + 2] \tag{3.43}$$

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 particle B_j." The reaction field factor of spherical molecules for the same conditions is given by

$$f_j = 2(\varepsilon - 1)/[3\varepsilon_0 V_j(2\varepsilon + 1)] \tag{3.44}$$

In a pure condensed medium of nonpolar molecules we have $\varepsilon_\infty = \varepsilon$. For isotropic spheres we have

$$\begin{aligned} g &= (\varepsilon + 2)/3 \\ \alpha &= 3\varepsilon_0 V_j(\varepsilon - 1)/(\varepsilon + 2) \end{aligned} \tag{3.45}$$

In the case of a pure liquid of nonpolarizable dipoles ($\mathbf{p}$) we have $\varepsilon_\infty = 1$; hence the conversion factor of the spherical permanent point dipoles is

$$g = 3\varepsilon/(2\varepsilon + 1) \tag{3.46}$$

Finally, the g factor of spherical molecules in the gas phase is

$$g = (\varepsilon + 2)/3 \tag{3.47}$$

and the polarizability is given by Eq. (3.45). The g factors for the various specific cases are compiled in Table 1.

3.3. *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 Eqs. (3.23) and (4.42) of part I. The electric field

TABLE 1. Conversion Factors (g, g_j) for Spherical Molecules, Relating Internal Field ($\mathbf{E}_{\text{int}}$), Directing Field ($\mathbf{E}_{\text{dir}}$), and Fröhlich Field ($\mathbf{E}_{\text{F}} = \mathbf{E}_{\text{dir}}$) to the (measured) Maxwell Field ($\mathbf{E}$)[a]

I. Pure liquids (ε)			
	g	$\mathbf{E}_{\text{int}}$	$\mathbf{E}_{\text{dir}}$
(a) Polarizable polar spheres (Onsager) ($p, \varepsilon_\infty, \varepsilon$)	$\dfrac{\varepsilon(\varepsilon_\infty + 2)}{2\varepsilon + \varepsilon_\infty}$	$\tilde{g}g\mathbf{E}_\parallel$	$g\mathbf{E}$
(b) Polarizable nonpolar spheres ($p = 0, \varepsilon_\infty = \varepsilon$)	$\dfrac{\varepsilon + 2}{3}$	$g\mathbf{E}_\parallel$	
(c) Polar nonpolarizable spheres ($p, \varepsilon_\infty = 1, \varepsilon$)	$\dfrac{3\varepsilon}{2\varepsilon + 1}$		$g\mathbf{E}$
(d) Polarizable permanent dipoles [Fröhlich, $p = p_G(\varepsilon_\infty + 2)/3; \varepsilon$]	$\dfrac{3\varepsilon}{2\varepsilon + \varepsilon_\infty}$		$g\mathbf{E}$
II. Molecules [$p_j, (\varepsilon_\infty)_j$] diluted			
	g_j	$(E_{\text{int}})_j$	$(E_{\text{dir}})_j$
(1) In nonpolar fluid medium (ε)			
(a) Polarizable polar spheres [$p_j, (\varepsilon_\infty)_j$]	$\dfrac{\varepsilon[(\varepsilon_\infty)_j + 2]}{2\varepsilon + (\varepsilon_\infty)_j}$	$\tilde{g}_j g_j\mathbf{E}_\parallel$	$g_j\mathbf{E}$
(b) Polarizable nonpolar spheres [$p_j, (\varepsilon_\infty)_j = 1; \varepsilon$]		$g_j\mathbf{E}_\parallel$	
(c) Polar nonpolarizable spheres [$p_j, (\varepsilon_\infty)_j = 1; \varepsilon$]	$\dfrac{3\varepsilon}{2\varepsilon + 1}$		$g_j\mathbf{E}$
(2) In polar fluid medium (ε) (Fröhlich, Kirkwood, g_K factor), $p_j = (p_j)_G[(\varepsilon_\infty)_j + 2]/3$	$\dfrac{3\varepsilon}{2\varepsilon + (\varepsilon_\infty)_j}$		$g_j\mathbf{E}$
(3) In gas phase (ε), polar polarizable spheres [$(p_j)_G, (\varepsilon_\infty)_j, \varepsilon$]	$\dfrac{(\varepsilon_\infty)_j + 2}{3}$	$g_j\mathbf{E}_\parallel$	$g_j\mathbf{E}$

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

dependence of the apparent equilibrium constant (concentration ratio) is given by

$$K(E) = K(0)e^{x} \tag{3.48}$$

where the following definition holds:

$$x = \int_0^E \Delta M \, dE/RT \tag{3.49}$$

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 $M_{(p)}$. It is therefore useful to follow Eq. (3.10) and to write ΔM in two terms:

$$\Delta M = \Delta M_{(\alpha)} + \Delta M_{(p)} \tag{3.50}$$

corresponding to a separation of the quantity x:

$$x = x_{(\alpha)} + x_{(p)} \tag{3.51}$$

The induced polarization is thus characterized by

$$x_{(\alpha)} = \int \Delta M_{(\alpha)} \, dE/RT \tag{3.52}$$

and the permanent dipole contribution is given by

$$x_{(p)} = \int \Delta M_{(p)} \, dE/RT \tag{3.53}$$

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. As to chemical and physical processes in the presence of applied electric fields, the solvent may 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. The calculation of the internal and the directing field in terms of an inhomogeneous Maxwell field is extremely intricate.(17) Therefore the classical relations between $\mathbf{E}_{\text{int}}$ and $\mathbf{E}_{\text{dir}}$ and the macroscopic average field (Maxwell field) of real molecular dielectrics are only approximations, related to the homogeneous part of the Maxwell field, **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. In the framework of this assumption the total polarization of a fluid dielectric and the chemical contributions to polarization changes may be treated in a similar way as the homogeneous approximation. In any case, the specific expressions of the reaction moment and the field dependence of the equilibrium constant reflect not only the different dielectric properties of the interacting molecules but also the reaction mechanism.

3.3.1. Nonpolar Polarizable Spheres

In the case of pure induced polarization the solute molecules B_j may be considered as polarizable spheres associated with a "molecular" permittivity $(\varepsilon_\infty)_j$ and $\mathbf{p}_j = 0$. When these molecules are immersed in a large quantity of nonpolar solvent the total permittivity of the solution ε is practically that of the solvent.

For particles of uniaxial anisotropic polarizability α_j, Eqs. (3.2) and (3.23) with $M = M_{(\alpha)}$ are combined with Eq. (3.29). Since $N_j = N_A n_j$, the resulting expression reads

$$\varepsilon_0(\varepsilon - 1)\, V = N_A \sum_j n_j \alpha_j (1 + 2\phi_j)\, g_j/3 \tag{3.54}$$

The appropriate relations of g_j and α_j are obtained from Eqs. (3.44) and (3.45), respectively. Assuming now that all $(\varepsilon_\infty)_j$ are equal such that all B_j have $(\varepsilon_\infty)_j = \varepsilon_\infty$, Eq. (3.54) may be formulated for the low-field limiting case $\phi_j \to 0$ as

$$\varepsilon_0(\varepsilon - 1)(2\varepsilon + \varepsilon_\infty)/\varepsilon = N_A \sum_j n_j \alpha_j (\varepsilon_\infty + 2)/3V \tag{3.55}$$

In this way the quantities which change upon a chemical transformation, the total permittivity ε and the mole 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 Eq. (3.1) of part I in the form $dn_j/d\xi = \nu_j$ we finally obtain

$$(\partial\varepsilon/\partial\xi)_{E,V} = N_A \varepsilon^2 \sum_j \nu_j \alpha_j (\varepsilon_\infty + 2)/[3V\varepsilon_0(2\varepsilon^2 + \varepsilon_\infty)] \tag{3.56}$$

Substitution of Eq. (3.56) into (3.4) leads to

$$\Delta M_{(\alpha)} = [N_A \varepsilon^2/(2\varepsilon^2 + \varepsilon_\infty)] \sum \nu_j \alpha_j [(\varepsilon_\infty + 2)/3]\, E \tag{3.57}$$

Insertion of Eq. (3.57) into (3.52) and integration yields the x quantity of the low-field range:

$$x_{(\alpha)} = [3\varepsilon^2/(2\varepsilon^2+\varepsilon_\infty)]\left\{\sum_j \nu_j \alpha[(\varepsilon_\infty+2)/3]/(6kT)\right\}E^2 \tag{3.58}$$

For isotropic polarizable spheres Eq. (3.27) applies and

$$x_{(\alpha)} = [3\varepsilon^2/(2\varepsilon^2+\varepsilon_\infty)]\left\{\sum_j \nu_j \alpha\,[(\varepsilon_\infty+2)/3]/2kT\right\}E^2 \tag{3.59}$$

In the limiting case of saturated induced dipole moments, Eq. (3.32) is used to obtain the reaction moment ΔM according to Eq. (3.4):

$$\Delta M_s = N_A \sum_j \nu_j (m_s)_j \tag{3.60}$$

The x quantity of this case is

$$x_s = \sum_j \nu_j (m_s)_j E/(kT) \tag{3.61}$$

Inspecting the Eqs. (3.58), (3.59), and (3.61) 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.$^{(9,10)}$

3.3.2. *Polar Nonpolarizable Spheres*

The special case of uniaxial nonpolarizable point dipoles refers to $(\varepsilon_\infty)_j = 1$ and a finite value of the permanent dipole moment $\mathbf{p}_j$ in the general expressions. The solvent is nonpolar and the total permittivity of the solution is ε. The combination of Eqs. (3.32) and (3.4) only leads to general analytical forms of $(\partial\varepsilon/\partial\xi)_{V,E}$ and of the $x_{(p)}$ factor if the g 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 Eq. (3.37) applies we derive from the general Eq. (3.2) that

$$\varepsilon_0(\varepsilon-1)V = N_A \sum_j n_j p_j^2\, g_j/(3kT) \tag{3.62}$$

The g factor of nonpolarizable spherical point dipoles is given by Eq. (3.46):

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

Term separation in Eq. (3.62) leads to

$$\varepsilon_0(\varepsilon - 1)(2\varepsilon + 1)/3\varepsilon = (N_A/V)\sum n_j p_j^2/(3kT) \tag{3.63}$$

and

$$(\partial\varepsilon/\partial\xi)_{V,E} = (N_A/\varepsilon_0 V)[3\varepsilon^2/(2\varepsilon^2 + 1)]\sum \nu_j p_j^2/(3kT) \tag{3.64}$$

Introducing now Eq. (3.64) into (3.4) we obtain for spherical point dipoles

$$\Delta M_{(p)} = N_A[3\varepsilon^2/(2\varepsilon^2 + 1)]\left\{\sum \nu_j p_j^2/(3kT)\right\} E \tag{3.65}$$

Substitution into Eq. (3.53) and integration yield the x quantity for the low-field range of chemically interacting point dipoles

$$x_{(p)} = [(3\varepsilon^2/(2\varepsilon^2 + 1))]\left\{\sum \nu_j p_j^2/[6(kT)^2]\right\} E^2 \tag{3.66}$$

In the high field strength range Eq. (3.38) applies and the orientational saturation is given by

$$\Delta M_s = N_A \sum_j \nu_j p_j \tag{3.67}$$

and

$$x_s = \sum_j \nu_j p_j E/kT \tag{3.68}$$

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 combination of the continuum expression (3.2) with the molecular representation in terms of the Langevin function $L[r_j]$ yields

$$\varepsilon_0(\varepsilon - 1)V = N_A \sum_j n_j p_j L[p_j g_j E/kT] \tag{3.69}$$

The differentiation with respect to ξ involves the term $\partial L[p_j g_j E/kT]/\partial\xi$ that cannot be treated in a closed analytical form. Even if the approximation $\Delta M_{(p)}=\sum_j v_j p_j L(r_j)$ could be applied, the integration of the function $L[r_j]$ to obtain the x quantity, according to

$$x_{(p)}=\frac{\sum_j v_j p_j \int L[r_j]\,dE}{kT}=\sum_j (v_j/g_j)\ln\frac{\sinh r_j}{r_j} \tag{3.70}$$

is valid only if the g_j factors can be considered independent of E, i.e., $(\partial\varepsilon/\partial E)=0$.

The assumption of constant ε is inherent in all integrations according to Eqs. (3.52) and (3.53). The ε values in the specific expressions for the x quantities refer to the actual value of ε in the presence of $\mathbf{E}$.

3.3.3. 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); the polarizability 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 electric fields which actually work on the molecules are given by Eqs. (3.18) and (3.34). The internal field of polar polarizable spheres is given by

$$\begin{aligned}(\mathbf{E}_{\text{int}})_j &= \tilde{g}_j g_j \mathbf{E}\\ &= [1+f_j(1-\alpha_j f_j)^{-1}\langle \mathbf{p}_j\rangle]\, g_j\mathbf{E}\end{aligned} \tag{3.71}$$

For spherical particle geometry the factors g_j, α_j, and f_j are obtained from Eqs. (3.42)–(3.44), respectively. The low-field approximation of $\langle \mathbf{p}_j\rangle$ is given by

$$\langle \mathbf{p}_j\rangle = p_j\langle\cos\vartheta_j\rangle = p_j^2\, g_j E/(3kT) \tag{3.72}$$

The total moment in Eq. (3.23) may be rewritten as

$$M = N_A\left\{\sum_j n_j\alpha_j(\mathbf{E}_{\text{int}})_j + \sum_j n_j\langle \mathbf{p}_j\rangle\right\} \tag{3.73}$$

In the low-field range we substitute Eqs. (3.71) and (3.72) into (3.73). Using now Eqs. (3.42)–(3.44) 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 equation for pure dipole liquids in the suggestive form

$$\varepsilon_0(\varepsilon-\varepsilon_\infty)(2\varepsilon+\varepsilon_\infty)/3\varepsilon = (N_A/V)\sum n_j p_j^2[(\varepsilon_\infty+2)/3]^2/(3kT) \tag{3.74}$$

Differentiation with respect to ξ yields

$$(\partial\varepsilon/\partial\xi)_{V,E} = (N_A/\varepsilon_0 V)[3\varepsilon^2/(2\varepsilon^2+\varepsilon_\infty)] \sum \nu_j p_j^2[(\varepsilon_\infty+2)/3]^2/(3kT) \tag{3.75}$$

and applying Eq. (3.4) results in

$$\Delta M = [N_A 3\varepsilon^2/(2\varepsilon^2+\varepsilon_\infty^2)] \left\{\sum \nu_j p_j^2[(\varepsilon_\infty+2)/3]^2\right\} E \tag{3.76}$$

With Eq. (3.49) the equilibrium shift by small fields is described by

$$x = [3\varepsilon^2/(2\varepsilon^2+\varepsilon_\infty^2)] \left\{\sum \nu_j p_j^2[(\varepsilon_\infty+2)/3]^2/[6(kT)^2]\right\} E \tag{3.77}$$

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

The Onsager approach appears quite adequate for the analysis of chemical reactions in nonpolar media.(14,19,25,26) For polar liquids like water, which is of particular interest for biochemical processes, a modified model developed by Fröhlich appears to be more adequate.(27,28)

3.4. *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. The fundamental polarization equation for such a polar dielectrics is

$$M = \varepsilon_0(\varepsilon_\infty - 1)\, VE + N_A \sum_j n_j \langle \mathbf{p}_j \rangle \tag{3.78}$$

Note that the induced part is formally separated from the permanent dipole moment contribution in a particular manner. In Fröhlich's version of the Onsager model the spherical dipoles have effective dipole moments

$$\mathbf{p}_j = (\mathbf{p}_j)_G[(\varepsilon_\infty)_j + 2]/3 \tag{3.79}$$

where $(\mathbf{p}_j)_G$ is the dipole moment of B_j in the gas phase.

The actual field working as the directing field is the Fröhlich field $\mathbf{E}_F$, which is given by

$$(\mathbf{E}_F)_j = g_j \mathbf{E} \tag{3.80}$$

where

$$g_j = 3\varepsilon/[2\varepsilon + (\varepsilon_\infty)_j] \tag{3.81}$$

In a medium of polar molecules specific intermolecular interactions such as, for instance, H-bridges in water may occur. The effect of this property is accounted for by the Kirkwood correlation factor g_K.

The average contribution of the "Fröhlich dipoles" to the total moment is, analogous to Eq. (3.72), given by

$$\langle p_j \rangle = p_j (g_K)_j L[p_j g_j E/kT] \tag{3.82}$$

The low-field approximation reads

$$\langle p_j \rangle = p_j^2 (g_K)_j g_j E/(3kT) \tag{3.83}$$

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

$$\langle p \rangle_s = p_j (g_K)_j \tag{3.84}$$

3.4.1. *Kirkwood–Fröhlich Equation*

For pure liquids where all $(\varepsilon_\infty)_j = \varepsilon_\infty$, the combination of Eqs. (3.2), (3.78), (3.81), and (3.83) leads to the familiar Kirkwood–Fröhlich equation, written here in the suggestive form of the separated variables ε and n_j:

$$\varepsilon_0(\varepsilon - \varepsilon_\infty)(2\varepsilon + \varepsilon_\infty)/3\varepsilon = (N_A/V) \sum n_j (g_K)_j (p_j)_G^2 [(\varepsilon_\infty + 2)/3]^2/(3kT) \tag{3.85}$$

Since in a pure liquid $\sum_j n_j (g_K)_j (p_j)_G^2 = n g_K p_G^2$, Eq. (3.85) is useful for the determination of dipole moments of polar liquids by dielectric measurements. The g_K factor must be calculated.(17)

3.4.2. *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 spheres we may use the approximation $(\varepsilon_\infty)_j = \varepsilon_\infty$ and $\varepsilon_\infty = 5(\pm 1)$ at 20°C.(28)

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 of ion-pair equilibria in water may be derived from Eq. (3.85):

$$\varepsilon_0(\varepsilon - \varepsilon_\infty)(2\varepsilon + \varepsilon_\infty)/3\varepsilon = (N_A/V) \sum_j n_j p_j^2/(3kT) \tag{3.86}$$

where p_j is given by Eq. (3.79). Substitution of

$$(\partial\varepsilon/\partial\xi)_{V,E} = (N_A/\varepsilon_0 V)[3\varepsilon^2/(2\varepsilon^2 + \varepsilon_\infty^2)] \sum_j \nu_j p_j^2/(3kT) \tag{3.87}$$

into (3.4) yields the reaction moment

$$\Delta M = N_A[3\varepsilon^2/(2\varepsilon^2 + \varepsilon_\infty^2)] \left[\sum \nu_j p_j^2/(3kT)\right] E \tag{3.88}$$

The use of Eq. (3.49) and integration result in the x factor of ion-pair equilibrium displacements at low field strengths:

$$x = [3\varepsilon^2/(2\varepsilon^2 + \varepsilon_\infty^2)] \left\{\sum_j \nu_j p_j^2/[6(kT)^2]\right\} E^2 \tag{3.89}$$

At high field strengths leading to orientational saturation $(\langle p_j \rangle = p_j)$, combination of Eq. (3.2) and (3.78) yields

$$\varepsilon_0(\varepsilon - \varepsilon_\infty) V \cdot E = N_A \sum_j n_j p_j \tag{3.90}$$

$$\Delta M = \varepsilon_0 V E (\partial\varepsilon/\partial\xi)_{V,E} = N_A \sum_j \nu_j p_j \tag{3.91}$$

$$x = \sum_j \nu_j p_j/(kT) \tag{3.92}$$

Note that in the Fröhlich version of the Onsager model due to Eq. (3.79), Eqs. (3.85) and (3.86) are identical to (3.74) and Eqs. (3.87) and (3.88) are identical to Eqs. (3.75)–(3.77), respectively. On the basis of of Eq. (3.87) the dipole moments of ion-pairs formed by Mg^{2+} and SO_4^{2-} in aqueous solution have been estimated.(7,8)

3.5. *Induced Dipole Moments in Polyionic Macromolecules*

As already mentioned, 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

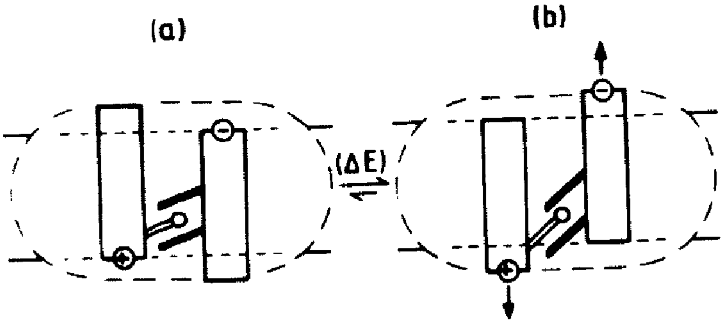

FIGURE 1. Principle of the saturatable induced dipole mechanism causing positional changes of side chains in helical membrane proteins. In bacteriorhodopsin helical parts with different net charge may move transversal to the membrane plane in opposite directions when the electric membrane field is increased, (a) → (b). The geometrically limited increase in the distance of the charge centers is equivalent to a saturatable induced dipole moment. The transversal displacement of at least one of the two helical parts can thereby cause a concerted rotational shift of the retinal (=o) and of aromatic amino acid side chains which may sandwich (T. H. Haines) the retinal chromophore.

polymer structure may restrict the local mobility of the charged groups and dipolar ion pairs. Restricted conformational displaceability may then lead to a saturable induced dipole moment$^{(30)}$; see Figure 1.

Consider a dipolar ion pair of 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 centers of this 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 (Figure 2). According to

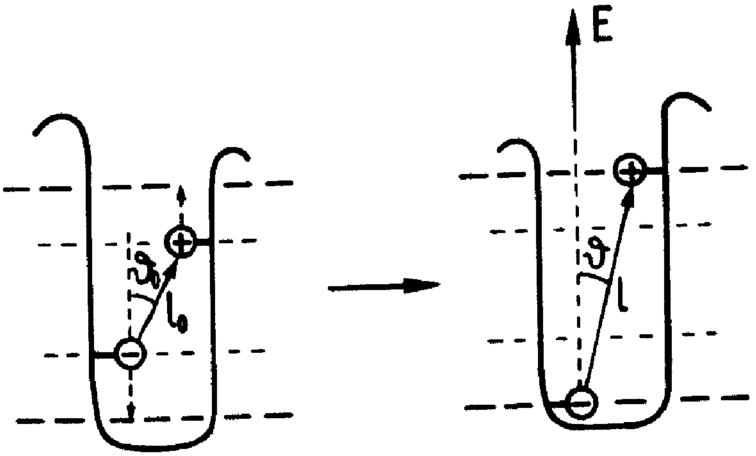

FIGURE 2. Scheme for the anisotropic mobility of a section in a membrane protein. Field-induced displacement of two oppositely charged groups (of an ion pair) along the membrane normal, leading to an increase of the apparently permanent dipole moment $\mathbf{m}_0 = e_0 \cdot \mathbf{l}_0$ to $\mathbf{m} = e_0 \cdot \mathbf{l}$, equivalent to an *induced* dipole moment of $\mathbf{m} - \mathbf{m}_0 = \mathbf{p}_{(\alpha)} = e_0 \delta\mathbf{l}$, where $\delta\mathbf{l} = \mathbf{l} - \mathbf{l}_0$. The dipole moment increase is accompanied by a rotation of the dipole axis $\mathbf{l}$ toward the membrane normal; the electric field vector is parallel to the normal.

this model an electric field $\mathbf{E}$ increases the dipole moment of an ion pair from a value $\mathbf{m}(0) = |\tilde{z}| e_0 r_0$ 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:

$$\mathbf{m}_{(\alpha)} = \mathbf{m}(r) - \mathbf{m}(r_0) = |\tilde{z}| e_0 \delta\mathbf{r} = \alpha \mathbf{E}_{\text{int}} \tag{3.93}$$

The limited conformational flexibility of (membrane) proteins will only permit motions of restricted extent. The membrane structure may further limit the motions in certain directions. It is likely that a major unidirectional charge displacement may only occur along the membrane normal.

Due to these limits the atomic polarizability α refers to the displaceability of charged protein groups in directions closely along the membrane normal. Furthermore, due to conformational restrictions, α will decrease from an initial value α at $\mathbf{E} = 0$, with increasing field strength until, at the saturation of the charge displacement, the limit $\alpha \to 0$ is reached.

Thus, in general, the induced dipole moment $\mathbf{m}_{(\alpha)}$ will reach a saturation value $\mathbf{m}_s$. As pictured in Figure 2, a restricted charge displacement may be accompanied by an orientational charge of the dipole axis. The electric field dependence of the total moment may be described in terms of coth functions as in the case of the counterion polarization in linear polyelectrolytes.(31) See also Yoshioka *et al.*(29)

The model outlined in Figure 1 for bacteriorhodopsin is suggestive not only for a possible control function of the electric field of the bacterial membrane during the photocycle of bacteriorhodopsin, but also for a possibly general, induced-dipole mechanism for electric-field-dependent

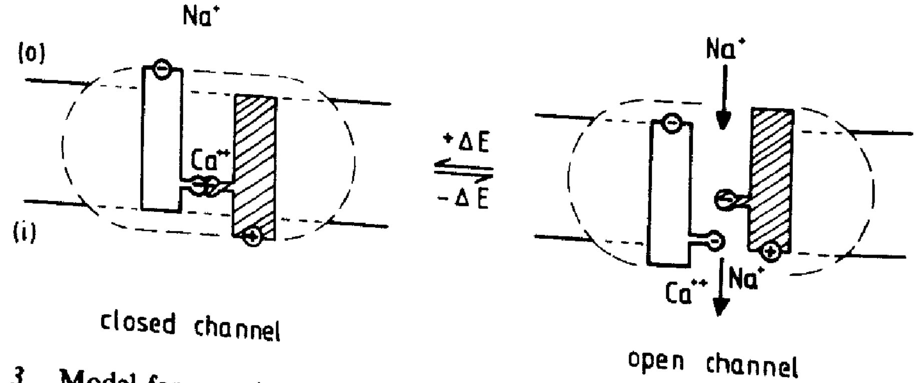

FIGURE 3. Model for a gating element of the axonal Na$^+$ channel. At high electric field (resting membrane potential) between the outside (o) and the inside (i) of the excitable membrane, the gating protein is in the closed conformation. Ca^{2+} ions are bound to anionic side chains of amino acids of helical parts, kept by the membrane field in a high dipole moment configuration (large distance between the cationic and anionic groups). Depolarization reduces the electric membrane field, which, in turn, reduces the electric polarization by transversal movements of the helical parts, decreasing the distance between the fixed charges. The Ca^{2+} ion of the former bridge is exchanged by Na$^+$ ions which flow through the open, "field-relaxed" conformation into the cell interior.

structural changes in membrane transport proteins such as the gating proteins in the excitable membranes of nerve and muscle cells. Analogous to Figure 1, we may suggest an induced-dipole gating mechanism for the axonal Na^+ channel in a schematic form (Figure 3), also incorporating ion exchange Ca^{2+}/Na^+.(32,33)

4. *Measurement of Electric Field Effects*

The majority of biological processes involves ionic species in aqueous environments. The stability of many biopolymer systems requires a finite ionic strength. Adequately, electric field effects in these systems have to be studied in conducting solutions and suspensions. Traditionally, dielectric measurements, conductivity relaxations, and electro-optic techniques were used to study electric field effects on chemical reactions.(18,19) In order to minimize Joule heating, high electric field strengths are applied in the form of short field pulses. 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 longlived and relax with time constants large compared to the field duration, Joule heating temperature jump spectrometers may be used.(2,31,33)

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. A general expression for chemical relaxation conditions may be found in terms of the appropriate chemical reaction affinity according to

$$A \ll RT \tag{4.1}$$

In terms of Eq. (2.1) the reaction rate v is

$$v = v_p - v_r \tag{4.2}$$

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

$$v_p = k_p^{\ominus} \prod a_r^{\nu_r}, \qquad v_r = k_r^{\ominus} \prod a_p^{\nu_p} \tag{4.3}$$

At equilibrium, $\bar{v} = 0$, thus $\bar{v}_p = \bar{v}_r$ and all a_j are given by the equilibrium values $\bar{a}_j$.

We now rewrite Eq. (4.2) as

$$v = v_p(1 - v_r/v_p) = v_r(v_p/v_r - 1) \tag{4.4}$$

and recall that $Q^\ominus = \prod a_p^{\nu_p}/\prod a_r^{\nu_r}$, $K^\ominus = \prod \bar{a}_p^{\nu_p}/\prod \bar{a}_r^{\nu_r}$. Since $A = RT\ln(K^\ominus/Q^\ominus)$, substitution into Eq. (4.4) leads to

$$v = v_p(1 - e^{-(A/RT)}) = v_r(e^{A/RT} - 1) \tag{4.5}$$

Near equilibrium where $A \ll RT$, we may set $v_p = \bar{v}_p$ and $v = \bar{v}_r$. Series expansion of the exponentials in Eq. (4.5) and neglect of higher-order terms result in an expression for the near-equilibrium chemical rate:

$$v_{\text{eq}} = \bar{v}_r A/RT = \bar{v}_p A/RT \tag{4.6}$$

4.1. Chemical Relaxations

In Eq. (4.6) the chemical reaction rate close to equilibrium 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 we may generally specify

$$\delta\xi = (\partial\xi/\partial z_i)_{z \neq z_i}\,\partial z_i = \xi(z_i) - \xi_{\text{ref}} \ll \xi_{\text{ref}} \tag{4.7}$$

We now recall Eq. (3.14) of Part I and express the chemical relaxation condition in electric fields in terms of the concentration of the reaction partner B_j:

$$\delta c_j = \nu_j \Gamma \Delta ME/(RT) \tag{4.8}$$

The condition $\delta c_j \ll c_j(0)$ must hold and the definition (3.15) applied to equilibrium at E is

$$\bar{\Gamma} = \left(\sum_j \nu_j^2/\bar{c}_j\right)^{-1} \tag{4.9}$$

If the thermodynamic activity coefficients depend on the extent of reaction, then the quantity Γ must be replaced by the term Γ^*; see Eq. (3.56) of part I. Therefore the amplitude of the E-induced concentration shift is

$$(\delta c_j)_\infty = \nu_j \bar{\Gamma}^* \Delta ME/(RT) \tag{4.10}$$

If an external perturbation is applied faster than the chemical equilibration time, the response to a step perturbation is a relaxation spectrum containing exponentials of time, t. The time course of the chemical relaxation with q (normal mode) processes is given by

$$\delta\xi(t) = \sum_q (\delta\xi)_{\infty,q}\, e^{-t/\tau_q} \tag{4.11}$$

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

An elementary step is always described by

$$\delta c_j(t) = (\delta c_j)_\infty e^{-t/\tau} \tag{4.12}$$

This expression is generally applicable for intramolecular elementary steps. It is applicable for bimolecular steps only if the perturbation is small such that $\delta\xi \ll \xi_{\mathrm{ref}}$ or $\delta c_j \ll (c_j)_{\mathrm{ref}}$.

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, $\bar{c}_j$.[9,34–36]

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 $c_\mathrm{L}^0 = 0$, passes then through a maximum at $(c_\mathrm{L}^0)_{\Gamma\mathrm{max}} = c_\mathrm{B}^0 + K$, and finally approaches zero again. Provided the conditions can be chosen such that $K < c_\mathrm{B}^0$, also the relaxation time passes through a maximum, at $(c_\mathrm{L}^0)_{\tau\mathrm{max}} = c_\mathrm{B}^0 - K$. We may therefore use the 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

$$K = [(c_\mathrm{L}^0)_{\Gamma\mathrm{max}} - (c_\mathrm{L}^0)_{\tau\mathrm{max}}]/2 \tag{4.13}$$

$$c_\mathrm{B}^0 = [(c_\mathrm{L}^0)_{\Gamma\mathrm{max}} + (c_\mathrm{L}^0)_{\tau\mathrm{max}}]/2 \tag{4.14}$$

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

It is recalled that the aim of chemical relaxation kinetics is the elucidation of reaction mechanisms: number and nature of elementary steps; identity and properties of reactants, intermediates, and products; kinetic and thermodynamic parameters (rate constants, reaction enthalpy, and entropy) characterizing individual equilibria and reaction pathways.[37,38]

4.2. *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

TABLE 2. Relaxation Parameters of Elementary Chemical Reactions (Kinetic Titration)[a]

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

[a] Superscript zero refers to total (analytical) concentration; the amplitude factor Γ is defined by Eq. (4.9) of the text.

Eq. (2.13), where the degree of dissociation may be written as $\Theta = c_\mathrm{L}/c_\mathrm{B}^0$, we have

$$\delta c_\mathrm{L}/c_\mathrm{L}(0) = \delta\kappa/\kappa(0) = \delta\Theta/\Theta^0 \tag{4.15}$$

With the help of Eqs. (4.15), (3.16), and (3.19) of part I(1) we may express the relaxation amplitude by

$$(\delta\kappa)_\infty = \kappa(0)[(1 - \Theta^0)/(2 - \Theta^0)](\partial \ln K/\partial E)_{P,T} E \tag{4.16}$$

In the linear range substitution of Eq. (2.21) into (4.16) leads to

$$(\delta\kappa)_\infty/\kappa(0) = [(1 - \Theta^0)/(2 - \Theta^0)][\gamma - \Delta M_a^{\ominus}/RT - (\partial \ln \tilde{Y}/\partial E)_\xi]E \tag{4.17}$$

In a similar way the relaxation time of an ionic process may be derived from the conductivity relaxation according to

$$\delta\kappa(t) = (\delta\kappa)_\infty e^{-t/\tau} \tag{4.18}$$

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., $\tau = \tau^{(\mathrm{rot})}$.(7,8)

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 chromophore position relative to rotation axis.(39,40)

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

$$A_\lambda = \sum_j (A_\lambda)_j = l \sum_j \varepsilon_j c_j \tag{4.19}$$

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 Eq. (4.19) we obtain the absorbance change per centimeter:

$$\delta A_\lambda^1 = \sum_j \varepsilon_j \, \delta c_j \tag{4.20}$$

at constant values of ε_j as a function of the concentration changes δc_j. On the same line, absorbance relaxations directly reflect concentration relaxations [Eq. (4.12)].

Experimentally, recent progress in instrumentation has opened the way for measuring field-induced, rotational and chemical relaxations in parallel, both optically and electrically in the nanosecond time range.(7,8)

4.3. *Component Contributions to Absorbance*

In general, the total optical signal change, δS, produced by a perturbation will contain several contributions as, for instance, demonstrated for electric-field-induced absorbance changes in ribosomal RNA.(41) The concentration shifts of the components B_j in a composite interacting system are summarized in a chemical term $\delta S^{(\mathrm{ch})}$. Density (volume) changes and changes in the intrinsic optical and electrical properties of the system constitute a physical term $\delta S^{(\mathrm{ph})}$.

In isotropic systems and at isotropic perturbations such as temperature and pressure changes, the term $\delta S^{(\mathrm{ph})}$ only reflects density (volume) changes.

In solutions and suspensions of anisotropic molecules directing external forces such as a hydrodynamic flow or electric field forces may cause signal changes $\delta S^{(\mathrm{rot})}$ originating from molecule rotations. In particular optically and electrically anisotropic (dipolar or polarizable) macromolecules exhibit major electric dichroism and electric birefringence.

In general, the total signal change is given by(9)

$$\delta S = \delta S^{(\mathrm{ch})} + \delta S^{(\mathrm{rot})} + \delta S^{(\mathrm{ph})}$$

There are numerous reviews on dichroism and birefringence as well as on the use of optical signals 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.(41,42)

We now recall the basic absorbance equation for a multicomponent system. Per centimeter light path

$$A^1_\lambda = \sum_j \varepsilon_j c_j \tag{4.21}$$

As outlined below it is of great practical relevance to use linearly polarized light. Furthermore, in electric field experiments it is customary to choose the direction (of the electric field of a parallel-plate capacitor measuring cell) as a reference for the light polarization plane.

When 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 the fundamental Eq. (4.21) may be field dependent. Note that the usual extinction coefficients of optically anisotropic molecules reflect random average values $\bar{\varepsilon}_j$ of all chromophore 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 (per centimeter) has two types of components:

$$dA^1_\lambda(E) = \left[\sum_j c_j \left(\frac{\partial \varepsilon_j}{\partial E}\right)_{c_j} + \sum_j \varepsilon_j \left(\frac{\partial c_j}{\partial E}\right)_{\varepsilon_j}\right] dE \tag{4.22}$$

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

$$\delta A_\sigma = A_\sigma(E) - A(0) \tag{4.23}$$

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 $E=0$, may not only involve orientational changes $\delta(\varepsilon_j)_\sigma$ but also concentration changes δc_j.

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

$$A^1(0) = \sum_j \bar{\varepsilon}_j c_j(0) \tag{4.24}$$

the absorbance in the field,

$$A^1_\sigma(E) = \sum_j (\varepsilon_j)_\sigma \, c_j(E) \tag{4.25}$$

is dependent on σ because of $(\varepsilon_j)_\sigma$.(43)

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

$$\delta(\varepsilon_j)_\sigma = (\varepsilon_j)_\sigma - \bar{\varepsilon}_j \tag{4.26}$$

and

$$\delta c_j = c_j(E) - c_j(0) \tag{4.27}$$

respectively, Eq. (4.23) is rewritten in terms of Eqs. (4.24) and (4.25):

$$\begin{aligned}\delta A^1_\sigma &= \sum_j \left[(\varepsilon_j)_\sigma \, c_j(E) - \bar{\varepsilon}_j c_j(0)\right] \\ &= \sum_j \left\{\delta(\varepsilon_j)_\sigma [c_j(0) + \delta c_j] + \bar{\varepsilon}_j \, \delta c_j\right\}\end{aligned} \tag{4.28}$$

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

Introducing the definitions

$$\delta A^{(\text{rot})}_\sigma = \sum_j \delta(\varepsilon_j)_\sigma [c_j(0) + \delta c_j] \tag{4.29}$$

$$\delta A^{(\text{ch})} = \sum_j \bar{\varepsilon}_j \, \delta c_j \tag{4.30}$$

Eq. (4.28) may be generally written in terms of a rotational and a chemical contribution(41,43):

$$\delta A^1_\sigma = \delta A^{(\text{rot})}_\sigma + \delta A^{(\text{ch})} \tag{4.31}$$

For axially symmetric measuring geometry like that of a parallel-plate capacitor cell, $\delta A^{(\text{ch})}$ 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

$$\delta A^{(\text{rot})}_{\parallel} + 2\delta A^{(\text{rot})}_{\perp} = 0 \tag{4.32}$$

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

$$\delta A^{(\text{ch})} = \delta A^1_{0.955} \tag{4.33}$$

On the other hand Eqs. (4.31) and (4.32) can be combined to

$$\delta A^{(\mathrm{ch})} = \tfrac{1}{3}(\delta A_{\parallel}^{l} + 2\delta A_{\perp}^{l}) \tag{4.34}$$

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, only be obtained from the analysis of the total time course of the relaxations. Owing 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 indispensable to determining the various components and their amplitudes.(9,41)

4.4. *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 deorientation relaxation of a system of identical particles, after termination of the step pulse, is monophasic, independently of the presence of permanent or induced dipoles. Table 3 summarizes the characteristic features of the rotational kinetics indicated by electric dichroism and birefringence for small perturbations.(39,40) We see that there are a number of specific relationships to differentiate between permanent and induced dipole mechanism. In particular, the technique of field-reversal is a sensitive indicator for the relative contributions of permanent or induced dipoles.(44,45)

The analysis of orientational changes faces problems when nonrigid molecules or molecules of nonhomogeneous 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

TABLE 3. Rotational Relaxation (Dichroism)[a]

$\delta A(t)/(\delta A)_\infty$	Signal build-up $(t_0 \leqslant t \leqslant t_e)$	$\left[\dfrac{d\delta A(\text{rot})}{dt}\right]_{t_0}$	Decay $(t_e \leqslant t \leqslant \infty)$	$\dfrac{\int_0^{t_e} \delta A(t)\,dt}{\int_{t_e}^{\infty} \delta A(t)\,dt}$
(a) General, $r \neq 0$	$\dfrac{3r\exp(-2D_rt)-(r-2)\exp(-6D_rt)}{2(r+1)}$	0	$\exp(-6D_rt)$	$\dfrac{4r+1}{r+1}$
(b) Permanent and saturated induced dipoles, $r=\infty$	$\exp(-2D_rt)+\exp(-6D_rt)$	0	$\exp(-6D_rt)$	4
(c) Induced dipoles, $r=0$	$\exp(-6D_rt)$	$(6D_r)^{-1}$	$\exp(-6D_rt)$	1

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[a] Rectangular field pulse starting at t_0 and ending at t_e in a solution of anisotropic molecules (B_j); $\delta A(t)/(\delta A)_\infty$, relative deviation from steady-state or equilibrium value, $(\delta A)_\infty$ being the maximum deviation at t_0 for the build-up of δA, and at t_e for the field-free relaxation, respectively; $(d\delta A/dt)_{t_0}$ represents the initial slope at t_0 of the signal build-up; D_r is the rotational diffusion coefficient, r is the ratio between permanent and induced dipole terms: $r=\beta/2\gamma$ where $\beta = g_jp_jE/(kT)$ $(g_j=1$ for elongated particles) and $\gamma=\frac{1}{2}\Delta\alpha_jg_jE^2/(kT)$, $\Delta\alpha_j$ being the excess polarizability; the integral ratio represents the area above the rise curve $(t_0 \leqslant t \leqslant t_e)$ and that below the zero-field relaxation curve $(t_e \leqslant t \leqslant \infty)$.(50,51) The g factors are listed in Table 1.

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.(39)

As already mentioned, dipolar or polarizable molecules aligning in the direction of an external field show linear dichroism at wavelengths corresponding to optical transitions the moments of which, in many examples, may be considered fixed with respect to the main dipole axis. The optical effects of rigid macromolecules of cylindrical symmetry such as linear (rodlike) polyelectrolytes in dilute solutions of low ionic strength are quantitatively analyzable. Owing to anisotropic counterion polarization, parallel to the long axis, the dipole axis of polyelectrolytes coincides with the long molecule axis. If, in addition, the polyions contain planar chromophores (ring structures such as the purine or pyrimidine bases in polynucleotides) with absorption bands resulting from $\pi \rightarrow \pi^*$ transitions with transition moments in the plane of the chromophore and eventually $n \rightarrow \pi^*$ transitions with moments perpendicular to this plane, these two components are associated with different absorption coefficients.(39)

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

$$\Delta A = A_{\parallel}^{(\text{rot})} - A_{\perp}^{(\text{rot})} = \delta A_{\parallel}^{(\text{rot})} - \delta A_{\perp}^{(\text{rot})} \tag{4.35}$$

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, independentley of whether there are chemical contributions present or not. Because $\delta A^{(\text{ch})}$ is independent of σ, i.e., $\delta A_{\parallel}^{(\text{ch})} = \delta A_{\perp}^{(\text{ch})}$, the application of Eq. (4.31) leads to the general form

$$\Delta A = A_{\parallel} - A_{\perp} = \delta A_{\parallel} - \delta A_{\perp} \tag{4.36}$$

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. For $E \rightarrow \infty$, $\Delta A = \Delta A_s$. The degree of orientation may then be defined by the orientation factor [Eq. (3.28)] as

$$\Phi = \Delta A / \Delta A_s = (\delta A^{(\text{rot})} / \delta A_s^{(\text{rot})})_{\sigma} \tag{4.37}$$

relative to the saturation values.(46)

The dependence of Φ on the electric field strength contains information on the electrical properties of the molecules: permanent dipole moment **p** and/or polarizability tensor α. General equations for the field dependence of ϕ are given by O'Konski *et al.*(24)

It should be remarked that Eq. (4.37) provides the basis for the rigorous analysis of chemical contributions of the induced or permanent dipole moments of the reaction partners, according to Eqs. (3.29) and (3.36). Note that the Langevin function can also be expressed in terms of Φ: $L[r] = (r/3)(1-\Phi)$, valid for $\Phi \ll 1$.

4.5. *Chemical Transition Factor*

The mechanism of electric field effects on a chemical equilibrium is reflected in the dependence of rate and equilibrium constants on the electric field strength. We recall Eq. (3.48): $K(E) = K(0)e^x$, $x = \int \Delta M \, dE/RT$. If the field-induced concentration shifts can be measured by absorbance changes, then Eq. (4.30) can be used. For example, the intramolecular transition $B_1 \rightleftharpoons B_2$ is described by

$$\delta A^{(\mathrm{ch})} = \bar{\varepsilon}_2 \, \delta c_2 + \bar{\varepsilon}_1 \, \delta c_1 \tag{4.38}$$

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 $\delta c_2 = c^0 \delta\Theta$ Eq. (4.38) is rewritten as

$$\delta A^{(\mathrm{ch})} = (\bar{\varepsilon}_2 - \bar{\varepsilon}_1) c^0 \delta\Theta \tag{4.39}$$

where

$$\delta\Theta = \Theta(E) - \Theta^0 \tag{4.40}$$

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,

$$\Theta(E) = K(0)\, e^x/[1 + K(0)e^x] \tag{4.41}$$

Substitution of Eqs. (4.40) and (4.41) into (4.39) leads to

$$\delta A^{(\mathrm{ch})} = (\bar{\varepsilon}_2 - \bar{\varepsilon}_1) c^0 \frac{(e^x - 1)(1-\Theta^0)}{e^x + (1 - \Theta^0/\Theta^0)} \tag{4.42}$$

providing a relationship between the field induced absorbance change (per centimeter) and the electric field factor x and Θ^0.(30)

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

$$\Phi^{(\mathrm{ch})} = \delta A^{(\mathrm{ch})}/\delta A_s^{(\mathrm{ch})} \tag{4.43}$$

where $\delta A_s^{(ch)}$ is the saturation value of the chemical absorbance amplitude at high field strengths. Equations (4.39) and (4.40) yield

$$\Phi^{(ch)} = [\Theta(E_0) - \Theta^0]/(1 - \Theta^0) \tag{4.44}$$

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

Finally, combination of Eqs. (4.41) and (4.44) yields

$$\Phi^{(ch)} = (e^x - 1)/[e^x + (1 - \Theta^0)/\Theta^0] \tag{4.45}$$

For calculational purposes, this is a very useful expression to describe chemical transitions as a function of the externally applied electric field.(30)

4.6. *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 processes by the rate of the orientational relaxations. As seen in Table 3, bimolecular chemical reactions exhibit a characteristic dependence of time constant and amplitude on concentration.

Independently of the time course of the absorbance changes there are a number of ways to differentiate between chemical and orientational contributions of anisotropic molecules and particles.

If chemical transformations are associated with isobestic or isochromic wavelengths, then at these wavelengths chemical contributions are zero, i.e., $\delta A^{(ch)} = 0$. When plane-polarized light is used at the light polarization angle σ^*, the rotational contributions cancel, i.e., $\delta A_{\sigma^*} = 0$. A purely chemical concentration shift in randomly distributed reaction partners is associated with an absorbance change which is independent of σ. The pure rotational contributions always obey Eq. (4.32). 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 at high field intensities. On the other hand, the second Wien effect and structural changes coupled to ionic dissociation–association processes may occur at already low field intensities.

5. *Macromolecular Cooperativity and Hysteresis*

Experimental experience demonstrates that in any case large reaction dipole moments (ΔM) are required to produce major displacements of dipolar equilibria; high ionic valencies are necessary for larger dissociation field effects in ionic association–dissociation reactions.(9)

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 biomembranes.

There is, however, another important feature of macromolecules and of organizations involving biopolymers, which mark them as attractive candidates for very efficient field effects. It is well known that macromolecular systems are very often capable of undergoing structural changes which are highly cooperative in nature. Among the immediate consequences of this cooperativity are far-reaching conformational changes produced by only small changes in the environmental conditions.

In a cooperative process, a larger sequence of residues in a polymer chain or an entire subunit (domain) of a macromolecular system are the reaction units which transform "as a whole." Returning to dipolar systems, even if the dipole moment of a single residue in such a sequence is small, the total cooperative unit may have a very large dipole moment. In this manner, cooperativity sums small reaction moments of elementary steps into concerted action; it thus represents a powerful amplification mechanism.

If a cooperative chemical transformation is coupled to an electric field effect, a relatively small change of the field intensity may suffice to cause a practically complete transition. Thus, electrical chemical coupling amplified by cooperativity is probably also a powerful mechanism for a direct and very efficient electrical control of biochemical reactivity. This principle is certainly very suggestive for the exploration of bioelectric mechanisms in general.

There is, however, an interesting alternative to strong equilibrium cooperativity for producing large structural changes by only small changes in external parameters. This alternative comprises thermodynamically metastable states and nonequilibrium transitions in cooperatively stabilized systems. The dissipative element of metastability and nonequilibrium processes endows the structures involved with threshold and trigger properties.(9,33) Electric field changes going beyond the stability point (threshold) of a metastable configuration will trigger abrupt nonequilibrium transitions to more stable (equilibrium) states.

It is well known that in certain cases the occurrence of structural metastability leads to pronounced hysteresis phenomena. Besides threshold and trigger features, hysteresis in structural transitions is a mechanism for chemical oscillations(47) and molecular memory.(33,48) In particular, the memory principle expressed in hysteresis is of appreciable cell-cybernetic interest in biology.(33)

In an electrical–chemical hysteresis, the nonequilibrium transitions underlying memory imprint on the one hand and erasure on the other hand, are triggered by electric field changes which go beyond the thresholds of the respective metastable states.

In an attempt to estimate the energetics and kinetics of nonequilibrium transformations in metastable biopolymers the physical chemical behavior of a model system exhibiting pronounced hysteresis loops was investigated.(33) The model hysteresis to be briefly discussed results from the acid–base titration of the polyelectrolyte complex poly(A) · 2poly(U). The overall process underlying the hysteresis loop is the cyclic transition between two helical structures: the triple helix poly(A) · 2poly(U) and the protonated double helix poly(A) · poly(A).

The overall reaction may be written in terms of the reactive residues:

$$2(\mathrm{U}\cdot\mathrm{A}\cdot\mathrm{U}) \rightleftharpoons (\mathrm{A}\cdot\mathrm{A}) + 4(\mathrm{U}) \tag{5.1}$$

It has been found that in the course of the acid titration, the (U · A · U) complex does not directly transform to the protonated (A · A) double helix, but is at first protonated (metastable protonation equilibrium) and then converts irreversibly to (A · A) and free (U) along the lower branch of the hysteresis. Reversing the direction of the pH change by adding alkali, a new curve is traced as the base branch of the loop.

Here, it is appropriate to note that electric impulses exceeding a threshold of about 20 kV cm^{-1} directly induce the conformational transition to (A · A) sequences in Eq. (5.1). The process that is primarily affected by the electric field is the helix-coil transition of base-paired (A · U) regions to the separated base residues according to

$$(\mathrm{U}\cdot\mathrm{A}\cdot\mathrm{U})_n = (\mathrm{A})_n + 2(\mathrm{U})_n + \Delta n\,\mathrm{C}^+ \tag{5.2}$$

The field effect on this reaction includes liberation of Δn counterions, C^+, from the ionic atmosphere of the triple helix. Under the experimental conditions of acid pH values the field-induced reaction is coupled to the protonation of the (A) residues

$$(\mathrm{A}) \rightleftharpoons (\mathrm{AH}^+) \tag{5.3}$$

and thus to the irreversible formation of the protonated poly(A) · poly(A) double helix. In this example the electric impulse acts as a trigger transiently opening the triple helix. The coupling with an irreversible process prevents relaxation to closed (U · A · U) base pairs after the impulse is terminated. It is thus possible to gradually cross the hysteresis from the acid toward the base branch. The fraction of triple helix converted per impulse is constant, suggesting that only a fraction of the randomly distributed complexes is favorably oriented to the external field pulse.(31) This key observation points to an end effect in this anisotropic system: at terminal regions counterion association is reduced and electric field effects decreasing the local ion pairing are most efficient. A particular end effect is caused by counterion displacement along the polyion.

A counterion polarization mechanism has been proposed to explain the electric induction of conformational changes in polyelectrolyte complexes such as the (U · A · U) triple helix. In accordance with this idea, the external electric field shifts the ionic atmosphere of the (U · A · U) complex and thereby induces a dipole moment. At the negative pole of the induced macrodipole, the screening by the ion cloud of the negative phosphate residues is reduced. This, in turn, causes repulsion between the ends of the polyanions and leads finally to the unwinding of the triple helix.(31) It later came to our attention that a polarization mechanism had already been proposed for strand separation of DNA by Pollak and Rein.(49)

It is now worth mentioning that the magnitudes of impulse intensity and duration, used in our investigations of the polynucleotides and membrane proteins, is well within the range of biological interest. This has revived the discussion on electrically induced conformational changes in macromolecules and membranes as a possible mechanism for electrically controlled regulatory processes in general and for a recording of electric signals in particular. With this in mind, we may consider directed structural transitions induced by electric impulses in biopolymers as model reactions for the process of imprinting the information of the nerve impulses in the neuronal network of the brain.

After these more speculative remarks, it finally appears appropriate to mention that the challenging field of bioelectric–chemical research requires a basic knowledge of the fundamental principles of electric field effects in elementary (bio)chemical reactions and molecular-rotational processes. The present elementary account on analytical aspects of chemical and orientational effects induced by electric fields in macromolecules summarizes some useful information as to how to investigate mechanisms of bioelectric phenomena on the macromolecular level.

Note Added in Proof

After the preparation of this review further progress in the physical chemical analysis of electric field effects in biological macromolecules(52–58) and in the membranes of isolated cells and organelles(59) has been documented. A few additional references are selected.(60–63)

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