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Principles of Electric Field Effects in Chemical and Biological Systems

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Contents

List of symbols

$\mathcal{A}$	chemical affinity (DEDONDER):
A	absorbance
δA_{ch}	chemical contribution to absorbance change
δA_{rot}	rotational contribution to absorbance change
δA_{inst}	instantaneous absorbance change
ΔA	dichroism
c_j	concentration of species *j* (mol dm^{-3})
c'_j	equilibrium concentration of species *j* (mol dm^{-3})
d	membrane thickness (cm)
e_0	electronic charge (C)
$\boldsymbol{E}$	external electric field intensity (V cm^{-1})
$\boldsymbol{E}_i$	inner electric field intensity (V cm^{-1})
f_j	thermodynamic activity coefficient of species *j*
G	Gibbs free energy (J)
ΔH	molar reaction enthalpy (J mol^{-1})
I	light intensity
I_0	intensity of the incident light
k	rate constant; BOLTZMANN constant (JK^{-1})
K	thermodynamic equilibrium constant
K'	apparent equilibrium constant (concentration ratio)
l	light path (cm)
$\boldsymbol{M}$	electric moment (J mol^{-1} cm V^{-1})
$\Delta\boldsymbol{M}$	electric reaction moment (J mol^{-1} cm V^{-1})
$\boldsymbol{m}_j$	induced dipole moment of species *j* (D)
$(m_j)_s$	m_j at saturating high electric fields
n	refractive index
N	AVOGADRO number (mol^{-1})
n_j	mole number of species *j*
p	pressure (N m^{-2})
$\boldsymbol{p}$	permanent dipole moment (D or C cm^{-1})
R	gas constant (J K^{-1} mol^{-1})
S	signal intensity
T	absolute temperature (K)
u_j	mobility of species *j* (cm^2 V^{-1} s^{-1})
V	volume (dm^3)
ΔV	partial volume change (dm^3 mol^{-1})
v	equilibrium exchange rate (mol s^{-1})
W	electric energy of a dipole (J)
z	intensive thermodynamic state variable; (p, T, . . .)
z_j	charge number of species *j*
Z	extensive state variable complementary to z
α_j	polarizability tensor of species *j*
β	degree of transition
Γ	amplitude factor of chemical relaxation; quantity defined by equation 54
γ	electric field factor defined by equation (47) and (46)
δ	small difference
∂	differential operator
ε	dielectric constant
ε_0	dielectric permittivity of the vacuum (CV^{-1} cm^{-1})

μ_j	chemical potential of species j (J mol^{-1})
ν_j	stoichiometric coefficient of species j
ξ	degree of advancement of a reaction (DEDONDER)
ρ	density (g cm^{-3})
τ	relaxation time (s)
ϕ	electric potential (V)

1. Introduction

Life and biological electricity appear to be unseparably coupled. Already on the lowest level of complexity, in algae and lower animals, electrical excitability constitutes an intrinsic dynamic attribute of life. It was probably electrical phenomena in biocellular systems that finally led two centuries ago to the invention of artificial, man-made electrical cells (GALVANI[1]) as a controllable source for electric forces. Subsequently, experimental physics and physical chemistry have traditionally applied electric field forces in order to probe electric–ionic properties of atoms and molecules and to study electronic and optical details of matter. In particular, the combination of electrical and optical techniques represents a powerful tool for the investigation of overall shape and structure geometrical and dynamic properties of molecules and molecular interactions.

In chemical kinetics it is well known that chemical reactions are dependent on physical state variables. Both reaction rates and the extent of a chemical transformation are generally affected by temperature, pressure or the presence of an electric (or a very strong magnetic) field. The elementary biochemical reactions of living organisms usually proceed at practically constant temperature and pressure. Therefore, temperature and pressure effects appear to be of minor relevance for the normal practically isothermal–isobaric cell reactivity. Electric field effects, on the other hand, play an important role in a number of vital cell processes. Phenomena as different as nerve excitation, electrogenic ion transport, neurostimulated secretory release of hormones and transmitter substances, or the photosynthesis of ATP include cell functions in which biochemical reactions are unseparably coupled to electric field forces.

It has been recognized that membranes appear to be the *sine qua non* condition for an effective coupling between high electric fields and biochemical reactivity. Electrical–chemical membrane processes are most evident in the rapid electric communication system of living entities. Here, not only the generation and rapid transmission of electric signals, such as nerve impulses, seem to be based on interactions between electric fields and macromolecular membrane organizations; but also the uptake and processing of external information, short-term storage and retrieval of learned experience in the central nervous system are believed to involve electric field changes coupled to structural transformations in the neuronal membranes (see, *e.g.*, O'KONSKI and STELLWAGEN;[2] KATCHALSKY and NEUMANN;[3] HYDEN[4]).

Despite many efforts, mechanistic details of the various bioelectric cell functions are not known. At present, all attempts at a physical–chemical description of bioelectric mechanisms are still at a very early stage. In many cases we don't even know the specific molecules or the specific chemical transformations involved.

In order to understand the role of electric fields in complicated biological systems, basic knowledge of field interactions with (bio)chemical reactivity in general is an essential prerequisite. This field of interest may be opened by asking three fundamental questions:

(1) What are the intensities of electric fields in biosystems and where are these fields operative?

(2) Which types of biomolecules and biostructures are particularly sensitive to electric field changes?

(3) What are the general principles of electric field effects in molecules and chemical reactions?

Answers to these questions may give physical insight into general principles valid also for specific bioelectric cell functions, an insight which may prove to be useful for further investigation of electric cell–cybernetic problems.

It will become apparent from the present account that only certain types of molecules, molecular structures, and reactions are suitable for an effective direct translation of electric field changes into functional changes of cell components.

The experimental and theoretical investigations of bioelectric phenomena cover various levels of complexity; cellular, subcellular, and molecular approaches provide complementary information. The various branches of physiology have traditionally investigated whole organisms, isolated organs, and more recently single cells. The biochemical and physico-chemical approach primarily deals with isolated cell fragments, membrane fragments and isolated molecules; in this field the reconstitution of isolated membrane components into membranes is of particular importance for the identification and functional characterization of, for instance, membrane proteins and protein complexes.

If the understanding of functional–mechanistic details is the primary aim of research, then kinetic techniques play a central role. Kinetic analysis is therefore touched on here in some theoretical as well as practical detail.

2. Electric field forces and molecules

Changes in temperature and external pressure usually represent isotropic perturbations for chemical or orientational distributions involving interacting particles in solution or suspension. An externally applied electric field is a vectorial perturbation. Unlike temperature and pressure effects on chemical transformations, direct sensitivity to electric fields is bound to certain electric properties of the molecules and of the molecular organizations involved. A necessary condition for major electric field induced chemical changes is the presence of permanent or induced dipoles, or ions, or ionized groups in macromolecules and membrane structures.

2.1. *Primary effects*

The primary effects of electric fields on molecules[5,6] involve:

(1) orientation of dipolar species,

(2) deformation of polarizable systems (and subsequent orientation of induced dipoles in electrically anisotropic particles),

(3) movement of ionic species.

These primary events may now be coupled to various chemical transformations, such as conformational transitions or dipolar and ionic association-dissociation equilibria or stationary states. We may briefly summarize the results of the electric field action on molecules and electrical-chemical coupling as follows:

(1) polar structures tend to orient in the direction of the applied electric field;

(2) conformations and molecules with large dipole moments increase in concentration at the expense of those configurations with smaller electric moments;

(3) the presence of electric fields increases the dissociation of weak acids and bases and promotes the separation of ion pairs into the corresponding dissociated ions or ionic groups (second WIEN effect). Free ions then move in the field direction (electrophoresis) and a field-dependent stationary state in the ion distribution is established.

In this account we shall not consider in detail the effects of external fields on electronic and atomic polarizabilities or electrochromic changes. These contributions to overall chemical transformations in solution or in membranes are usually negligibly small (see section 5.2.4.), they determine however the effective field intensity (internal field) which is actually experienced by the chemically interacting molecules.

It should be mentioned that even in the absence of dipolar, polarizable, or ionic reaction partners, high electric fields may cause shifts in chemical distributions.[7] Such a field effect requires, however, that the solvent phase has a finite temperature coefficient of the dielectric permittivity or a finite coefficient of electrostriction; an additional condition is that the chemical reaction proceeds with a finite reaction enthalpy (ΔH) or a finite partial volume change (ΔV). Electric field-induced temperature and pressure effects of this type are usually very small; they may, however, gain importance for isochoric reactions in the membrane phase.

2.2. *Elementary chemical reactions*

When molecules carrying permanent or induced dipole moments interact with each other undergoing chemical transformations three types of electrical chemical coupling have to be considered: permanent dipole equilibria, induced dipole equilibria, and ionic association–dissociation processes.

Changes in the concentration of the reaction partners arise from two types of elementary chemical reactions: intramolecular and bimolecular elementary processes.

Intra(mono)molecular steps. Suppose the molecules B are able to equilibrate between two alternative conformations:

$$\mathrm{B}(\uparrow) \rightleftharpoons \mathrm{B}'(\Uparrow) \tag{1}$$

where B′ has a higher dipole moment than B, as indicated by the longer arrow. In an electric field this equilibrium will be shifted to the side of higher dipole moment.

A thermodynamic analysis of electric field effects will deal with the dependence of equilibrium and steady-state distribution constants, K, on the field intensity $\boldsymbol{E}$. The thermodynamic equilibrium constant is defined as

$$K = \Pi(c_j')^{\nu_j} f_j^{\nu_j} \tag{2}$$

where ν_j is the stoichiometric coefficient, being negative for reactants and positive for products, c_j' is the equilibrium concentration and f_j is the activity coefficient of species j. In dilute solutions where usually $f_j \cong 1$, $K \cong K' = \Pi(c_j')^{\nu_j}$.

The reaction in equation (1) is specified by $\nu_{\mathrm{B'}} = 1$, $\nu_{\mathrm{B}} = -1$ and $K' = c'_{\mathrm{B}}/c'_{\mathrm{B'}}$.

Bimolecular reaction steps. A dimerization equilibrium such as

$$\mathrm{B}(\uparrow) \cdot \mathrm{B}(\downarrow) \rightleftharpoons 2\mathrm{B}(\uparrow) \tag{3}$$

where, for instance, the dipole moments [arrows in equation (3)] compensate each other upon complex formation, is particularly sensitive to electric fields because the reaction moment (see Section 5) is in the order of the dipole moment of the monomer. The reaction in equation (3) is characterized by $K' = c'^2_{\mathrm{B}}/c'_{\mathrm{BB}}$ being larger in the presence of an electric field compared to the field free state.

For the bimolecular reaction

$$(\mathrm{A^+B^-}) \rightleftharpoons \mathrm{A^+} + \mathrm{B^-} \tag{4}$$

where the association step involves ion pair formation to $(\mathrm{A^+B^-})$ or neutralization to the complex AB, we have $K' = c'_{\mathrm{A^+}} c'_{\mathrm{B^-}}/c'_{(\mathrm{A^+B^-})}$. As is discussed in Section 5, K of reaction (4) is larger in an electric field than in the absence of a field.

The exchange reaction

$$\mathrm{A} + \mathrm{CB} \rightleftharpoons \mathrm{AB} + \mathrm{C} \tag{5}$$

may proceed via an intermediate ABC and/or involve the bimolecular reactions $CB \rightleftharpoons C+B$ and $AB \rightleftharpoons A+B$; on an elementary scale only bimolecular steps characterize equation (5) with the overall equilibrium constant $K' = c'_{ABC}c'_C/c'_{AC}c'_{BC}$.

3. Biological and experimental electric fields

In living organisms electric fields of sufficiently high intensity and drastic field changes of duration sufficiently long to affect chemical processes (see Section 5), are encountered only within membrane phases and probably also across certain cell–cell junctions and synapses. In the excitable membranes of nerve and muscle we find stationary potential differences $\Delta\phi$ which are about −70 mV, in some cases as high as −100 mV (see, *e.g.* COLE[8]). These membrane potentials have negative values because they are traditionally referred to the electric potential outside the cell, which is arbitrarily chosen as zero. If we formally relate $\Delta\phi$ to an average membrane thickness, d, of about 5 to 10 nm, we may use the relationship $E = \Delta\phi/d$ to estimate electric field intensities, E, across homogeneous dielectric parts of the membrane. Since there are fixed surface-charges, probably asymmetrically distributed, and ions in the membrane, the membrane field is not homogeneous. Therefore, we can estimate only average values and presume that the maximum field intensities in biomembranes are between 100 and 150 kV/cm. In the majority of cell membranes under (resting) steady state conditions, the electric field vector points (perpendicular to the membrane surface) from the outer interface of the membrane to the interface adjacent to the cytoplasma.

Changes in the electric field are most pronounced in excitable membranes during impulse activities. During an action potential or nerve impulse, the membrane potential transiently changes to about +40 mV (see, *e.g.* HODGKIN[9]). It is important to realize that the electric field in the membrane changes not only its magnitude, but also its direction.[10,11] The transient field changes during excitation last about 1 to 5 ms. This means that during an action potential all components within the membrane experience an impulse-like change in the electric field.

In postsynaptic parts of excitable membranes the field changes are in the order of 50 to 100 $\mathrm{kV\,cm^{-1}}$ and may last up to one second.

3.1. *Polyionic field effects*

Besides the powerful field changes occurring within membranes, there are inhomogeneous electric fields originating from the surface of polyionic macromolecules and membranes. The electric potential $\phi(r)$ in the environment of these structures decays with increasing distance r from the surface of fixed ionized groups (or absorbed ions). The corresponding electric field forces

$E = -\text{grad}\,\phi(r)$, however, are at physiological ionic strengths (0.1 to 0.15 mol dm^{-3}) largely screened by counterion atmospheres. An effective direct interaction of these inhomogeneous fields with chemical reactions is limited to a short range of about 1 nm at 0.1 mol dm^{-3} ionic strength and can involve only low molecular weight species. The electric fields of polyionic surfaces may, however, indirectly affect chemical reactions by accumulating small ionic species in their immediate environment. In these regions of higher ionic strengths, the rate and extent of chemical reactions between ionic reaction partners will be different from the behavior in the bulk solution. This catalytic effect will be very pronounced for polyelectrolyte structures; in particular, the extent of protonation reactions will increase if anionic molecules enter the area of higher H^+ concentration near polyanionic surfaces. In this way transport of ionic species across membranes may be increased just by increasing the concentration of the uncharged membrane-permeable form of that species in the range of high ionic strength near polyionic membrane surfaces.[11]

Theoretical approaches aimed at understanding *polyelectrolyte catalysis* are advancing;[12] for instance, partial dehydration of ionic reaction partners in the high local electric field close to a polyionic surface appears to be one of the important factors. A point not considered so far is that a kind of orientational *fixation* of a reactant in the locally high electric field of the polyion may either favour or disfavour a reaction. Furthermore, what has been named *polyelectrolyte catalysis* should be called more precisely a *polyionic electric field effect* on reactions taking place in the immediate vicinity of polyionic structures.

3.2. *Experimental limitations*

From a practical point of view, the field intensities which are experimentally accessible are limited by dielectric break-down. In aqueous solutions, fields up to 150–200 kV cm^{-1} may be controlled over distances in the cm range. It is an additional limitation that in ionic solutions, electric fields cannot be maintained for a long time. Due to ionic currents the field will decrease and JOULE heating may cause appreciable temperature increase. These problems can be minimized by applying field pulses of limited duration to ionic solutions and suspensions. For the study of electric field effects in planar lipid membranes, potential differences up to 200 mV (corresponding to about 400 kV cm^{-1}) or more may be used. Thus the maximum homogeneous fields which can be experimentally achieved are comparable to the maximum values of electric fields encountered in biomembranes.

The electric fields across the interface between a metal electrode of high electric potential and an aqueous solution are limited to a very short distance and may only affect adsorbed (and thereby probably distorted) molecules. This topic is not covered here.

4. Biopolymers

Among the most convincing examples of a successful use of electric fields to probe ionic structures and electrical and optical anisotropies are the linear polyelectrolytes. Basic information about macromolecular dimensions, size and shape have been derived from the relaxation of field-induced changes in optical properties[13] and electric parameters.[14,15] The analysis of electric conductivity measurements has demonstrated that linear polyelectrolytes are electrically anisotropic.[16-18] It can be established that the extremely large dipole moments, which are induced by the electric field via displacement of the counterion atmosphere parallel to the long axis of the polyions, are responsible for the orientation in the direction of the external field.

The interest in electric field effects on macromolecules revived appreciably when it was found that electric fields are capable of producing structural-conformational changes in biopolymers and membranes. Here, too, optical properties are a convenient indicator of field-induced processes. First hints at presumably chemical contributions to field-induced changes in the birefringence were reported for DNA solutions of low ionic strength.[2] Dielectric measurements have shown that polypeptides in viscous organic solvents may undergo intramolecular helix-coil transitions in the presence of electric fields.[19] In the meantime there are many reports on field-induced conformational changes in multi-stranded as well as in single-stranded polyelectrolytes.[20-29]

4.1. *Threshold effects*

Of particular interest for the discussion of electric field effects in biological structures is the observation of threshold phenomena. It has been found that electric impulses above a certain threshold intensity, are capable of triggering conformational transitions in metastable polynucleotide structures.[20,21,26] A similar threshold effect is associated with electric-field-induced permeability changes in vesicle membranes.[30,31] Recently, non-linear field dependencies of base-stacking in single-stranded polynucleotides have been discussed as a threshold effect.[28]

4.2. *Optical anisotropies*

In the majority of examples optical and electrical properties have been used to indicate structural and orientational changes induced by electric fields in macromolecules. It has been established that many biopolymers such as nucleic acids and a number of protein nucleic acid complexes are linear polyelectrolytes or contain linear polyionic regions. In addition to electrical anisotropy, polynucleotides and various polypeptides are also optically anisotropic giving rise to flow birefringence and dichroism as well as to electric dichroism and electric birefringence.

5. Chemical transformations in electric fields

Electric-field-induced chemical transformations in macromolecules and macromolecular organizations such as membranes cannot be analyzed satisfactorily in all cases because adequate theoretical approaches are lacking. The observed dependencies of biopolymer reactions on the electric field intensity seem, however, to be the same as for small molecules. It appears therefore justified to open the discussion of field-induced macromolecular changes with relationships which have been derived for the description of field effects in reactions of small molecules. These relations will be used for quantitative estimates of chemical displacements which electric fields are capable of producing in dipolar and ionic equilibria.

5.1. *General thermodynamics*

The equilibrium constant or the steady-state distribution constant, $K(z)$, of a chemical equilibrium is dependent on intensive variables, z, such as temperature, T, pressure, p, or an external electric field, $\boldsymbol{E}$. As is specified in equation (2), K' is a ratio of concentrations and it is desirable to express shifts of K' with z in terms of a general concentration variable, *e.g.* the degree of advancement of a reaction $\xi(z)$ which is defined by

$$\xi = \frac{n_j}{\nu_j} = \frac{c_j}{\nu_j} V \tag{6}$$

where n_j is the number of moles of the component j and V is the volume (in dm^3).

Furthermore, it is usually sufficient to consider diluted systems where $K' \cong K$. For linear ranges of $K(z)$ [or $\xi(z)$] or equivalently, when the shifts in K and ξ produced by the external perturbation δz_i are small, we may use the linear approximations:

$$\delta K = \left(\frac{\partial K}{\partial z_i}\right)_{z \neq z_i} \delta z_i \quad \text{and} \quad \delta \xi = \left(\frac{\partial \xi}{\partial z_i}\right)_{z \neq z_i} \delta z_i \tag{7}$$

where the index $z \neq z_i$ means constant values of z except z_i; δ is used as the sign for (small) differences.

As is appropriate for laboratory conditions the state of zero electric field is taken as a general reference state. Thus at $\boldsymbol{E} = 0$, $K = K_0$ and $\xi = \xi_0$. When $K(\boldsymbol{E})$ and $\xi(\boldsymbol{E})$ are the respective values in the presence of an electric field, the conditions

$$\delta K = K(\boldsymbol{E}) - K_0 \ll K_0 \quad \text{and}$$

$$\delta \xi = \xi(\boldsymbol{E}) - \xi_0 \ll \xi_0$$

are the criteria for linear approximations, generally valid for small perturbations. Under such *linear conditions* we have

$$\frac{\delta K}{K}=\left(\frac{\partial \ln K}{\partial z_i}\right)_{z\neq z_i}\delta z_i$$

and

$$\frac{\delta \xi}{\xi}=\left(\frac{\partial \ln \xi}{\partial z_i}\right)_{z\neq z_i}\delta z_i$$

and the combination of both equations applied to dilute systems ($K \cong K'$) yields

$$\frac{\delta \xi}{\xi}=\left(\frac{\partial \ln \xi}{\partial \ln K'}\right)_{z\neq z_i}\frac{\delta K'}{K'} \tag{8}$$

connecting K' with the degree of advancement of the reaction ξ.

In order to permit a general treatment a dimensionless fractional extent of reaction, β, is introduced which, depending on the elementary chemical reaction, is the degree of a structural transition, the degree of dissociation or dimerization, etc. If a suitable component j is chosen, β is given by

$$\beta=\frac{c_j}{c_j^0}=\frac{\xi}{\xi^0}$$

where c_j^0 is the total concentration and ξ^0 represents the total number of moles of j. Thus, we see that $\beta \propto \xi$ and that $0 \leqslant \beta \leqslant 1$.

Now, equation (8) is rewritten in terms of β using K_0 and β_0 as the reference terms for K' and β at $\boldsymbol{E}=0$, respectively,

$$\frac{\delta \beta}{\beta_0}=\left(\frac{\partial \ln \beta}{\partial \ln K'}\right)_{z\neq z_i}\frac{\delta K'}{K'_0} \tag{9}$$

The differential quotient

$$\frac{\partial \ln K'}{\partial \ln \beta}=\frac{\beta}{K'}\frac{\partial K'}{\partial \beta} \tag{9a}$$

is now applied to the elementary steps in equations (1), (3) and (4). For $\mathrm{B}\rightleftharpoons\mathrm{B}'$ (with c_B^0 = the total concentration of B), $K'=c'_\mathrm{B}/c'_{\mathrm{B}'}=(1-\beta)/\beta$. We now insert this expression into equation (9a) and differentiate K' with respect to β and obtain $\partial K'/\partial\beta=-\beta^{-1}-(1-\beta)\beta^{-2}$. With reference to the zero field state where $\beta=\beta_0$ and with $\delta\beta=\beta(\boldsymbol{E})-\beta_0$ we derive from equation (9) the expression

$$\frac{\delta \beta}{\beta_0}=-(1-\beta_0)\frac{\delta K'}{K'_0} \tag{10}$$

It can be seen that the relative concentration shift is maximal at vanishing β_0.

For the bimolecular reactions in equations (3) and (4) we define $\beta = c'_B/c^0_B$ with $c^0_B = 2c^0_{BB}$ for reaction (3) and for the case where in reaction (4) all total concentrations are equal: $c^0_A = c^0_B = c^0_{AB}$, then $\beta = c'_A/c^0_{AB}$. Both cases can be treated analogous to the monomolecular transition $B \rightleftharpoons B'$. Note that for equation (3) $K' = [2\beta^2/(1-\beta)]c^0_B$ it follows

$$\frac{\partial K'}{\partial \beta} = c^0_B[4\beta(1-\beta)^{-1} + 2\beta^2(1-\beta)^{-2}]$$

and for equation (4)

$$K' = \frac{\beta^2}{1-\beta}c^0_B; \qquad \frac{\partial K'}{\partial \beta} = [2\beta(1-\beta)^{-1} + \beta^2(1-\beta)^{-2}]\, c^0_B$$

The result for both reactions is

$$\frac{\delta\beta}{\beta_0} = \frac{1-\beta_0}{2-\beta_0}\,\frac{\delta K'}{K'_0} \tag{11}$$

If the exchange reaction in equation (5) is specified by $c^0_A = c'_A + c'_{AB} = c^0_c = c'_{CB} + c'_C$, with $\beta = c'_{AB}/c^0_A = c'_C/c^0_C$ and $K' = (1-\beta)^2/\beta^2$, then, as for the previous examples

$$\frac{\delta\beta}{\beta_0} = -\frac{1-\beta_0}{2}\,\frac{\delta K'}{K'_0} \tag{12}$$

Also for equations (11) and (12), the relative concentration displacement is maximal at very small β_0 and becomes zero at $\beta_0 \rightarrow 1$.

5.2. *Dipole equilibria*

Electric field effects on chemical equilibria of permanent dipoles or polarizable molecules can be subjected to thermodynamic analysis.[5,7] The dependence of the chemical equilibrium constant, K, on the electric field, $\boldsymbol{E}$, can be cast in a relationship which is analogous to the dependence of K on the pressure, p, and the absolute temperature, T.

We recall that at constant pressure and electric field, the temperature dependence of the equilibrium constant is given by the VAN'T HOFF relationship:

$$\left(\frac{\partial \ln K}{\partial T}\right)_{p,E} = \frac{\Delta H}{RT^2} \tag{13}$$

where ΔH is the molar reaction enthalpy; R is the gas constant equal to $\mathrm{N} \cdot k$, with $k = 1.38 \times 10^{-23}\ \mathrm{JK^{-1}}$ is the BOLTZMANN constant and $\mathrm{N} = 6.02 \times 10^{23}\ \mathrm{mol^{-1}}$ is the AVOGADRO number.

In a similar manner the isothermal pressure dependence at constant electric field follows the relationship

$$\left(\frac{\partial \ln K}{\partial p}\right)_{T,\boldsymbol{E}} = -\frac{\Delta V}{RT} \tag{14}$$

where ΔV_r is the molar partial volume change for one stoichiometric transformation.

For the description of the isothermal–isobaric field dependence of the equilibrium constant we start with the GIBBS free energy change, dG, of a chemical transformation at constant p, T:

$$(\mathrm{d}G)_{p,T} = -\mathcal{A}\,\mathrm{d}\xi - \boldsymbol{M}\,\mathrm{d}\boldsymbol{E} \tag{15}$$

In equation (15), $\mathcal{A}$ and ξ are DEDONDER's reaction variables; ξ is defined by equation (6) and the affinity is defined by

$$\mathcal{A} = -\sum \nu_j \mu_j \tag{16}$$

where as usual the stoichiometric coefficients are negative for the reactants and positive for the products. The chemical potential is conventionally expressed in terms of concentrations and activity coefficients:

$$\mu_j = \mu_j^0 + RT \ln f_j + RT \ln c_j \tag{17}$$

where μ_j^0 is the standard reference; $\mu_j^0 = \mu_j$ at $c_j = 1$ mol dm^{-3} and $f_j = 1$.

Inserting equation (17) into equation (16) the affinity $\mathcal{A} = -(\partial G/\partial \xi)_z$ is expressed as:

$$\mathcal{A} = -\sum \nu_j \mu_j^0 - RT \sum \nu_j \ln f_j - RT \sum \nu_j \ln c_j \tag{18}$$

where $\sum \nu_j \mu_j^0 = \Delta G^0$ is the GIBBS free energy change for one stoichiometric transformation.

At equilibrium $\mathcal{A} = 0$ and all c_j are equilibrium concentrations. From equation (18) then follows that at equilibrium $\Delta G^0 = -RT \sum \nu_j \ln c_j' f_j = -RT \ln K$.

Since our interest is in field-induced concentration changes, equation (18) is rewritten as

$$\mathcal{A} = RT(\ln K' - \sum \nu_j \ln c_j) \tag{19}$$

where $K' = K \Pi f_j^{-\nu_j}$ or

$$\ln K' = \ln K - \sum \nu_j \ln f_j \tag{20}$$

Recalling now that dG is a total differential twofold differentiation yields

$$\left(\frac{\partial \mathcal{A}}{\partial \boldsymbol{E}}\right)_{p,T,\xi} = \left(\frac{\partial \boldsymbol{M}}{\partial \xi}\right)_{p,T,\boldsymbol{E}} \equiv \Delta \boldsymbol{M} \tag{21}$$

where $\Delta \equiv \partial/\partial\xi$ and the electric reaction moment

$$\Delta \boldsymbol{M} = \sum \nu_i \boldsymbol{M}_i \tag{22}$$

represents the difference in the macroscopic dipole moments $\boldsymbol{M}_i$ for one stoichiometric transformation, *e.g.*, for the reactions in equations (1), (3) and (4) proceeding from left to right.

At constant ξ (*i.e.* constant concentrations) differentiation of equation (19) results in

$$\left(\frac{\partial \ln K'}{\partial \boldsymbol{E}}\right)_{p,T,\xi} = \frac{1}{RT}\left(\frac{\partial \mathcal{A}}{\partial \boldsymbol{E}}\right)_{p,T,\xi} \tag{23}$$

For dilute solutions where $f_i \cong 1$, and if effects of $\boldsymbol{E}$ on f_i are negligibly small, equation (20) reads $\ln K' \cong \ln K$. As outlined in detail by SCHWARZ[7] equations (21) and (23) can be used to describe the isothermal-isobaric displacement of dipolar equilibria in electric fields by

$$\left(\frac{\partial \ln K}{\partial \boldsymbol{E}}\right)_{p,T,} = \frac{\Delta \boldsymbol{M}}{RT} \tag{24}$$

Equation (24) is generally applicable provided that field-induced temperature and pressure effects are negligible compared to larger values of $\Delta \boldsymbol{M}$. This is practically always the case.

It is obvious from equation (24) that electric fields will shift chemical dipole equilibria if $\Delta \boldsymbol{M}$ has a finite value; *i.e.*, if there is a *difference* in either the permanent or the induced dipole moments of products and reactants. This difference is particularly large if the reaction involves compensation or mutual enhancement of the dipole moments. For instance, dipolar reactants may form a non-polar complex [see equation (3)].

If the equilibrium shift, δK, induced by the application of the electric field is small ($\delta K \ll K$), we derive from equation (24) a simple expression for the relative change in the equilibrium constant. For linear ranges $\delta \ln K = \boldsymbol{E}(\partial \ln K/\partial \boldsymbol{E})_{p,T}$ and

$$\frac{\delta K}{K_0} = \frac{1}{RT}\int_0^{\boldsymbol{E}} \Delta \boldsymbol{M}\, \mathrm{d}\boldsymbol{E} \tag{25}$$

Equation (25) can be used as a basis for quantitative estimates of field-induced changes in K; see Table 1. As is well known, dipolar equilibrium shifts in general are very small for small electric field intensities ($\boldsymbol{E} < 10^3$ V cm^{-1}). Therefore, explicit numbers are given for the field intensity change of 10 and of 100 kV cm^{-1}, and for the case where the reaction moment factor $\Delta \boldsymbol{M}'$ (see Table 1) of the reaction partners is 5 D and 100 D (1 D = 3.34×10^{-28} C cm), respectively, and the temperature is 20 °C = 293 K. In this context it is noted that a number of proteins have permanent dipole moments of several hundred

Debyes; the induced dipole moments of linear polyelectrolytes may be as large as 10^5D (EIGEN and SCHWARZ[16]). The number values of the following estimates are compiled in Tables 1 and 2. It is remarked that using equations (8)–(12) $\delta K/K_0$ values for diluted systems ($K_0 \cong K'_0$) may be readily expressed in terms of concentration changes.

5.2.1. *Permanent dipoles*

Let us return to the dimerization equilibrium $\mathrm{B \cdot B = 2\,B}$, equation (3), of a polar species B in a non-polar environment. Equation (22) specifies for this case that the reaction moment is

$$\Delta \boldsymbol{M} = 2\boldsymbol{M}_{\mathrm{B}} - \boldsymbol{M}_{\mathrm{B \cdot B}} > 0 \tag{26}$$

For dilute solutions the molar moment (parallel to the external electric field) of each reacting component j can be computed according to

$$\boldsymbol{M} = \mathrm{N} \cdot [\alpha \boldsymbol{E}_i + \boldsymbol{p}L(r)] \tag{27}$$

In equation (27), α is the atomic-electronic polarizability, $\boldsymbol{E}_i$ is the internal field,[32] and $L(r) = \coth r - (1/r)$ is the LANGEVIN function of the ratio $r = \boldsymbol{p}\boldsymbol{E}_d/kT$ where $\boldsymbol{p}$ is the permanent dipole moment and $\boldsymbol{E}_d$ is the directing (orienting) field. If $r < 1$, we can approximate $L(r)$ by $\boldsymbol{p}\boldsymbol{E}_d/(3kT)$.[32]

Inserting equation (27) into equation (26) and using ONSAGER's theory[33,34] we obtain:

$$\Delta \boldsymbol{M} = \mathrm{N} \cdot f(\varepsilon, n) \frac{\sum \nu_j \boldsymbol{p}_j^2}{3kT} \boldsymbol{E} \tag{28}$$

with

$$f(\varepsilon, n) \cong \frac{\varepsilon^2 (n^2 + 2)^2}{3(2\varepsilon^2 + n^4)} \tag{29}$$

where ε is the dielectric constant and n is the refractive index of the medium. It is remarked that equation (29) contains the internal field correction factor $B = (\varepsilon + 2)/3$ and other approximations valid only for non-polar molecules in non-polar solvents.[32,34] For polar molecules in polar solvents, $f(\varepsilon, n)$ is more complicated[32,34] and the use of equation (29) gives only very rough estimates.

For our example in equation (3), $(\Delta M')^2 = \sum \nu_j \boldsymbol{p}_j^2 = 2\boldsymbol{p}_{\mathrm{B}}^2 - \boldsymbol{p}_{\mathrm{BB}}^2$; see Table 1.

We now insert equation (28) into equation (25) and after integration we obtain

$$\frac{\delta K}{K_0} \cong f(\varepsilon, n) \left(\frac{\sum \nu_j \boldsymbol{p}_j^2}{6(kT)^2} \right) \boldsymbol{E}^2 \tag{30}$$

It is seen that the dependence of the relative equilibrium shift on the field

TABLE 1. Estimates for the relative shifts $\delta K/K_0$ induced by electric fields in the equilibrium constant K_0 (at zero field) of dipolar equilibria at 293 K

$\Delta M'$(D)	$f(\varepsilon, n)$ $\varepsilon \cong 80$ $n \cong 1,33$	 $\varepsilon \cong 2$ $n \cong 1,5$	E(V cm^{-1})	$\frac{\delta K}{K_0}$
(*a*) Permanent dipoles				
5		1.84	10^4	5.2×10^{-6}
5		1.84	10^5	5.2×10^{-4}
100		1.84	10^4	2.1×10^{-3}
100		1.84	10^5	0.21
(*b*) Induced dipoles				
5			10^4	4.1×10^{-3}
5			10^5	0.041
100			10^4	0.083
100			10^5	0.83
(*c*) Saturated induced dipoles				
100	2.4		10^4	2.7×10^{-3}
	2.4		10^5	0.27

$\Delta M'$ reaction moment parameter (defined for convenient comparison, see text).
(*a*) for permanent dipoles: $\Delta M' = \sqrt{\sum \nu_j p_j^2}$,
(*b*) for induced dipoles: $\Delta M' = \sum \nu_j m_j = \frac{1}{2} \Delta \alpha \cdot E$,
(*c*) for saturated induced dipoles: $\Delta M' = \sqrt{\sum \nu_j (m_j)_s^2}$.
Note that $\Delta M'$ is a difference term.

intensity is quadratic. For high electric fields, however, where the function $L(r)$ becomes practically independent of E, orientational saturation causes $\delta K/K_0$ to be linear in E. The field intensities for which orientational saturation appears, are the lower the larger the term $\sum \nu_j p_j^2$. As long as $r < 1$ is valid, *i.e.* $E \leqslant 10^5$ V cm^{-1} and $p_j \leqslant 100$ D; equations (28) and (30) may be used.

If the reaction takes place in the lipid phase of a membrane where $\varepsilon \cong 2$ and $n \cong 1.5$, we obtain from equation (29) the term $f(\varepsilon, n) \cong 1.84$. If, for instance, $\Delta M' = 100$ D (see Table 1), $T = 293$ K and the field intensity is 10^5 V cm^{-1}, application of equation (30) yields $\delta K/K_0 \cong 0.21$. This means, that for a moment difference of 100 D and a field change of 100 kV cm^{-1} the shift in the equilibrium of the permanent dipoles is about 21 per cent. This shift is large in comparison to field-displacements in systems where small dipoles interact. For a difference of only 5 D the respective equilibrium shift is only about 0.05 per cent.

5.2.2. *Induced dipoles*

Another type of (dipolar) electrical-chemical coupling occurs when the induced dipole moments of the products are different from those of the reactants. As for the chemical transformations of permanent dipoles, the reaction moments for chemical processes involving molecules with small induced dipole moments are also very small. Characteristic values for the polarizability, α, of small organic molecules are in the order of 10^{-23} cm^3, corresponding to 10^{-35} C cm^2 V^{-1} (BÖTTCHER *et al.*[32]). The induced dipole moment, $\boldsymbol{m} = \alpha \cdot \boldsymbol{E}_i$, of such a molecule in an electric field which causes an internal field $\boldsymbol{E}_i$ of 10^5 V cm^{-1} is only 10^{-30} C cm ($\cong$0.003 D).

The reaction moment for the case of purely induced dipole moments of small isotropic molecules j is:

$$\Delta \boldsymbol{M} \cong \mathrm{N} \cdot \sum \nu_j \alpha_j (\boldsymbol{E}_i)_j \tag{31}$$

If equation (25) can be applied, the relative equilibrium shift caused by the electric field is given by

$$\frac{\delta K}{K_0} \cong \frac{1}{kT} \int_0^{\boldsymbol{E}} \sum \nu_j \alpha_j (\boldsymbol{E}_i)_j \, \mathrm{d}\boldsymbol{E} \tag{32}$$

For the purpose of obtaining an estimate we assume that $\boldsymbol{E}_i \cong \boldsymbol{E}$ for all species j. Then $\Delta \boldsymbol{M} \cong \mathrm{N} \left(\sum \nu_j \alpha_j\right) \boldsymbol{E}$, and equation (32) is reduced to

$$\frac{\delta K}{K_0} \cong \frac{\Delta \alpha}{2kT} \boldsymbol{E}^2 \tag{33}$$

where $\Delta \alpha = \sum \nu_j \alpha_j$ and the difference in the induced dipole moments is given by $\Delta \boldsymbol{m} = 0.5 \, \Delta \alpha \cdot \boldsymbol{E}$.

As for permanent dipoles, the field dependence of chemical equilibria involving small induced dipoles is also quadratic. If the difference in the polarizability $\Delta \alpha$ associated with a stoichiometric transformation is in the order of α itself (*i.e.* 10^{-35} C cm^2 V^{-1} for small molecules) and $\boldsymbol{E} = 10^5$ V cm^{-1}, hence $\Delta \boldsymbol{m} \cong 0.005$ D we estimate that at 293 K, $\delta K / K_0 \cong 4 \times 10^{-5}$. We see that even for high electric fields the equilibrium shifts are extremely small when low molecular weight species, *i.e.* small dipoles interact.

In contrast to small molecules, the induced dipole moments of macromolecules, in particular of linear polyelectrolytes, may become very large. These dipole moments depend strongly on the length of the polymer and on the ionic strength. For rod-like polyelectrolytes having a length of 10^{-5} cm, at low ionic strength ($\leqslant 10^{-3}$ mol dm^{-3}), induced dipole moments of up to 10^5 D have been estimated from conductivity measurements.[18]

The induced dipole moment of an individual linear polyelectrolyte depends on the electric field according to a LANGEVIN-like function.[21,23] Furthermore, the saturation of counterion polarization sets in at already moderately high electric fields. As a consequence of saturation in the counterion polarization, the induced dipole moment becomes independent of the external electric field and the molecules behave like permanent dipoles.[35]

Let us now consider, as an instructive example. the overall equilibrium of a linear polyelectrolyte system consisting of polyriboadenylate, poly(A), and polyribouridylate, poly(U) and the 1 : 2 complex poly(A) · 2 poly(U):

$$\text{poly(A)} \cdot 2\,\text{poly(U)} \rightleftharpoons \text{poly(A)} + 2\,\text{poly(U)} \tag{34}$$

Owing to different linear charge densities, the polarizability (and thus the induced dipole moment) of the single polymers is different from that of the complex. The reaction moment for the process in equation (34) is $\Delta \boldsymbol{M} = \boldsymbol{M}_{\mathrm{A}} + 2\boldsymbol{M}_{\mathrm{U}} - \boldsymbol{M}_{\mathrm{U \cdot A \cdot U}}$. Particularly large values of $\Delta \boldsymbol{M}$ should occur at saturating electric fields where we may approximate the induced dipole moment by $\boldsymbol{m}_j = (\boldsymbol{m}_j)_s \cong$ constant, where the subscript s refers to polarization saturation at high electric fields. Now we may use equation (28) and replace $\boldsymbol{p}_j$ by $(\boldsymbol{m}_j)_s$. Recalling that for very long polyelectrolytes the internal field $\boldsymbol{E}_i \cong \boldsymbol{E}$, we obtain a quadratic field dependence of the relative change in the equilibrium constant.

$$\frac{\delta K}{K_0} \cong f(\varepsilon, n)\left(\frac{\sum \nu_j (\boldsymbol{m}_j)_s^2}{6(kT)^2}\right)\boldsymbol{E}^2 \tag{35}$$

The validity of equation (35) is restricted to the condition that $r = (\boldsymbol{m}_j)_s \cdot \boldsymbol{E}_d/(kT) \leqslant 1$; (see KIKUCHI and YOSHIOKA[23]).

In aqueous solution where $\varepsilon \cong 80$ and $n \cong 1.33$, we estimate with equation (29) that $f(\varepsilon, n) \cong 2.4$. Assuming again that the reaction moment factor of the saturated induced dipoles of products and reactions $\Delta \boldsymbol{M}' = (\sum \nu_j (\boldsymbol{m}_j)_s^2)^{1/2}$ is about 100 D, *i.e.* $\sum \nu_j (\boldsymbol{m}_s)_j^2 = 1.1 \times 10^{-51}\ \mathrm{C^2\,cm^2}$, we calculate for $\boldsymbol{E} = 10^5\ \mathrm{V\,cm^{-1}}$ at 293 K that $\delta K/K_0 \cong 0.27$. This result means that for the above specifications of 100 D dipole moment difference and 100 kV $\mathrm{cm^{-1}}$ an equilibrium shift of 27 per cent is obtained.

These estimates show that, as in the case of permanent dipoles, major equilibrium shifts in systems involving induced dipoles require the presence of macromolecular structures and high electric field intensities; large reaction moments of induced dipoles may result from interactions between polyelectrolytic macromolecules.

5.2.3. *Intramolecular dipole orientation*

Of particular biocybernetic significance are presumably intramolecular conformational transitions in biopolymers, which lead to a variation of some cell function coupled to the macromolecule. For instance, if an active and an inactive state of a membrane protein are associated with different dipole moments, a cell function involving this membrane protein can be controlled directly or indirectly by changes in the electric field of the membrane. This type of electrical–chemical coupling may be realized by intramolecular dipole rotations.

A model reaction for such an intramolecular dipole reorientation induced by electric fields in biopolymers is the rapid helix-coil transition of a polypeptide in a viscous solution. Since in this example the overall rotation of the macromolecule is slow as compared to the rate of conformational transitions, dipolar helical sequences rapidly form in the direction of the electric field and helices lying unfavourably to the field vector dissolve to coil regions.[19]

We shall now briefly touch on the case where certain modes of overall rotation are hindered or extremely slow and only intramolecular orientational changes within a macromolecule are functionally significant. Suppose that the membrane permeability to smaller molecules or ions is determined by such a biopolymer embedded in the membrane. We readily understand that macromolecular rotations which would expose nonpolar parts of the molecule to the aqueous environment or which would bring ionic groups into the lipid phase, are energetically disfavoured. On the other hand, dipolar (or polarizable) side groups or larger portions of the macromolecule, which are sufficiently flexible, may change the orientation under the impact of a varying membrane field. We consider now a two-state model where two conformations of a *permeability-control protein*, or a *gating protein*, are characterized by two different angles θ between the dipole axis of a rotationally flexible side group or subunit and the direction of the electric field of the membrane. Furthermore, we specify that at large electric fields the dipole axes are largely aligned parallel to the field vector ($\theta \cong 0$) thereby closing the pathways for permeants. The open configuration of pathways permitting transport is assumed to be associated with a very narrow distribution of the dipole axes around an angle $\theta = \theta_0$. The transition between the closed and open structure is seen as a rotation of dipolar side groups or subunits from $\theta = 0$ to $\theta = \theta_0$.

To obtain a quantitative estimate of a field effect on such a rotational equilibrium we recall that the electric energy W of a dipole $\boldsymbol{p}$ in a directing electric field is given by[32]

$$W = -\boldsymbol{p} \cdot \boldsymbol{E}_d \cdot \cos\theta \tag{36}$$

Now, the reaction moment for the conformational transition in the two state model specified above is:

$$\Delta \boldsymbol{M} = \mathrm{N} \cdot \boldsymbol{p} \cdot \Delta \cos\theta \tag{37}$$

We assume that for the local environment of the membrane reaction the factor $f(\varepsilon, n) \cong 2$, and that for the dipole distribution equation (25) can be applied. With $\boldsymbol{E}_d \cong f(\varepsilon, n) \cdot \boldsymbol{E}$, the relative equilibrium shift of the rotational equilibrium is approximated by

$$\frac{\delta K}{K_0} \cong \frac{\boldsymbol{p} \cdot \Delta \cos\theta}{kT} f(\varepsilon, n) \cdot \boldsymbol{E} \tag{38}$$

It is seen that the applied model predicts a linear dependence of the equilibrium shift on the electric field intensity.

If we assume that the dipole moment of the subunit is $\boldsymbol{p} = 100$ D and that $\boldsymbol{E} = 10^5$ V cm^{-1}, then, for $\theta_0 = 45°$ (such that $\Delta \cos \theta = 0.29$), we calculate with equation (38) for 293 K and $f(\varepsilon, n) \cong 2$ that $\delta K/K_0 \cong 0.46$. In case the rotation of the dipolar subunit spans a larger angle, say $\theta_0 = 90°$, $\Delta \cos \theta = 1$ and $\delta K/K_0 = 1.56$. These estimates show that under proper conditions electric field changes may cause very large shifts in dipolar rotational equilibria. Thus the rotation model considered above suggests an effective mechanism for the electric control of permeability changes in membranes. The characteristic feature of the model is the linear field dependence as expressed by equation (38). In this context it is remarked that ionic conductivity changes in the excitable membranes of nerves show larger ranges of linear dependence on the membrane potential (electric field); (see, *e.g.*, GOLDMANN and SCHAUF[36]). It is not yet known whether this electric property indicates a rotatory dipole mechanism for the field-dependent conductivity changes in excitable membranes.

By inspection of the estimates given in Table 1, we may summarize that major electric field effects on (simple) dipolar reaction systems require macromolecular components. This condition appears to be a general prerequisite in all cases of permanent or induced dipolar equilibria which can be described in the framework of a simple mass action law.

5.2.4. *Indirect field effects*

As seen from equation (24), field-induced displacements of K require finite values for the reaction moments $\Delta \boldsymbol{M}$. It has already been mentioned that, even if $\Delta \boldsymbol{M} = 0$, an electric field may indirectly affect chemical equilibria *via* temperature or pressure effects (see, *e.g.* SCHWARZ[7]). Here, we shall briefly inspect the electrostriction effect, which may be of importance for biomembrane reactions.

There is evidence that membranes are elastic but largely incompressible (see, *e.g.*, REQUENA *et al.*[37]). We may now formulate the isothermal field-induced pressure effect by

$$\left(\frac{\partial \ln K}{\partial \boldsymbol{E}}\right)_T = \left(\frac{\partial \ln K}{\partial \boldsymbol{p}}\right)_{T,\boldsymbol{E}} \left(\frac{\partial \boldsymbol{p}}{\partial \boldsymbol{E}}\right)_{T,V} \tag{39}$$

Inserting equation (14) into equation (39) yields:

$$\left(\frac{\partial \ln K}{\partial \boldsymbol{E}}\right)_T = -\frac{\Delta V_r}{RT} \left(\frac{\partial \boldsymbol{p}}{\partial \boldsymbol{E}}\right)_{T,V} \tag{40}$$

The coefficient $(\partial \boldsymbol{p}/\partial \boldsymbol{E})_{T,V}$ is related to the energy of a dielectric medium in the external electric field. The pressure developed in the direction parallel to the electric field is given by the approximation

$$\left(\frac{\partial p}{\partial \boldsymbol{E}}\right)_{T,V} = \varepsilon_0\varepsilon(\varepsilon-1)\boldsymbol{E} \tag{41}$$

where $\varepsilon_0 = 8.85 \times 10^{-14}$ C V^{-1} cm^{-1} is the dielectric permittivity of the vacuum and ε is the dielectric constant of the medium. Combining equations (40) and (41) yields

$$\left(\frac{\partial \ln K}{\partial \boldsymbol{E}}\right)_{p,T} = -\frac{\Delta V_r}{RT}\varepsilon_0\varepsilon(\varepsilon-1)\boldsymbol{E} \tag{42}$$

For small shifts in K we find that

$$\frac{\delta K}{K_0} = -\frac{\Delta V_r}{2\,RT}\varepsilon_0\varepsilon(\varepsilon-1)\boldsymbol{E}^2 \tag{43}$$

Thus the electrostriction effect yields also a quadratic field dependence. The molar volume change resulting from reactions between non-ionic species is usually very small. Assuming $\Delta V_r = 5$ cm^3 mol^{-1} and the dielectric constant of the lipid phase $\varepsilon \cong 2$ we calculate for $\boldsymbol{E} = 10^5$ V cm^{-1} at 293 K that $\delta K/K_0 \cong 10^{-6}$. Thus the electrostriction effect is extremely small; measurable chemical changes are therefore only to be expected when the reactions with larger molar volume changes occur in regions of larger dielectric constants.

5.3. *Ionic equilibria*

Whereas the coupling of chemical transformations to the dielectric polarization of permanent or induced dipoles is accessible to an equilibrium-thermodynamic analysis, the dissociation field effect (or second WIEN effect) of ionic association-dissociation equilibria requires a non-equilibrium (kinetic) approach.

5.3.1. *Simple electrolytes*

According to ONSAGER it is the dissociation rate constant, k_d, of the separation of an ion pair that is affected by the electric field; the association rate coefficient k_a remaining practically unchanged.[33] Since $K = k_d/k_a$, we may formally express the dissociation field effect by a change of the equilibrium constant K_0 at zero field, to a field dependent steady-state distribution $K(\boldsymbol{E})$. The actual dependence of K on $\boldsymbol{E}$ begins non-linearly and reaches a range where the distribution of the reaction partners of an ionic dissociation-association system like $(A^+ \cdot B^-) = A^+ + B^-$, depends linearly on the absolute value of the applied external electric field. For this range ONSAGER's theory for diluted weak electrolytes yields

$$\frac{\partial \ln K}{\partial |\boldsymbol{E}|} = \frac{z_A u_A - z_B u_B}{u_A + u_B} \cdot \frac{|z_A z_B| e_0^3}{8\pi\varepsilon_0\varepsilon (kT)^2} \tag{44}$$

In equation (44), $e_0 = 1.6 \times 10^{-19}$ C is the electron charge, z_j is the charge number (with sign), and u_j is the ionic mobility ($\cong 10^{-4}\ \mathrm{cm}^2\ \mathrm{V}^{-1}\ \mathrm{s}^{-1}$).

It is seen from equation (44) that the magnitude of the dissociation field effect depends on the valencies of both cation and anion.

For small equilibrium displacements $\delta K = K(\boldsymbol{E}) - K_0$ we obtain from equation (44):

$$\frac{\delta K}{K_0} = \gamma |\boldsymbol{E}| \tag{45}$$

For symmetric electrolytes where $z_A = -z_B = |z|$, the factor γ is reduced to

$$\gamma = \frac{|z|^3 e_0^3}{8\pi\varepsilon_0\varepsilon (kT)^2} \tag{46}$$

Note the dependence of γ on the third power of z. For instance, in aqueous solution where $\varepsilon \cong 80$, we calculate for a 1:1 electrolyte at 293 K that $\gamma = 1.4 \times 10^{-6}\ \mathrm{cm\ V}^{-1}$. If $\boldsymbol{E} = 10^5\ \mathrm{V\ cm}^{-1}$, we find from equation (45) that for this case $\delta K/K_0 \cong 0.14$. The corresponding estimate for a 2:2 electrolyte is $\delta K/K_0 \cong 1.1$ (see Table 2). Thus the equilibrium constant of a 1:1 weak electrolyte is increased by an electric field of 100 kV cm^{-1} to about 14 per cent, that of a 2:2 system to about 110 per cent. Compared to simple dipolar

TABLE 2. Relative shifts $\delta K/K_0$ in stationary distribution constants K_0 (at zero field) at 293 K; second WIEN effect for $z_A : z_B$ ionic associations; see text

$z_A : z_B$	ε	γ (cm V^{-1})	$\boldsymbol{E}$ (V cm^{-1})	$\delta K/K_0$
a) simple electrolytes				
1:1	80	1.4×10^{-6}	10^4	0.014
			10^5	0.14
1:1	2	5.6×10^{-5}	10^4	0.56
			10^5	5.64
2:2	80	1.13×10^{-5}	10^4	0.11
			10^5	1.1
b) polyelectrolytes				
$z_A^{\mathrm{eff}} : 1$	80	1.4×10^{-5}	10^4	0.14
			10^5	1.4
$z_A^{\mathrm{eff}} : 2$	80	5.6×10^{-5}	10^4	0.56
			10^5	5.6

equilibria of small molecules where electric field effects are negligibly small, we conclude that the dissociation of simple ion pairs is associated with a relatively large field effect.

Due to the low dielectric constant, ion pair formation is expected to be favored in the lipid phase of membranes. Assuming that $\varepsilon = 2$, for a 1:1 electrolyte $\gamma = 5.64 \times 10^{-5}$ cm V^{-1}. It is now readily estimated that a change in the electric field of 100 kV cm^{-1} yields $\delta K/K_0 = 5.6$, *i.e.* a relative shift in K by a factor of about 6. Ionic dissociation–association equilibria within membranes therefore appear to be very sensitive to electric field changes.

It should be mentioned that intramolecular ion pairs between oppositely charged groups of a protein or of protein subunits may also be sensitively affected by electric fields. In particular, flexible membrane proteins will be able to change conformation due to field changes affecting intramolecular ion pairs. Ionic protein lipid interactions may be influenced in a similar manner by field changes in the membrane.

5.3.2. *Polyelectrolytes*

It is well established that the second WIEN effect is particularly large in polyelectrolytes. As in simple electrolytes, extended regions of a linear field dependence have also been observed in polyelectrolytes. See Fig. 1. Because of this linearity in **E**, ONSAGER's equation for the dissociation field effect has

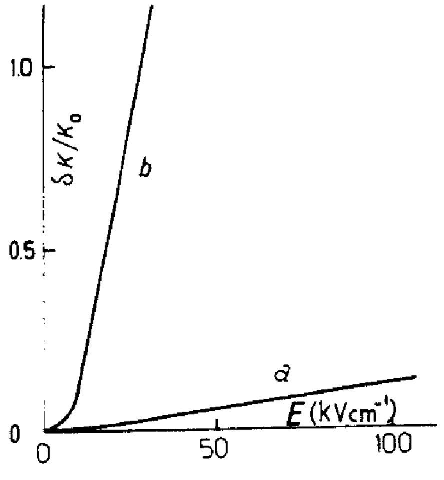

Fig. 1. Relative shift $\delta K/K_0$ of the equilibrium constant K_0 of ionic dissociation-association equilibria to a steady-state distribution as a function of the electric field intensity **E**: (*a*) diluted 1:1 electrolyte, *e.g.*, CH_3COOH; (*b*) polyelectrolyte, *e.g.*, K-polyphosphate (see, *e.g.*, SCHÖDEL *et al.*[38]).

been used as the basis for a *qualitative* discussion of the second WIEN effect in polyelectrolytes.[38] For this purpose we shall first rewrite the factor γ in equation (45).

Denoting by A_n the polyion and by B the counterions, the large mass difference justifies the approximation $u_{A_n} \ll u_B$, 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. Due to the neighbouring fixed charges the effective charge, $z_A^{eff} e_0$ per residue, A, is larger than $z_A e_0$ 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 is additionally favored by the lower (local) dielectric constant as compared to that of the bulk aqueous solution.[18]

If the diffuse *counterion binding* is viewed in terms of one residue of the polyion, we may define an average γ-factor by

$$\bar{\gamma}_{res} = \frac{(z_B e_0)^2 z_A^{eff} e_0^3}{8\pi\varepsilon_0\varepsilon (kT)^2} \tag{47}$$

and thus quantify the influence of counterion valency and effective polyionic charges per residue. It is remarked that $\bar{\gamma}$ and thus the effective charge can be experimentally determined [see equation (45)].

It has been found that the dissociation field effect in polyelectrolytes is generally larger by a factor of about 10 to 100 as compared to simple electrolytes. Thus the values for the relative displacements of the distribution constant, $\delta K/K_0$ 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 $\boldsymbol{E} = 10^4$ V cm^{-1} and 14 for $\boldsymbol{E} = 10^5$ V cm^{-1}. We conclude from these estimates, summarized in Tab. 2, that already for moderately large changes in the field intensity the degree of counterion binding may change to a large extent. Since usually the conformation and the degree of stretching of flexible polyelectrolytes depends on counterion binding, external electric field changes may readily affect structural changes. Due to the efficiency of dissociation field effects in polyelectrolytes, 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.

Recently, a theoretical approach for the second WIEN effect has been started in terms of the OOSAWA–MANNING counterion condensation model.[39] Although the treatment covers the linear field dependence, further development of this promising approach is required.

The results of the general analysis of electric-field-induced chemical overall shifts provide some thermodynamic criteria for the identification of type and extent of electrical–chemical coupling. For instance, equilibria of permanent dipoles show, at low fields, a dependence of $\delta K/K_0$ on $(\boldsymbol{E}/T)^2$. At larger field intensity the function $L(r)$ in equation (27) saturates for $r = \boldsymbol{p} \cdot \boldsymbol{E}_d/kT \gg 1$ where then $L(r) = \coth r - (1/r) \cong 1$. When the corresponding value of $\Delta \boldsymbol{M} \cong \mathrm{f}(\varepsilon, n) \sum \nu_i \boldsymbol{p}_i$ is inserted into equation (25) we find that $\delta K/K_0$ is proportional to $\boldsymbol{E}/T$. On the other hand, induced dipolar equilibria at low field intensity show a proportionality between $\delta K/K_0$ and $\boldsymbol{E}^2/T$. At high fields where the induced dipole moments and thus also the reaction moments are saturated, the field dependence is that of a permanent dipole system. The second WIEN effect is characterized by a linear dependence of $\delta K/K_0$ on $\boldsymbol{E}/T^2$ after a non-linear region at small field intensities. Thus the dependence of the relative displacements $\delta K/K_0$ on field intensity and temperature may be utilized to differentiate the type of process underlying the measured electric field effect.

6. Measurement of electric field effects

The general relationships in equations (8) and (9) and the specific expressions of equations (10)–(12) show how changes in chemical distributions $\delta K/K_0$ are connected with the (measurable) concentrations of the reaction partners. For the measurement of electric field induced concentration changes in biochemical systems it is important to realize that the majority of biological processes involves ionic species in aqueous environments. Furthermore, the structural stability of many biopolymer systems requires a finite ionic strength. Electric-field-sensitive processes in these systems have to be studied in conducting solutions and suspensions. In order to minimize JOULE heating, field pulses are applied. The analysis of field induced changes is particularly straightforward for rectangular pulses. However, if the system is simple and only one process occurs or if the field-induced changes are long-lived and relax with time constants large compared to the field duration, JOULE heating temperature jump spectrometers may be used.[20,21,30,40,41]

6.1. *Chemical relaxations*

6.1.1. *Chemical relaxation amplitudes*

When an intensive state variable z is changed, a chemical distribution equilibrium will adjust to the varied conditions. For linear ranges or small perturbations we rewrite equation (7) for constant affinity $\mathscr{A}$ as

$$\delta\xi = \left(\frac{\partial \xi}{\partial z}\right)_{\mathscr{A}} \delta z \tag{48}$$

The total differential of $\mathscr{A}(\xi, z)$ is given by

$$\mathrm{d}\mathscr{A}(\xi, z) = \left(\frac{\partial \mathscr{A}}{\partial \xi}\right)_z \mathrm{d}\xi + \left(\frac{\partial \mathscr{A}}{\partial z}\right)_\xi \mathrm{d}z \tag{49}$$

At equilibrium, $\mathscr{A} = 0$ (*i.e.* constant) and we derive for this condition from equation (49) that

$$\left(\frac{\mathrm{d}\xi}{\mathrm{d}z}\right)_{\mathscr{A}=0} = -\frac{(\partial \mathscr{A}/\partial z)_\xi}{(\partial \mathscr{A}/\partial \xi)_z} \tag{50}$$

We use now equation (18) with equation (6) in the form

$$\mathscr{A} = RT\left[\ln K - \sum \nu_i \ln\left(f_i \nu_i \frac{\xi}{V}\right)\right] \tag{51}$$

and determine the differential terms of equation (50). If the activity coefficients f_i can be considered constant for small changes in z, differentiation of equation (51) with respect to z, at constant ξ yields

$$\left(\frac{\partial \mathscr{A}}{\partial z}\right)_\xi = \left(\frac{\partial \mathscr{A}}{\partial \ln K} \frac{\partial \ln K}{\partial z}\right)_\xi = \Delta Z \tag{52}$$

where

$$\Delta Z = RT\left(\frac{\partial \ln K}{\partial z}\right)_\xi$$

is the extensive variable complementary to z; see below.

Since at constant z, $K(z)$ is a constant, the differentiation of $\mathscr{A}$ with respect to ξ results in:

$$\left(\frac{\partial \mathscr{A}}{\partial \xi}\right)_z = -\frac{RT}{V\Gamma}\left\{1 + V\Gamma\left(\frac{\sum \nu_i \partial \ln f_i}{\partial \xi}\right)_z\right\} \tag{53}$$

where the amplitude factor Γ is defined by

$$\Gamma = \left(\sum \frac{\nu_i^2}{c_i}\right)^{-1} \tag{54}$$

For small changes in ξ produced by a small change in z, the activity coefficients may be considered independent of ξ. If the reaction system is diluted, the f_i terms may be determined by other components in higher but constant concentration; thus the activity coefficients may be treated as independent of ξ. For these conditions equation (53) simplifies to

$$\left(\frac{\partial \mathscr{A}}{\partial \xi}\right)_z = -\frac{RT}{V\Gamma}$$

The total shift produced by δz in the degree of advancement of the reactions for $t \to \infty$, $\delta\xi(\infty)$, is called relaxation amplitude. Insertion of equations (52) and (53) into equations (48) and (50) leads to an expression for the relaxation amplitude:

$$\delta\xi(\infty) = \frac{V\Gamma}{1 + V\Gamma\left(\frac{\sum \nu_i\, \partial \ln f_i}{\partial \xi}\right)_z} \frac{\Delta Z}{RT}\,\delta z \tag{55}$$

For the case of constant activity coefficients equation (55) reduces to

$$\delta\xi(\infty) = V\Gamma \frac{\Delta Z}{RT}\,\delta z \tag{56}$$

Recalling equation (54) it is seen that the specific expression for the amplitude factor Γ depends on the type of elementary reaction; see below. The term $\Delta Z \delta z$ classifies the external perturbation. Using equations (13) and (14) a temperature change is specified by $\delta z = \delta T$ and $\Delta Z = \Delta H/T$, a pressure jump by $\delta z = \delta p$ and $\Delta Z = -\Delta V$, and finally, an electric field jump by $\delta z = \delta \boldsymbol{E}$ and $\Delta Z = \Delta \boldsymbol{M}$.

The total equilibrium shift caused by an electric field pulse applied to the system (at zero field) at constant p, T under conditions where equation (56) is valid, is given by

$$\frac{\delta\xi(\infty)}{\xi_0} = \frac{V\Gamma}{\xi_0} \cdot \frac{\Delta \boldsymbol{M}}{RT} \cdot \boldsymbol{E} \tag{57}$$

where $\delta z = \boldsymbol{E}$ and reference is made to the zero field values K_0 and ξ_0.

Introducing the fractional transformation variable β and using $\delta \ln K' = \delta K'/K'_0$, we obtain $\delta\beta = (\partial\beta/\partial \ln K')_{p,T} \cdot (\delta K'/K'_0) = \Gamma(\beta) \cdot \Delta \boldsymbol{M} \cdot \boldsymbol{E}/(RT)$. Using equation (10), an intramolecular step, equation (1), is characterized by $\Gamma(\beta) = -\beta_0(1-\beta_0)$ having a maximum at $\beta_0 = 0.5$. Thus, for this case, field effects are maximal at the *half-point* of transformation.

Similarly, for bimolecular steps and the conditions specified in equations (11) and (12), the respective amplitude factors are $\Gamma(\beta) = \beta_0(1-\beta_0)/(2-\beta_0)$ with a maximum at $\beta_0 = 2 - \sqrt{2} = 0.586$ and $\Gamma(\beta) = -\beta_0(1-\beta_0)/2$ with a maximum at $\beta_0 = 0.5$.

For practical purposes we utilize equation (55) in terms of the concentration of one reaction partner j. Since from equation (6),

$$\mathrm{d}c_j = \frac{1}{V}\nu_j\,\mathrm{d}\xi$$

equations (55) and (56) lead to specific expressions for the concentration relaxation amplitude:

$$\delta c_j(\infty) = \frac{\nu_j \Gamma}{1+\Gamma\left(\nu_j^2 \frac{\partial \ln f_j}{\partial c_j}\right)_z} \frac{\Delta Z}{RT} \delta z$$

and for concentration independent activity coefficients,

$$\delta c_j(\infty) = \nu_j \Gamma \frac{\Delta Z}{RT} \delta z \tag{58}$$

where ν_j is positive when j is a reaction product, but is negative, when j is a reactant.

Analogous to equation (56), the total equilibrium shift or relaxation amplitude of an electric field induced change is given by

$$\delta c_j(\infty) = \nu_j \Gamma \cdot \frac{\boldsymbol{\Delta M}}{RT} \cdot \boldsymbol{E} \tag{59}$$

6.1.2. *Chemical relaxation times*

If an external perturbation is applied faster than the chemical equilibration time, the response to such a step perturbation is a chemical relaxation spectrum containing exponentials of time, t. The time course of the chemical relaxation of a composite chemical system with i (normal mode) processes can be expressed in terms of deviations $\delta\xi_i(t) = \xi(t) - \xi$ from the new equilibrium ξ:

$$\delta\xi(t) = \sum \delta\xi_i(\infty) \exp\left(-\frac{t}{\tau_i}\right)$$

where τ_i is the relaxation time and $\delta\xi_i(\infty)$ is the amplitude respectively, of relaxation mode i.

For an elementary step, the chemical relaxation can be expressed in terms of one reaction partner j as

$$\delta c_j(t) = \sum \delta\xi_i(\infty) \exp\left(-\frac{t}{\tau_i}\right) \tag{60}$$

Equation (60) is generally applicable for intramolecular elementary steps. It is applicable for bimolecular steps only if the perturbation is small such that $\delta\xi \ll \xi$ (or $\delta c_j \ll c_j$).

For elementary steps, relaxation time, τ, and amplitude factor, Γ, are related in a simple way (see, *e.g.* CASTELLAN,[42] JOVIN[43]):

$$\Gamma = \tau \upsilon \tag{61}$$

where υ is the equilibrium exchange rate.

When systems with several coupled reaction steps have to be considered the calculation of relaxation times and amplitudes is more elaborate; the main aim is then to calculate the normal modes characterizing the kinetics of the complex system. For details it is recommended to consult one of the numerous reviews and books on theory and practice of chemical relaxation kinetics (see, *e.g.*, EIGEN and DEMAEYER,[44,45] YAPEL and LUMRY,[41] JOVIN[43]).

Recent theoretical developments offer a simple formalism to evaluate time constants and amplitudes in terms of total concentrations rather than equilibrium concentrations (WINKLER,[46] see, *e.g.*, NEUMANN and CHANG[47]).

For instance, let us consider the binary association-dissociation reaction

$$\mathrm{A}+\mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{AB} \tag{62}$$

with $K'=c'_{\mathrm{A}}c'_{\mathrm{B}}/c'_{\mathrm{AB}}=k'_{-1}/k'_1$ where k'_1 is the apparent rate constant for the association step and k'_{-1} is the apparent dissociation rate constant; the activity coefficients contained in the apparent rate constants are assumed to be constant.[17,38]*

The relaxation time τ for the reaction equation (62) is given by[44,45] $\tau^{-1}=k'_1(c'_{\mathrm{A}}+c'_{\mathrm{B}})+k'_{-1}=k'_1(c'_{\mathrm{A}}+c'_{\mathrm{B}}+K')$. Applying equation (58) we obtain $\Gamma=c'_{\mathrm{A}}c'_{\mathrm{B}}(c'_{\mathrm{A}}+c'_{\mathrm{B}}+K')^{-1}$ and see with $v=k'_1c'_{\mathrm{A}}c'_{\mathrm{B}}=k'_{-1}c'_{\mathrm{A}}$ that equation (61) is fulfilled.

Introducing now the mass conservation equations $c'_{\mathrm{A}}=c^0_{\mathrm{A}}-c'_{\mathrm{AB}}$ and $c'_{\mathrm{B}}=c^0_{\mathrm{B}}-c'_{\mathrm{AB}}$ (where the index zero indicates total concentration) into K', we derive for equation (62) that

$$c'_{\mathrm{AB}}=\tfrac{1}{2}\{c^0_{\mathrm{A}}+c^0_{\mathrm{B}}+K'-\sqrt{(c^0_{\mathrm{A}}+c^0_{\mathrm{B}}+K')^2-4c^0_{\mathrm{A}}c^0_{\mathrm{B}}}\}.$$

Since $c'_{\mathrm{A}}+c'_{\mathrm{B}}+K'=\sqrt{(c^0_{\mathrm{A}}+c^0_{\mathrm{B}}+K')^2-4c^0_{\mathrm{A}}c^0_{\mathrm{B}}}$, relaxation time and amplitude factor can be expressed as

$$\begin{aligned}\tau&=\{k'_1\sqrt{(c^0_{\mathrm{A}}+c^0_{\mathrm{B}}+K')^2-4c^0_{\mathrm{A}}c^0_{\mathrm{B}}}\}^{-1},\\ \Gamma&=\frac{K'}{2}\left\{\left(1-\frac{4c^0_{\mathrm{A}}c^0_{\mathrm{B}}}{(c^0_{\mathrm{A}}+c^0_{\mathrm{B}}+K')^2}\right)^{-1/2}\quad\right\}\end{aligned} \tag{63}$$

In a kinetic titration where, for instance, c^0_{B} is kept constant, $\Gamma=0$ at $c^0_{\mathrm{A}}=0$; passes through a maximum at $c^0_{\mathrm{A}}(\Gamma_m)=c^0_{\mathrm{B}}+K'$ with

$$\Gamma_m=\frac{K'}{2}\left\{\left(\frac{c^0_{\mathrm{B}}+K'}{K'}\right)^{1/2}-1\right\}$$

and is again zero for $c^0_{\mathrm{A}}\to\infty$. The relaxation time at $c^0_{\mathrm{A}}=0$ is given by $\tau=\{k'_1(c^0_{\mathrm{B}}+K')\}^{-1}$. Provided that $c^0_{\mathrm{B}}>K'$, τ passes through a maximum at $c^0_{\mathrm{A}}(\tau_m)=c^0_{\mathrm{B}}-K'$ with $\tau_m=\{2k'_1(K'c^0_{\mathrm{B}})^{1/2}\}^{-1}$, and is zero at $c^0_{\mathrm{A}}\to\infty$. Similar characteristic features can be derived for other types of elementary chemical reactions. The results are summarized in Table 3.

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* The primes indicate equilibrium conditions.

TABLE 3. Relaxation parameters of elementary chemical reactions (kinetic titration)

Reaction	Relaxation time	Amplitude factor
$A+B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB$	$\tau=\dfrac{1}{k_1'\sqrt{(c_A^0+c_B^0+K')^2-4c_A^0c_B^0}}$	$\Gamma=\dfrac{K'}{2}\;\dfrac{1}{\sqrt{1-\dfrac{4c_A^0c_B^0}{(c_A^0+c_B^0+K')^2}}}-1$
$c_B^0=\text{const.},\ c_A^0=0$	$\tau_0=\dfrac{1}{k_1'(c_B^0+K')}$	$\Gamma_0=0$
$c_B^0=\text{const.},\ c_A^0$	$\tau_m=\dfrac{1}{2k_1'\sqrt{K'c_B^0}},$	$\Gamma_m=\dfrac{K'}{2}\left(\sqrt{\dfrac{c_B^0+K'}{K'}}-1\right)$
	at $c_A^0(\tau_m)=c_B^0-K',\ c_B^0>K'$	at $c_A^0(\Gamma_m)=c_B^0+K'$
$2B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BB$	$\tau=\dfrac{1}{k_1'\sqrt{K'(K'+8c_B^0)}}$	$\Gamma=\dfrac{K'}{8}\left(\dfrac{K'+4c_B^0}{\sqrt{K'(K'+8c_B^0)}}-1\right)$
$c_B^0=0$	$\tau_0=k_1'K'=k_{-1}'$	$\Gamma_0=0$
$B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B'$	$\tau=\dfrac{1}{k_1'+k_{-1}'}=\dfrac{1}{k_1'(1+K')}$	$\Gamma=\dfrac{c_B^0K'}{(1+K')^2}$

It is recalled that the aim of chemical 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.

6.1.3. *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 for following concentration as well as orientation changes in electrically anisotropic molecules.[16–18] The second WIEN effect is directly accessible from the determination of the relative conductivity changes $\delta\kappa/\kappa_0 = \delta\beta/\beta_0$, β_0 being the degree of ion pair dissociation at $\boldsymbol{E} = 0$.

In general, the total signal change, δS, produced by a perturbation will contain several contributions as, for instance, demonstrated for electric field induced absorbance changes in ribosomal RNA (REVZIN and NEUMANN[26]; see also DOURLENT *et al.*[40]; PÖRSCHKE[28,29]). The concentration shifts of the components j in a composite interacting system are summarized in a chemical term $\delta S^{ch} = \sum \delta S_j^{ch}$. Density (volume) changes and changes in the intrinsic optical and electrical properties of the system constitute a physical term δS^{ph}.

In isotropic systems and for isotropic perturbations such as temperature and pressure changes the term δS^{ph} only refers to density (volume) changes and to variations in the intrinsic optical coefficients of the molecules; for this case $\delta S^{ph} = \delta S^{in}$.

In solutions and suspensions of anisotropic molecules directing external forces such as a hydrodynamic flow or electric field forces may cause signal changes δS^{rot} originating from molecule rotations. In particular optically and electrically anisotropic (dipolar or polarizable) macromolecules show large values of the electric dichroism and electric birefringence.

Thus, in general, the total signal change may be written as

$$\delta S = \delta S^{ch} + \delta S^{rot} + \delta S^{in} \qquad (64)$$

6.2. ***Light absorption***

There are numerous reviews on dichroism and birefringence as well as on the use of optical signals (light transmission, fluorescence emission light scattering, optical rotation, circular dichroism) to indicate concentration changes. Less frequent, however, are accounts where it is emphasized that *both chemical and* *orientational* changes may contribute to measured optical signals,[26,40] see Fig. 2.

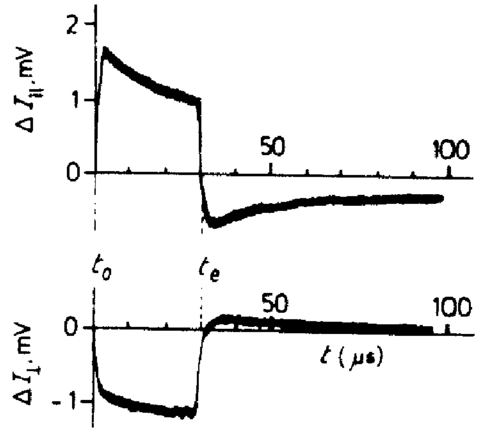

Fig. 2. Electric dichroism of ribosomal RNA at 293 K, 5×10^{-4} *M* Na^+-cacodylate, pH 7; rRNA concentration of 2×10^{-4} *M* phosphate residues. A 44 kV cm^{-1} pulse was applied for 30 μs; the impulse begins at t_0 and ends at t_e. $\Delta I_{\parallel}$ and $\Delta I_{\perp}$ are the separately measured intensity changes at 280 nm for light polarized parallel and perpendicular to the field direction, respectively. REVZIN and NEUMANN.[26]

In the field of optical techniques light transmission and fluorescence emission appear to cover, in general, both concentration changes and rotational contributions in anisotropic systems; linear dichroism seems to yield maximum information on molecular shape, chromophor position relative to rotation axis, etc. (see, *e.g.*, TRICOT and HOUSSIER[15]).

Directly correlated to the concentration of light absorbing molecules (and to absorption anisotropy) is the absorbance, defined by the LAMBERT–BEER law:

$$A = \sum A_j = l \sum \varepsilon_j c_j \tag{65}$$

where ε_j is the (decadic) absorption coefficient of component j in a composite system and l is the optical pathway. I is measured by the transmitted light

$$I = I_0 \exp[-2.3\,A] = I_0 \times 10^{-A} \tag{66}$$

where I_0 is the incident light intensity. The factor 2.303 accounts for the decadic absorbance scale of conventional spectrophotometers.

If an external perturbation causes a change δI we derive from equation (66) that

$$\delta I = I(\exp[-2.3\,\delta A] - 1 = I(10^{-\delta A} - 1) \tag{67}$$

By series expansion of the exponential function in equation (67) the relative change in the transmitted light is given by:

$$\frac{\delta I}{I} = -2.3\,\delta A + \tfrac{1}{2}(2.3\,\delta A)^2 - \tfrac{1}{6}(2.3\,\delta A)^3 + \cdots \tag{68}$$

For relaxation conditions of small perturbations, $\delta I \ll I$, and equation (68) reduces to

$$\delta I = -2.3\,I\,\delta A \tag{69}$$

6.2.1. *Stray light and background absorption*

The actually measured signal, S, before any external perturbation may be expressed as:

$$S = C I_0 (\exp[-2.3(A + A^b)] + A_s) \tag{70}$$

where C is a machine constant, I_0 is the incident light intensity, A^b is the background absorbance (measuring cell, solvent, solutes other than those giving rise to A), and A_s is the stray light factor assumed to be constant. The stray light may be corrected for by electronic offset and the corrected signal is suitably represented as

$$S - S_\infty = C I_0 \exp[-2.3(A + A^b)] \tag{71}$$

where S_∞ is the signal for infinite high absorbance.[43]

The signal change produced by a perturbation is then:

$$\delta S = (S - S_\infty)\{\exp[-2.3\,\delta(A + A^b)] - 1\} \tag{72}$$

For small perturbations, *i.e.* $\delta S \ll (S - S_\infty)$, equation (72) is reduced to:

$$\frac{\delta S}{S - S_\infty} = -2.3\,\delta(A + A^b) \tag{73}$$

Note that the absorbance is determined by a signal ratio; therefore the factor $C I_0$ in equation (70) drops out when δA is determined.

6.3. *Component contributions to absorbance*

For the electric-field-induced signal changes, δI (and δA) are specified as:

$$\delta I = I_E - I \quad \text{and} \quad \delta A = A_E - A \tag{74}$$

where I_E and A_E refer to the transmitted light in the presence, and I and A in the absence of the electric field, respectively.

In a composite system the total absorbance change is expressed as a sum of the component contributions,

$$\delta A = \sum \delta A_j = l\,\delta\left(\sum \varepsilon_j c_j\right) \tag{75}$$

and, analogous to equation (64), we obtain:

$$\delta A = \delta A^{ch} + \delta A^{rot} + \delta A_{inst} \tag{76}$$

The individual terms are specified as a chemical, a rotational and an instantaneous contribution, respectively:

$$\begin{aligned}\delta A^{ch} &= l \sum \varepsilon_j\, \delta c_j \\ \delta A^{rot} &= \sum \delta A_j^{rot} \\ \delta A_{inst} &= l \sum \varepsilon_j c_j \left(\frac{\sum \varepsilon_j c_j \delta \ln \varepsilon_j}{\sum \varepsilon_j c_j} + \delta \ln \rho \right) + \delta A_b\end{aligned} \tag{77}$$

where δA_b is the background contribution and ρ is the density of the system[43]; note that $\delta \ln \rho = -\delta V/V$. Density changes may arise from electrostriction or from a volume change caused by a (JOULE) temperature change in solutions of finite ionic conductivity; electrostriction effects are usually extremely small.

The intrinsic changes in ε, ρ and A_b are in general rapid compared to chemical and rotational relaxations, and are therefore taken together as an instantaneous change δA_{inst} that constitutes a preamplitude preceding slower chemical and rotational relaxations. If only one component j contributes to absorption, $\delta A_{inst} = l\varepsilon_j c_j[(\delta\varepsilon_j/\varepsilon_j) - (\delta V/V)] = lc_j[\delta\varepsilon_j - \varepsilon_j(\delta V/V)]$.

6.4. *Anisotropy effects* (*linear dichroism*)

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 are 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 fairly well 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 chromophors (ring structures such as the purine or pyrimidine bases in polynucleotides) with absorption bands resulting from $\pi - \pi^*$ 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.

6.4.1. *Polarized light*

It is of great practical relevance to use linearly polarized light and to choose the direction of the electric field vector of the external field as a reference. (In the following analysis we omit, where appropriate, the superscript *rot* from the symbols δS^{rot} characterizing the orientational contributions).

For practical purposes the incident polarized light, I_0, with the polarization plane inclined at the angle γ to the field direction, may be decomposed into a component parallel to $\boldsymbol{E}$ and a component perpendicular to $\boldsymbol{E}$ (see Fig. 3):

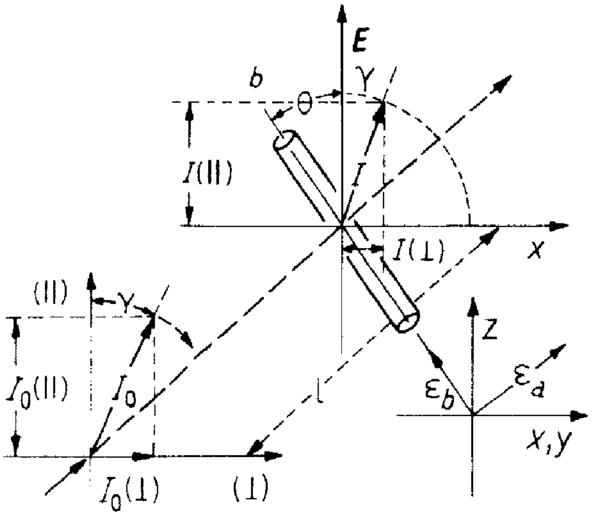

Fig. 3. Polarization modes for the measurement of electrically induced chemical and orientational changes in solutions of interacting anisotropic molecules. As an example a $(z-x, y)$-symmetric system of cylindrical symmetry with the main polarizability axis, b (characterized by the extinction coefficient ε_b) is considered; the dipole axis is oriented under the angle θ to the direction (z) of the electric field $\boldsymbol{E}$. The dichroism contribution relative to the saturation value for this angle is given by $(\varepsilon_z - \varepsilon_{x,y})/(\varepsilon_b - \varepsilon_a) = \frac{1}{2}(3\cos^2\theta - 1)$; (see, *e.g.*, ALLEN and VAN HOLDE[49]).

$$I_0 = I_0(\|) + I_0(\perp) \tag{78}$$

Similarly, the absorbance of optically anisotropic molecules may be decomposed in two component contributions $A_\|$ and $A_\perp$, respectively, because in the wavelength range of the absorption bands the two (formal) components of the linearly transmitted polarized light will be absorbed differently by the anisotropic particles.

The difference between the two components is defined as dichroism:

$$\Delta A = A_{\parallel} - A_{\perp} \tag{79}$$

The absorbance, A, of a solution of optically anisotropic molecules of cylindrical symmetry which are randomly distributed is the mean of the component contributions.

$$A = \tfrac{1}{3}(A_{\parallel} + 2A_{\perp}) \tag{80}$$

Following equation (74) the electrically induced absorbance change δA_{γ} for any angle of polarization γ relative to $\boldsymbol{E}$ is given by

$$\delta A_{\gamma} = A_{\gamma,\boldsymbol{E}} - A \tag{81}$$

The actual values δA_{γ} depend on the degree of orientation, defined by

$$\frac{\delta A_{\gamma}}{(\delta A_{\gamma})_s} = F(\alpha, \boldsymbol{p}, \boldsymbol{E}, T) \tag{82}$$

where the saturation value $(\delta A_{\gamma})_s$ corresponds to maximum orientation. The value of $\boldsymbol{F}$ is a function of the electro-ionic properties of the molecules: permanent dipole moment $\boldsymbol{p}$, polarizability tensor α, external field and absolute temperature (see, *e.g.* STOYLOV[48]).

Three polarization angles are of particular interest for the analysis of electric field effects.

At $\gamma = 0$, we have the parallel mode of the electric field experiment; the absorbance change, $\delta A_{\parallel}$, produced by the field may be expressed with equations (79)–(81) as:

$$\delta A_{\parallel} = A_{\parallel,\boldsymbol{E}} - A = \tfrac{2}{3}\Delta A \tag{83}$$

The perpendicular mode specified by $\gamma = 90°$ is represented by:

$$\delta A_{\perp} = A_{\perp,\boldsymbol{E}} - A = -\tfrac{1}{3}\Delta A \tag{84}$$

These relationships show the experimental access to the dichroism term ΔA.

Comparison of equations (83) and (84) demonstrates that the rotational contributions for axially symmetric systems are characterized by:

$$\delta A_{\parallel}^{rot} = -2\,\delta A_{\perp}^{rot} \tag{85}$$

This means that the absorbance change measured with light polarized parallel to the field is twice as large and opposite in sign compared to the perpendicular mode.

The conventional parameter used to express anisotropy is the reduced dichroism $\Delta A/A$ which is related to $\delta A_{\parallel}$ and $\delta A_{\perp}$ by

$$\frac{\Delta A}{A} = \frac{A_{\parallel} - A_{\perp}}{A} = \frac{\delta A_{\parallel} - \delta A_{\perp}}{A} \tag{86}$$

as is readily derived from equations (79), (83) and (84). It is remarked that the reduced dichroism $(\Delta A/A)_s$ at orientational saturation (extrapolation to infinitely high field intensity) permits determination of the angle between the chromophore's transition moment and the main dipole axis of the aligned molecule.[49]

Returning to equation (85) we conclude that upon a variation of the polarization angle between the parallel and the perpendicular mode, there must exist an angle γ^* at which $\delta A_\gamma = 0$. At γ^* parallel and perpendicular components just cancel each other and $(\Delta A/A) = 0$; or, at this angle, rotational changes do not contribute to (electric field induced) optical changes.

In order to determine this angle we also decompose, analogously to equation (78), the transmitted light I_γ into a parallel, $I(\parallel)$, and a perpendicular component $I(\perp)$. Inserting equations (66) and (78) into

$$I_\gamma = I(\parallel) + I(\perp) = I_0 \exp(-2.3A) \tag{87}$$

we obtain

$$I_\gamma = I_0(\parallel) \exp(-2.3A'_\parallel) + I_0(\perp) \exp(-2.3A'_\perp) \tag{88}$$

Since in general $I = c\mu^2$, where c is a constant and μ is the transition moment, the components in equation (88) are expressed as

$$I_0(\parallel) = I_0 \cos^2 \gamma \quad \text{and} \quad I_0(\perp) = I_0 \sin^2 \gamma \tag{89}$$

Introducing now these terms and equation (87) into equation (88) we obtain

$$I_\gamma = I_0[\cos^2 \gamma \exp(-2.3A'_\parallel) + \sin^2 \gamma \exp(-2.3A'_\perp)] \tag{90}$$

Corresponding to equation (81) the electrically induced change of the transmitted light $\delta I_\gamma = I_E - I$ can be expressed in terms of equations (79), (80), (83), (84) and (90) by equation (91) provided that $A'_\parallel = A_\parallel$ and $A'_\perp = A'_\perp$.

$$\left(\frac{\delta I}{I}\right)_\gamma = \cos^2 \gamma[\exp(-\tfrac{2}{3}2.3\Delta A)] + \sin^2\gamma[\exp(\tfrac{1}{3}2.3\Delta A)] - 1 \tag{91}$$

By inspecting the relationships between $\delta A_\parallel$ and $\delta A_\perp$ in equations (83) and (84), the expressions for the changes in the transmitted light consistently result from equation (91). For the parallel polarization mode, specified by $\gamma = 0$, hence $\cos^2 \gamma = 1$ and $\sin^2 \gamma = 0$ we obtain:

$$\left(\frac{\delta I}{I}\right)_\parallel = \exp(-2.3\,\delta A_\parallel) - 1 \cong -2.3\,\delta A_\parallel \tag{92}$$

and, correspondingly, for the perpendicular mode where $\gamma = 90°$ we have

$$\left(\frac{\delta I}{I}\right)_\perp = \exp(-2.3\,\delta A_\perp) - 1 \cong -2.3\,\delta A_\perp \tag{93}$$

the approximation sign being valid for $\delta I \ll I$, *i.e.* for small perturbations.

Series expansion of equation (91) for $\gamma^* = 54.75°$ where $\cos^2 \gamma = 1/3$ and $\sin^2 \gamma = 2/3$ leads to:

$$\left(\frac{\delta I}{I}\right)_{\gamma^*} = \tfrac{1}{9}(2.3\Delta A)^2 - \tfrac{1}{81}(2.3\Delta A)^3 + \cdots \tag{94}$$

For $\delta I \ll I$, *i.e.* $2.3\Delta A \ll 1$, we obtain

$$\left(\frac{\delta I}{I}\right)_{\gamma^*} = -\delta A^{rot}_{\gamma^*} = 0 \tag{95}$$

corresponding to zero dichroism at $\gamma^* = 54.75$.

In summary, equations (85) and (95) express characteristic features of the rotational contributions, δA^{rot}, and are useful in experimental procedures to differentiate between chemical and orientational contributions to electrically induced absorbance changes in solutions of electro-optically anisotropic molecules. Provided that field induced intensity changes are small, chemical relaxations are directly measurable at $\gamma^* = 54.75°$ without interference from orientational changes.

It should, however, be mentioned that apparatus factors such as, *e.g.*, depolarizing windows of the measuring cell, may cause the actual experimental value of γ^* to be different from 54.75° (see, *e.g.* DOURLENT *et al.*[40]). Furthermore, there are absorbance values where the sensitivity of δI_γ with respect to δA_γ is optimal (KUHN *et al.*[50]; see, *e.g.*, FREDERICQ and HOUSSIER[14]).

6.4.2. *Orientational relaxations*

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 in the presence of a constant electric field builds up with zero slope and has two modes whereas the build-up of induced dipole orientation starts with maximum slope and is characterized by only one time constant. The deorientational relaxation after termination of the step pulse is monophasic, independently of the presence of permanent or induced dipoles. Table 4 summarizes the characteristic features of the rotational kinetics indicated by electric dichroism and birefringence for small perturbations (cf. TRICOT and HOUSSIER[15]). 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.[13,51]

The analysis of orientational changes faces problems when non-rigid molecules or molecules of non-homogeneous length distribution are present. The

TABLE 4. Rotational relaxation (dichroism) at small perturbations caused by a field pulse starting at t_0 and ending at t_e ($\boldsymbol{E}$ constant):

Signal build-up	$\dfrac{\delta A(t)}{\delta A(\infty)}$, $t_0 \leq t \leq t_e$	$\left(\dfrac{d\delta A}{dt}\right)$	Decay, $t \geq t_e$	Integral ratio: $\dfrac{\int_0^{t_e} \delta A(t)\,dt}{\int_{t_e}^{\infty} \delta A(t)\,dt}$
(*a*) general, $r \neq 0$	$\dfrac{3r \exp(-2D_r t) - (r-2)\exp(-6D_r t)}{2(r+1)}$	0	$\exp(-6D_r t)$	$\dfrac{4r+1}{r+1}$
(*b*) permanent and saturated induced dipoles, $r = \infty$	$\exp(-2D_r t) + \exp(-6D_r t)$	0	$\exp(-6D_r t)$	4
(*c*) induced dipoles, $r = 0$	$\exp(-6D_r t)$	$\dfrac{1}{6D_r}$	$\exp(-6D_r t)$	1

$\delta A(t)/\delta(A)\infty$, 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; $(d\,\delta A/dt)_0$ represents the initial slope at t_0 of the signal build-up; D_r is the rotational diffusion coefficient [$\propto L^3$, L being the length of the (dipolar) molecule]; r is the ratio between permanent and induced dipole terms: $r = \beta^2/2\gamma$ where $\beta = B \cdot \boldsymbol{p} \cdot \boldsymbol{E}/kT$ ($B \cong 1$ for elongated particles) and $\gamma = 0.5\Delta\alpha \boldsymbol{E}^2/kT$ ($\Delta\alpha$ being the excess polarizability); the integral ratio represents the ratio between the area above the rise curve ($t_0 \leq t \leq t_e$) and that below the zero-field relaxation curve ($t_e \leq t < t_\infty$). (See, *e.g.*, BENOIT[52]; NISHINARI and YOSHIOKA.[53])

quantitative treatment of field-induced changes in molecule shape is still very difficult. Chain bending or stretching, structural changes, dimer formation, or multimeric aggregation will change the anisotropy components arising from long-range optical interactions as well as short range interactions with the solvent having, in general, a refractive index different from that of the absorbing molecules considered (form anisotropy). Such contributions, however, are small for solutions of low turbidity, *i.e.* if the size of the aggregates remains small compared to the wavelength of the monochromatic light used (see, *e.g.*, TRICOT and HOUSSIER[15]).

6.5. *Differentiation between component contributions*

As is seen in Table 5 there are a number of possibilities of differentiating between chemical and orientational contributions to absorbance changes produced by electric fields in solutions of anisotropic molecules and particles. If chemical transformations are associated with isosbestic or isochromic wavelengths, λ_{iso} (at λ_{iso} the absorption coefficients of all absorbing reaction partners are equal, *i.e.* $\sum \varepsilon_j = 0$), chemical contributions to absorbance are zero at λ_{iso}. In the same line, rotational contributions cancel when plane polarized light is used at the angle $\gamma^*(=54.75°$, or γ^* equals to the corresponding apparatus-specific angle) to the field direction. The parallel and perpendicular modes of polarization serve to further identify rotational contributions.

TABLE 5. Characteristic features of component contributions to absorbance changes in solutions of anisotropic molecules, at three polarization modes γ of the polarized (monochromatic) light. See also Fig. 4

	Chemical	Rotational
$\lambda_{iso}{}^{a}$	$\delta A^{ch} = 0$	$\delta A_{\parallel}^{rot} = -2\,\delta A_{\perp}^{rot}$
$\gamma = 0°$	$\delta A_{\parallel}^{ch} = l \sum \varepsilon_j(\parallel)\,\delta c_j$	
$\gamma = 90°$	$\delta A_{\perp}^{ch} = l \sum \varepsilon_j(\perp) \cdot \delta c_j$	
$\gamma^* = 54.75°{}^{b}$	$\delta A_{\gamma^*} = l \sum \varepsilon_j \cdot \delta c_j$	$\delta A_{\gamma^*}^{rot} = 0$
Limit cases:		
(*a*) $\tau^{rot} \gg \tau^{ch}$	$\delta A_{\parallel}^{ch} = \delta A_{\perp}^{ch} = \delta A_{\gamma^*}$	$\delta A_{\parallel}^{rot} = -2\,\delta A_{\perp}^{rot}$
(*b*) $\tau^{rot} \ll \tau^{ch}$	$\delta A_{\parallel}^{ch} = l \sum \varepsilon_j(\parallel)\,\delta c_j$ $\delta A_{\perp}^{ch} = l \sum \varepsilon_j(\perp)\,\delta c_j$ $\cdot\,\delta A_{\gamma^*} = l \sum \varepsilon_j\,\delta c_j$	$\delta A_{\parallel}^{rot} = -2\,\delta A_{\perp}^{rot}$

[a] λ_{iso}, isosbestic (or isochromic) wavelength.

[b] $\gamma^* \neq 54.75°$, if the polarized light passes through depolarizing windows of the measuring cell, or if the electro-dichroism is not small; in general the parallel component, $\varepsilon_j(\parallel)$, is different from the perpendicular one, $\varepsilon_j(\perp)$, and thus $\delta A_{\parallel}^{ch} \neq \delta A_{\perp}^{ch}$.

From a practical point of view, the analysis of the time course of the measured signal is indispensable to determine the various components and their amplitudes. As is demonstrated in Fig. 2 and 4 the component contributions may have opposite signs and instantaneous signal changes must usually be considered. Some criteria for limiting cases are now briefly touched on. If the time constant of the chemical process is small compared to the rotation times of the interacting molecules ($\tau^{ch} \ll \tau^{rot}$), the chemical relaxation is rapidly established before the orientational changes occur. Thus for the three polarization modes we have $\delta A_{\parallel}^{ch} = \delta A_{\perp}^{ch} = \delta A_{\gamma^*}$. This case is illustrated in Fig. 4(*a*). The rotational contributions always obey equation (85); it is obvious that they must show the same time constants in all three polarization modes. The same is true for the chemical contribution at the various polarization modes.

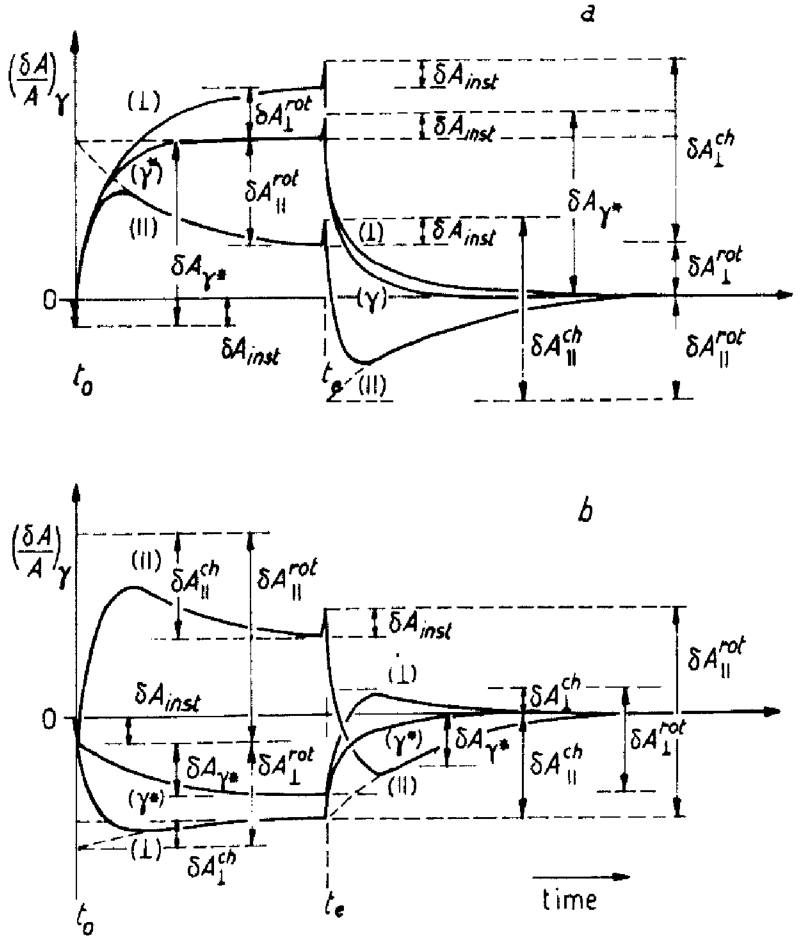

Fig. 4. Schematic representation of the relative absorbance change $(\delta A/A)_\gamma$ as a function of time at three different polarization modes; ∥, $\gamma = 0°$; ⊥, $\gamma = 90°$; $\gamma^*(=54.75°)$ for the two limiting cases: (*a*) $\tau^{ch} \ll \tau^{rot}$, with $\delta A_{\parallel}^{ch} = \delta A_{\perp}^{ch} = \delta A_{\gamma} < \delta A_{\parallel}^{rot}$, $\delta A_{\perp}^{rot}$; $\delta A_{\parallel}^{rot} = -2\delta A_{\perp}^{rot}$. (*b*) $\tau^{ch} \gg \tau^{rot}$ with $\delta A_{\parallel}^{ch} > \delta A_{\perp}^{ch}$; $\delta A_{\parallel}^{rot} = -2\,\delta A_{\perp}^{rot}$. Note that an initial rapid absorbance change δA_{inst}, due to a density change and/or a change in the intrinsic optical properties of the system is taken into account.

For the limiting case that $\tau^{ch} \gg \tau^{rot}$ the rotational changes are established before the chemical change. Therefore the more rapid signal change follows equation (85). The chemical contribution of the parallel mode $\delta A_{\parallel}^{ch}$ will in general be different from $\delta A_{\perp}^{ch}$ but the time constant of both modes are the same. Fig. 4(*b*) illustrates this limiting case. It is recalled that in kinetic titrations the time constants and amplitudes of chemical relaxations show characteristic concentration dependences (see Table 3).

After having considered these analytical details we turn again to bioelectrochemical questions.

7. Cooperativity and hysteresis

The estimates summarized in Tables 1 and 2 demonstrate that, in any case, large reaction moments are required for major displacements of dipolar equilibria; high ionic valencies are necessary for larger dissociation field effects in ionic association-dissociation reactions.

These conditions generally require that the reaction partners themselves have either large dipole moments or large polarizabilities or a high density of fixed ionic groups. The structures which fulfil these conditions are macromolecules and macromolecular organizations such as 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 (see, *e.g.*, SCHWARZ and SEELIG[19]). 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.

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 non-equilibrium transitions in cooperatively stabilized systems. The dissipative element of metastability and non-equilibrium processes endows the structures involved with threshold and trigger properties.[10] Electric field changes going beyond the stability point (threshold) of a metastable configuration will trigger abrupt non-equilibrium 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 (KATCHALSKY and SPANGLER[54]) and molecular memory (see, *e.g.*, KATCHALSKY and NEUMANN[3]). In particular, the memory principle expressed in hysteresis is of appreciable cell-cybernetic interest in biology.[10,11]

In an electrical-chemical hysteresis, the non-equilibrium 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 non-equilibrium transformations in metastable biopolymers, the physical chemical behavior of a model system exhibiting pronounced hysteresis loops has been investigated.[10,55,56] The model hysteresis to be briefly discussed results from the acid-base titration of the polyelectrolyte complex poly(A) · 2 poly(U) formed according to equation (34). The overall process underlying the hysteresis loop is the cyclic transition between two helical structures: the triple helix poly(A) · 2 poly(U) and the protonated double helix poly(A) · poly(A).

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

$$2(\mathrm{U \cdot A \cdot U}) \rightleftharpoons (\mathrm{A \cdot A}) + 4(\mathrm{U}) \tag{96}$$

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; see Fig. 5. Reversing the direction of the pH change by adding alkali, a new curve is traced as the base branch of the loop.

It is important to note in the present account on electrical-chemical coupling, that impulses exceeding a threshold of about 20 kV cm^{-1} directly induce the conformational transition to (A · A) sequences in equation (96).

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

$$\mathrm{X}_k^+(\mathrm{U \cdot A \cdot U})_n = (\mathrm{A})_n + 2(\mathrm{U})_n + m\mathrm{X}^+ \tag{97}$$

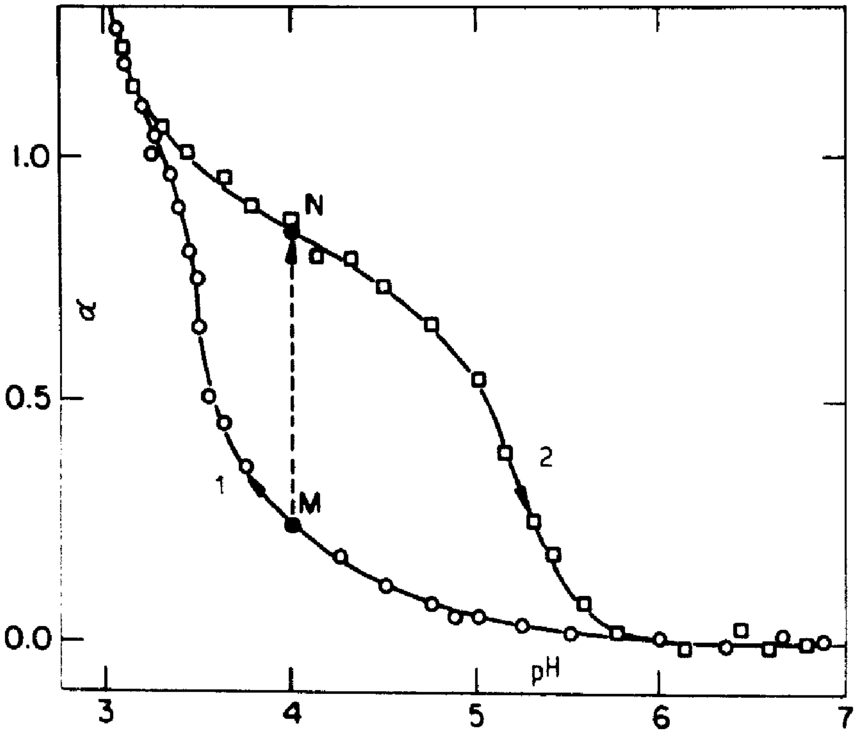

Fig. 5. Degree of protonation, α, as a function of pH in a cyclic potentiometric titration of poly(A) · 2 poly(U) in 0.1 *M* NaCl; pH 7; 293 K. ○, acid titration (from pH 7 to pH 3); the lower curve 1 represents the acid branch; □, subsequent base titration (from pH 3 to pH 7); the upper curve 2 is the base branch of the hysteresis loop. Transition from point M toward point N, induced by electric field pulses with the initial intensity of 20 kV cm^{-1} and decaying exponentially with time (time constant 10 μs); see, *e.g.*, NEUMANN and KATCHALSKY.[21]

The field effect on this reaction includes *liberation* of $m(<k)$ counterions, X^+, from the ionic atmosphere of the triple helix containing a total of k counterions X^-. Under the experimental conditions of Fig. 5, the field-induced reaction is coupled to the protonation of the (A) residues

$$(A)+H^+=(AH^+) \tag{98}$$

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 favourably oriented to the external field pulse (see Fig. 6). This key observation points to an *end effect* in this anisotropic system; at terminal

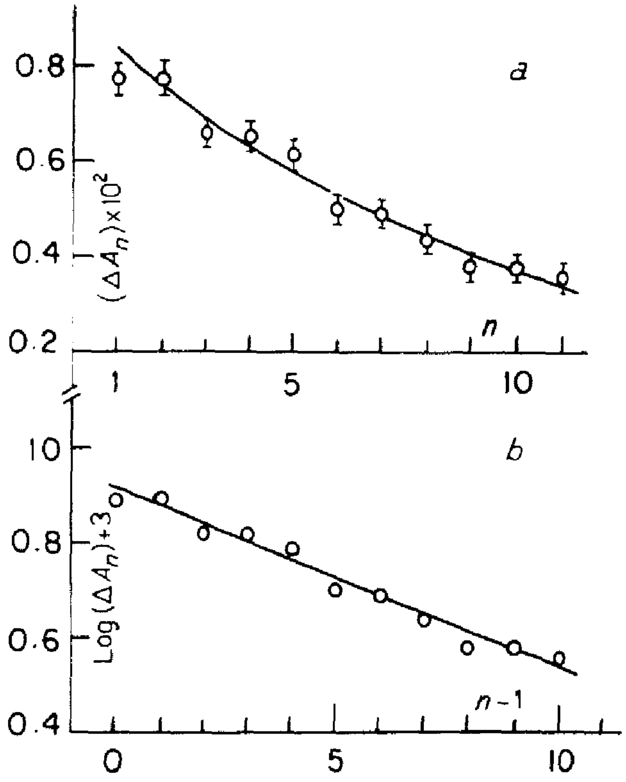

Fig. 6. Helix-coil transition in poly(A) · 2 poly(U); electrically induced change in the absorbance ΔA at 260 nm as a function of impulse number n (NEUMANN and KATCHALSKY[21]).

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 according to equation (35) 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.[21] It later came to our attention that a polarization mechanism had already been proposed for strand separation of DNA by POLLAK and REIN.[57]

It is now worth mentioning that the magnitudes of impulse intensity and duration, used in our investigations of the polynucleotides and membranes,[10,20,21,30] lie 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 nerve impulses in the neuronal network of the brain.

8. Effects of weak electric fields

After the more speculative remarks made in closing the previous chapter, it appears appropriate to comment on some interesting reports on the action of weak electric fields. It is well known that certain fish, insects and birds have specialized organs to sense small changes in the electric state of their environment. Further the behaviour of monkeys or the release of Ca^{2+} ions from brain tissue are also reported to be influenced by very weak electric fields (mV cm^{-1}). For a recent summary of these interesting topics see ADEY and BAWIN.[58] Of great medical interest are observations in which the application of weak electric currents or electric fields led to healing of otherwise uncurable bone fractures.[59]

It is fair to say that none of these weak-field phenomena is understood, neither on the cellular level nor in molecular terms. In the light of the estimates summarized in Tables 1 and 2 the observations of weak electric field effects are above all puzzling. Even if highly cooperative and highly polarizable structures like polyionic complexes are involved, only very *high* electric intensities (kV cm^{-1}) can produce measurable changes in structural equilibria or in ion binding; any direct action of *weak* electric fields (mV cm^{-1}) on ionic binding distributions or structural conformational changes is orders of magnitude weaker than thermal motion. It should also be realized that ions and ionic structures such as polyionic macromolecules and membranes are associated with locally high electric fields which are (probably) practically not influenced by *weak external field* perturbations.

The extremely small bias a weak electric field exerts may, of course, involve *in vivo* large assemblies of subunits which may be synchronized by an external force to cooperative behaviour. Nevertheless, special mechanisms must be claimed to channel the very small bias exerted on one subunit and to amplify the initial *input* such that the signal develops out of the thermal noise. At the present low level of knowledge on such mechanisms adequate cellular model systems may offer a promising step toward understanding the observed bioelectrical phenomena; they are most probably associated with macromolecular organizations such as membranes. It is obvious that also in this challenging field a basic knowledge of the fundamental principles of electric field effects in elementary (bio)chemical reactions and molecular-rotational processes appears to be a necessary prerequisite for the study of organized and complicated systems. The present account may provide some useful information as to how to investigate mechanisms of bioelectric phenomena.

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