Principles of Membrane Electroporation and Transport of Macromolecules

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1. Introduction

The phenomenon of membrane electroporation (ME) methodologically comprises an electric technique to render lipid and lipid–protein membranes porous and permeable, transiently and reversibly, by electric voltage pulses. It is of great practical importance that the primary structural changes induced by ME, condition the electroporated membrane for a variety of secondary processes, such as, for instance, the permeation of otherwise impermeable substances.

Historically, the structural concept of ME was derived from functional changes, explicitly from the electrically induced permeability changes, which were indirectly judged from the partial release of intracellular components (1) or from the uptake of macromolecules such as DNA, as indicated by electrotransformation data (2–4). The electrically facilitated uptake of foreign genes is called the direct electroporative gene transfer or electrotransformation of cells. Similarly, electrofusion of single cells to large syncytia (5) and electroinsertion of foreign proteins (6) into electroporated membranes are also based on ME, that is, electrically induced structural changes in the membrane phase.

For the time being, the method of ME is widely used to manipulate all kinds of cells, organelles, and even intact tissue. ME is applied to enhance iontophoretic drug transport through skin—see, for example, Pliquett et al. (7)—or to introduce chemotherapeutics into cancer tissue—an approach pioneered by L. Mir (8).
Medically, ME may be qualified as a novel microsurgery tool using electric pulses as a microscalpel, transiently opening the cell membrane of tissue for the penetration of foreign substances (4,9,10). The combination of ME with drugs and genes also includes genes that code for effector substances such as interleukin-2 or the apoptosis proteins p53 and p73. Therefore, the understanding of the electroporative DNA transport is of crucial importance for gene therapy in general and antitumor therapy in particular.

Clearly, goal-directed applications of ME to cells and tissue require knowledge not only of the molecular membrane mechanisms, but potential cell biological consequences of transient ME on cell regeneration must be also elucidated, for instance, adverse effects of loss of intracellular compounds such as Ca\textsuperscript{2+}, ATP, and K\textsuperscript{+}. Due to the enormous complexity of cellular membranes, many fundamental problems of ME have to be studied at first on model systems, such as lipid bilayer membranes or unilamellar lipid vesicles. When the primary processes are physicochemically understood, the specific electroporative properties of cell membranes and living tissue may also be quantitatively rationalized.

Electrooptical and conductometrical data of unilamellar liposomes showed that the electric field causes not only membrane pores but also shape deformation of liposomes. It appears that ME and shape deformation are strongly coupled, mutually affecting each other (4,11,12). The primary field effect of ME and cell deformation triggers a cascade of numerous secondary phenomena, such as pore enlargement and transport of small and large molecules across the electroporated membrane. Here we limit the discussion to the chemical—structural aspects of ME and cell deformation and the fundamentals of transport through electroporated membrane patches. The theoretical part is essentially confined to those physicochemical analytical approaches that have been quantitatively conceptualized in some molecular detail, yielding transport parameters, such as permeation coefficients, electroporation rate coefficients, and pore fractions.

2. Theory of Membrane Electroporation

The various electroporative transport phenomena of release of cytosolic components and uptake of foreign substances, such DNA or drugs are indeed ultimately caused by the external voltage pulses. It is stressed again that the transient permeability changes, however, result from field-induced structural changes in the membrane phase. Remarkably, these structural changes comprise transient, yet long-lived permeation sites, pathways, channels, or pores (3,13–17).
2.1. The Pore Concept

Field-induced penetrations of small ions and ionic druglike dyes are also observed in the afterfield time period, that is, in the absence of the electrodiffusive driving force (Fig. 1). Therefore, the electrically induced permeation sites must be polarized and specifically ordered, local structures which are potentially “open for diffusion” of permeants. As indicated by the longevity of the permeable membrane state, these local structures of lipids are long-lived (milliseconds to seconds) compared to the field pulse durations (typically, 10 μs to 10 ms). Thus, the local permeation structures may be safely called transient pores or electropores in model membranes as well as in the lipid part of cell membranes. The special structural order of a long-lived, potential permeation site may be modeled by the so-called inverted or hydrophilic (HI) pore (Fig. 2) (17–19). On the same line, the massive ion transport through planar membranes, as observed in the dramatic conductivity increase when a voltage (≥100–500 mV) is applied, can hardly be rationalized without field-induced open passages or pores (17).

The afterfield uptake of substances like dyes or drug molecules, added over a time period of minutes after the pulse application, suggests a kind of interactive diffusion, probably involving the transient complex formation between the permeant and the lipids of the pore wall to yield leaky, but transiently occluded, pores (9).

2.1.1. Pore Visualization

Up to now there is no visible evidence for small electropores such as electrornicrographs. But also the movement of a permeant through an electroporated membrane patch has also not been visualized. The large porelike crater structures or volcano funnels of 50 nm to 0.1 μm diameter, observed in electroporated red blood cells, most probably result from specific osmotic enlargement of smaller primary pores, invisible in microscopy (14). Voltage-sensitive fluorescence microscopy at the membrane level has shown that the transmembrane potential in the pole caps of sea urchin eggs goes to a saturation level or even decreases, both as a function of pulse duration and external field strength, respectively. If the membrane conductivity would remain very low, the transmembrane potential linearly increases with the external field strength. Leveling off and decrease of the transmembrane potential at higher fields indicate that the ionic conductivity of the membrane has increased, providing evidence for ion-conductive electropores (15). On the same line, in direct current (DC) electric fields the fluorescence images of the contour of
Fig. 1. Pore resealing kinetics indicated by dye uptake. The fraction $f_C$ of colored cells as a function of the time $t = t_{add}$ of dye addition after the pulse. B-lymphoma cells (line IIA1.6) were exposed to one rectangular electric field pulse ($E = 1.49$ kV cm$^{-1}$; pulse duration $t_E = 110$ $\mu$s) in the presence of the dye SERVA blue G ($M_r = 854$). (From ref. 9, with permission.)

Fig. 2. Specific chemical state transition scheme for the molecular rearrangements of the lipids in the pore edges of the lipid vesicle membrane. $C$ denotes the closed bilayer state. The external electric field causes ionic interfacial polarization of the membrane dielectrics analogous to condenser plates (+, −). $E_m = E_{ind}$ is the induced membrane field, leading to water entrance in the membrane to produce pores (P); cylindrical hydrophobic (HO) pores or inverted hydrophilic (HI) pores. In the pore edge of the HI pore state, the lipid molecules are turned to minimize the hydrophobic contact with water. In the open condenser the ion density adjacent to the aqueous pore ($\varepsilon_w$) is larger than in the remaining part ($\varepsilon_L$) because of $\varepsilon_w >> \varepsilon_L$.

Elongated and electroporated giant vesicle shows large openings in the pole caps opposite to the external electrodes (20). Apparently, these openings are appearing after coalescence of small primary pores invisible in microscopy. Theoretical analysis of the membrane curvature in the vesicle pole caps suggests that vesicle elongation under Maxwell stress must facilitate both pore formation and enlargement of existing pores.
2.1.2. Born Energy and Ion Transport

Membrane electroporation for small ions and larger ionic molecules cannot be simply described by a permeation across the densely packed lipids of an electrically modified membrane (17). Theoretically, a small monovalent ion, such as Na⁺(aq) of radius \( r_i = 0.22 \text{ nm} \) and of charge \( z_i e \), where \( e \) is the elementary charge and \( z_i \) the charge number of the ion \( i \) (with sign), passing through a lipid membrane encounters the Born energy barrier of

\[ \Delta G_B = \frac{z_i^2 \cdot e^2}{(1/\varepsilon_m - 1/\varepsilon_w)/(8 \cdot \pi \cdot \varepsilon_0 \cdot r_i)}, \]

where \( \varepsilon_0 \) the vacuum permittivity, \( \varepsilon_m \approx 2 \) and \( \varepsilon_w \approx 80 \) are the dielectric constants of membrane and water, respectively. At \( T = 298 \text{K} \) (25°C), \( \Delta G_B = 68 \cdot kT \), where \( k \) is the Boltzmann constant and \( T \) is the absolute temperature. To overcome this high barrier, the transmembrane voltage \( |\Delta \phi| = \Delta G_B / |z_i \cdot e| \) has to be 1.75 V. An even larger voltage of 3.5 V is needed for divalent ions such as Ca²⁺ or Mg²⁺ (\( z_+ = 2 \), \( r_i = 0.22 \text{ nm} \)). Nevertheless, the transmembrane potential required to cause conductivity changes of the cell membrane usually does not exceed 0.5 V (16,17). The reduction of the energy barrier can be readily achieved by a transient aqueous pore. Certainly, the stationary open electropores can only be small (about \( \leq 1 \text{ nm} \) diameter) to prevent discharging of the membrane interface by ion conduction (4,9,18).

2.2. Transmembrane Field

In line with the Maxwell definition of the electric field strength as the negative electric potential gradient, we define the membrane field strength by

\[ E_m = -\Delta \phi_m / d, \]  \hspace{1cm} (1)

where \( \Delta \phi_m \) is the intrinsic cross membrane potential difference and \( d \approx 5 \text{ nm} \) the dielectric membrane thickness. This inner-membrane potential difference may generally consist of several contributions.

2.2.1. Natural Membrane Potential and Surface Potential

All living cell membranes are associated with a natural, metabolically maintained, (diffusion) potential difference \( \Delta \phi_{\text{nat}} \), defined by \( \Delta \phi_{\text{nat}} = \phi^{(i)} - \phi^{(o)} \) as the difference between cell inside (i) and outside (o) (see Fig. 3). Typically, this resting potential amounts to \( \Delta \phi_{\text{nat}} \approx -70 \text{ mV} \), where \( \phi^{(o)} = 0 \) is taken as the reference potential (21).

Biomembranes usually have an excess of negatively charged groups at the interfaces between membrane surfaces and aqueous media. The contribution of these fixed charges and that of the screening small ions are covered by the surface potentials \( \phi^{(o)}_s \) and \( \phi^{(i)}_s \). If cells are exposed to low ionic strength, the inequality \( |\phi^{(o)}_s| > |\phi^{(i)}_s| \) may apply. Therefore, there will be a finite value for
Fig. 3. Electric membrane polarization of a cell of radius $a$. (A) Cross section of a spherical membrane in the external field $E$. The profiles of (B) the electrical potential $\phi$ across the cell membranes of thickness $d$, where $\Delta\phi_{\text{ind}}$ is the drop in the induced membrane potential in the direction of $E$ and (C) the surface potential $\phi_s$ at zero external field as a function of distance, respectively; (D) $\Delta\phi_{\text{nat}}$ is the natural (diffusive) potential difference at zero external field, also called resting potential.

the surface potential difference $\Delta\phi_s = \phi_s^{(i)} - \phi_s^{(o)}$ (defined analogously to $\Delta\phi_{\text{nat}}$), which in this case is positive and therefore opposite to the diffusion potential $\Delta\phi_{\text{nat}}$ (see Fig. 3). Provided that additivity holds the field-determining potential difference is $\Delta\phi_m = \Delta\phi_{\text{nat}} + \Delta\phi_s$. At larger values of $\Delta\phi_s$, the term $\Delta\phi_{\text{nat}}$ may be compensated by $\Delta\phi_s$ and therefore $\Delta\phi_m \approx 0$. If lipid vesicles containing a
surplus of anionic lipids are salt-filled and suspended in low ionic strength medium, the surface potential difference \( \Delta \varphi_s > 0 \) is finite, but \( \Delta \varphi_{\text{nat}} = 0 \). Generally, even in the absence of an external field, there can be a finite membrane field \( E_{m} = |\Delta \varphi_{\text{nat}} + \Delta \varphi_s| / d \) (21). Here we may neglect the locally very limited, but high (150–600 mV) dipole potentials in the boundary between lipid head groups and hydrocarbon chains of the lipids (22,23).

2.2.2. Field Amplification by Interfacial Polarization

In static fields and low-frequency alternating fields dielectric objects such as cells, organelles, and lipid vesicles in electrolyte solution experience ionic interfacial polarization (Fig. 3A) leading to an induced cross-membrane potential difference \( \Delta \varphi_{\text{ind}} \), resulting in a size-dependent amplification of the membrane field. For spherical geometry with cell or vesicle radius \( a \) the induced field \( E_{\text{ind}} = -\Delta \varphi_{\text{ind}} / d \) at the angular position \( \theta \) relative to the external electric field vector \( E \) (Fig. 3B) is given by

\[
E_{\text{ind}} = \frac{3}{2} \frac{a}{d} \cdot E \cdot f(\lambda_m) \cdot \cos \theta l,
\]

where the conductivity factor \( f(\lambda_m) \) can be expressed in terms of \( a \) and \( d \) and the conductivities \( \lambda_m, \lambda_i, \lambda_0 \) of the membrane, the cell (vesicle) interior and the external solution, respectively (21). Commonly, \( d \ll a \) and \( \lambda_m \ll \lambda_0, \lambda_i \) such that

\[
f(\lambda_m) = \left[ 1 + \lambda_m(2 + \lambda_i / \lambda_0) / (2\lambda_i d/a) \right]^{-1}.
\]

At \( \lambda_m \approx 0 \) or for negligibly small membrane conductivity we have \( f(\lambda_m) = 1 \).

The field amplification factor \( (3 \cdot a / 2 \cdot d) \) is particularly large for large cells and vesicles; for typical values such as \( a = 10 \mu m \) and \( d = 5 \) nm, we have a field amplification of \( (3 \cdot a / 2 \cdot d) = 3 \cdot 10^3 \). For elongated cells like bacteria aligned by the field in the direction of \( E \), the contribution of \( E_{\text{ind}} \) at the pole caps, where \( \cos \theta l = 1 \), amounts to

\[
E_{\text{ind}} = (L / 2 \cdot d) \cdot E,
\]

where the amplification factor \( (L / 2 \cdot d) \) is proportional to the bacterium length \( L \) (24).

2.2.3. Vesicles and Cells in Applied Fields

In the case of lipid vesicles there is no natural membrane potential, that is, \( \Delta \varphi_{\text{nat}} = 0 \). However, for charged lipids and unequal electrolyte concentrations within and outside the vesicle, the surface potentials are different from zero, and therefore \( \Delta \varphi_m = \Delta \varphi_{\text{ind}} + \Delta \varphi_s \) (25). Hence at the angle \( \theta \) we obtain (21,26):

\[
E_{\text{m}}^0 = E_{\text{ind}}^0 + \Delta \varphi_s \cdot \cos \theta l / (d \cdot \cos \theta).
\]
Note that $|\cos \theta|/\cos \theta = +1$ for the right hemisphere and $-1$ for the left one. Therefore, at the right hemisphere $\Delta \phi_s/d$ adds to the applied field and at the left hemisphere $\Delta \phi_s/d$ reduces the induced field.

For living cells, there is always a finite $E_m$ field, because $\Delta \phi_{nat} \neq 0$ (Fig. 3D). Generally, the stationary value of the transmembrane field at the angular position $\theta$ for cells with finite natural and surface potential membrane potentials relative to the direction of the external field, can be expressed as:

$$E_m = \left\{ \frac{3 \cdot a}{2 \cdot d} \cdot E \cdot f(\lambda_m) + \frac{\Delta \phi_{nat} + \Delta \phi_s}{d \cdot \cos \theta} \right\} \cdot |\cos \theta|. \quad (3)$$

Normally, $\Delta \phi_{nat}$ and $\Delta \phi_s$ are independent of $\theta$. For the special case when $\Delta \phi_{nat}$ and $\Delta \phi_s$ have equal signs, there can be a major asymmetry. At the left pole cap the sum $\Delta \phi_{nat} + \Delta \phi_s$ is in the same direction as $\Delta \phi_{ind}$, whereas at the right pole cap $\Delta \phi_{nat} + \Delta \phi_s$ is opposite to $\Delta \phi_{ind}$. For example, if $\Delta \phi_{nat} = -70$ mV and $\Delta \phi_{ind} = -500$ mV, one has at the left pole cap $\Delta \phi_m = -570$ mV and at the right one $\Delta \phi_m = -430$ mV. Therefore membrane electroporation will start at the left hemisphere where the field $E_m = -(\Delta \phi_{ind} + \Delta \phi_{nat} + \Delta \phi_s)/d$ is larger than $E_m = -(\Delta \phi_{ind} - \Delta \phi_{nat} - \Delta \phi_s)/d$ at the right hemisphere. In the case of opposite signs of $\Delta \phi_s$ and $\Delta \phi_{nat}$ the natural potential $\Delta \phi_{nat}$ may be compensated by $\Delta \phi_s$, the asymmetry in the two hemispheres of cells gets smaller.

2.2.4. Condenser Analog

The redistribution of ions in the electrolyte solution adjacent to the membrane dielectrics results in charge separations which are equivalent to an electrical condenser with capacity

$$C_m = \varepsilon_m \cdot \varepsilon_0 \cdot S_m/d,$$

where $S_m$ is the membrane surface area (Fig. 2). However, unlike conventional solid state dielectric condensers, the lipid membrane and adjacent ionic layers are highly dynamic phases of mobile lipid molecules in contact with mobile water molecules and ions. The lipid membrane is hydrophobically kept together by the aqueous environment. Such a membrane condenser with both mobile interior and mobile environment favors the entrance of water molecules to produce localized cross-membrane pores (P) with higher dielectric constant $\varepsilon_w \approx 80$ compared with $\varepsilon_\infty \approx 2$ of the replaced lipids (state C).

In the case of charged membranes there are two additional condensers due to the electrical double layers of fixed surface charges and mobile counterions on the two sides of the charged membrane, represented by the capacities

$$C_i = \varepsilon_w \cdot \varepsilon_0 \cdot S_m / \ell_D^{(i)} \quad \text{and} \quad C_o = \varepsilon_w \cdot \varepsilon_0 \cdot S_m / \ell_D^{(o)},$$

where $\ell_D^{(i)}$ and $\ell_D^{(o)}$ are the Debye screening lengths inside and outside the cell (vesicle), respectively (Fig. 3C).
In the absence of an external field the total potential difference across the membrane is defined solely by the condenser charge \( q = q_+ = |q_-| \) due to the natural diffusion potential and charged surface groups:

\[
\Delta \varphi_m = \Delta \varphi_{\text{nat}} + \Delta \varphi_s,
\]

with

\[
\Delta \varphi_s = q / (1/C_i - 1/C_o).
\]

Explicitly the contribution by the surface charge potential is given by:

\[
\Delta \varphi_s = \frac{1}{F} \sqrt{\frac{RT}{2 \cdot \varepsilon_0 \cdot \varepsilon_w}} \left( \frac{\sigma_i}{\sqrt{J_i}} - \frac{\sigma_o}{\sqrt{J_o}} \right),
\]

where \( F \) is the Faraday constant, \( R \) the gas constant, \( \sigma_i = q_i / S_m \) and \( \sigma_o = q_o / S_m \) are the charge densities on the inner and outer membrane surfaces, respectively, and \( J_i \) and \( J_o \) are the molar ionic strengths of the inside and the outside bulk electrolyte, respectively. Note that

\[
J_{i(o)} = (\sum_j z_j^2 \cdot c_j)_{i(o)}/2,
\]

where \( j \) refers to all mobile ions and fixed ionic groups; frequently \( J \) is determined by the salt ions of the buffer solution. When the salt concentrations inside and outside are largely different, \( \Delta \varphi_s \) may appreciably contribute to \( E_m \).

### 2.3. Electroporation–Resealing Cycle

#### 2.3.1. Chemical Scheme for Pore Formation

The field-induced pore formation and resealing after the electric field is viewed as a state transition from the intact closed lipid state \((C)\) to the porous state \((P)\) according to the reaction scheme (2I):

\[
\begin{align*}
\text{C} & \iff \text{P}. \\
\end{align*}
\]

The state transition involves a cooperative cluster \((L_n)\) of \( n \) lipids \( L \) forming an electropore \((19)\). The degree of membrane electroporation \( f_p \) is defined by the concentration ratio

\[
\begin{align*}
f_p = \frac{[P]}{[P] + [C]} = \frac{K}{1 + K},
\end{align*}
\]

where \( K = [P] / [C] = k_1 / k_{-1} \) is the equilibrium distribution constant, \( k_1 \) the rate coefficient for the step \( \text{C} \rightarrow \text{P} \) and \( k_{-1} \) the rate coefficient for the resealing step \( \text{C} \leftarrow \text{P} \). In an external electric field, the distribution between \( C \) and \( P \) states is shifted in the direction of increasing \([P]\). Note, the frequently encountered observation of very small pore densities means that \( K << 1 \). For this case \( f_p = K \). Hence the thermodynamic, field-dependent quantity \( K \) is directly obtained from the experimental degree of poration.
2.3.2. Reaction Rate Equation

Kinetically, the reaction rate equation for the time course of the electroporation-resealing cycle describes the differential increase $d[P]$ in pore concentration at the expense of lipids outside the pore wall, $d[C]$, in the form of the conventional differential equation (4):

$$\frac{d[P]}{dt} = -\frac{d[C]}{dt} = k_1[C] - k_{-1}[P].$$

(7)

Mass conservation dictates that the total concentration is $[C_0] = [P] + [C]$. Substitution into Eq. 7 and Eq. 6, integration yields the time course of the degree of pore formation:

$$f_p^{C \rightarrow P} = \frac{K}{1 + K} \cdot \left(1 - e^{-it/\tau}\right),$$

(8)

where the practical assumption that $f_p(0) = 0$ at $E = 0$ and $t = 0$ was applied. The relaxation time is given by:

$$\tau = (k_1 + k_{-1})^{-1} = [k_{-1} (1 + K)]^{-1}.$$  

(9)

For the after-field time range $t > t_E$ where $k_{-1} \gg k_1$ and

$$f_p(t_E) = K/(1 + K) \cdot (1 - e^{-t_E/\tau}),$$

integration of Eq. 7 yields:

$$f_p^{P \rightarrow C} = f_p(t_E) \cdot e^{-k_{-1}(t-t_E)}.$$  

(10)

It is readily seen that the experimentally accessible quantities $\tau$ and $K$ yield both rate coefficients $k_1$ and $k_{-1}$. The symbol $P$ may include several different pore states. If, for instance, we have to describe the pore formation by the sequence $C \Leftrightarrow$ HO $\Leftrightarrow$ HI, then $(P)$ represents the equilibrium HO $\Leftrightarrow$ HI between hydrophobic (HO) and hydrophilic (HI) pore states (Fig. 2). In this case normal mode analysis is required and $k_{-1}$ in the expressions for $f_p$ must be replaced by $k_{-1}/(1 + K_2)$, where $K_2 = [HI]/[HO]$ is the equilibrium constant of the second step HO $\Leftrightarrow$ HI (19).

2.3.3. $\theta$ Averages

For the curved membranes of cells and organelles, the dependence of the induced potential difference $\Delta \varphi_{\text{ind}}$ and thus the transmembrane field $E_{\text{ind}} = -\Delta \varphi_{\text{ind}}/d$ on the positional $\theta$ angle leads to the shape-dependent $\theta$ distribution of the values of $K$ and $k_1$; $k_{-1}$ is assumed to be independent of $E$ and thus independent of $\theta$. Therefore, all conventionally measured quantities ($f_p$ and $\tau$) are $\theta$ averages. The stationary value of the actually measured $\theta$-average fraction $\bar{f}_p$ of porated area is given by the integral:
Fig. 4. The fraction, $f_p^\theta$, of membrane surface area covered by electropores as a function of the positional angle $\theta$. The $\theta$ average $\bar{f}_p$ of membrane electroporation is by a factor of 4 smaller than $f_p^\theta$ in the cell pole caps opposite to the electrodes ($\theta = 0^\circ$, $\theta = 180^\circ$, respectively).

$$\bar{f}_p = \frac{1}{2} \int_0^\pi \frac{k_1(\theta)}{k_{-1} + k_1(\theta)} \sin \theta \, d\theta.$$  \hfill (11)

The actual pore density $f_p^\theta$ in the cell pole caps, where $\theta \approx 0^\circ$ and $180^\circ$, respectively, can be a factor of 4 larger than the $\theta$ average fraction $\bar{f}_p$ (Fig. 4). It is found that $\bar{f}_p$ is usually very small (11,12), for example, $\bar{f}_p \leq 0.003$, that is, 0.3%. Even the pole cap values $f_p^\theta (0^\circ, 180^\circ) = 4 \cdot \bar{f}_p = 0.012$ certainly correspond to a small pore density.

3. Thermodynamics of Membrane Electroporation

As already mentioned, the lipid membrane in an external electric field is an open system with respect to $\text{H}_2\text{O}$ molecules and surplus ions, charging the membrane condenser. Therefore, to ensure the minimization of the adequate Gibbs energy with respect to the field $E_m$, we have to transform the normal Gibbs energy $G$ with $dG$ proportional to $E_m \cdot dM$, where $M$ is the global electric dipole moment, to yield the transformed Gibbs energy $\dot{G} = G - E_m M$ with $d\dot{G}$ proportional to $-M dE_m$ (27). Now, $E_m$ in $dE_m$ is the explicit variable and membrane electroporation can be adequately described in terms of $E_m$ and the induced electric dipole moment $M$ of the pore region.

The global equilibrium constant $K$ of the poration–resealing process is directly related to the standard value of the transformed reaction Gibbs energy $\Delta_\text{r}\dot{G}^\circ$ by (28):
\[ K = e^{-\Delta_f \tilde{G}^0_{\text{irr}}} \]  

The molar work potential difference

\[ \Delta_f \tilde{G}^0 = \tilde{G}^0(P) - \tilde{G}^0(C), \]

between the two states \(C\) and \(P\) in the presence of an electric field generally comprises chemical and physical terms (18):

\[ \Delta_f \tilde{G}^0 = \sum_{\alpha} \sum_{j} (v_j \cdot \mu_{j,\alpha})^a + \int_{0}^{L} \Delta_f \gamma dL + \int_{0}^{S} \Delta_f \Gamma dS + \int_{0}^{H} \Delta_f \beta dH - \int_{0}^{m} \Delta_f \lambda dE_m. \]  

Note that \(\Delta_f = d / d\xi\), where \(d\xi = d n_j / v_j\) is the differential molar advancement of a state transition, \(n_j\) is the amount of substance and \(v_j\) is the stoichiometric coefficient of component \(j\), respectively. The single terms of the right-hand side of Eq. 13 are now separately considered.

### 3.1. Chemical Contribution, Pore Edge Energy, and Surface Tension

The first term is the so-called chemical contribution. The pure concentration changes of the lipid \((j = L)\) and water \((j = W)\) molecules involved in the formation of an aqueous pore with edges are described by \(v_j^\alpha\) and the conventional standard chemical potential \(\mu_{j,\alpha}^0\) of the participating molecule \(j\), constituting the phase \(\alpha\), either state \(C\) or state \(P\) (27); here,

\[ \Delta_f \tilde{G}^0 = \sum_{\alpha} \sum_{j} (v_j \cdot \mu_{j,\alpha}^0) = (v_w \cdot \mu_{w,\alpha}^0 + v_L \cdot \mu_{L,\alpha}^0)^P - (v_w \cdot \mu_{w,\alpha}^0 + v_L \cdot \mu_{L,\alpha}^0)^C. \]

In Eq. 13, \(\gamma\) is the line tension or pore edge energy density and \(L\) is the edge length, \(\Gamma\) is the surface energy density and \(S\) is the pore surface in the surface plane of the membrane. Explicitly, for cylindrical pores (HO-pore, Fig. 2) of mean pore radius \(\bar{r}_p\) the molar pore edge energy term reads:

\[ \int_{0}^{L} \Delta_f \gamma dL = N_A \int_{0}^{L} \left( \gamma_p - \gamma_C \right) dL = 2 \cdot \pi \cdot N_A \cdot \gamma \cdot \bar{r}_p, \]

where \(\gamma_p = \gamma\) (because \(\gamma_C = 0\), no edge) and \(L = 2 \pi \cdot \bar{r}_p\) is the circumference line; \(N_A = R/k\) is the Avogadro constant.

The surface pressure term for spherical bilayers in water:

\[ \int \Delta_f \Gamma dS = N_A \int_{0}^{S} \left( \Gamma_p - \Gamma_C \right) dS \]

is usually negligibly small because the difference in \(\Gamma\) between the states \(P\) and \(C\) is in the order of \(\leq 1.2\) mN m\(^{-1}\) for phosphatidylcholine in the fluid bilayer state (29).
3.2. Curvature Energy Term

The explicit expression for the curvature energy term of vesicles of radius $a$ and membrane thickness $d$ is given by (18,30):

$$\int \Delta \beta \, dH = N_A \int (\beta_p - \beta_C) \, dH$$

$$= - \frac{64 \cdot N_A \cdot \pi^2 \cdot \kappa \cdot \frac{r_p^2}{\pi} \cdot \zeta \cdot \left( \frac{1}{a} + \frac{H_0^{cl}}{2\pi \cdot \alpha} \right),$$

where differently to reference (30) here the total surface area difference refers to the middle of the two monolayers (31). Note that the aqueous pore part has no curvature, hence the curvature term is reduced to $\beta_p - \beta_C = -\beta_C$. \(H = H_0 + 1/a\) is the membrane curvature inclusively the spontaneous curvature \(H_0 = H_0^{chem} + H_0^{cl}\), where $H_0^{chem}$ is the mean spontaneous curvature due to different chemical compositions of the two membrane leaflets and $H_0^{cl}$ is the electrical part of the spontaneous curvature, for example, at different electrolyte surroundings at the two membrane sides. If $H_0 = 0$, then, in the case of spherical vesicles, we have $H = 1/a$. Further on, $\kappa$ is the elastic module, $\alpha (=1)$ is a material constant (31), $\zeta$ is a geometric factor characterizing the pore conicity (18). It appears that the larger the curvature and the larger the $H_0^{cl}$ term, the larger is the energetically favorable release of the (transformed) Gibbs energy during the pore formation. The curvature term $\int \Delta \beta \, dH$ can be as large as a few kT per one pore (30). For small vesicles or small organelles and cells the curvature term is particularly important for the energetics of ME.

The effect of membrane curvature on ME has been studied with dye-doped vesicles of different size, that is, for different curvatures. At constant transmembrane potential drop (e.g., $\Delta \varphi_m = -0.3$ V), an increased curvature greatly increases the amplitude and rate of the absorbance dichroism, characterizing the extent of pore formation (Figs. 5A,B) (19,30). This observation was quantified in terms of the area difference elasticity (ADE) energy resulting from the different packing density of the lipid molecules in the two membrane leaflets of curved membranes (Fig. 5C) (31,32). Strongly curved membranes appear to be electroporated easier than planar membrane parts (4).

Different electrolyte contents on the internal and external sides of membranes with charged lipids cause different charge screening. This has become apparent when salt-filled vesicles were investigated by electrooptical and conductometrical methods. The larger the electrolyte concentration gradient across the membrane, the larger the turbidity dichroisms, characterizing the extent of pore formation and vesicle deformation (19). The effect of different charge screening on ME is theoretically described in terms of the surface
Fig. 5. The effect of vesicle size on the extent and rate of electroporation. The amplitudes of the absorbance dichroism $\Delta A/A_0$ (A) and (B) the relaxation rate $\tau^{-1}$ as functions of the vesicle curvature $H = 1/\alpha$ at constant total lipid concentration $[L_i] = 1.0 \text{ mM}$ and the same nominal transmembrane voltage drop $\Delta \phi_m^N = -1.5 \cdot \alpha \cdot E = -0.3 \text{ V.}$ The unilamellar vesicles are composed of L-α-phosphatidyl-L-serine (PS) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in the molar ratio PS : POPC of 1:2 doped with 2-[(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine (β - DPH pPC, $M_i = 782$); total lipid concentration $[L_T] = 1.0 \text{ mM}$; [β - DPH pPC$_T$] = 5 μM; 0.66 mM HEPES (pH = 7.4), 130 μM CaCl$_2$; vesicle density $\rho_V = 2.1 \cdot 10^{15} \text{ L}^{-1}$. Application of one rectangular electric pulse of the field strength $E$ and pulse duration $t_E = 10 \mu s$ at $T = 293 \text{ K (20°C)}$. (C) The membrane curvature is associated with a lipid packing difference between the two membrane leaflets and a lateral pressure gradient across the membrane. Membrane electroporation, causing conical hydrophobic (HO) pores, reduces the lipid packing density difference between the two monolayers and, consequently, the gradient of lateral pressure across the membrane.
potential drop $\Delta \phi$, see Eq. 4, and the electrical part of membrane spontaneous
curvature $H_0^{el}$.

Extending previous approaches (33,34), we obtain for a thin membrane
($d \ll a$), 1:1 electrolyte and for small values of the dimensionless parameter

$$s_{(i(o)} = e \cdot \sigma_{(i(o)} \cdot \frac{\ell_D^{(i)}}{(4\pi \cdot \varepsilon_{(i(o)} \cdot kT)} \ll 1,$$

that $H_0^{el}$ is given by:

$$H_0^{el} = \frac{2}{3} \cdot (s_i^2 - s_o^2) / (s_i^2 \cdot \ell_D^i + s_o^2 \cdot \ell_D^o),$$

where in the SI notation

$$\ell_D^{(i)} = \left[ \varepsilon_o \cdot \varepsilon_{(i(o)} \cdot kT / (2 \cdot \varepsilon^2 \cdot \ell_{(i(o)} \cdot N_A) \right]^{1/2}$$

is the explicit expression for the Debye screening length, $\varepsilon_{(i(o)}$ the
dielectric constant of the inner (i) and outer (o) medium, respectively. It has been found
that large salt concentration gradients across strongly curved charged mem-
branes permit electroporative efflux of electrolyte ions at surprisingly low
transmembrane potential differences, for instance $|\Delta \phi_m| = 37.5$ mV at a vesicle
radius of $a = 50$ nm and pulse durations of $t_E = 100$ ms compared with $|\Delta \phi_m|
\approx 500$ mV for planar noncurved membranes (11,35).

### 3.3. Electric Polarization Term

In the electric polarization term $\int \Delta r_M \cdot dE_m$, the electric reaction moment
$\Delta r_M = M_m(P) - M_m(C)$ refers to the difference in the molar dipole moments
$M_m$ of state C and P, respectively. The field-induced reaction moment in the
electrochemical model is given by (21):

$$\Delta r_M = N_A \cdot V_p \cdot \Delta r_P$$

(17)

where $V_p = \pi \cdot r_p^2 \cdot d$ is the average (induced) pore volume of the assumed
cylindrical pore.

Inspired by the physical analysis of Abidor et al. (35), we define the chemi-
ical reaction polarization as (19):

$$\Delta r_P = \varepsilon_0 \cdot (\varepsilon_W - \varepsilon_L) \cdot E_m,$$

(18)

The difference $\varepsilon_W - \varepsilon_L$ in the dielectric constants of water and of lipids, respec-
tively, refers to the replacement of lipids by water. Note that the possible dif-
fERENCE in the values of $E_m(C)$ and $E_m(P)$ is not too essential for the calculation
of $\Delta r_P$, because usually $\varepsilon_W \gg \varepsilon_L$, and $E_m(C) \approx E_m(P)$, thus we may approxi-
mate $\varepsilon_0 \cdot (\varepsilon_W(P) - \varepsilon_L \cdot E_m(C)) = \varepsilon_0 \cdot (\varepsilon_W - \varepsilon_L)E_m(P)$. In general, this approxima-
tion is valid only for small pores of radius <1 nm, which are not yet too conductive. Since $\varepsilon_W \gg \varepsilon_L$, the formation of aqueous pores is strongly favored.
in the presence of a cross-membrane potential difference $\Delta \phi_m = \Delta \phi_{\text{ind}} + \Delta \phi_e + \Delta \phi_{\text{nat}}$, in particular when the contribution $\Delta \phi_{\text{ind}}$ is large; see Eq. 3.

The final expression of the electrical energy term is obtained by sequential insertions and integration; explicitly at the angle $\theta$, we obtain (18,19):

$$
\int_0^{E_m} \Delta \phi \, dE_m = \frac{9 \pi \varepsilon_0 \cdot a^2 \cdot (\varepsilon_w - \varepsilon_L) \cdot \bar{r}_p^2 \cdot N_A \cdot f^2(\lambda_m) \cdot \cos^2 \theta \cdot E^2}{8 \cdot d},
$$

(19)

where we see that the polarization energy depends on the square of the field strength.

If the relation between $K$ and $E$ can be formulated as $K = K_0 \exp \left[ \frac{\Delta \phi_{\text{ind}}}{RT} \right]$, where $K_0$ refers to $E = 0$, Eq. 19 can be used to calculate the mean pore radius $\bar{r}_p$ from the field dependence of $K$ or of $f_p$ (the degree of poration). Typically, at $\Delta \phi_{\text{ind}} = -0.42$ V and pulse duration $t_E = 10 \mu$s, we obtain $\bar{r}_p = 0.35$ nm (19).

4. Membrane Electroporation and Cell Deformation

Besides direct visualization of porous patches and elongations of vesicles and cells in the direction of the external field, there are many electrooptical and conductometrical data on lipid vesicles filled with electrolyte which convincingly show that the external electric field causes membrane electroporation and electromechanical vesicle elongation (18). In the case of these vesicles the overall shape deformation under the field-induced Maxwell stress is associated with at least two kinetically distinct phases (11,12).

4.1. Electroporative Shape Deformation at Constant Volume

The initial very rapid phase (microsecond time range) is the electroporative elongation from the spherical shape to an ellipsoid in the direction of the field vector $E$. In this phase, previously called phase 0 (Fig. 6A) (4), there is no measurable release of salt ions. Hence the internal volume of the vesicle remains constant. Elongation is therefore only possible if, in the absence of membrane undulations in small vesicles, the membrane surface can be increased by ME. The formation of aqueous pores means entrance of water and thus increase in the overall membrane volume and surface. Thus, vesicle elongation is rapidly coupled to ME according to the scheme: $C \Rightarrow P < \Rightarrow >$ (elongation).

It is important, that the characteristic time constant $\tau_{\text{def}}$ of vesicle deformation is usually smaller than the $\theta$ average time constant of ME ($\tau \approx 0.5$ to 1 $\mu$s). Actually, for vesicles of radius $a = 50$ nm, a typical membrane bending rigidity of $\kappa = 2.5 \times 10^{-20}$ J and the viscosity of water $\eta = 10.05 \times 10^{-4}$ kg m$^{-1}$ s$^{-1}$ at 20°C, the upper limit of the shape deformation time constant at zero field is (36):
Fig. 6. Electroporative deformation of unilamellar lipid vesicles (or biological cells). (A) Phase 0: fast (µs) membrane electroporation rapidly coupled to Maxwell deformation at constant internal volume and slight (0.01–0.3%) increase in membrane surface area. Phase I: slow (milliseconds to minutes) electromechanical deformation at constant membrane surface area and decreasing volume due to efflux of the internal solution through the electropores. Maxwell stress and electrolyte flow change the pore dimension from initially \( r_p = 0.35 \pm 0.05 \) nm to \( r_p = 0.9 \pm 0.1 \) nm. (B) Membrane electroporation and shape deformation in cell tissue subjected to an externally applied electric field. The electrical Maxwell stress “squeezes” the cells, permitting drug and gene delivery to electroporated cells through the interstitial pathways between the cells into electroporated cells distant from the site of application of drug or genes. At \( E = 0 \), resealing and return to original shape occurs slowly.

\[
\tau_{\text{def}}(0) = 0.38 \cdot \eta \cdot \alpha^3/\kappa = 0.9 \, \mu\text{s}.
\]

It can be shown that in electric fields of typically \( 1 \leq E/\text{MVm}^{-1} \leq 8 \), the shape relaxation time constant \( \tau_{\text{def}}(E) \) is 100-fold smaller than \( \tau_{\text{def}}(0) \), say 10 ns (Kakorin et al., unpublished). Therefore, because \( \tau \gg \tau_{\text{def}}(E) \), it is the structural change of pore formation, inherent in ME, that controls not only the extent, but also the rate of the vesicle deformation in the phase 0. Vesicle and cell deforma-
tions, and thus ME, can be easily measured by electrooptic dichroism, either turbidity dichroism or absorbance dichroism. Proper analysis of the respective electrooptic data provides the electroporative deformation parameter \( p = c/b \), where \( c \) and \( b \) are the major and minor ellipsoid axis, respectively, of the vesicle or cell. Specifically, from \( p \) we obtain the \( \theta \) average degree \( \bar{\theta}_p \) of ME (4).

### 4.2. Shape Deformation at Constant Surface

In the second, slower phase (millisecond time range), previously called phase I (Fig. 6A) (4), there is an efflux of salt ions under Maxwell stress through the electropores created in phase 0, leading to a decrease in the vesicle volume under practically constant membrane surface (including the surfaces of the aqueous pores). The increase in the suspension conductivity, \( \Delta \lambda \lambda / \lambda_0 \), in the phase I reflects the efflux of salt ions under the electrical Maxwell stress through the electropores. The kinetic analysis in terms of the volume decrease yields the membrane bending rigidity \( \kappa = 3.0 \pm 0.3 \times 10^{-9} \) J. At the field strength \( E = 1.0 \) MV m\(^{-1}\) and in the range of pulse durations of \( 5 \leq t_E / \mu s \leq 60 \), the number of water-permeable electropores is found to be \( N_p = 35 \pm 5 \) per vesicle of radius \( a = 50 \) nm, with mean pore radius \( \bar{r}_p = 0.9 \pm 0.1 \) nm (11). This pore size refers to the presence of Maxwell stress causing pore enlargement from an originally small value (\( \bar{r}_p = 0.35 \pm 0.05 \) nm) under the flow of electrolyte through the pores.

### 4.3. Electroporative Deformation of Cells in Tissue

The kinetic analysis developed for vesicles may be readily applied to tissue cells. The external electric field in tissue produces membrane pores as in isolated single cells and the electric Maxwell stress squeezes the cells (Fig. 6B) (12). The electromechanical cell squeezing can enlarge preexisting, or create new, pathways in the intercellular interstitial spaces, facilitating the migration of drugs and genes from the periphery to the more internal tissue cells. The results of single vesicles or vesicle aggregates finally aim at physicochemical guidelines to optimize the membrane electroporation techniques for the direct transfer of drugs and genes into tissue cells.

### 5. Electroporative Transport of Macromolecules

It is emphasized again that the ion efflux from the salt-filled vesicles in an electric field is caused by membrane electroporation and by the hydrostatic pressure under Maxwell stress and that the electrooptic signals reflect electroporative vesicle deformations coupled to ME. The analysis of electrooptic dichroisms yields characteristic parameters of ME such as electri-
Fig. 7. The average fraction $\bar{f}_p$ of the electroporated membrane area, (■) at a large NaCl concentration difference (in the vesicle interior $[\text{NaCl}]_i = 0.2$ M, in the medium $[\text{NaCl}]_o = 0.2$ mM, osmotically balanced with 0.284M sucrose), (▲) at equal concentrations ($[\text{NaCl}]_i = [\text{NaCl}]_o = 0.2$ mM, smoothly increases with the field strength $E$, whereas the massive conductivity increase $\Delta \lambda^1 / \lambda_0$, (●) of the suspension of the salt filled vesicles of radius $a = 160 \pm 30$ nm ($\lambda_0 = 7.5$ $\mu$S cm$^{-1}$, $T = 293$ K (20°C)) (18) indicates an apparent threshold value $E_{\text{thr}} = 7$ MV m$^{-1}$. The ratio $\bar{f}_p = S(t_F) / S_m$ was calculated from the electrooptic relaxations, yielding characteristic rate parameters of the electroporation-resealing cycle in its coupling to ion transport.

cal pore densities for ion transport across the electroporated membrane patches. The fraction $\bar{f}_p$ of the electroporated membrane surface (derived from electrooptics) smoothly increases with the field strength (Fig. 7). In terms of the chemical model there is no threshold of the field strength (4,18). Experimentally there is always a trivial threshold when the actual data points emerge out of the margin of measuring error. The conductivity increase ($\Delta \lambda^1 / \lambda_0$) in the suspension of the salt filled vesicles however appears to have a “threshold value” of the field strength (Fig. 7). The large pore dimensions refer to the pores maintained by medium efflow under Maxwell stress or reflect fragmentation of a small (<1%) fraction of vesicles (U. Brinkmann et al., unpublished data).

5.1. Electroporative Transport of Ionic Macromolecules

The transport kinetics of larger macromolecules such as drugs and DNA indicates that there are several kinetically distinct stages. Transport is greatly facilitated if there is at first adsorption of the macromolecules to the membrane surface (10,24). For charged macromolecules, adsorption is followed by elec-
Fig. 8. Scheme for the coupling of the binding of a macromolecule (D), either a
dyelike drug or DNA (described by the equilibrium constant $K_D$ of overall binding),
electrodiffusive penetration (rate coefficient $k_{pen}$) into the outer surface of the membrane and translocation across the membrane, in terms of the transport coefficient $k_0$;
and the binding of the internalized DNA or dye molecule ($D_{in}$) to a cell component $b$
(rate coefficient $k_b$) to yield the interaction complex $D_b$, as the starting point for the
actual genetic cell transformation or cell coloring, respectively.

...trophoretic penetration into the surface of electroporated membrane patches.
Further steps are the afterfield diffusion, dissociation from the internal membrane surface and, finally, binding with cell components in the cell interior
(Fig. 8) (9,10).

5.1.1. Surface Adsorption

The transient adsorption of potential permeants on the membrane surface may change both the local surface structure and the local membrane composition (phase separation) in the outer membrane leaflet. The alterations of the molecular structure and redistributions of membrane components can lead to local changes in the membrane’s spontaneous curvature, bending rigidity and surface tension, respectively (31,32). Increased spontaneous curvature can either hinder or facilitate ME (30). For instance, the Ca$^{2+}$ mediated adsorption of the protein annexinV to anionic lipids increases the lipid packing density by insertion of the tryptophan side chain into the membrane surface. This in turn, reduces the electroporatability of the remaining membrane parts (30).
Alternatively, the adsorption of plasmid DNA on the membrane surface, mediated by calcium or sphingosine, obviously facilitates ME and thus the transport of small ions (leak) and DNA itself across the membrane (10,37,38).

The degree of transformation $f_T$ of yeast cells by plasmid DNA as a function of pulse duration is characterized by a long “delay phase” (Fig. 9A) (10). The delay phase gets shorter with increasing field strength. The degree $f_C$ of cell coloring of B cells by dye SERVA blue G exhibits a similar functional dependence as $f_T$ of yeast cells (Fig. 9B) (9).
Fig. 9. Kinetics of the electroporative uptake of DNA and dye. (A) Degree of transformation $f_T$ of yeast cells by plasmid DNA ($M_r = 3.5 \cdot 10^6$) and (B) degree of coloring $f_C$ of mouse B cells by druglike dye SERVA blue G ($M_r = 854$) as a function of pulse duration at different field strengths: $E_0 / \text{kV cm}^{-1} = 2.5$ (●); 3.0 (○); 3.25 (□); 3.5 (●); 4.0 (■), for cell transformation, and $E / \text{kV cm}^{-1}$: (○) 0.64; (●) 0.85; (□) 1.06; (■) 1.28; (▲) 1.49; (▲) 1.7; (▲) 1.91; (▲) 2.13, for cell coloring, respectively. $E_0$ is the amplitude and $\tau_{E_0}$ is the characteristic time constant of an exponential pulse used for the transformation of yeast cells by plasmid DNA ($M_r = 3.5 \cdot 10^6$). $E$ is the amplitude and $t_E$ is the duration of the rectangular pulse used for the coloring of mouse B cells by the (druglike) dye SERVA blue G ($M_r = 854$).

5.1.2. Flow Equation for Drug and DNA Uptake

The similarities of cell transformation and cell coloring suggest that the mechanism for the electroporative transport of both genes and drugs into
the cell interior has essential features in common. Therefore a general formalism was developed for the electroporative uptake of drug and genes.

In line with Fick’s first law, the radial inflow (vector) of macromolecules is given by:

$$\frac{dn^i_c}{dt} = -D_m \cdot S_m \cdot \frac{dc_m}{dx},$$

(20)

where $n^i_c$ is the molar amount of the transported molecule in the compartment volume $V_c$, $c_m$ and $D_m$ are the concentration and the diffusion coefficient of the permeant in the membrane phase, respectively, $S_m$ is the membrane surface through which the diffusional translocation occurs. The concentration gradient within the membrane is usually approximated by:

$$dc_m/dx = (c^o_m - c^i_m)/d,$$

(21)

where $c^o_m$ and $c^i_m$ are the concentrations of the permeant in the outer and inner membrane/medium interfaces, respectively (Fig. 10). The partition of the permeant between the bulk solution and the membrane surfaces may be quantified by a single distribution constant according to: $\gamma = c^o_m/c^o = c^i_m/c^i$, where $c^o_m$ and $c^i_m = n^i_c/V_c$ are the bulk concentrations inside and outside the cell (or vesicle), respectively. We now define a flow coefficient $k_f$ for the transmembrane transport:

$$k_f = \frac{\gamma \cdot D_m \cdot S_m}{d} = \frac{P_m \cdot S_m}{V_c},$$

(22)

where the permeability coefficient $P_m$ for the perfused membrane patches is given by:

$$P_m = \frac{\gamma \cdot D_m}{d} = k_f \cdot \frac{V_c}{S_m}.$$  

(23)

$P_m$ can be calculated from the experimental value of $k_f$, provided $S_m$ is known. Substitution of Eqs. 21 and 23 into Eq. 20 yields the linear inflow equation:

$$dc^{in}/dt = -k_f \cdot (c^{out} - c^{in}).$$

Frequently, the external volume $V_0$ is much larger than the intracellular or intravesicular volume, that is, $N_c \cdot V_c << V_0$, where $N_c$ is the number of cells or vesicles in suspension. Mass conservation dictates that the amount $n^{out}$ of permeant in the outside volume is given by $n^{out} = n^i_c - n^{in}_c \cdot N_c$. Hence the inequality $N_c \cdot V_c << V_0$ yields: $c^{out} = n^{out}/V_0 = c_0 - c^{in} \cdot N_c \cdot V_c / V_0 \approx c_0$, where $n_0$ and $c_0 = n_0/V_0$ are the initial amount and the initial total concentration of
Fig. 10. Profile of concentration of a lipid-soluble or surface adsorbed permeant across the lipid plasma membrane of the thickness $d$, between the outer (out) and inner (in) cell compartments, respectively, in the direction $x$. Because of adsorption of permeant on the cell surface, the bulk concentrations $c_{\text{out}}$ and $c_{\text{in}}$ of the permeant are smaller than $c_{m}^{\text{out}}$ and $c_{m}^{\text{in}}$, respectively; $c_{m}$ refers to the very small volume of a shell with thickness $\emptyset$, where $\emptyset$ is given by the diameter of the flatly adsorbed DNA, sketched as double-helical backbones. For the data in Fig. 9A, the distribution constant is $\gamma = c_{m}^{\text{out}}/c_{m}^{\text{in}} = 1.3 \cdot 10^{3}$.

the permeant in the outside volume, respectively. Substitution of the approximation $c_{\text{out}} = c_{0}$ into the flow equation yields the simple transport equation:

$$\frac{dc_{\text{in}}}{dt} = -k_{f} \cdot (c_{0} - c_{\text{in}})$$

(24)

If the effective diffusion area $S_{m}$ changes with time, for instance, due to electroporation-resealing processes, the flow coefficient $k_{f}(t)$ is time-dependent. In this case we may specify $S_{m}(t)$ with the degree of electroporation $f_{p}$ according to $S_{m}(t) = f_{p}(t) \cdot S_{c}$, where $S_{c} = 4\pi \cdot a^{2}$ is the total area of the outer membrane surface. The explicit form of the pore fraction $f_{p}(t)$ is dependent on the model applied. The time dependent flow coefficient can now be expressed as: $k_{f}(t) = k_{f}^{0} \cdot f_{p}(t)$, where the characteristic flow coefficient for the radial inflow is defined by
\[ k_f^0 = \frac{P_m \cdot S_e}{V_e} = \frac{3 \cdot P_m}{a}. \]  

(25)

Note that \( k_f^0 \) and thus \( P_m \) are independent of the electrical pulse parameters \( E \) and \( t_E \). Hence these transport quantities are suited to compare vesicles and cells of different size and different lipid composition. Substitution of \( k_f(t) = k_f^0 \cdot f_p(t) \) into Eq. 24 and integration yields the practical equation for the increase in the internal permeant concentration with time:

\[ c^{\text{in}} = c_0 \cdot \left( 1 - \exp \left[ -k_f^0 \int_0^{t_E} f_p^C \to P(t) \, dt + \int_{t_E}^{t_{\text{obs}}} f_p^P \to C(t) \, dt \right] \right). \]  

(26)

If the transported molecules are added before the pulse, we have \( t_0 = 0 \). For the postfield addition the first integral for \( f_p^C \to P \) in Eq. 26 cancels and we set \( t_E = t_0 = t_{\text{add}}, \) where \( t_{\text{add}} \) is the time point of adding the molecules after pulse termination (\( t_E \)). Usually, the appearance of the transported molecules becomes noticeable at observation times \( t_{\text{obs}} \) which are much larger (min) than the characteristic time of pore resealing \((k_{-1})^{-1}\) which is in the milliseconds to seconds time range. For these cases the approximation \( t_{\text{obs}} \to \infty \) holds (9,10). Note that the integrals in Eq. 26 contain implicitly the pulse duration \( t_E \) and the field strength \( E \) in the degree of poration \( f_p(t,t_E,E) \).

In the case of charged macromolecules like DNA or the dye SBG, the presence of an electric field across the membrane causes electrodifusion. The enhancement of the transport of a macroion only refers to that side of the cell or vesicle where the electric potential drop \( \Delta \Phi_m \) is in the favorable direction. The electrodifusive efflux of the macromolecules from the cell cytoplasm is usually negligibly small compared with the influx and may be neglected. Formally, for the boundaries \( t_0 \) and \( t_E \), \( D_m \) in Eq. 26 must be replaced by the electrodifffusional coefficient (10):

\[ D_m(E) = D_m \cdot \left( \frac{1 + |z_{\text{eff}}| e_0 \cdot \Delta \Phi_m}{kT} \right), \]  

(27)

where \( \Delta \Phi_m = -(3/8) a \cdot f(\bar{\lambda}_m) \) is the \( \theta \) average transmembrane potential drop, \( \bar{\lambda}_m \) the angular average of the membrane conductivity and \( z_{\text{eff}} \) the effective charge number (with sign) of the transported macromolecule.

On the same line, the permeability coefficient with respect to electrodiffusion is given by:

\[ P_m(E) = \frac{\gamma \cdot D_m(E)}{d}. \]  

(28)

It is instructive to compare the present analysis of (electro) diffusion through porous membrane patches characterized by the quantities \( k_f^0, P_m, \) and \( f_p \) with the conventional approach with the permeability coefficient \( P \) in the context of
formally \( f_p = 1 \). The conventional coefficient \( P \) is related to \( P_m \) of the present analysis by: 
\[
P = f_p(t_E) \cdot P_m. \tag{29}
\]
The analysis of the kinetic data of cell transformation and cell coloring by dyes (Fig. 9) suggests that the rate-limiting step is the binding of the permeants to intracellular components. The simplest binding scheme is given by (see Fig. 8):
\[
D^{in} + b \xrightarrow{k_b} D_b, \tag{29}
\]
where \( D^{in} \) symbolizes the macromolecules in the cell interior \( (c^{in}) \), \( b \) the yet unoccupied binding sites in the cell and \( k_b \) is the overall rate coefficient of binding. The degree of binding of molecule \( D \) is defined by:
\[
f_b = \frac{[D_b]}{[b_0]}, \tag{30}
\]
where \([D_b] \) is the concentration of bound maromolecules and \([b_0] \) is the total concentration of binding sites in the cell interior.

The integration of the binding rate equation \( d[D_b]/dt = k_b \cdot c^{in} \cdot [b] \) for the Eq. 29, and substitution of Eq. 26 yields (10):
\[
f_b(t_E, t_{obs}) = \frac{c^{in} \cdot [1 - \exp A]}{[b_0] - c^{in} \cdot \exp A}, \tag{31}
\]
where the dependence on \( t_E \) and \( t_{obs} \) is explicitly in \( c^{in}(t_E, t_{obs}) \) and
\[
A(t_E, t_{obs}) = k_b \cdot t_{obs} \cdot (c^{in}(t_{obs}, t_E) - [b_0]).
\]

For the cell transformation the time of observation is \( t_{obs} \approx 2 \) hours. Note that \( c^{in}(t_E, t_{obs}) \) refers to the total amount of the transported molecule which enters the cell interior in the time interval \( t_0 \leq t \leq t_{obs} \) when a pulse of duration \( t_E \) was applied. In a previous study the equation for \( f_b \) contains a misprint (10).

As previously suggested (24), the degree of transformation \( f_T = T/T_{max} \), where \( T_{max} \) is the maximum number of transformants, may be equated with the degree of bound molecules \( f_b \). Hence the data analysis uses \( f_{T/C} = f_b \) and Eq. 31. Obviously, at least one binding site \( b \) has to be occupied with DNA to permit transformation. In the following we present the reevaluation of previous data in terms of the transport parameters \( k_0^0, P_m, \) and \( f_p \).

5.2.1. Uptake of DNA by Yeast Cells

For an efficient uptake, DNA should be present, preferably adsorbed already before pulse application. Both the adsorption of DNA, directly measured with \(^{32}\)P-dC DNA, and the number of transformants are collinearly enhanced with increasing total concentrations \([D_i]\) and \([Ca_i]\) of DNA and of \(Ca^{2+}\), respectively. At the total bulk concentration \([D_i] = 2.7\) nM, the molar concentration of DNA bound to the membrane surface amounts to \([D_b] = 2\) nM.
(10). At the cell density \( \rho_c = 10^9 \text{ cm}^{-3} \), there are \( N_{\text{DNA}} = N_A \cdot \frac{[D_b^S]}{\rho_c} = 1.2 \cdot 10^3 \) DNA molecules per cell of radius \( a = 2.7 \mu\text{m} \). Presumably all adsorbed DNA is located in the head group region of the outer leaflet of membrane bilayer. The actual concentration of DNA in the membrane surface refers to a thin layer of thickness \( \theta = 2.37 \text{ nm} \), where \( \theta \) is the diameter of the \( \beta \) helix of DNA. We obtain \( c_{\text{out}} = \frac{[D_b^S]}{(\rho_c \cdot S_c \cdot \theta)} = 9.2 \mu\text{M} \) (Fig. 10). Since the bulk concentration of DNA is \( c_{\text{out}} = [D_b^S] = 0.7 \text{ nM} \), the partition coefficient amounts to \( \gamma = c_{\text{out}} / c_{\text{out}} = 1.3 \cdot 10^3 \); that is, the concentration of the absorbed DNA is about \( 10^3 \)-fold larger than the bulk concentration. This feature was not considered so far and requires a partial reevaluation of previous data (10). Fig. 9A, where it was found that the direct electroporative transfer of plasmid DNA (YEp 351, 5.6 kbp, supercoiled, \( M_r = 3.5 \cdot 10^6 \)) in yeast cells (Saccharomyces cerevisiae, strain AH 215) is basically due to (electro) diffusive processes. At the field strength \( E_0 = 4.0 \text{ kV cm}^{-1} \), the diffusion coefficient ratio is \( D_{\text{in}}(E) / D_{\text{m}} \approx 10.3 \). Hence electrodiffusion of DNA is about 10 times more effective than simple diffusion. Addition of DNA after the field pulse only occasionally leads to transformants. The most decisive stage in the cell transformation is the electrodiffusive surface penetration of DNA followed either by further electrodiffusive, or by passive (after field) diffusive, translocation of the inserted DNA into the cell interior (Fig. 8).

Actually, the rather long sigmoid phase of \( f_T(t_E) \), Fig. 9A, requires a description in terms of an at least two-step process: \( C \xrightarrow{k_p} P_1 \xrightarrow{k_p} P_2 \), where the state \( P_1 \) denotes pore structures of negligible permeability for DNA; \( P_2 \) is the porous membrane state of finite permeability for DNA. The electroporation rate coefficient \( k_p \) is assumed to be the same for both steps, associated with the same reaction volume \( \Delta_r V_p \). This assumption is theoretically justified by the corresponding minima in the hydrophobic force profiles as a function of pore radius (39). Pore resealing, that is, the reverse reaction steps \( (P_2 \rightarrow P_1 \rightarrow C) \), may be neglected for the time range \( 0 \leq t \leq t_E \) in the presence of the external field. We recall that \( k_p \) explicitly occurs in the integral:

\[
\int_0^{t_E} f_{P}^{\rightarrow P}(t) \, dt = f_{P}^{0} \cdot \{ t_E + k_p^{-1} [(2 + k_p \cdot t_E) \cdot e^{-k_p \cdot t} - 2] \},
\]

where \( f_{P}^{\rightarrow P} = f_{P}^{0} \cdot \{ 1 - (1 + k_p \cdot t) \cdot e^{-k_p \cdot t} \} \) for the reaction \( (P_2 \rightarrow P_1 \rightarrow C) \) and \( f_{P}^{0} \) is the amplitude value of \( f_{P}^{\rightarrow P}(t) \). Applying Eq. 31 for the exponential pulse of the initial field strength \( E_0 = 4.0 \text{ kV cm}^{-1} \) and the decay time constant \( \tau_E = 45 \text{ ms} \), we find with \( t_E = \tau_E \) that \( k_p = 7.2 \text{ s}^{-1} \).

The mean minimum radius of DNA-permeable pores has been calculated from the field dependence of \( k_p(E_0) \): \( \bar{r}_p(P_2) = 0.39 \pm 0.05 \text{ nm} \) (10). If we assume that deviations of the data points from the relationship
\[ \ln \left( \frac{k_p}{k_p(E = 0)} \right) = b^* \cdot \cos^2 \theta \cdot E^2, \]

where

\[ b^* = (9/8) \cdot \pi \cdot \varepsilon_0 \cdot a^2 \cdot (\varepsilon_w - \varepsilon_l) \cdot \bar{r}_p^2 \cdot N_A \cdot f^2(\bar{\lambda}_m) \cdot E^2 / (d \cdot kT) \]

at higher field strengths is due to the increase in the average transmembrane conductivity by \( \Delta \bar{\lambda}_m = 2.5 \cdot 10^{-7} \text{ S cm}^{-1} \) from \( \bar{\lambda}_m(E_0 = 0) \) to \( \bar{\lambda}_m(E_0 = 4 \text{ kV cm}^{-1}) = \bar{\lambda}_m(E_0 = 0) + \Delta \bar{\lambda}_m \). This conductivity increase corresponds to a replacement of 0.0025% of the membrane area by pores filled with the intracellular medium of conductivity \( \lambda_i = 1.0 \cdot 10^{-2} \text{ S cm}^{-1} \) under Maxwell stress. The fractional increase in the transport area for small ions (Na\(^+\), Cl\(^-\)) is given by \( f_p^i = \Delta \bar{\lambda}_m / \lambda_i = 2.5 \cdot 10^{-5} \) (15). For these conditions the mean number of conductive pores per cell is \( \bar{N}_p = S_c \cdot f_p / \pi \cdot \bar{r}_p^2 = 4.8 \cdot 10^3 \), corresponding to an average minimum distance between the pore centers \( \bar{r}_p = (S_c / N_p)^{1/2} = 138 \text{ nm} \). In order to estimate the permeability coefficient \( P_m \) of DNA, one may identify the fraction \( f_p \) of DNA permeable membrane area (pore state \( P_2 \)) with that of small ions: \( f_p = f_p^i \). If the DNA permeable membrane area is smaller than the area of ion permeable pores: \( f_p < f_p^i \), we obtain only an upper limit of \( P_m \) for DNA.

Apparently, the mean radius \( \bar{r}_p(P_2) = 0.39 \text{ nm} \) of the pores in DNA-permeable pore patches is too small for free diffusion of large plasmid DNA. Such a small pore radius is not even sufficient for the entrance of a free end of a linear DNA molecule, because the diameter of the type B-DNA is \( \varnothing \approx 2.37 \text{ nm} \). Nevertheless, small parts of the adsorbed DNA may interact with many small pores, and the DNA-polymer may penetrate part by part into the membrane. The total length of a 6.5 kbp DNA is about \( \ell_{DNA} = 6.5 \cdot 10^3 \cdot 0.34 \text{ nm} = 2.2 \cdot 10^3 \text{ nm} \) and the corresponding surface area on the membrane is \( S_{DNA} = a_{DNA} \cdot \varnothing = 5.2 \cdot 10^3 \text{ nm}^2 \). On average, one totally adsorbed DNA may cover only \( 4 \cdot N_p \cdot S_{DNA} / S_c \approx 1 \text{ membrane electropore in the cell pole caps} \) (see Fig. 4). Since the DNA is probably only partially inserted into porous patches, the regions can be considered as closed, but leaky. If the occlusions locally decrease the membrane conductivity, the transmembrane field gets larger such that the membrane somewhere in the vicinity of the inserted DNA part is electroporated. As a consequence, a neighboring part of DNA can penetrate into the newly porated membrane patch. In any case the interaction of the adsorbed DNA with the lipid membrane appears to largely facilitate ME, yielding larger transiently occluded pores. Leaky porelike channel structures are indicated by ionic current events if DNA interacts with lipid bilayers. Furtheron, if DNA is present in the medium, there is a sharp increase in the membrane permeability of Cos-1 cells to fluorescent dextrin molecules in the electric field (40).
The reevaluation of the data (Fig. 9) for $E_0 = 4.0$ kV cm$^{-1}$ and $t_E = \tau_E = 45$ ms yields $k_f = 2 \cdot 10^2$ s$^{-1}$. With $f_p(t_E) \approx f_p^i = 2.5 \cdot 10^{-5}$ the characteristic flow coefficient is $k_f^0 = \gamma \cdot D_m(E) \cdot S_c / d \cdot V_c = 8.0 \cdot 10^6$ s$^{-1}$ at $T = 293$ K. From Eq. 23 we obtain the corresponding permeability coefficient $P_m = k_f^0 \cdot a / 3 = 7.2 \cdot 10^2$ cm s$^{-1}$. Because $D_m(E) = D_m \cdot 10.3$, we see that at $E = 0$ formally $P_m = P_m / 10.3 = 70$ cm s$^{-1}$. Note that the conventional membrane permeability coefficient $P_0$ refers to the total membrane surface area by $P_0 = P_m \cdot f_p(t_E) = 1.8 \cdot 10^{-3}$ cm s$^{-1}$. With $\gamma = 1.3 \cdot 10^3$ and $d = 5$ nm, the electrodiffusion coefficient $D_m(E)$ of DNA in the electroporated membrane patches at $E = 4$ kV cm$^{-1}$ is $D_m(E) = P_m d / \gamma = 2.8 \cdot 10^{-7}$ cm$^2$ s$^{-1}$, and at $E = 0$ we have $D_m = D_m(E) / 10.3 = 2.7 \cdot 10^{-8}$ cm$^2$ s$^{-1}$. If the diffusion of DNA is formally related to the total membrane surface (electroporated patches and the larger nonelectroporated part), $D = D_m \cdot f_p(t_E) = 6.7 \cdot 10^{-13}$ cm$^2$ s$^{-1}$. Compared with the diffusion coefficient of free DNA in solution $D^\text{free} = 5 \cdot 10^{-8}$ cm$^2$ s$^{-1}$ (41), the bulk diffusion is about $7 \cdot 10^4$-fold faster than the interactive diffusion of DNA through the electroporated membrane, reflecting the occluding interaction of DNA with perhaps many small membrane electropores.

For practical purposes of optimum transformation efficiency, 1 mM Ca$^{2+}$ is necessary for sufficient DNA binding and the relatively long pulse duration of 20–40 ms is required to achieve efficient electrodiffusive transport across the cell wall and into the outer surface of electroporated cell membrane patches.

5.2.2. Uptake of Druglike Dyes by Mouse B Cells

The color change of electroporated intact FcγR$^+$ mouse B cells (line II A1.6, cell diameter 25 μm) after direct electroporative transfer of the drug-like dye Serva Blue G (SBG) ($M_r = 854$) into the cell interior is shown to be prevalently due to diffusion of the dye after the electric field pulse (9). The net influx of the dyes ceases, even if the pores stay open, when the concentration equality $c_{\text{in}} = c_0$ is attained. For this limiting case, the fraction $f_c = c_{\text{in}} / c_0$ of the colored cells equals unity. The data in (Fig. 9) suggest that at least three different pore states ($P$) in the reaction cascade $C \iff P_1 \iff P_2 \iff P_3$ are required to model the sigmoid kinetics of pore formation as well as the biphase pore resealing. The rate coefficient for pore formation $k_p$ was taken equal for all the three steps: $C \iff P_1$, $P_1 \iff P_2$, and $P_2 \iff P_3$. At $E = 2.1$ kV cm$^{-1}$ and $T = 293$ K, we find from the respective integrals $\int f_p^C(t) \, dt$ that $k_p = 2.4 \pm 0.2 \times 10^3$ s$^{-1}$. The resealing rate coefficients are $k_{-2} = 4.0 \pm 0.5 \times 10^{-2}$ s$^{-1}$ and $k_{-3} = 4.5 \pm 0.5 \times 10^{-3}$ s$^{-1}$, independent of $E$ as expected for $E = 0$. Analysis of the field dependence of $k_p(E)$ yields the mean radius of the dye permeable pore state $r(P_3) = 1.2 \pm 0.1$ nm (9).

The maximum value of the fractional surface area of the dye-conductive pores is approximated by the fraction of conductive pores: $f_p = \Delta \lambda_m / \lambda_i =$
1.0 \cdot 10^{-3}$, where $\Delta \lambda_m = 1.3 \cdot 10^{-5}$ S cm$^{-1}$ is the increase in the transmembrane conductivity at $E = 2.1$ kV cm$^{-1}$ and $\lambda_i = 1.3 \cdot 10^{-2}$ S cm$^{-1}$. Hence the maximum number of dye permeable pores is $N_p = S_c \cdot f_p / \pi \cdot r_p^2 (P_3) = 4.4 \cdot 10^5$ per average cell, where $S_c = 4 \cdot \pi \cdot a^2 = 2.0 \cdot 10^{-5}$ cm$^2$. Data reevaluation yields $k_f = 1 \cdot 10^{-2}$ s$^{-1}$. From $k_f(t) = k_f^0 \cdot f_p(t)$ we obtain the characteristic flow coefficient $k_f^0 = (1.0 \pm 0.1) \cdot 10^1$ s$^{-1}$. Since there is no evidence for adsorption of SBG on the membrane surface, the partition coefficient was assumed to be $\gamma = 1$. The corresponding permeability coefficient of dye in the pores is: $P_m = k_f^0 \cdot a/3 = 4.2 \cdot 10^{-3}$ cm s$^{-1}$. If the permeability coefficient is related to the total membrane surface area, we obtain $P = P_m \cdot f_p = 4.2 \cdot 10^{-6}$ cm s$^{-1}$. The diffusion coefficient of SBG is $D_m = P_m \cdot d = 2.1 \cdot 10^{-9}$ cm$^2$ s$^{-1}$ and $D = D_m \cdot f_p = 2.1 \cdot 10^{-12}$ cm$^2$ s$^{-1}$, respectively. It is seen that $D_m$ is by the factor $D_{\text{free}} / D_m = 2.4 \cdot 10^{-5}$ smaller than $D_{\text{free}} = 5 \cdot 10^{-6}$ cm$^2$ s$^{-1}$ estimated for free dye diffusion. This large difference apparently indicates transient interaction of the dye with the pore lipids during translocation and partial occlusion of the pores.

### 5.3. Field–Time Relationship for the Electroporative Transport

Obviously the two pulse parameters $E$ and $t_E$ are of primary importance to control extend and rate of the transmembrane transport. Within certain ranges of $E$ and $t_E$ a relationship of the type $E^2 \cdot t_E = c$ holds (Fig. 11), where $c$ is a constant ($9,10,26$). However, very large field strengths or very long pulse durations may lead to secondary effects like bleb formation ($9$) or fragmentation of the vesicles and cells under Maxwell stress. Therefore in the range of massive cell deformation and fragmentation the constant $c$ has a different value than in the range of short pulse durations. In any case, the empirical correlation $E^2 \cdot t_E = \text{constant}$ is theoretically rationalized in terms of the interfacial polarization mechanism of ME ($24,26$).

### 6. Summary and Conclusions

Since the electroporative transport of permeants is caused by ME, the transport quantities $f_T(t)$ and $f_C(t)$ are closely connected to the degree $f_p(t)$ of ME, permitting to investigate the mechanism of formation and development of membrane pores by the electric field. The results of our theoretical approach, based on electrooptical data of vesicles, as well as on the kinetics of cell electrotransformation and cell coloring, can be used to specify conditions for the practical purposes of gene transfer and drug delivery into the cells. In electrochemotherapy, for instance, the optimization of the electroporative channeling of the cytotoxic drugs into the tissue cells may be refined by using the electroporative transport theory ($4,42–44$). Future work may include optical probes like DPH in cell plasma membranes to elucidate the sequence of events of the electroporative DNA and protein transfers as well as to investigate
Fig. 11. Field strength/pulse duration relationship. The data refer to the selected fraction $f$ of (A) transformed ($f_T = 0.5$) and (B) colored cells ($f_C = 0.5$). Experimental parameters as in Fig. 9. The linear dependencies are consistent with the interfacial electric polarization mechanism ($E^2 \cdot t_E = c$) preceding cell membrane electroporation.

molecular details of other electroporation phenomena such as electrofusion and electrotclusion.

In conclusion, the theory of ME has been developed to such a degree that analytical expressions are available for the optimization of the ME techniques in biotechnology and medicine, in particular in the new fields of electroporative drug delivery and gene therapy. The electroporative gene vaccination is certainly a great challenge for modern medicine.

**Acknowledgments**

We thank the Deutsche Forschungsgemeinschaft for grant Ne 227/9-2 to E. Neumann.

**Glossary**

<table>
<thead>
<tr>
<th>SBG</th>
<th>Serva Blue G</th>
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<td>$[\text{Ca}_t]$</td>
<td>total concentration of Ca</td>
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\([\mathcal{D}_d] = c^0\) total concentration of DNA
\([\mathcal{D}_b]\) concentration of bound DNA
\([\mathcal{P}_2]\) concentration of DNA-permeable pores
\([\mathcal{P}_3]\) concentration of SBG-permeable pores
\(a\) cell/vesicle radius
\(c^\text{out}_{m}, c^\text{in}_{m}\) molar concentrations of the permeant in the outer and inner membrane/medium interfaces, respectively
\(c^\text{out}, c^\text{in}\) bulk concentrations of the permeant inside and outside the cell (or vesicle), respectively
\(c_0\) initial total concentration of permeant in the outside medium
\(D_m\) diffusion coefficient in electroporated membrane patches
\(D_m(E)\) electrodiffusion coefficient in electroporated membrane patches
\(D\) diffusion coefficient related to the total membrane surface area
\([\mathcal{D}_b]\) concentration of bound macromolecules to the intracellular sites
\([\mathcal{D}_b]\) concentration of bound macromolecules to the membrane surface
\(E\) electric field strength
\(E_m\) transmembrane field strength
\(e\) elementary charge
\(\varepsilon_0\) vacuum permittivity
\(\varepsilon_w\) dielectric constant of water
\(\varepsilon_L\) dielectric constant of the lipid phase
\(f_T\) degree of cell transformation
\(f_C\) degree of cell coloring
\(f_B\) degree of binding of permeants to intracellular sites
\(f_P\) fraction of porated membrane area
\(f(\lambda_m)\) conductivity factor
\(\gamma\) partition coefficient of permeant between membrane and solution
\(\Delta\phi_{\text{in}}\) electrical potential difference across the electroporated membrane patches
\(k_1\) rate coefficient for the step \(\text{C} \rightarrow \text{P}\)
\(k_{-1}\) rate coefficient for the step \(\text{P} \rightarrow \text{C}\)
\(k\) Boltzmann constant
\(k_b\) rate coefficient for intracellular permeant binding (\(\text{M}^{-1} \text{s}^{-1}\))
\(k_p\) electroporation rate coefficient (\(\text{s}^{-1}\))
\(k_f\) flow coefficient for cross-membrane transport (\(\text{s}^{-1}\))
\(k_f^\text{ch}\) characteristic flow coefficient (\(\text{s}^{-1}\)), independent of \(E\) and \(t_E\)
\(\lambda_m\) transmembrane conductivity (\(\text{S} \text{m}^{-1}\))
\(\lambda_0\) conductivity of bulk solution
\(\lambda_i\) conductivity of cell interior
\(N_p\) number of electropores per cell
\(n_c\) molar amount of DNA or SBG in one cell
\[ n_{\text{out}} \] molar amount of DNA or SBG in the bulk solution

\[ P_m \] permeability coefficient for the electroporated membrane patches

\( P \) conventional permeability coefficient (related to the total membrane)

\( \bar{r}_p \) mean pore radius

\( \rho_c \) cell density

\( S_c \) cell surface area

\( S_m \) electroporated area of cell surface

\( S_p \) surface area of the average pore

\( \tau_E \) electrical pulse duration

\( \tau_E \) decay time constant of an exponentially decaying field pulse

\( V_c \) volume of an average cell

\( V_0 \) external volume

\( z_i \) charge number (with sign) of ion \( i \)

\( z_{\text{eff}} \) effective charge number of the DNA-phosphate group

References


27. Neumann, E. (1986) Elementary analysis of chemical electric field effects in


