I. Introduction

Membrane electroporation describes the transient, reversible permeabilization of the membranes of cells, organelles, or lipid bilayer vesicles by electric field pulses; for review see Neumann et al. (1989). Electroporation not only renders membranes transiently permeable and leads to material exchange across membranes, but also induces and facilitates fusion of membranes in contact; for review see Sowers (1987). There is an increasing number of practical applications of the electroporation technique, for instance:
- Direct transfer of genes, other nucleic acids, proteins, and other molecules into all types of cells and microorganisms.
- Electrofusion of cells.
- Electrostimulation of cell growth and proliferation.
- Electroinsertion of membrane proteins.

Contrary to the impressive success of the electroporation techniques in cell biology, biotechnology, and medicine (gene therapy), the molecular membrane processes of electroporation are not yet well understood. Data analysis and optimization of conditions for the electrotransfer of DNA and of proteins, electrofusions, electrostimulation, and electroinsertion are still primarily empirical.

Since it is primarily the lipid part of biological membranes that is electroporated, lipid bilayer vesicles may be used as a model system to study the elementary processes of the electroporation phenomena.

Recent electrooptic and conductometric data are consistent with electric field-induced changes in the membrane structure of the vesicles. The lipids rearrange in such a way that optically anisotropic light scattering centers appear in the lipid phase of the vesicles. These scattering centers may be identified with geometrically anisotropic pores, which conduct alkali salt ions and other substances if, at given field strengths and pulse durations, a critical pore size is reached.

The relaxation kinetic data are the basis for the interpretation of a variety of observations accumulated in previous studies on membrane permeabilization, cell electrotansfection, and electrofusion. Deeper insight is gained on the stimulus/duration curve, on the electrosensitivity of cells, on the dependences of cell transformations on DNA and cell concentrations, and various other parameters.

II. Electrooptic and Conductometric Relaxations of Lipid Bilayer Vesicles

Recent progress in relaxation kinetics in the presence of high electric field strengths has provided new ways to study structural changes in membrane systems. We have developed an electric field-jump relaxation spectrometer that permits the simultaneous measurements of field pulse-induced changes in electric conductance and in optical properties of solutions and suspensions in the nanosecond to millisecond time range with high resolution. Rectangular field pulses up to 150 kV cm\(^{-1}\) and of variable pulse duration (0.5 µs to 1 ms) can be applied by cable discharge in a single DC-pulse mode.
A. Relaxation Data

Figure 1 shows that there are up to three conductance modes $\Delta\lambda/\lambda(0)$ and up to two electrooptic scattering–dichroitic relaxation modes.

Mode I of the conductance relaxation clearly has the feature of a displacement current, as expected for the interfacial ionic polarization preceding, as an exponen-
Figure 2 Linear scattering dichroism and relaxation rates ($\tau^{-1}$) as a function of the field strength: $\Delta S = \Delta S_\parallel - \Delta S_\perp$, where $\Delta S_\parallel$ is the light intensity change observed with polarized light parallel to the direction of the applied electric field and $\Delta S_\perp$ is the signal of the perpendicular light polarization mode. See legend of Fig. 1. Note the positive value of $\Delta S$ of mode I and the negative value of $\Delta S$ of mode II. The relaxation rates of the conductivity relaxation mode I are the same as those of the optical model I, and the rates of mode III and the $S(\Pi)$ mode are roughly the same.

\[ \Delta S = \Delta S_\parallel - \Delta S_\perp \]

Eventually decaying forcing function, the subsequent conductance relaxations II and III.

Mode I of the light-scattering dichroism, defined as $\Delta S = \Delta S_\parallel - \Delta S_\perp$ (see Fig. 1), has the same relaxation rate ($\tau^{-1}$) as the interfacial polarization current, at all field strengths (Fig. 2). The optical change thus seems to be caused by, and rate-limited by, the buildup of the electric potential difference across the membrane.

At higher field strengths a second relaxation with opposite amplitude compared to mode I is visible. This optical mode (II) is slightly faster than the relaxation mode (III) of the conductance relaxations, but both modes become observable at about the same field intensity, $E_c$ of the 10-µs pulse.
B. Structural Changes

The conservative scattering dichroism suggests that the field-induced, interfacial ionic polarization of the vesicle causes solvent to enter the membrane. If these aqueous scattering centers are pores, the shape of the pore must be asymmetric as indicated by the light scattering anisotropy ($\Delta S \neq 0$). We may model these pores as narrow hydrophobic (HO) pores ($P_{HO}$), of radius $r$. If the field strength $E$ reaches a critical value $E_c$ at a given pulse duration $\Delta t$, a different pore quality appears. This pore type is associated with a negative value of the scattering dichroism and with a larger conductance. We model these pores as broader hydrophilic (HI) pores ($P_{HI}$), which are formed when a critical pore radius $r_c$ is reached. The formation of these pores is slower ($\tau^{-1} = k_{HI} \approx 10^3 \text{ s}^{-1}$) compared to the HO pores ($\tau^{-1} = k_{HO} \approx 10^6 \text{ s}^{-1}$) because the transition $P_{HO} \Leftrightarrow P_{HI}$ involves rotation of lipid molecules in the pore wall.

1. Sequence of Membrane Changes

Since the light-scattering signal $\Delta S(1)/S(0)$ increases continuously with increasing field intensity, it is presumed that the number and size of hydrophobic (HO) pores increase continuously up to a saturation value (Fig. 2). The sequence of membrane changes from the poreless state (M) to HO pores ($P_{HO}$), and HI pores ($P_{HI}$), is modeled in Fig. 3.

![Figure 3](image)

Figure 3  Sequence of events in membrane electroporation, modeled in terms of aqueous pores: M is the closed bilayer state, and ($P_{HO}$), is the hydrophobic pore of radius $r$. The HO pores convert to hydrophilic (HI) pores, ($P_{HI}$), if a critical radius $r_c$ is reached; $P_{CR}$ refers to crater-like pores. The geometrically anisotropic pores $2r \neq d$ describe the change of sign of the dichroic signal $\Delta S = \Delta S_\| - \Delta S_\perp = S_\| - S_\perp$ with respect to the direction of the applied electric field $E$. 
2. Model for the $\text{HO} \rightleftharpoons \text{HI} \text{ Transition}$

For geometrical reasons of lipid packing, water will enter to a certain degree between the individual lipids of the pore wall of a hydrophilic pore. The transition

$$(\text{P}_{\text{HO}})_c \rightleftharpoons (\text{P}_{\text{HI}})_c$$

changes the geometry of the light scattering center. The HI pore ($2r' > d$) is broadened compared to the HO pore ($2r < d$); see Fig. 3.

3. Field Strength and Pulse Duration

The scheme of the structural transitions depicted in Fig. 3 is very instructive for the interpretation of the stimulus/duration curves: the higher the field strength, the shorter can be the pulse length in order to achieve the same membrane permeabilization. A low-intensity pulse may need a pulse duration of milliseconds to reach the same extent of structural transitions as a high intensity pulse applied for only microseconds. Thus even very small field intensities may cause DNA transfer, provided the field application is long enough (seconds). It is thus obvious that the specification of a critical field intensity $E_c$ is only meaningful if the pulse duration $\Delta t$ is also given.

4. Pulse Number

The return to the closed membrane state M (Fig. 3) after switching off the field at the end of the pulse occurs in the absence of the external field. In particular, the return transition $\text{P}_{\text{HO}} \leftarrow \text{P}_{\text{HI}}$, involving reorientation of the wall lipids, may face major activation barriers and thus is slow (seconds, minutes).

If now a second pulse hits the membrane patch not in the M state but in the remaining $\text{P}_{\text{HI}}$, states, the change induced by the second pulse is facilitated, because the transitions $\text{P}_{\text{HO}} \rightarrow \text{P}_{\text{HI}}$ have already been caused by the first pulse. Thus in terms of membrane structure and lipid rearrangements the pulses of a pulse train are not equivalent.

III. Electroporation and Membrane Permeability

Among the various practical aspects, the electrotransformation of intact bacteria and other microorganisms requires media with low conductivity to reduce Joule heating, although that by itself can be favorable for efficient gene transfer (Wolf et al., 1989). In any case, the interpretation of the low-conductivity data necessitates an extension of the analytical framework in terms of the external solution conductivity (Neumann and Boldt, 1989).
Using the green algae cells of the species *Chlamydomonas reinhardtii* (*Cb. reinh.*) as an example, a simple procedure is outlined to determine the critical electroporation voltage ($\Delta \Phi_{M,c}$) and the membrane/envelope conductivity ($\lambda_m$). Finally, a strategy is presented that permits the determination of optimum values of electric field strength, pulse length, and pulse number for the transient permeabilization of viable cells, thus providing a useful basis of optimization strategies for cell biological and medical applications.

A. Permeabilization–Resealing Cycle

It was recently recognized that the transient membrane electroporation may be viewed as a cycle of electropore formation and resealing resembling a relaxation hysteresis (Neumann, 1989). In brief, in the presence of the field pulse of limited duration ($\Delta t = 5\ \mu s$ to 10 ms), the cell membrane (initial states C, representing the actual state equilibria $M \rightleftharpoons \Sigma_{\text{eq}}^c (P_{\text{HOS}})$) becomes more porous (states P representing the state equilibria $\Sigma_{\text{eq}}^c (P_{\text{HOS}})$) and thereby also fusogenic. The state transitions $C \rightleftharpoons P$ in the presence of the electric field occur unidirectionally and may lead to irreversible rupture at long pulse durations. If, however, the electric field pulse is switched off before rupture or lysis of the cells occurs, the electro pores or electrocracks anneal at $E = 0$, with $C \leftarrow P$ being unidirectional, too. The electroporation–resealing cycle may be represented by the scheme

$$
C \xrightleftharpoons[k_p(E)]{k_R} P
$$

where $k_p(E)$ and $k_R$ are overall rate coefficients for electroporation and resealing, respectively. The electroporated (and fusogenic) membrane states $P$ usually are long-lived, in particular at low temperatures (4°C).

If the electroporation hysteresis is coupled to other processes (states X), such as material release or uptake, or lysis, Scheme (1) has to be extended to

$$
C \xrightleftharpoons{k_p(E)} P \rightarrow X
$$

Usually for short pulse times $\Delta t$, the reverse transition $P \leftarrow C$ and the transition $P \rightarrow X$ occur at $E = 0$; they are after-field pulse effects.

B. Electrosensitivity

It is known that a biological cell population is inhomogeneous in cell size, in the state of growth, or in metabolic conditions. Nonspherical cells such as bacterium rods have different positions relative to the external electric field direction. Because of the dominantly negative surface charges of the cell wall, the rods will orient with their longest axis into the direction of the electric field. Both oriented rods and the
Figure 4  Electrosensitivity $G(\%)$ of a suspension of green algae cells *Chlamydomonas reinhardtii* (wild type 11-32 c, Göttingen) to quasirectangular electric pulses of the initial field intensity $E_0$ and of the pulse length $\Delta t = 0.2$ ms at different medium conductivities $\lambda_0$: •, $\lambda_0 = 3.5 \pm 0.1 \times 10^{-8}$ S cm$^{-1}$; ■, $\lambda_0 = 1.5 \pm 0.1 \times 10^{-4}$ S cm$^{-1}$; ▲, $\lambda_0 = 5.6 \pm 0.5 \times 10^{-3}$ S cm$^{-1}$. $G(\%)$ is the percentage of cells that were critically permeabilized such that the (lethal) dye serva blue $G$ ($M_r = 854$, largest dimension 2.5 nm) was taken up, visibly coloring these cells.

oriented polyelectrolyte DNA will migrate with different velocities along the electric field lines. All these factors cause a distribution of the critical electroporation field strength $E_c$.

Figure 4 shows examples of such a distribution. Here, the state X in Scheme (2) is the colored cell (G), obtained by the uptake of a dye by the electroporated cell. The percentage $G(\%)$ of the G colored cells is given by: $G(\%) = 100 \times G/C_T$, where $C_T$ is the total number of the cells. $G(\%)$ is a measure for the critically permeabilized cells.

For practical purposes we may take the field strength $E_0$ (50%) where $G(\%) = 50$ as representative for the cell population and define a mean value by

$$\overline{E}_c = E_0(50\%)$$ (3)

The width of the electrosensitivity of the cell population may be given in terms of a "variance" ($\pm \Delta E_c$). Since $E_c$ depends on the cell size, the mean value $\overline{E}_c$ corresponds to a mean value $\overline{\alpha}$ of the effective radius of spherical cells. Here, too, we have a "variance": $\overline{\alpha} \pm \Delta \overline{\alpha}$.

C. Ionic Interfacial Polarization

The electroporation data suggest that the membrane electroporpermabilization results from an indirect electric field effect. The structural changes of the membrane phase are preceded by the ionic interfacial polarization (Neumann, 1989).
Usually in electroporation experiments the polarization time constant $\tau_p$ (Schwan, 1957) is small compared with the pulse duration $\Delta t$, the buildup of the stationary value $\Delta \varphi(E)$ of the interfacial potential difference across the membrane is practically instantaneous.

For low-conductivity membranes of thickness $d$ of cells of radius $a$, the stationary value is given by

$$\Delta \varphi(E) = -1.5f(\lambda)aE|\cos \delta|$$

(4)

where $\delta$ is the angle between the membrane site considered and the direction of $E$. The conductivity factor $f(\lambda)$ is a function of the specific conductances or conductivities of the external solution ($\lambda_o \approx 10^{-4} \text{ S cm}^{-1}$), of the cell interior ($\lambda_i \approx 10^{-2} \text{ S cm}^{-1}$), and of the membrane ($\lambda_m \approx 10^{-7} \text{ S cm}^{-1}$), respectively, and of the ratio $d/a$.

Usually $\lambda_m \ll \lambda_i$, $\lambda_o$ and $d \ll a$ such that (Neumann, 1989)

$$f(\lambda) = \frac{1 + \lambda_m(2 + \lambda_i/\lambda_o)/(2 \lambda_i d/a)}{1}$$

(5)

If in low-conductivity media $\lambda_o \ll \lambda_i$, but still $\lambda_o \gg \lambda_m$, Eq. (5) reduces to a form that is particularly useful for graphical data evaluation:

$$[f(\lambda)]^{-1} = 1 + (\lambda_m a/2d) \lambda_o^{-1}$$

(6)

Obviously, $E_c$ corresponds to a critical cross-membrane electroporation voltage $\Delta \varphi_{M,c}$; $\Delta \varphi_{M,c} \approx 1 \text{ V}$ (Sale and Hamilton, 1968) for short repetitive pulses of 20 $\mu$s. When the contributions of fixed surface charges and the associated ionic atmospheres can be neglected, the total trans-membrane voltage $\Delta \varphi_m(E)$ in the presence of the externally applied field $E$ is a function of the intrinsic membrane potential $\Delta \varphi_m$ (e.g., $\Delta \varphi_m = -70 \text{ mV}$) and of the interfacial polarization term $\Delta \varphi(E)$:

$$\Delta \varphi_m(E) = fct[\Delta \varphi_m, \Delta \varphi(E)]$$

(7)

The total potential profile in the direction of $E$ is explicitly given by (Neumann, 1989)

$$\Delta \varphi_m(E) = -[1.5f(\lambda)aE + \Delta \varphi_m/cos \delta]|\cos \delta|$$

(8)

Often $|\Delta \varphi(E)| \gg |\Delta \varphi_m|$, hence $\Delta \varphi_m(E) \approx \Delta \varphi(E)$. The pole caps of spherical membranes are the sites of maximum interfacial polarization. With $|\cos \delta| = 1$, Eq. (8) can be used to relate the mean values of $E_c$ and $\bar{a}$ by the expression

$$\bar{E}_c = -\Delta \varphi_{M,c}/[1.5a f(\lambda)]$$

(9)

Applying Eq. (6) for low-conductivity media we obtain

$$\bar{E}_c = -(\Delta \varphi_{M,c}/3\bar{a} [1+ (\lambda_m \bar{a}/2d) \lambda_o^{-1}$$

(10)

When $\bar{a}$ is given by the geometrical radius of the most abundant cell size, the data in Fig. 5 can be used to determine $\Delta \varphi_{M,c}$ and $\lambda_m$. For the Ch. reinh. cells, $\bar{a} = 3.5 \mu\text{m}$: $\Delta \varphi_{M,c} = -0.8 \text{ V}$, $\lambda_m = 5 \times 10^{-7} \text{ S cm}^{-1}$. The critical voltage 0.8 V at $\Delta t = 0.2 \text{ ms}$ compares well with the value of 1 V estimated by Sale and Hamilton.
Figure 5  The population mean value $\bar{E}_c = E_0$ (50%) of *Ch. reinhardtii* cells (Fig. 4) decreases with increasing conductivities $\lambda_0$ of the pulsing medium. The $\lambda_0$ value at which $\bar{E}_c = 2\bar{E}_{c,\text{min}}$ [i.e., $f(\lambda) = 0.5$] is given by $(\lambda_0)_{0.5} = \lambda_m \bar{a}/2d$ and permits a simple graphical estimate of the $\lambda_m$ value. From $\bar{E}_{c,\text{min}}$ we obtain $\Delta \phi_{M,c} = -1.5 a\bar{E}_{c,\text{min}}$.

(1968) for $\Delta t = 20 \mu s$. The dependence of $E_c$ on $\lambda_0$ is quantitatively consistent with the concept of ionic interfacial polarization, being reduced at low medium conductivity ($\lambda_0$), that is, low electrolyte concentration. The data at low $\lambda_0$ values suggest that the membrane conductivity $\lambda_m$ cannot be neglected; instead of $f(\lambda) = 1$, Eq. (6) applies.

D. Lifetime of Membrane Electroporation

The longevity of the electroporated cells is dependent on the temperature and may be measured by the after-plus addition of a dye (which colors the cell) at various times $t_{\text{add}}$ after the electroporation pulse. Due to annealing processes more cells lose the permeability property at larger after-pulse addition times (Fig. 6). For the data analysis Scheme (2) is specified to

\[ C \xrightarrow{k_R} P \rightarrow G \]

where $k_R$ and $k_D$ are the rate coefficients for resealing (R) and dye uptake (D) and $X = G$ is the (lethally) colored cell state.

The data suggest that $k_D \gg k_R$, such that $G$ is a quantitative measure of the cells in the critically electroporated states $P (= G)$. The rate equation for Scheme (11) under these conditions is given by

\[ -d[G]/dt = -d[P]/dt = -k_R[P] \]

Integration for the boundary conditions $t = 0$: $[P] = [P_0]$, and $t \to \infty$: $[P] =$
Figure 6  Time course of the recovery of the electroporated cells (resealing), measured by the uptake of the dye serva blue G, which was added at various times $t_{add}$ after the electroporation pulse. For this example $k_R = 2.6 \pm 0.9 \times 10^{-2}$ s$^{-1}$ at $T = 298$ K (25°C).

$[P_\infty]$ yields

$$[P(t)] - [P_\infty] = \frac{[P_0] - [P_\infty]}{\exp(-k_R t)}$$

Equation (13) permits the evaluation of the coefficient $k_R$ of cell recovery from the observed exponential decay of $G(\%)$ in Fig. 6. Note that $G(\%)$ is proportional to $P$ such that, for instance, $([P(t)] - [P_\infty])/([P_0] - [P_\infty]) = (G(t) - G_\infty)/(G_0 - G_\infty)$.

The rate coefficient $k_R$ is a measure of the mean lifetime $\tau_e$ of the electroporated cell state: $\tau_e = k_R^{-1}$. In the example given in Fig. 6, $\tau_e \approx 40$ s; the electroporation state is thus called long-lived compared to the pulse duration of 0.2 ms (Fig. 4).

E. Physical Electroporation Parameters

The dye method of coloring electroporated cells can be used to determine the pulse strength–duration $(E_c/\Delta t)$ relationship and the dependence of $E_c$ on medium conductivity ($\lambda_0$), on temperature, and on pulse number. The $E_c/\lambda_0$ dependence yields $\lambda_m$ as a membrane-specific electric parameter. By $G_m = \lambda_m/a$, the membrane conductance, $G_m$ can be determined. For *C. reinb.,* $G_m \approx 1.4 \times 10^{-3}$ S cm$^{-2}$, comparing well with other biomembrane systems.

It is of particular practical importance that the values $G_0(\%)$ and $G_\infty(\%)$ can be used to determine, at a given pulse length and pulse number, the optimum range of $E_0$ for the electroporation experiments (Fig. 7). The optimum field strength range shifts when the medium conditions ($\lambda_0$, temperature, etc.) are changed; $\Delta E_0$ and $\Delta G(\%) = G_0(\%) - G_\infty(\%)$ must be explored for every particular cell type.
In summary, it is shown that the dye method, originally applied as a qualitative tool to estimate electroporated cells (Neumann et al., 1980, 1982) can be quantified to yield useful information for the optimization of the electroporation technique.

IV. Electroporative DNA Transfer

One of the more recent developments in the technique of the electroporative gene transfer is the electrotransformation of intact bacteria, without pretreatment to remove the cell wall (see, for instance, Wolf et al., 1989).

A. DNA Surface Adsorption

The number \( T \) of transformants per milliliter cell suspension increases with increasing DNA concentration [DNA] and with increasing cell density (Wolf et al., 1989). Both correlations, \( T(\text{DNA}) \) at constant cell density and \( T(\text{cell density}) \) at constant DNA concentration, are of the Langmuir-type adsorption isotherm. We may model these observations with a scheme in which the electroporation hysteresis \( C \rightleftharpoons P \) [see Eq. (1)] is coupled to the adsorptive, (electro)diffusive approach of DNA (D) to the surface of the cell (C), yielding the surface bound states \( D \cdot C \) and \( D \cdot P \):

\[
\begin{align*}
    D + mC & \rightleftharpoons D \cdot C \\
    \downarrow & \downarrow \\
    D + mP & \rightleftharpoons D \cdot P \rightleftharpoons P \cdot D \rightarrow D_{m} \rightarrow TC
\end{align*}
\]

In Scheme (14), the process \( D \cdot P \rightleftharpoons P \cdot D \) denotes the crossmembrane transport of DNA, \( D_{m} \) is the DNA that entered the cytoplasm, and TC denotes transformed cell; \( m \) is the maximum number of surface binding sites for DNA per cell.
B. Analysis of DNA Surface Binding

It appears reasonable to assume that the probability of cell transformation increases with an increase in the concentration of the state \( D \cdot P \). If \([TC] \sim [D \cdot P]\), then

\[
T/T_{\text{max}} = \frac{[TC]}{[TC]_{\text{max}}} = \frac{[DP]}{[DP]_{\text{max}}}
\]

(15)

In terms of Scheme (14) the extent of “adsorption” depends on DNA and cell concentration, respectively, according to

\[
\frac{[DP]}{[DP]_{\text{max}}} = \frac{[D]}{[D] + \overline{K}_D} = \frac{m[C]}{m[C] + \overline{K}_c}
\]

(16)

where \( \overline{K}_D = \overline{K}_c \) is given by

\[
\overline{K}_D = \frac{[D](m[C] + m[P])}{([DC] + [DP])}
\]

\[
= K_1(1 + K_0)/(1 + K_0')
\]

(17)

and \( K_0 = [P]/[C] \), \( K_1 = ([D](m[C])/[DC] \), \( K_0' = [D \cdot P]/[D \cdot C] \), and \( K_2 = K_1K_0/K_0' \) are the equilibrium constants of the individual steps.

Figure 8  Electrotransformation of intact Corynebacterium glutamicum cells. Cell density \( 10^{10}/\text{ml} \) at \( 4^\circ \text{C} \). Quasirectangular pulse, \( E_0 = 10 \text{ kV cm}^{-1} \), \( \Delta t = 5 \text{ ms} \); medium: \( 0.272 \text{ M sucrose, } 0.01 \text{ M HEPES buffer, } 1 \text{ mM MgCl}_2 \), KOH adjusted to yield pH 7.4 at \( 4^\circ \text{C} \), plasmid DNA pUL-330-plasmid (5.2 kb). The Langmuir adsorption type relationship between the number of transformants \( T \) per milliliter and the total concentration of DNA, \([D_T]\), is represented in terms of reciprocal quantities; \( T^{-1} = T_{\text{max}}^{-1}(1 + \overline{K}_D/[D_T]) \). The dashed line refers to the linear dependence on \([D]^{-1} \); \([D] \) is the concentration of free DNA, \( M = 5.43 \times 10^6 \text{ g/mol.} \) The abscissa and ordinate intercepts yield \( \overline{K}_D = 2 (\pm 1) \times 10^{-9} \text{ M (DNA)} \) and \( T_{\text{max}} = 3.3 \times 10^5/\text{ml} \).
Equations (15) and (16) can be combined and rewritten as

$$T^{-1} = T_{\text{max}}^{-1} (1 + \overline{K}_D(D))$$  \hspace{1cm} (18)$$

providing a convenient relationship for a graphical data evaluation. Note that $[D]$ refers to the free, unbound DNA. The data representation in terms of the total DNA concentration $[D_T]$ is nonlinear in $1/[D_T]$, but it is suited to evaluate $T_{\text{max}}$ and $\overline{K}_D$; see Fig. 8. For intact *Corynebacterium glutamicum* cells we obtain $\overline{K}_D = 2(\pm 1) \times 10^{-9} \text{M (DNA)}$, $m = 25$, and $T_{\text{max}} = 3.3 \times 10^3 \text{ml}^{-1}$ at $E = 10.5 \text{kV cm}^{-1}$ and $\Delta t = 5 \text{ms}$.

The electroporative cell transformation of intact *Corynebacterium glutamicum* cells can thus be quantitatively described in terms of preceding surface adsorption of the applied plasmid DNA.

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**References**


