Electrooptics of membrane electroporation and vesicle shape deformation

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Electrooptical and conductometric methods continue to reveal new and more detailed information on the dynamic properties of membranes in electric fields. In particular, the electric pore formation in lipid vesicles, doped with optical probes, has been successfully investigated with electrooptical techniques thus providing new insight into the lipid rearrangements underlying membrane electroporation (ME) and vesicle deformation. Progress in understanding the molecular mechanism of ME and related phenomena, such as electrofusion of cells or electroinsertion of foreign proteins into membranes, is crucially important for the numerous applications of ME, for example, direct electroporative gene transfer and drug delivery in the new medical discipline of electroporative chemotherapy.

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Abbreviations
DPh diphenyl-1,3,5-hexatriene
HI hydrophilic
HO hydrophobic
ME membrane electroporation
OD optical density

Introduction
Methodologically, membrane electroporation (ME) defines an electrical technique which renders lipid membranes porous and permeable, transiently and reversibly, by external voltage pulses [1]. ME conditions the membrane for a variety of secondary processes which may be coupled to the electric pore formation and resealing cycle. The concept of ME was derived from the electrically-induced permeability changes, as judged from the release of intracellular material [2] and/or from the electrodiffusive uptake of macromolecules such as DNA (called direct electroporative gene transfer or electrotransformation of cells) [1–7,8**, 9–11]. Similarly, electrofusion of single cells to large syncyia [12] and electroinsertion of foreign proteins into (electroporated) membranes [13] are structurally based on ME.

In the meantime there are numerous applications of ME to manipulate cells, organelles and tissue in cell biology and medicine. The method has been recently applied to enhance iontophoretic drug transport through skin [14–16] and to introduce chemotherapeutica into cancer tissue [17–20]. On the other hand, ME has been used as an analytical tool to quantify the effect of membrane curvature on the surface adsorption and partial insertion of proteins such as annexin V (K Tönsing, S Kakorin, E Neumann, unpublished data).

Despite these attractive features of the ME phenomena, the molecular mechanism of ME itself and the mechanisms of the various secondary processes coupled to ME are not yet clarified to the extent that reliable directives can be given for analytical and cell-manipulative applications.

Clearly, the observed transport phenomena of the release and uptake of substances reflect transient membrane permeability changes, ultimately caused by the voltage pulses. Yet, these permeability changes must result from structural changes of the membrane phase leading to permeation sites, pathways or pores. Up to now, the permeation of a permeant through a porated membrane patch has not been visualized. The microscopically visible local reduction, or even disappearance, of optical density from the membrane contour of giant vesicles, however, suggests the formation of larger porous patches; concomitant with pore formation, particle shape deformation was directly seen in long-lasting voltage applications [21,22]. On the same line, the position of fluorescent, voltage-dependent membrane probes visibly indicated that external fields (of several µs duration) lead to regions which are free of fluorescence response; this suggested the existence of porous patches [23,24]. In addition, there are clearly visible shape changes of the electroporated particles at larger (ms) field durations [22,25].

In this critical review of data and concepts derived from electrooptical and conductometric data of lipid bilayer vesicles as model systems, we shall confine ourselves to the discussion of results obtained with optical diphenyl-1,3,5-hexatriene (DPh)-membrane probes (Fig. 1). Basically, we shall consider structural changes leading to chemically specific pore states in the membrane, that is, transitions from the closed (C) state to hydrophobic (HO) and hydrophilic (HI) pore states. We shall also discuss shape deformations coupled to the electrical pore formation (i.e. electroporation) in external electric fields. One of the main results is that the structural changes according to C ≡ (HO) ≡ (HI) increase continuously with time and electric field strength. Massive permeability changes, for instance, reflected in large conductivity changes or in the release and uptake of larger molecules are, however, associated with threshold field strengths (Fig. 2) and delay times.
Optical probes for lipid rearrangements

The hydrophobic optical probe DPH and DPH-labeled lipids have been widely used to determine lipid order and fluidity in lipid bilayers and natural membranes in the gel and fluid phases [26,27]. These probes are uniaxial molecules with optical anisotropy; the optical transition dipole moment lies in the direction of the long axis. Therefore positional changes of the molecular axis can be monitored with polarized light, leading, for instance, to linear absorbance dichroism. It is a great advantage that the probe molecules are dominantly (up to 97%) oriented parallel to the lipid hydrocarbon chains, as demonstrated, for instance, by DPPC vesicle membranes in the fluid phase. The experimentally suggested prevalent parallel orientation along the membrane normal also agrees with the results from computer simulations using the Monte Carlo method [28]. Unexpectedly, the 'free' DPH molecules in the membrane phase exhibit narrower angular distributions around the membrane normal when compared to the DPH-labeled lipids such as β-DPHePC, β-DPHePC, β-DPHpPC in which the DPH group is attached at different distances (c.e and p) from the head group of the β chain of the lipid molecule [27].

Contrary to the membrane normal-parallel orientation, the prevalent surface-parallel orientations of DPH, suggested by neutron scattering experiments on solid supported thin bilayer films, have been questioned by the authors themselves [29]. The observations refer to lipid layers on microscope slides at the extremely high DPH concentrations (20 mole%) corresponding to a mole fraction \( x_{DPH} = \frac{[DPH]}{[DPH] + [lipid]} = 0.2 \) (where [DPH] and [lipid] denote the amounts (in mole units) of DPH and lipid, respectively). In vesicle membranes, the mole fraction of DPH cannot be higher than 0.005 (or 0.5 mole%), because of the finite ability of the membrane surface to incorporate the DPH molecules [T. Liss, E. Neumann, unpublished data]. In a lipid vesicle suspension where the mole fraction of DPH is 0.2, the majority of the DPH probes are probably associated to hydrophobic colloidal aggregates. If such aggregates are also present in slide-supported lipid layers, the DPH aggregates could be partially attached to the head group region of the lipid molecules after the evaporation of the chloroform, causing 80–90% of the DPH labels to be lying practically in the plane of the lipid/water interface [29]. It is therefore hardly justifiable to relate the angular distribution of the highly concentrated DPH labels in a lipid film, prepared on the solid substrate, to the diluted DPH distribution in the membranes of the suspended vesicles.

A dominant orientation of the DPH molecules parallel to the membrane surface is not consistent with the majority of other data, for instance, with the negative absorbance dichroism of vesicles [30,31]. The negative sign and the kinetics of the absorbance dichroism observed in the
DPH-doped lipid vesicles as well as in vesicles containing β-DPhpPC (1:200) at fluid phase temperatures are consistently explained only by the DPH labels being oriented dominantly along the membrane normal. The detailed analysis of the theory of absorbance dichroism, caused either by membrane electroporation or by vesicle shape deformation in an electric field, showed that the small fraction (=3%) of DPH labels oriented parallel to the membrane plane should only slightly decrease the amplitude of the dichroism, but should not change the characteristic time course of the optical signal in the μs time range; see the section on shape deformation below. Therefore, DPH labels can be effectively used to investigate field-induced structural transitions in the membrane phase as well as the shape deformation of vesicles or cells in an electric field.

**Field-induced absorbance changes of DPH**

DPH inserted into lipid bilayers absorbs light in the wavelength range 300–400 nm. Even at the low DPH concentrations which can be achieved in bilayers, the absorbance, A, of DPH is the larger contribution to the measured optical density, OD = A + τt, where t is the light path and τ is the turbidity term arising from the light scattering of the vesicles of radius, a, 60 nm ≤ a ≤ 160 nm, investigated so far electrooptically. In polarized light, a change in the absorbance,

\[ \Delta A^\sigma = \Delta A^\sigma_{CH} + \Delta A^\sigma_{OR} \tag{1} \]

at the polarization angle σ of the light vector, relative to the direction of the external electric field vector E, may have two contributions [32]. The term \( \Delta A^\sigma_{CH} \) accounts for the change of the immediate environment of the chromophore, whereas \( \Delta A^\sigma_{OR} \) is a measure of the rotational position change. The chemical term \( \Delta A^\sigma_{CH} \) and the dichroic term \( \Delta A^\sigma_{OR} \) can be obtained with high accuracy from the dependence of \( \Delta A^\sigma \) on the polarization angle σ [33]. See also the legends to Figures 1 and 3.

The dichroism of single DPH molecules dispersed in the lipid bilayer has been interpreted as arising from the passive rotation of the lipids and DPH molecules to minimize hydrophobic contact sites with water, and this has led to an understanding of the hydrophilic or inverted pore state HI. The rotational displacement is more directly indicated by the dichroic data of lipid-coupled DPH, for instance β-DPhpPC as used in the experiments discussed in the section on protein–membrane interactions.

The analysis of the field-induced absorbance changes of DPH in the vesicular membrane has to take into account a particular feature of the vesicle geometry. The electrooptic and conductometric relaxation spectrometric techniques using high voltage pulses of up to 50 µs pulse duration, have provided evidence that the primary field effect on the dielectric membrane shell is the ionic interfacial polarization of the Maxwell–Wagner type. The accumulation of small ions at the membrane interfaces leads to large transmembrane electric potential differences Δφ across the membrane thickness d. The magnitude and the direction of the induced transmembrane field strength (stationary value),

\[ E_m = -\Delta \varphi / d = 1.5 \cdot E \cdot (a/d) \cdot f(\lambda_m) \cdot |\cos \theta| \tag{2} \]

depends on the positional angle θ; see Figure 1. Therefore the field \( E_m \) acting on the membrane phase is greatly amplified relative to the external field. \( E_m \) is largest at the two pole caps in the direction of the external field vector E. As seen in Equation (2) all measured field dependencies reflect |cosθ| averages. Furthermore, the dichroitic signals due to the specific HI pore state (Fig. 1), exhibiting reversals with time (Fig. 3) and with external field strengths [30] are readily rationalized in terms of the shell geometry of the vesicle membrane [31].
Vesicle shape deformation

Conceptually, the electric-field induced deformation of a DPH-doped lipid vesicle to an ellipsoidal shape (Fig. 4) causes a dichroic absorbance change. In recent years, the electric deformation of vesicles has been studied extensively, both experimentally [22,25] and theoretically [34–37]. So far, giant vesicles have been used during experimental studies because these particles can be easily observed with the light microscope. The electrically induced vesicle deformation was shown to be accompanied by membrane electroporation [21,22]. Surprisingly, as judged from the measured principal axes, the electric deformation of giant vesicles to ellipsoids was associated with an enormous increase of the membrane area (more than 15%) at constant volume [25]. The large increase in area is too large to originate from an apparent stretching or flattening of the thermal membrane undulations, or from the real stretching of the membrane itself.

In line with this observation, our kinetic data (conductivity and light scattering) on the deformation of small (a=45 nm) salt-filled vesicles in electric fields of 0.5 MV m⁻¹ < E < 1 MV m⁻¹, and pulse durations of 10 ms ≤ t ≤ 100 ms are consistently described in terms of a volume decrease due to vesicle elongation at constant surface area (E. Redeker, E. Neumann, unpublished data). The membrane bending rigidity derived from the vesicle volume change is k_{2}=2.3 \times 10⁻²⁰ J. This value is in good agreement with all other estimates for phosphatidylethanolamine membranes [25,42**]. The number of permeable electro pores, estimated from Poiseuille's formula applied to the electrolyte flow from the vesicle, is N=36 per vesicle and the mean pore radius under flow conditions is r_p=1 nm, also consistent with previous estimates [43].

Chemical thermodynamics of membrane electroporation

Specific chemical model

All electrooptic and conductometric relaxation data are consistently described with an apparently simple, yet specific reaction scheme:

\[
\frac{k_1}{k_{-1}} C \rightarrow (HO) \rightarrow (HI)
\]

The state transition of a cluster (L_n) of n lipids L_i from the closed bilayer state (C) to (HO) pore states accounts for the electrically induced entrance of water and ions to form cylindrical HO pores. Because of the large volume of the buffer solution, the reaction step is buffered with respect to H₂O and ions and is therefore considered to be of pseudo-first order. The data suggest that this state transition is rate-limiting for a more rapid second process: the transition of (HO) pore states to (HI) pore states (Fig. 1). The normal mode relaxation rate (τ⁻¹) for the coupled reaction system, Equation 3, is given by τ⁻¹=k₁+k₂−(1+K₂⁻¹), where k₁ and k₋₁ are the rate coefficients and K₁=[(HO)]/[C] is the equilibrium constant of the first step; and K₂=[(HI)]/[HO] is the equilibrium constant for the rapid cluster transition (HO) → (HI) constituting the second step [31].

Electrochemical energetics

The charge accumulations by ionic interfacial polarization on both sides of the membrane dielectrics are equivalent to electric condenser plates. The membrane is a highly
dynamic phase of mobile lipid molecules in contact with water and hydrophobically held together by the aqueous environment. Such a charged condenser with both mobile interior and mobile environment favors the entrance of water molecules to produce HO pores with a higher dielectric constant (ε=80) compared with εx=2 of the replaced lipids (state C). In this sense, the lipid membrane is an open system with respect to H2O molecules and ions charging the condenser to yield the total charge g.

The purely electrical aspect of ME may be described in terms of a change dGel of the electrical part (Gel) of the electromechanical Gibbs energy change, here dG = dGel, by dGel = Δφdψx -Fd where Δφ is the transmembrane potential difference, F is a generalized force acting on the dielectrics and tending to change the water content x by dx, replacing lipids and thereby forming pores. At zero membrane conductivity (βm = 0), the external field maintains Δφ constant. Therefore, ME proceeds at constant Δφ and the replacement of lipids by water means dGel = (Δφdψx/2) > 0. In contrast to this physically rigorous picture, the previous formal treatments [43-48,49,50,51] take the condenser energy term (equivalent to Δφdψx/2) with a negative sign; formally, the difference between the (positive) mechanical energy and the (positive) condenser energy (equivalent to Fdx - Δφdψx) is physically not justified.

The chemical, thermodynamic description of ME in terms of the transformed Gibbs energy, ̃G = G - E_m M, is the more general approach to the energetics of ME. Here, M is the (induced) electric dipole moment of the pore regions and G is the Gibbs energy (containing the chemical potentials of the H2O and lipid molecules constituting a pore). Note that at E_m = 0, we have ̃G = G.

At constant pressure and temperature, a field-induced state transition is characterized by d ̃G ≤ 0. The thermodynamic equilibrium distribution constant K of a two state process is then determined by

$$Δ_R G^θ = -RT \ln K,$$

where the standard Gibbs reaction energy is given by

$$Δ_R G^θ = Δ_R G^θ + \int_0^{HΔR} Δ_R G^θ dA + \left[\int_0^{HΔR} ΔR \beta dH - \int_0^{HΔR} ΔR M dE_m\right]$$

Note that Δ_R = d/dξ, where ξ is the molar advancement of the two state process. In Equation 5, γ is the line tension (or pore edge energy density) and L is the edge length, G is the surface energy density and A is the pore surface in the surface plane of the membrane, β is the curvature energy term and H is the vesicle curvature.

In the case of the spherical vesicle, the curvature H is given by the radius a of the midsurface of the bilayer: H = a⁻¹. The term ΔR G = ΣΔH (VjH) is the conventional standard Gibbs reaction energy (without tension, curvature and electric terms) where Vj and Hj are the stoichiometric coefficients and the standard chemical potentials of the molecules, respectively, constituting the phase α.

**Application to the HO pore formation**

The C = HO state transition, for instance, is characterized by

$$\int_0^{HΔR} ΔR γdL = N_A \int_0^{H(γ_HO - γ_c)} dL = N_A \gamma \pi r_p$$

where N_A is Avogadro's constant, γ = γ_HO is the pore line tension, L = 2πr_p is the circumference, and r_p the radius of the HO pore; note that γ_c = 0.

For small vesicles, the curvature term is very important for the membrane electroportability. As the surface pressure term is very small, it is presently not considered.

The curvature energy is attributed to the deviation Δ(ΔA) = ΔA - ΔA_0 of the area difference between the two monolayer leaflets from its equilibrium value ΔA_0. At an approximately equal number of lipid molecules within the outer and the inner leaflets, and neglecting the alterations in the interfacial energy with changing curvature [42,50,51], the curvature term is given by:

$$\int_0^{HΔR} ΔR β dH = N_A \int_0^{H(β_HO - β_c)} dH =$$

$$\frac{-64π^2 - α/k_c r_p^2 \cdot \xi}{N_A}$$

where, β_HO = 0, β = β_c, k_c is the elastic module and α(1 ≤ α ≤ 6) is a material parameter [42,51].

Different to cylindrical HO pores of volume V = πr_p^2d, the conical HO pores in the vesicle membrane reduce the lipid packing density difference and, consequently, the free energy of the electroportated vesicle system. Equation 7 indicates that the larger vesicle curvature H, the larger the Gibbs energy released by the pore formation.

The mean pore radius r_p of the conus-shaped HO pore is defined by r_p = (r_0 + r_in)/2 where r_out and r_in are the pore radii in the outer and inner leaflets of the bilayer, respectively. The factor ζ = (r_out - r_in)(r_out + r_in) characterizes the pore conicity. For r_p = 0.35nm, there are seven lipid molecules in the outer pore edge and five lipid molecules in the inner pore edge, hence ζ = 0.352.

The electric polarization term at the positional angle θ is expressed as [31]:

$$\text{Polarization term} = \text{constant} \times \sin(θ)$$
\[ \int_0^{E_m} \Delta R M dE_m = \frac{9 \pi \varepsilon_0 a^2 (\varepsilon_w - \varepsilon_L) \gamma}{8d} f^2(\lambda_m(\theta)) \cos^2 \theta \cdot E^2, \]  

where \( \varepsilon_0 \) is the vacuum permittivity and \( f(\lambda_m) \) the conductivity factor. The difference \( \varepsilon_w - \varepsilon_L \) refers to the replacement of lipids by water constituting the C = (HO) transitions. Applying Equations 4 and 5 we obtain for the first step in Equation 3:

\[ K_1 = K_1^0 \exp(-N_A(2\pi \Gamma \gamma - \beta_{\text{HO}-H})) + \int_0^{E_m} \Delta R M dE_m/(RT), \]

where \( K_1^0 = \exp(-\Delta R C^0/RT) \).

The data suggested that \( K_2 \) is independent of the field strength and of the vesicle curvature.

**Figure 5**

Transmembrane electric potential drop (\( \Delta \psi \)) in the direction of the external field vector as a function of the external field strength \( E \), stationary value \( \Delta \psi_{\text{ss}} \) and \( \psi \), transient transmembrane voltage \( \Delta \psi = \Delta \psi_{\text{ss}} + \Delta \psi_{\text{tp}} \). The dashed straight line indicates the transmembrane voltage calculated for the hypothetical case of zero membrane conductivity: \( \lambda_m = 0 \) and \( f(\lambda_m) = 1 \).

The chemical thermodynamic concept has turned out to be generally applicable to ME data analysis. In summary, the main results of DPH-labeled lipid vesicles are, first, the peculiar feature of the observed reversals in the time course and in the field course of the \( \Delta \Lambda^- \) (here being practically equal to the absorbance dichroism) consequent to the spherical geometry of vesicles. Specifically, the induced transmembrane electric field \( E_m \) is position-dependent such that \( E_m \) is largest at the pole caps. On the other hand, the equatorial area is larger than the pole cap areas and becomes dominant at higher fields. Second, the time course of the pore formation reflects the ‘spreading’ of rapidly (\( \leq 0.1 \mu s \)) formed individual pores from the pole caps to the equatorial region of the vesicle. The spreading time constants decrease from 4 \( \mu s \) to 1 \( \mu s \) with increasing field strengths. Third, the mean pore radius of the pores formed within the pulse duration (\( \leq 10 \mu s \)) can be derived from the chemical thermodynamic model; here \( r_p = 0.35 \pm 0.05 \text{ nm}, \) suggesting a cluster size \( \pi = 12 \pm 2 \) of lipids in the pore edge [31]. At higher field strengths (\( E \geq 4 \text{ MV m}^{-1} \)), vesicle radius \( a = 160 \text{ nm} \) larger pores appear: \( r_p = 0.35 \pm 0.005 \text{ nm}, \) corresponding to a minimum in the hydrophobic interaction free energy. Fourth, pore size and pore density in the vesicle membrane appear to be limited by the conductivity of the HI pores for small ions which prevents further interfacial polarization and concomitant further increase in the stationary values of the transmembrane voltage beyond \( |\Delta \psi_{\text{ss}}| = 0.53 \text{ V}; \) see Figure 5. Finally, higher voltages \( |\Delta \psi_{\text{Q}}| \geq 0.6 \text{ V} \) can only build up transiently (for \( \leq 0.5 - 2 \mu s \)) in the pole caps. An occasionally larger conductivity at one of the pole caps gives rise to a potential difference at the other pole cap, being approximately twice as large; this in turn causes larger pores (\( r_p = 0.80 \pm 0.05 \text{ nm} \)). These additional transient pores are described by the parallel Q-reaction \( C = H_{\text{Q}}; \) see the data in Figure 6. The asymmetry between the two pole caps relaxes with time constants of about 2 \( \mu s \) to yield \( \Delta \psi_{\text{ss}} \), equal in both caps [31].

**Vesicle poration or vesicle elongation?**

Interestingly, the dependence of the electrooptical absorbance changes on \( H \) can be used to answer the question ‘vesicle poration or vesicle deformation?’ The comparison must be made at a constant ratio \( E/H \); here taken to be \( 0.2 \text{ V}. \) The nominal transmembrane potential difference for this example is \( \Delta \psi^N = -1.5 \text{ E}/H = -0.3 \text{ V}; \) it is constant regardless of \( E \) and \( H \). The actual field-induced transmembrane potential drop is given by: \( \Delta \psi = \Delta \psi^N f(\lambda_m) \cdot \cos \theta \), where \( f(\lambda_m) \) is the conductivity term. The extent of the electroporation is small such that we can use \( f(\lambda_m) = 1 \) for all vesicle sizes [31]. For a given value of \( \Delta \psi^N \) the fraction of potated membrane area is constant irrespective of the size of the spherical unilamellar vesicles. From Equation 7 and Equation 9 we obtain the fraction of HI pores \( |\Delta \Lambda^-|/\Delta \Lambda^0 \) being proportional to \( |\Delta \psi^N|/|\Delta \psi^0| \).

At small vesicle deformations, the axis ratio \( p \) of the spheroid is given by \( p = 1 + (3 \varepsilon_0 \varepsilon_w E^2)/(64 \kappa_0 H^3) \).

Therefore, when \( E/H \) is constant, \( p \sim 1/H \). If the de-
formational model was valid, the difference $\Delta A^- / A^0$ should decrease with increasing vesicle curvature. In Figure 7 it is seen that the experimental data are only consistent with the membrane electroporation model but not with vesicle shape deformation. The data refer to unilamellar vesicles composed of L-α-phosphatidyl-L-serine (PS) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in the molar ratio of PS:2 POPC, doped with 2(3)-diphenylhexatrienylpropanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine (β-DPHPC, $M_r = 782$), and 5 μM and 0.66 mM Na-HEPES-buffer (pH=7.4) containing 130 μM CaCl$_2$. $T = 293$ K (20°C).

In summary, the vesicle shape deformation in the electrical field is of minor importance for the amplitudes of the absorbance relaxations of the electroporated vesicle, if the pulse duration is ≤10 μs. The characteristic deformation time constants for small vesicles and for field strengths 1 MV m$^{-1} \leq E \leq 10$ MV m$^{-1}$ are in the minute time range. Therefore, it is only membrane electroporation that can explain the absorbance relaxation data of the DPH-labeled vesicles.

Finally, the elastic curvature energy of the vesicle membrane is crucially important for the description of the increase in the extent and rate of the absorbance relaxations with increasing vesicle curvature.

Electroporative transport and cooperativity

Both electrically induced release and uptake of substances by cells and vesicles is only observed beyond a certain threshold field strength $E_T$. $E_T$ decreases with increasing pulse duration. It is recalled that the structural changes underlying ME proceed continuously with increasing field strength. Transport appears to require a certain critical pore density. In addition, it appears that larger molecules such
as dyes or DNA transiently interact with the membrane lipids. Indeed, the data suggest that the macromolecule X interacts with n pores (P) according to the cooperative scheme

$$X + nP = XP_n$$

during translocation through porated membrane patches. We found that for $X = \text{DNA}$, $n = 6$. This means that at least six small pores are involved in the contact region of a part of the DNA molecule with a porous membrane patch. The interaction model rationalizes the observation of relatively small pore sizes ($0.3 \text{ nm} \leq r_p \leq 0.8 \text{ nm}$) with the electroportive transport of large molecules. Larger stationary pores are conductive and would locally discharge the interfacial condenser, thereby reducing the extent of poration. Larger pores may, however, exist under massive osmotic flow or when vesicle deformation leads to volume flow through the electropores. These volume flows are dependent on the fourth power of $E$ (S. Kakorin, unpublished data), such that an apparent threshold behavior may be seen in the data.

A real threshold is associated with the so-called Q-reaction, Figure 6, arising from the asymmetrical increase in the membrane conductivity in one of the vesicle pole caps due to the asymmetrical pore formation, beginning at a certain field strength. The transient dichroism associated with the transient additional pore formation is concomitant with a transient increase in transmembrane voltage in one of the vesicle pole caps (Fig. 5). These data are consistent with the data on voltage-sensitive fluorescent membrane probes [23,24].

**Protein–membrane interactions**

One of the more recent analytical applications of the electroportation method refers to the interaction of proteins with membrane surfaces. The adsorption of annexin V to the vesicle membrane does not change the light absorbance spectrum of the membrane probe β-DPhpPC. Yet, extent and rate of electroportation of the vesicle membrane, covered by annexin V, are increasingly reduced with increasing protein adsorption up to an annexin V (AN) concentration of $[\text{AN}] = 4 \mu M$, where the protein adsorption appears to saturate (K. Tönsing, S. Kakorin, E. Neumann, unpublished data). Detailed analysis of the absorbance relaxation in the electroportated vesicles (Fig. 7) suggests that the annexin V partially penetrates the outer leaflet of the lipid layer of the membrane, thereby reducing the local curvature and, in turn, reducing the gradient in the lateral pressure across the vesicle membrane. The degree of the annexin replacements in the membrane is about 0.01%. Therefore, it is most likely that only a molecular group of the protein, perhaps only the tryptophan residue, is inserted in the membrane. In summary, ME and the chemical thermodynamic analysis of the electroportive data provide new insight into the modes of protein–membrane interactions.

**Conclusion**

Electrooptical relaxation methods using optical membrane probes and polarized light have provided new insights into the molecular mechanisms of electric pore formation in lipid membranes as well as electric shape deformations of lipid vesicles. The specific chemical state transition model $C = (\text{HO}) = (\text{HI})$ for the electroporative lipid rearrangements causing HO and HI pore states, induced by ionic interfacial polarization and water entrance, has proven to be instrumental not only for the determination of mean pore numbers and mean pore radii but also for the description of extent and rate of vesicle shape deformations and curvature effects enhancing the electroportability.

Remarkably, the pore radii are relatively small and the density of the electropores increases continuously with time and electric field strength. There are apparent threshold field strength values and delay times, however, for the electroportation-induced massive permeability changes to small ions, dye molecules, proteins and nucleic acids, such as DNA in electroporative gene transfer. The data suggest that the cross-membrane transport of macromolecules transiently involves cooperative adsorption complexes, with several membrane pores progressively coalescing in contact with the (electro-)diffusing polymer and annealing after the passage of the permeants.

Future work aims at using optical DPH probes in cell plasma membranes to elucidate the sequence of events of the electroporative DNA and protein transfers as well as to investigate molecular details of electofusion and electroinsertion phenomena.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


New step towards a quantitative theory of direct transfer of plasmid DNA by membrane electroporation of cells. Gene uptake is mainly due to electrodiffusion of DNA molecules across electroporated membrane patches. A biomolecular DNA-binding interaction in the cell interior is the rate-limiting step for the cell transformation. The kinetic data analysis yields practical instructions for optimal transformation efficiency of yeast cells.


The current theoretical understanding of the concept of the membrane bending elasticity and curvature model is presented in this review article. The morphology and changes in the morphology of vesicles are considered using this concept. In many cases, links between the theory and the experiment have been established.

43. Abidin IG, Arakelyan VB, Chumakova LV, Chizmadzhev YA, Pastushenko VP, Tarasevich MI: Electric breakdown of bilayer


This review paper summarizes theoretical and experimental achievements in electroporation science and contains a comprehensive list of references to the literature of this quickly expanding field.


A nice review article on phenomenological physical properties of lipid vesicles. Special attention was paid to the structural dynamics and elastic properties of fluid and solid bilayer vesicles, together with the thermodynamics of the thermotropic transitions.