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Lipid membranes in external electric fields: Kinetics of large pore formation causing rupture

Mathias Winterhalter

School of Engineering and Science, Jacobs University Bremen, Germany

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ABSTRACT

About 40 years ago, Helfrich introduced an elastic model to explain shapes and shape transitions of cells (Z Naturforsch C, 1973; 28:693). This seminal article stimulated numerous theoretical as well as experimental investigations and created new research fields. In particular, the predictive power of his approach was demonstrated in a large variety of lipid model system. Here in this review, we focus on the development with respect to planar lipid membranes in external electric fields. Stimulated by the early work of Helfrich on electric field forces acting on liposomes, we extended his early approach to understand the kinetics of lipid membrane rupture. First, we revisit the main forces determining the kinetics of membrane rupture followed by an overview on various experiments. Knowledge on the kinetics of defect formation may help to design stable membranes or serve for novel mechanism for controlled release.

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1. Introduction

To characterize the shape or shape transitions of complex cells under external forces, viscoelastic models have been successfully applied [1–8]. In 1973, Wolfgang Helfrich suggested his elastic model in a seminal article [3]. In analogy to smectic liquid crystals, he derived a functional relationship of the elastic energy on deformation and the related material parameters like area expansion modulus, Gaussian modulus and bending elasticity. Giving a rational estimation of the expected range of the effects, he was able to explain changes in the shape. His theoretical predictions could be experimentally verified surprisingly well [4–8]. This approach has been particular successful to study shapes as well as shape transitions and stimulated many research groups to continue in various direction. For example, mechanical tension or external magnetic fields can induce shape transitions [1,9]. In a further publication, Helfrich [10] extended his model to the possible order of magnitude of vesicle deformation in a homogeneous external electric field. Subsequently, Helfrich and coworkers [11] verified his prediction experimentally. Increasing electric field strength caused the deformation of an originally spherical vesicle up to lysis. Two holes on each pole can be formed and stabilized by an external electric field. This tubelike shape is an equilibrium structure given by the bending energy and the external forces and allows subsequently for an estimate of the bending energy [11]. Further experiments revealed that this particular sequence of action depends very much on the actual mechanical properties [12–23]. Lengthy calculations accounting for the detailed electric field distribution revealed only minor corrections [12]. More recently, sophisticated calculations supported the earlier predictions

E-mail address: m.winterhalter@jacobs-university.de.

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and were able to solve experimental contradictions [20]. In particular, accounting for possible differences in solvent conductances inside and outside of the vesicle allows to obtain deformation from a spherical to an oblate vesicle under high AC fields [18,20].

The above-described work initiated an investigation on the pore forming mechanism. First, we will revisit an earlier work by Helfrich and coworkers on the electric field forces at large pores. In the following sections, we present the charge pulse technique allowing the rapid detection of membrane rupture. This technique has been applied to characterize the rupture of a broad range of modified lipid membranes.

1.1. Electric field effect on pore stability

To understand the pore formation of vesicles under high electric field pulses, Helfrich and coworkers [21] revisited the underlying mechanism. In absence of electric fields, the stability of a membrane may be explained by two competing energies (Deryagin-Gutop relation) [24]:

$$E_{\text{pore}} = 2\pi \cdot a \cdot \Gamma - \pi \cdot a^2 \tau \tag{1}$$

wherein the first term describes the energy needed to cut intermolecular interaction (represented by the line tension Γ) and to build up the edge of a cylindrical pore of radius a. The second term represents the energy gained by the release of the pore membrane area under the membrane tension τ. Combining both contributions yields a critical radius above which irreversible rupture occurs [25–30].

Accounting for an external electric field requires additional considerations (see schematically Fig. 1): initially, the membrane is a perfect insulator; the formation of a pore dramatically changes the boundary conditions, enhancing the electric field effect. If we denote the applied electric potential across the membrane by V_0, the electric line tension $\Gamma_{\text{el}} = -\varepsilon_w \varepsilon_0 V_0 2/2\pi$ leads to an opening of the pore, and the contribution to the membrane tension $\tau_{\text{el}} = \varepsilon_l \varepsilon_0 V_0 2/2d$ emanating from the field along the membrane surface tends to close it again. Here, d denotes the membrane thickness, $\varepsilon_0 = 8.810^{-12}$ As/Vm stands for the permittivity of vacuum, $\varepsilon_w = 80$ is the relative dielectric constant of the aqueous (w) and $\varepsilon_l = 2$ for the lipid phase (l). Incorporating both contributions into Eq. (1) by replacing the purely mechanical parameters by an effective Γ_{eff} edge energy and an effective τ_{eff} tension, we obtain

$$E_{\text{pore}} = 2\pi \cdot a \cdot \Gamma_{\text{eff}} - \pi \cdot a^2 \tau_{\text{eff}} = 2\pi \cdot a \cdot \left(\Gamma - \varepsilon_w \cdot \varepsilon_0 \frac{V_0^2}{2\pi}\right) - \pi \cdot a^2 \cdot \left(\tau - \varepsilon_1 \cdot \varepsilon_0 \frac{V_0^2}{2d}\right) \tag{2}$$

Inspection of Eq. (2) reveals two possible electric field effects on pore formation: increasing the applied potential V_0 in Eq. (2) may either yield $\Gamma_{\text{eff}} = 0$ or $\tau_{\text{eff}} = 0$. In the first case, an enhancement of the field reduces the critical radius and promotes rupture. In the second case, an increase of the applied external voltage V_0 stabilizes the membrane and causes stable pore formation at higher voltages.

It is interesting to note that most authors neglect the deformation of the electric field after pore formation. In a simplification, only the condenser energy corresponding to an exchange of the dielectric medium lipid against water $(\varepsilon_l - \varepsilon_w)\ \varepsilon_0 V_0 2/2d$ has been added [32–34]. During the early stage, a so-called hydrophobic pore was postulated [34–40]. However, even for hydrophobic pores, the electric potential will be altered [21].

The above-mentioned equations describe the stability of a pore in a film using two global parameters: membrane tension versus intermolecular forces, which keep the film together. Below, we extend this approach to cover kinetic phenomena under the influence of additional external forces pushing the film out of equilibrium, for example, the wetting of a solid surface by lipid membranes, the rupture of free-standing lipid membranes or the bursting of soap films [41–43]. Earlier studies have been done on the bursting of soap films [43]. Large soap films were spanned across a rim, defects were induced by electrical discharges and the kinetics of film rupture were recorded using a high speed camera [43]. The velocity of the pore rim can range from several meters per second down to micrometers per second [43–51]. The observed velocities represent the balance between the mechanical forces exceeding those driving the opening (or closing) of the pore as well as the damping forces. The latter depend on the velocity and are typically dominated by viscosity, or in fast pore widening, by inertia.

Inspection of Eq. (2) yields a force, which is either directed outwards, causing further widening, or inward, causing resealing [44]. The unbalanced part of both forces will cause a movement of the lipid. A general solution of the motion of the thin film requires a solution of the Navier–Stokes equation. To be able to perform such calculations, knowledge of the experimental boundaries and the exact material flow profile is needed. A closer inspection of the particular experimental conditions reveals that during the "opening process" of the pore, lipid flows radially away from the center to the torus of the membrane. The exact solution of this membrane material flow requires knowledge of the local stress distribution after breakdown. To reduce the complexity, we select experimental conditions in our experiment in a manner that the effect of voltage is only to induce the initial critical pore size and decays very rapidly due to the discharge across the pore. Beyond the critical radius membrane, tension remains the major driving force for the opening process. In this limit, the kinetic of pore widening is determined by either the membrane viscosity or the inertia of the accelerated material flow. As shown [44], a damping force acting at the boundary and working against the movement of the pore rim must be added to the mechanical forces derived from Eq. (2). The viscous force is given by

$$f^a{}_{\text{vis}} = -8\pi\eta da/dt \tag{3}$$

where η stands for the solvent viscosity. This force has to be balanced with

$$f^a{}_{\text{tension}} = 2\pi a \tau \tag{4}$$

The combination of both terms readily yields an exponential time-dependent increase in the rim of the pore

$$a(t) = a_0 e^{\left(\frac{\tau}{4\eta}\right)t} \tag{5}$$

The decay time $t_{\text{vis}} = 4\eta/\tau$ is about 2×10^{-6} s for reasonable values [44] of η (10^{-9} Ns/m) and of τ ($= 2 \times 10^{-3}$ N/m). The exponential increase in the pore widening will result in a rapid flow of the rim material. At larger velocities, the kinetic energy of the moving lipid material has to be included

$$E_c = \pi a^2 d\rho \left(\frac{da \cdot}{dt}\right)^2 \tag{6}$$

causing an inertia based opposing force to the pore widening

$$f_{\text{inertia}} = 2\text{d a}(\text{da/dt})^2 \tag{7}$$

with ρ as the lipid density. Balancing the force from the membrane tension and that from inertia, we obtain the time dependency of the pore radius as [44]

$$a(t) = a_0 + \sqrt{\frac{\Phi \cdot \tau}{d\rho}} \cdot t = a_0 + u \cdot t \tag{8}$$

Here, Φ is a parameter depending on the unknown material flow as well as on dissipation effects and u is the velocity of the pore radius.