Undulation instability of lipid membranes under an electric field

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Abstract

The influence of an electric field on a poorly conductive membrane such as a lipid bilayer is studied theoretically. The unbalanced electric stress created by an ionic current across a non-perfectly flat membrane gives rise to a destabilizing surface energy enhancing undulations. The deformation of a membrane attached to a frame and the subsequent force on the frame are derived and the electrohydrodynamic instability of a free floating membrane is also studied. We find a most unstable mode of undulation, of wavelength in the μm range, connected to the crossover between membrane and solvent dominated dissipations.

87.16.Dg Membranes, bilayers, and vesicles
47.20.-k Hydrodynamic stability
87.50.Rr Electric fields
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Due to their low permeability to electrolytes, biological or other lipid membranes are strongly influenced by applied electric fields. Researchers are actively investigating phenomena such as *electroporation* (creation of long-lived pores in a lipid membrane under the action of a strong electric field) [1,2], in part because of very promising applications for gene therapy. Similarly, the *electroformation* of lipid vesicles is a widely used technique to form large unilamellar vesicles under the action of an electric field [3]. Despite its importance, there is no clear understanding of this experimental tool, which remains mostly empirical. Many other situations (electrofusion [4], electro-injection of macromolecules in vesicles and cells [5]) require a better understanding of the action of electric fields on lipid bilayers. This paper addresses this issue theoretically, and discusses the effect of an electric field on *i)* the force exerted on a rigid frame by an attached membrane, and *ii)* the dynamical instability of a free floating membrane and its relevance for the electroformation of neutral liposomes. Before reaching these two topics, we study the stability of an almost flat membrane under electric field.

The force acting on an interface in an electric field E can be calculated by evaluating the discontinuity of the Maxwell stress tensor $\sigma_{ij} = \epsilon(E_i E_j - \frac{1}{2}E^2\delta_{ij})$ across the interface [6]. We consider an infinite membrane of dielectric constant $\epsilon_m(\simeq 2\epsilon_0)$ and conductivity $\chi_m^{-1}(\simeq 10^{-6}S/m)$ in a solvent of dielectric constant $\epsilon_1(\simeq 80\epsilon_0)$ and conductivity $\chi_1^{-1}(\simeq 10^{-1}S/m)$ (numbers are typical for a lipid membrane in water, ϵ_0 is the permitivity of vacuum). A fixed electric field E (in practice, a fixed electric current j) is applied across the membrane, and we note E_m the electric field *inside* the membrane.

If the membrane is *perfectly* flat, the electric stress is symmetrically balanced on both sides of the membrane (i.e., $\sigma_{el} \simeq \epsilon_m E_m^2 - \epsilon_m E_m^2 = 0$), which experiences a mere compression [7]. As already pointed out in [2], this perfect cancellation of the net electric stress is however accidental since any membrane curvature leads to an unbalanced net stress whose order of magnitude can be expressed as $\sigma_{el} \simeq \epsilon_m E_m^2 d/R$, where d is the thickness of the membrane and R its local radius of curvature (a curvature of the membrane leads to a non-uniform surface charge density - see Fig.1). In the case of a closed vesicle [2], E_m is given by the potential drop at the vesicle scale (i.e., $E_m \sim ER/d$) as most of the electric field goes around the finite sized object, whereas the current continuity across the (infinite) membrane dictates the expression of E_m here, namely $E_m \sim E\chi_m/\chi_1$. This resulting stress σ_{el} tends to *enhance* the (small) local undulations of the membrane $u(r) = \sum_q u_q e^{iqr}$ (Monge representation in real and Fourier space). After a short transient time $\tau_e \simeq (\epsilon_m/d)\chi_1 H \simeq 10^{-6} - 10^{-5}$s ($H$ is the distance between the electrodes), the solution of the Poisson equation (in the limit $\epsilon_m\chi_m^2 \gg \epsilon_1\chi_1^2$) yields the following expression for a given Fourier mode: $\sigma_{el} = 2\epsilon_m(E\chi_m/\chi_1)^2(e^{qd}-1)/(e^{qd}+1)u(\mathbf{r})$. Hence, integrating the work of the electric stress (in the large wavelength limit $qd \ll 1$) yields a net decrease in energy

$$F_{el} = -\frac{\Gamma_{el}}{2}\int dS(\nabla u)^2 \qquad \Gamma_{el} = \epsilon_m\left(\frac{\chi_m}{\chi_1}\right)^2 E^2 d \tag{0.1}$$

This amounts to an effective *negative* surface tension Γ_{el} acting on the membrane. In the presence of a lipid reservoir (or at the interface between two immiscible fluids of different conductivities), this term leads to an electric field induced decrease of the interfacial tension, known as the *electrocapillary effect* [8]. On the contrary, for a fixed number of surfactants,

enhancing membrane undulations under electric field builds a concomitant mechanical tension because of deviation from the nominal area per molecule. Hence we expect a tense yet floppy-looking membrane in this case. For typical values of the electric field used in the electroporation and electroformation experiments $E \sim 10^3 V/m$, the electrostatic surface tension reaches $\Gamma_{el} = 10^{-3} J/m^2$, which is of order the mechanical tension needed to rupture a lipid membrane [1].

Two consequences of this destabilizing effect are studied below: *i*) a membrane on a fixed frame is deformed until the electric stress is balanced by an opposing mechanical stress and *ii*) a free membrane undergoes strong undulations under electric field.

$\diamond$ Case of a bilayer attached to a fixed frame

The elastic behavior of a lipid membrane is generally characterized by a bending modulus κ ($\sim 5 \times 10^{-20} J$), and a stretching modulus K_s ($\sim 0.1\ J/m^2$) [9]. Typically, bending a lipid membrane involves energies of order $1 - 10 k_B T$, while stretching it requires much larger energies. To simplify the description below, we will not include the bending rigidity in the treatment of the static deformation of the membrane, nor will we include the thermal fluctuations of the membrane, restricting ourselves to large electric fields.

We write a linear theory for small deformations of the membrane $q u_q \ll 1$. The membrane is characterized by its total area S, its projected area S_p, and its optimum area for which it is not stretched S_0. We use the two small parameters $\delta \equiv (S_p - S_0)/S_0$ and $\mathcal{I} \equiv (S - S_p)/S_0 \simeq (1+\delta)\frac{1}{2}\sum q^2 |u_q|^2 \simeq \frac{1}{2}\sum q^2 |u_q|^2$. The electric-field-induced undulation term involves the parameter $\mathcal{I}$ only (Eq.(0.1)), and the stretching energy involves the total increase of area with respect to S_0:

$$F = S_0 \left(\frac{1}{2} K_s (\delta + \mathcal{I})^2 - \Gamma_{el} \mathcal{I} \right) \tag{0.2}$$

Note that the electric-field-induced undulation term (Γ_{el}) is quadratic in the small quantity $q u_q$, while the stretching term (K_s) corresponds to an expansion to the fourth order, consistent with the large value of the ratio of the stretching over the electrostatic parameters $\beta \equiv K_s/\Gamma_{el} > 100$.

The minimization of this energy with respect to the out-of-plane membrane deformation $\mathcal{I}$ leads to an equilibrium class of membrane shapes corresponding to a total area difference Δ_S

$$\Delta_S = \delta + \mathcal{I}_{eq} = \frac{\Gamma_{el}}{K_s} \sim 10^{-2} \tag{0.3}$$

The energy of the membrane only is $F_m = \frac{1}{2} S_0 \frac{\Gamma_{el}^2}{K_s}$, and the energy of the system, including the electrostatic energy, is $F = F_m + F_{el} = -\frac{1}{2} S_0 \frac{\Gamma_{el}^2}{K_s} + S_0 \Gamma_{el} \delta$.

The surface tension of the lipid membrane at equilibrium is given by $\gamma = \partial F_m / \partial S$ with $S = S_0(1 + \delta + \mathcal{I})$

At equilibrium, the mechanical tension is equal, but opposite in sign, to the electrostatic energy per unit area: $\gamma = \Gamma_{el}$. The building of a mechanical tension in a membrane under electric field is due to an increase of its area.

Beyond the electroporation threshold, holes in the membrane reduce the mechanical tension by decreasing the membrane area and strongly increasing the membrane electrical conductivity $1/\chi_m$. The effect of the electric field could be measured via the setup depicted in Fig.2. The force measured by the spring is $f = \partial F_{tot}/\partial L_p = \Gamma_{el} L_y$ ($\sim 10^{-3} N/m$), where L_p and L_y are the membrane projected size in the direction of and perpendicular to the spring respectively. The membrane pulls on the spring ($f > 0$) if $\mathcal{I} > -\delta$. The force in the absence of field is simply: $f_0 = K_s L_y \delta$.

It is clear from Eq.(0.3) that the balance between electric-field-induced undulation and membrane stretching does not select a particular equilibrium membrane shape, as both effects depend on the global increase of membrane area only. Including the bending energy of the membrane selects the shape of lowest curvature, namely the first harmonic $q_1 = \pi/L_p$. The energy gap between the different harmonics is however small (of order $k_B T$) for $q\lambda_\kappa < 1$, where $\lambda_\kappa = 2\pi\sqrt{\kappa/\Gamma_{el}}$. For large electric fields ($E \sim 10^3 V/m$), λ_κ is very small ($\sim 10nm$). For small electric fields, the thermal fluctuations dominate both the membrane shape and tension. We postpone the study of the interesting crossover between these two limits to a future publication.

There are some dynamical issues connected to force measurements on lipid membranes (Fig.2). Lipid membranes spread on a frame are generally connected to the frame by meniscii which are large compared to the membrane thickness, and from which lipid molecules may flow toward the deformed membrane (a phenomenon reminiscent of the Marangoni effect [10]). In this case, the membrane elastic stress consequent to the action of an electric field is rapidly released by migration of lipid molecules, and might not be detectable experimentally. The kind of force measurement depicted on Fig.1 should however be feasible on polymeric membranes, which have shown sensitivity to electric field as well [11], and for which the migration of molecules along the membrane, if any, is very slow. An alternative experimental setup for lipid membranes involves measurements on lipid membrane strongly bound to a solid substrate, which are expected to exhibit a fairly slow lateral dynamics.

$\diamond$ Instability of free floating membranes under E-field

We now study the case of a free floating membrane under an electric field. This study is partly motivated by the experimental technique of electroformation of vesicles, which allows a controlled swelling of an electrode deposit of lipids to form vesicles of fairly well controlled sizes under electric field [3]. Interestingly, this technique works for charged and nonionic lipids, although the optimal conditions vary widely with the nature of the lipids.

The dynamics of a free membrane under electric field can be decomposed into two mechanisms. *i*) The normal deformation of the membrane (described in the previous section), which creates a tension γ in the membrane and saturates when $\gamma = \Gamma_{el}$. *ii*) The lateral sliding (contraction) of the membrane, in an attempt to release this tension. The characteristic time for the normal deformation for a given undulation mode of wavelength λ is $\tau_\perp \sim \eta\lambda/\Gamma_{el}$ ($\sim 10^{-6} s$ for $\lambda = 1\mu m$), while the sliding motion involves the whole membrane $\tau_\parallel \sim \eta L/\Gamma_{el}$ ($\sim 10^{-3} s$ for $L \sim 1mm$). The two mechanisms occur at very different timescales, and can be treated separately.

A thorough treatment of the undulation modes of a film immersed in a solvent can be found in the literature [12]. The electrohydrodynamic instability of a layer of non-conducting fluid between two semi-infinite conducting fluids has been studied in [13], where special

attention is given to the peristaltic deformation modes (the two interfaces undulating in antiphase), as these modes lead to the destruction of the film when the two interfaces make contact. In the case of a lipid membrane, these peristaltic modes are suppressed because of the very low compressibility of the film. We study below the bending instability (interfaces undulating in phase) of the membrane.

i) The normal displacement of the membrane involves viscous dissipation in and around the membrane [14,15]. There are three main dissipation mechanisms, namely the dissipation in the solvent (viscosity $\eta = 10^{-3} Pa.s$), which dominates the dynamics of large wavelengths deformation, the friction between the two monolayers (friction coefficient $b_{fr} = 10^8 Pa.s/m$), dominant at intermediate wavelengths, and the membrane surface dissipation (surface viscosity $\mu = 10^{-10} Pa.s.m$). For undulating membranes, the latter mechanism is relevant at very small wavelength of the order of the bilayer thickness $d = 5nm$ only, and will be neglected in what follows. We present below a simplified description of the interplay between external and internal dynamics. For a thorough treatment of membrane dynamics, see Seifert [14].

Neglecting inertia, a normal deformation of the membrane (of typical lateral size $2\pi/q$ and typical velocity $\dot{u}_q$) creates a motion in the surrounding fluid which propagates to a distance $\sim 1/q$. The curvature of the membrane leads to a velocity difference of order $\delta v = q d \dot{u}_q$ between the two monolayers. The power dissipated by viscous effect around and in the membrane can be written respectively:

$$\begin{aligned} P_\eta &= \eta \int dV \, (\nabla v)^2 = S\eta \sum_q q \dot{u}_q^2 \\ P_{fr} &= b_{fr} \int dS \, (\delta v)^2 = S b_{fr} \sum_q d^2 q^2 \dot{u}_q^2 \end{aligned} \tag{0.4}$$

This dissipated power must compensate the power stored in the membrane $P_m = \partial_t F$ (the energy F is given by Eq.(0.2)). This condition leads to an evolution equation for each deformation mode:

$$\begin{aligned} &(\eta q + b_{fr} d^2 q^2) \dot{u}_q(t) = \\ &\left[\Gamma_{el} q^2 \left(1 - \beta(\delta(t) + \mathcal{I}(t))\right) - \kappa q^4\right] u_q(t) \end{aligned} \tag{0.5}$$

with $\beta \equiv K_S/\Gamma_{el} > 100$. The left hand side of this equation describes the viscous dissipation in and around the membrane and the right hand side consists of the electric-field-induced undulation term (Γ_{el}) with a stretching saturation (β term). Note that the bending rigidity of the membrane has been added to the membrane energy (κ term), for it is mandatory for the description of the small wavelength deformations $q > \sqrt{\Gamma_{el}/\kappa}$. This equation is non-linear, since the saturation involves the total increase of area $\mathcal{I} = \frac{1}{2} \sum_q q^2 |u_q|^2$. Thanks to the different time scales for normal and lateral motions, the projected area of the membrane $S_p(t) \propto \delta(t)$ can be considered as constant for the short time evolution.

This non-linear equation can be solved, but for our purpose, it is sufficient to consider its linearized form, and to treat the saturation dynamics separately. The short time evolution is described by the linear equation

$$(\eta q + b_{fr} d^2 q^2) \dot{u}_q = (\Gamma_{el} q^2 - \kappa q^4) u_q \tag{0.6}$$

The amplitude of a given Fourier mode has a time evolution $u_q(t) \sim e^{\alpha_q t}$ with

$$\alpha_q = \frac{\Gamma_{el}}{\eta} q \left(\frac{1 - (q/qstat)^2}{1 + q/q_{dyn}} \right) \tag{0.7}$$

with the two characteristic wavevectors:

$$q_{stat} = \sqrt{\frac{\Gamma_{el}}{\kappa}} \sim 5.10^7 m^{-1} \quad q_{dyn} = \frac{\eta}{bd^2} \sim 5.10^5 m^{-1} \tag{0.8}$$

The evolution rate presents a sharp maximum at

$$q^* = \left(q_{dyn} q_{stat}^2 \right)^{1/3} = 8.10^6 \ m^{-1} \tag{0.9}$$

for $q_{stat} \gg q_{dyn}$. This defines a particular lengthscale which grows exponentially faster than the others: $\lambda^* = 2\pi/q^* \sim 1 \ \mu m$. The corresponding growth rate is $\alpha_q^* \sim \frac{\Gamma}{bd^2} \sim 5.10^5 s^{-1}$. The evolution saturates similarly for all lengthscales when $\beta \left[\delta(t) + \mathcal{I}(t) \right] = 1$, at which point a mechanical tension Γ_{el} is established, leading to a contraction of the membrane.

ii) The lateral motion of the membrane occurs at an almost constant surface tension, because the time needed to build up the tension $\tau_\perp$ is much smaller than the time $\tau_\parallel$ over which it could be released. Since the contraction of the membrane involves solvent flow over large lengthscales (mm), inertial effects must be included [16]. The flow created by the lateral motion of the membrane (of size L) propagates over a size $L_z = L/(\sqrt{1 + L^2/(\nu t)})$ ($\nu = \eta/\rho$ is the kinematic viscosity $\sim 10^{-6} m^2/s$ for water - ρ is the density of water). The comparison of the power dissipated by the sliding motion of velocity $\dot{L}$: $P_{diss} = \eta \int dV (\nabla v)^2 = \eta L^2 \dot{L}^2 / L_z$, with the power stored in the membrane $P_{stor} = \Gamma_{el} L \dot{L}$, leads to the dynamical equation for the membrane size:

$$\dot{L} = -\frac{\Gamma_{el}}{\eta} \frac{L_z}{L} = -\frac{\Gamma_{el}}{\eta} \frac{1}{\sqrt{1 + L^2/(\nu t)}} \tag{0.10}$$

The short time evolution ($t < L_{t=0}^2/\nu \sim 1s$) is dominated by the diffusion of the solvent flow. Assuming that $L_{t=0} = L_0$ (No stretching without electric field), the evolution equation for short time is

$$\begin{aligned} L(t) &= L_0 \sqrt{1 - 4/3 \sqrt{t^3/(\tau_\Gamma^2 \tau_\nu)}} \\ \tau_\Gamma \equiv \eta L_0/\Gamma_{el} \simeq 10^{-3} s &\qquad \tau_\nu = L_0^2/\nu \simeq 1s \end{aligned} \tag{0.11}$$

from which emerges a characteristic time scale $\tau_{slide} = \left(\tau_\Gamma^2 \tau_\nu \right)^{1/3} \sim 10^{-3} s$. This result is reminiscent of the dynamics of bursting of a soap film in a viscous environment [17]. The evolution of the variable $\delta = L_p/L_0 - 1$ which appears in Eq.(0.5), is given by $\delta(t) \simeq 2/3 (t/\tau_{slide})^{3/2}$.

In many practical situations, fixed boundaries or walls (the electrodes in the case of the electroformation of vesicles) may strongly modified the hydrodynamics. The solvent viscous dissipation (Eq.(0.4)) is modified by the presence of a wall at a distance h from the membrane, and becomes $P_{\eta,h} = S\eta \sum_q \dot{u}_q^2/(q^2 h^3)$ for $qh < 1$. The left hand side of Eq.(0.6)

is modified accordingly, and shows an optimal wavevector $q_h^* \sim \sqrt{q^*/h}$ where q^* is given by Eq.(0.9). For $h = 10nm$, the fastest growing wavelength is of order $2\pi/q_h^* \sim 0.1\mu m$. The sliding motion is strongly affected by the wall. For times larger than h^2/ν, the time dependent length L_z should be replaced by the distance h in Eq.(0.10), and the dynamics is described by $L(t) = L_0\sqrt{1 - t/\tau_h}$ with $\tau_h = \tau_\Gamma L_0/h \sim 10^2 s$ instead of $\tau_{slide} = 10^{-3}s$ (note that solvent permeation through lipid membrane becomes relevant near a wall)!

To conclude this paper, we would like to propose that the electrohydrodynamic instability described above may play an important role in the first stage of the electroformation of liposomes. A remarkable feature of this technique is that it produces vesicles of fairly well defined size. We find a fastest growing undulation mode of wavelength in the μm range (Eq.(0.8,0.9)). This mode might be the precurssor of large scale deformations of the membrane which, after a complex process partly sketched in Ref [3] and involving coalescence of neighboring blisters, lead to the formation of closed vesicles (the size of which can reach 50 μm for nonionic lipids [18]). Future developments will include the treatment of small pores which are expected to be present in a membrane under tension, and their influence on both the membrane electrical conductivity (hence Γ_{el}) and dynamics (solvent permeation through the membrane).

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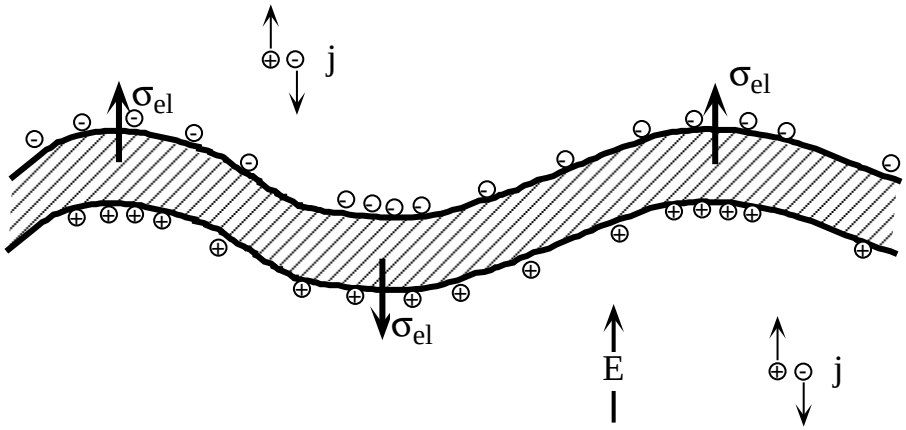

Fig.1 Net accumulation of conduction charges near
a curved lipid membrane under electric field

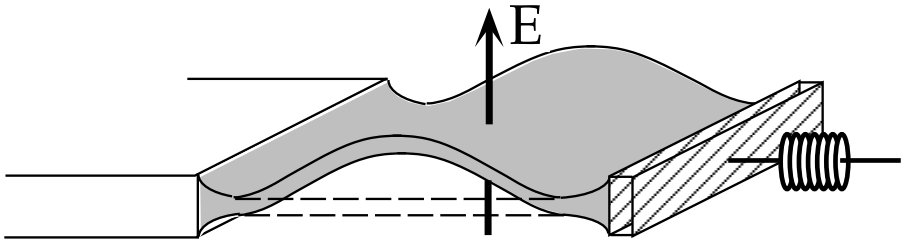

Fig.2. Possible experimental setup for the force measurement