Transient and Steady-State Deformations of a Vesicle with an Insulating Membrane in Response to Step-Function or Alternating Electric Fields

Hiroyuki HYUGA, Kazuhiko KINOSITA Jr. and Nobuyoshi WAKABAYASHI

Department of Physics, Faculty of Science and Technology, Keio University,
Hiyoshi 3-14-1, Kohoku-Ku, Yokohama 223

Deformation of a vesicle with an insulating membrane under an external electric field is considered. Application of a step-function field, under which the eventual form is prolate, is shown to transientiy induce an oblate deformation, provided that the conditions of the surrounding medium are chosen properly. It is further demonstrated that alternating electric fields induce deformation in a frequency-dependent manner; the deformation at low frequencies is always a prolate one, whereas the steady-state deformation at high frequencies is either prolate or oblate depending on the medium conditions. If this frequency dependence is experimentally observable, the capacitance and curvature elastic modulus of the membrane as well as the intravesicular conductance can be estimated directly from the data. The field-induced deformation may serve as a novel experimental method in cell biology.

KEYWORDS: vesicle deformation, electric field, cell, liposome, membrane capacitance, curvature elastic modulus

§1. Introduction

Closed-membrane vesicles such as cells or liposomes undergo deformations when they are exposed to an electric field. Analysis of the pattern and extent of the deformation should yield information on electrical and mechanical properties of the membrane and of the vesicle interior. The theoretical basis for such analyses, however, appears rather limited.

Helfrich[1] has shown that, when the electrical conductance of the membrane is negligibly small, the deformation of a spherical vesicle will always be toward a prolate ellipsoid with its major axis parallel to the field. Ionic conductance of normal cell membranes and lipid bilayers are in fact very small.[2] Under an intense electric field, on the other hand, a dramatic increase in the membrane conductance may take place,[3,4] a phenomenon called electroporation. For the highly conductive membrane, theory[5-8] predicts both prolate and oblate deformations; the direction of the deformation is determined by the ratio of the specific conductivity of the medium inside the vesicle to that outside.

None of the above theories take into account the finite time constant with which the membrane capacitance is charged. During this transient phase, as we show below, the direction of the force acting on the membrane can be different from the steady-state force. A vesicle with an insulating membrane, for which the eventual form is prolate, is initially subjected to a force leading to oblate deformation, provided the internal conductivity is smaller than the external one. This requirement for the conductivity ratio is fulfilled by most cells under physiological conditions.

In this paper, we first calculate the transient behavior of a vesicle, with an insulating membrane, in response to a step-function electric field. Then we calculate the steady-state deformation achieved under an alternating (sinusoidal) electric field. In this case, the direction and extent of deformation depend on the frequency of the alternating field. Oblate deformation expected at high frequencies is much larger than that which occurs during the short transient period under a step-function field of similar magnitude. For the purpose of the experimental estimation of the intracellular conductance, therefore, stimulation by an alternating field is the method of choice. From the frequency dependence, in addition, one can directly estimate the time constant for charging the membrane capacitance. The extent of deformation may also be related to the elastic modulus of the membrane.

Deformation of individual cells (or liposomes) can be observed under a microscope. We believe that the analysis of field-induced deformation will prove useful in assessing the electrical and mechanical properties of a single, intact cell without penetrating it with a microelectrode and without causing electroporation.

The remainder of this paper is organized as follows. In §2, we derive dynamical equations that determine the electric field in and around the vesicle, eq. (2.17), and the initial conditions, eq. (2.20), taking into account the capacitance and conductance of the membrane. In §3, these equations are solved and the electric force acting on the cell is derived for the case of a homogeneous membrane for which the capacitance (or dielectric constant) and conductance are assumed to be constant and to not vary with time. The deformations under step-function fields are discussed in subsection 3.2. In subsection 3.3, the deformations under alternating electric fields are discussed in detail, and in 3.4, their implications on experimental determination of cellular properties are explained.

§2. Fundamental Equations for Electric Potentials

2.1 Fundamental equations

We consider the situation in which a vesicle is placed in

an aqueous solution. The conductivity and dielectric constant of the medium inside the vesicle are, respectively, σ_i and ε_i, and those of the external medium are σ_e and ε_e. The conductivity of the membrane of the vesicle is denoted as σ_m and the dielectric constant as ε_m. In the following, the suffices i, e, and m are used to distinguish quantities related, respectively, to the internal, external and intramembrane regions of the vesicle.

Fundamental equations to determine the electric field are the continuity equation for the electric charge and current, $\partial \rho / \partial t + \mathrm{div}\, J = 0$, the relationship between the current and the field, $J = \sigma E$, and one of the Maxwell equations, $4\pi \rho = \mathrm{div}\, \varepsilon E$. Since we consider only the case of an applied electric field, we neglect the small effects of the induced magnetic field. Therefore the electric field can be regarded as irrotational, $\mathrm{rot}\, E = 0$, and it is expressed in terms of the electric potential ϕ as $E = -\mathrm{grad}\, \phi$. Combining all together, we immediately arrive at the fundamental equation,

$$\frac{1}{4\pi}\frac{\partial}{\partial t}\mathrm{div}\,(\varepsilon\,\mathrm{grad}\,\phi) + \mathrm{div}\,(\sigma\,\mathrm{grad}\,\phi) = 0. \tag{2.1}$$

We consider only the case in which the quantities related to the aqueous media, σ_i, σ_e, ε_i and ε_e, are constant. In this section, ε_m and σ_m are allowed to vary with time and position in order for the theory to be also applicable to the case in which electroporation takes place. By the use of operators defined as

$$L_\beta \equiv \frac{1}{4\pi}\frac{\partial}{\partial t}\varepsilon_\beta + \sigma_\beta \quad (\beta = \mathrm{e, i, m}), \tag{2.2}$$

eq. (2.1) reduces to

$$L_\beta \Delta \phi_\beta = 0 \quad (\beta = \mathrm{e, i}) \tag{2.3}$$

for the aqueous medium. In usual experimental setups, it can be assumed that there is no local charge in the medium. We therefore use a more restrictive equation than eq. (2.3),

$$\Delta \phi_\beta = 0 \quad (\beta = \mathrm{e, i}), \tag{2.4}$$

for the potentials inside and outside the vesicle.

If we denote the strength of the applied field by $E_0(t)$, the asymptotic form of the potential ϕ_e at infinity is

$$\phi_e \longrightarrow -E_0(t)z. \tag{2.5}$$

Here the z-axis is taken along the direction of the applied field. One of the boundary conditions for the potentials on the vesicle surfaces is related to $\mathrm{rot}\, E = 0$ and it is the continuity of the potentials given by

$$\phi_e = \phi_m \quad \text{on the outer surface} \tag{2.6a}$$

and

$$\phi_m = \phi_i \quad \text{on the inner surface.} \tag{2.6b}$$

The other condition is related to the continuity equation, $\partial \rho / \partial t + \mathrm{div}\, J = 0$, and is expressed as

$$L_e E_{en} = L_m E_{mn} \quad \text{on the outher surface} \tag{2.7a}$$

and

$$L_i E_{in} = L_m E_{mn} \quad \text{on the inner surface.} \tag{2.7b}$$

Here $E_{\beta n}$ is the normal component of the electric field E_β on the surface. These boundary conditions (2.7) are valid only if the charge carrier density in the surrounding medium is negligible.

2.2 Equations in the multipole expansion

The shape of the vesicle is assumed to be axisymmetric around the z-axis. Then the outer and inner surfaces of the vesicle are, respectively, expressed in polar coordinates as

$$r(\theta, t) = f_{\mathrm{out}}(\theta, t) = a(t)[1 + g_{\mathrm{out}}(\theta, t)] \tag{2.8a}$$

for the outer surface and

$$r(\theta, t) = f_{\mathrm{in}}(\theta, t) = b(t)[1 + g_{\mathrm{in}}(\theta, t)] \tag{2.8b}$$

for the inner surface. The membrane of the vesicle is assumed to be very thin in comparison with the radius of the vesicle. Thus it is reasonable to approximate $g_{\mathrm{out}}(\theta, t) = g_{\mathrm{in}}(\theta, t)$, which we write simply as $g(\theta, t)$. In the present work, we consider only small deformations of quadrupole type so that $g(\theta, t)$ is written as

$$g_{\mathrm{out}} = g_{\mathrm{in}} = g(\theta, t) \approx g_2(t)P_2(\cos\theta). \tag{2.8c}$$

Furthermore, it is assumed that the vesicle is a sphere of outer radius a_0 and inner radius b_0 at the onset of the electric field. Therefore we expand relevant quantities associated with the electric fields in terms of g_2 and take into account only the lowest order, $\mathrm{O}((g_2)^0)$. In this approximation, $a(t)$ and $b(t)$ in eqs. (2.8) remain constant, namely $a(t) = a_0$ and $b(t) = b_0$. Higher order calculations[7,8] are extremely complicated when the membrane interior is taken into consideration, as in the present work.

The potentials ϕ_e and ϕ_i satisfy eq. (2.4) and are thus expressed as

$$\phi_e = -E_0(t)r\cos\theta + \sum_l A_l(t)\frac{a_0^{l+2}}{r^{l+1}}P_l(\cos\theta) \tag{2.9a}$$

and

$$\phi_i = \sum_l D_l(t)\frac{r^l}{a_0^{l-1}}P_l(\cos\theta), \tag{2.9b}$$

where $A_l(t)$'s and $D_l(t)$'s are functions of time yet to be determined. The intramembrane potential ϕ_m is expressed as

$$\phi_m = \sum_l M_l(r, t)P_l(\cos\theta) \tag{2.10}$$

where $M_l(r, t)$'s are functions of both radius r and time t. This potential must satisfy eq. (2.1). Inserting eq. (2.10) into eq. (2.1) and multiplying $P_l(\cos\theta)$ and integrating over the angle θ, we arrive at somewhat complicated coupled equations for M_l. But it can be shown that in the limit of a very thin membrane, there is no need to solve the equations. The functions $M_l(r, t)$ in eq. (2.10) are completely determined by boundary conditions, as is discussed below.

The boundary conditions (2.6) give rise to the following relations in the lowest order with respect to the deformation $g(\theta, t)$:

$$-E_0(t)\delta_{l1} + A_l(t) = \frac{1}{a_0}M_l(a_0, t) \tag{2.11a}$$

and

$$D_l(t)\left(\frac{b_0}{a_0}\right)^l = \frac{1}{a_0} M_l(b_0, t). \tag{2.11b}$$

On the other hand, the boundary conditions (2.7) yield, in the same approximation,

$$L_e\left(\frac{\partial\phi_e}{\partial r}\right)_{r=a_0} = L_m\left(\frac{\partial\phi_m}{\partial r}\right)_{r=a_0} \tag{2.12a}$$

and

$$L_i\left(\frac{\partial\phi_i}{\partial r}\right)_{r=b_0} = L_m\left(\frac{\partial\phi_m}{\partial r}\right)_{r=b_0}, \tag{2.12b}$$

where L_β is defined in eq. (2.2). Inserting eqs. (2.9) and (2.10) into these equations and projecting onto each partial wave, we obtain

$$-\delta_{l1}L_eE_0(t) - (l+1)L_eA_l(t)$$
$$= \sum_L \langle l|L_m|L\rangle\left(\frac{\partial M_L(r, t)}{\partial r}\right)_{r=a_0} \tag{2.13a}$$

and

$$\left(\frac{b_0}{a_0}\right)^{l-1} lL_iD_l(t) = \sum_L \langle l|L_m|L\rangle\left(\frac{\partial M_L(r, t)}{\partial r}\right)_{r=b_0}, \tag{2.13b}$$

where

$$\langle l|L_m|L\rangle = \left\langle l\left|\frac{\varepsilon_m}{4\pi}\right|L\right\rangle\frac{d}{dt} + \langle l|\sigma_m^{eff}|L\rangle \tag{2.13c}$$

$$\left\langle l\left|\frac{\varepsilon_m}{4\pi}\right|L\right\rangle = \frac{2l+1}{2}\int_0^\pi \frac{\varepsilon_m(\theta, t)}{4\pi}P_l(\cos\theta)P_L(\cos\theta)$$
$$\times \sin\theta\, d\theta \tag{2.13d}$$

and

$$\left\langle l\left|\sigma_m^{eff}\right|L\right\rangle = \frac{2l+1}{2}\int_0^\pi\left[\sigma_m(\theta, t) + \frac{1}{4\pi}\frac{\partial\varepsilon_m(\theta, t)}{\partial t}\right]$$
$$\times P_l(\cos\theta)P_L(\cos\theta)\sin\theta\, d\theta. \tag{2.13e}$$

Since the membrane is very thin, we expand the functions $M_l(r, t)$ around $r=a_0$ as

$$M_l(r, t) = a_0B_l(t) + C_l(t)(a_0-r) + O((a_0-r)^2). \tag{2.14}$$

The two functions of time, $B_l(t)$ and $C_l(t)$, are necessary to satisfy the boundary conditions, since the equation for ϕ_m is a second-order differential equation with respect to r. In the limit of a thin membrane, higher-order terms in the above expansion, eq. (2.14), can be shown to make no contribution to the physical quantities to be calculated in the following. Therefore the complicated differential equation for ϕ_m need not be solved in this limit.

We denote the ratio of the membrane thickness to the vesicle radius as δ,

$$\delta = \frac{a_0-b_0}{a_0} = 1 - \frac{b_0}{a_0}, \tag{2.15}$$

and we retain only the terms of the lowest order in δ in the subsequent calculation. The functions B_l and C_l are obtained from eqs. (2.11), (2.14) and (2.15) as

$$B_l(t) = A_l(t) - E_0(t)\delta_{l1} \tag{2.16a}$$

and

$$C_l(t) = (D_l(t) - A_l(t) + E_0(t)\delta_{l1})/\delta + O(\delta^0). \tag{2.16b}$$

Thus the intramembrane potential ϕ_m is completely determined. By using these results to eliminate B_l and C_l in eq. (2.13), we arrive at differential equations for A_l and D_l,

$$\delta_{l1}L_eE_0(t) + (l+1)L_eA_l(t)$$
$$= \sum_L\left\langle l\left|\frac{L_m}{\delta}\right|L\right\rangle(D_L(t) - A_L(t) + E_0(t)\delta_{l1}) \tag{2.17a}$$

and

$$(l+1)L_eA_l(t) + lL_iD_l(t) = -\delta_{l1}L_eE_0(t). \tag{2.17b}$$

Subsequent discussions are based on these equations. The effect of the membrane appears in eq. (2.17a) in the form of L_m/δ as

$$\frac{L_m}{\delta} = \frac{1}{4\pi}\frac{\varepsilon_m}{\delta}\frac{\partial}{\partial t} + \frac{\sigma_m}{\delta} + \frac{1}{4\pi}\left(\frac{\partial}{\partial t}\frac{\varepsilon_m}{\delta}\right). \tag{2.18}$$

Since δ is actually a very small number, $\varepsilon_m/4\pi\delta$ can be very large for cells and lipid vesicles.

2.3 Initial conditions

Equations (2.17) are first-order differential equations with respect to time. To solve these equations completely, we need initial conditions. In the present problems, the initial time should be regarded as the onset time when the external field is applied. We assume that there is no initial charge distribution at either the inner or outer surface of the membrane. We call the solution obtained under this assumption the induced electric field. If there is intrinsic charge distribution on the membrane, we should add the electric field associated with this charge to the induced field. As we consider only spherical vesicles, such an intrinsic electric field is a radially directed vector. The electric force calculated by the use of the Maxwell tensor consists of the force originating purely from the intrinsic field (which should be balanced by the internal force of the vesicle), the force originating purely from the induced field (which is our present concern) and the force originating due to the interference between the two fields. In the case of a homogeneous membrane, this interference force is of a dipole type and gives rise to the translational motion of the vesicle. However, since we are only interested in the quadrupole deformation, this type of motion will be ignored. If the initial state of the membrane is inhomogeneous, namely, the initial values of ε_m and/or σ_m depend on θ, forces of a quadrupole and higher multipole types can be generated from the intrinsic electric field. We expect such inhomogeneity to be rather small and neglect these forces.

The condition that there is no initial charge on the surface leads to the equations

$$\varepsilon_eE_{en} = \varepsilon_mE_{mn} \quad \text{and} \quad \varepsilon_iE_{in} = \varepsilon_mE_{mn} \tag{2.19}$$

on the outer and inner surfaces, respectively, at the initial time ($t=0$). These conditions become, in the lowest order of deformation $g(\theta, t)$,

$$\varepsilon_e \delta_{l1} E_0 + \varepsilon_e(l+1)A_l = \sum_L \left\langle l \left| \frac{\varepsilon_m}{\delta} \right| L \right\rangle (D_L - A_L + \delta_{L1} E_0)$$

$$\text{(2.20a)}$$

$$\varepsilon_e(l+1)A_l + \varepsilon_i l D_l = -\varepsilon_e \delta_{l1} E_0. \qquad \text{(2.20b)}$$

Here all the quantities are evaluated at $t=0$.

2.4 Electric force and transmembrane potential

The forces acting on the vesicle are calculated from the Maxwell stress tensor. The outward normal component of the force per unit area of the vesicle surface is given as

$$F_n = (a_0^2/8\pi)[\varepsilon_e(E_{en}^2 - E_{et}^2) - \varepsilon_i(E_{in}^2 - E_{it}^2)]. \qquad \text{(2.21)}$$

Here the limit $(\delta \to 0)$ is already taken and $E_{\beta n}$ and $E_{\beta t}$ are, respectively, the normal and tangential components of the electric field $E_\beta(\beta = i, e)$ evaluated on the surface of the vesicle. The tangential component of the force is assumed to be balanced by the stretching force of the membrane and not to contribute to the deformation.[1,7,8] The presence of the vesicle membrane imposes the constraint that the surface area remain constant. This constraint appears to justify the above assumption concerning the force balance. In refs. 5 and 6, which deals with the case of liquid droplets, the local balance of forces is not explicitly considered, and the static deformation is determined by minimizing the sum of the electric and surface energies. However, it may be rather difficult to extend this approach to calculate the static deformation of a vesicle and even more difficult to apply it to time-dependent cases.

The transmembrane potential V is given, in the thin membrane limit, by

$$V \equiv \phi_e(r=a_0) - \phi_i(r=b_0)$$

$$= a_0 \sum_{l=1}^{\infty} [-E_0(t)\delta_{l1} + A_l(t) - D_l(t)]P_l(\cos\theta). \qquad \text{(2.22)}$$

§3. Dynamics of Homogeneous Membrane

3.1 General formulation

In the present work, we discuss only the case in which the membrane of the vesicle is homogeneous and ε_m and σ_m are the same everywhere in the membrane. Furthermore, ε_m and σ_m are assumed to be independent of time. The more complicated situation in which electroporation takes place will be discussed elsewhere.

The assumption of constant ε_m and σ_m leads to great simplification, since contributions from higher multipoles other than the dipole vanish ($A_l = D_l = 0$ for $l > 1$). This is because the off-diagonal matrix elements of ε_m and σ_m vanish (i.e., $\langle l | \varepsilon_m | L \rangle = \varepsilon_m \delta_{lL}$ and $\langle l | \sigma_m | L \rangle = \sigma_m \delta_{lL}$), hence equations determining A_l and D_l for $l > 1$ in eqs. (2.17) and (2.20) are decoupled from those involving A_1, D_1, and the external field $E_0(t)$. By omitting the suffix $l = 1$ ($A \equiv A_1$ and $D \equiv D_1$) for simplicity, we rewrite eq. (2.17) as follows:

$$S \begin{pmatrix} dA/dt \\ dD/dt \end{pmatrix} + \Sigma \begin{pmatrix} A \\ D \end{pmatrix} = \begin{pmatrix} -L_e E_0(t) \\ (\delta^{-1}L_m - L_e)E_0(t) \end{pmatrix} \qquad \text{(3.1)}$$

with

$$S = \frac{1}{4\pi} \begin{pmatrix} 2\varepsilon_e, & \varepsilon_i \\ 2\varepsilon_e + \varepsilon_m/\delta, & -\varepsilon_m/\delta \end{pmatrix} \qquad \text{(3.2a)}$$

and

$$\Sigma = \begin{pmatrix} 2\sigma_e, & \sigma_i \\ 2\sigma_e + \sigma_m/\delta, & -\sigma_m/\delta, \end{pmatrix}. \qquad \text{(3.2b)}$$

The solutions of the homogeneous equation associated with this equation are easily found by making the substitution $A(t) = \exp(-\lambda t)$ and $D(t) = d \cdot \exp(-\lambda t)$ in eq. (3.1) and solving the simple eigenvalue problem given by

$$\det(-\lambda_j S + \Sigma) = 0, \quad (-\lambda_j S + \Sigma)\begin{pmatrix} 1 \\ d_j \end{pmatrix} = 0. \qquad \text{(3.3)}$$

Then the general solution of eq. (3.1) is written as

$$\begin{pmatrix} A(t) \\ D(t) \end{pmatrix} = \begin{pmatrix} A_s(t) \\ D_s(t) \end{pmatrix} + C_1 \begin{pmatrix} 1 \\ d_1 \end{pmatrix} \exp(-\lambda_1 t) + C_2 \begin{pmatrix} 1 \\ d_2 \end{pmatrix}$$

$$\times \exp(-\lambda_2 t), \qquad \text{(3.4)}$$

where $A_s(t)$ and $D_s(t)$ are a special solution of eq. (3.1). The unknown coefficients C_1 and C_2 are determined by the initial conditions, eqs. (2.20). These conditions are expressed as

$$A(0) = \frac{(\varepsilon_i - \varepsilon_e)(\varepsilon_m/\delta) - \varepsilon_i \varepsilon_e}{(\varepsilon_i + 2\varepsilon_e)(\varepsilon_m/\delta) + 2\varepsilon_i \varepsilon_e} E_0(0) \qquad \text{(3.5a)}$$

$$D(0) = \frac{-3\varepsilon_e(\varepsilon_m/\delta)}{(\varepsilon_i + 2\varepsilon_e)(\varepsilon_m/\delta) + 2\varepsilon_i \varepsilon_e} E_0(0). \qquad \text{(3.5b)}$$

The electric force calculated from eq. (2.21) consists only of monopole and quadrupole components in the case of a spherical vesicle having a homogeneous membrane. Since we attempt only the lowest perturbative calculations concerning the deformation and since the monopole deformation is regarded to be of a higher order, as was discussed in ref. 8, only the quadrupole component of the force is of relevance here and is given by

$$F_n = (a_0^2 \varepsilon_e/6\pi)Q(t)P_2(\cos\theta) + \text{monopole}, \qquad \text{(3.6a)}$$

where

$$Q(t) = E_0(t)^2 + E_0(t)A(t) + (5/2)A(t)^2 - (\varepsilon_i/\varepsilon_e)D(t)^2. \qquad \text{(3.6b)}$$

This force is then inserted into the equation of motion of the vesicle, as was discussed in detail in ref. 7. We obtain the equation of motion for g_2 defined in eqs. (2.8a)–(2.8c) as

$$d^2 g_2/dt^2 + 2\Gamma dg_2/dt + K_2 g_2 = kQ(t), \qquad \text{(3.7a)}$$

where Γ and K_2 represent, respectively, the fluid resistance and the restoring force divided by the inertial term. The explicit forms of these coefficients are given in ref. 7. The coefficient k in eq. (3.7a) is given as

$$k = \varepsilon_e/2\pi \nu \rho_w a_0^2, \qquad \text{(3.7b)}$$

where ν is the mass factor and ρ_w is the mass density of the medium (see ref. 7 for details).

3.2 Deformation in a step-function field

In this subsection, we consider the vesicle deformation when a constant field $E_0(t)=E_0$ is applied at time $t=0$. Since the electroporation is not considered here, we treat only the situation of a weak field or the initial stage under a strong field. It is important to realize that the electric properties of the membrane appear only in the form of ε_m/δ and σ_m/δ. Since δ is defined as the ratio of the membrane thickness and the radius of the vesicle [eq. (2.15)], it is actually very small; consequently, ε_m/δ is much larger than ε_e and/or ε_i. In fact, the ratio $(\varepsilon_m/\delta)/\varepsilon_e$ turns out to be of the order of 10^2 or greater for most cells and large liposomes.

In the limit $\varepsilon_m/\delta \gg \varepsilon_e(\varepsilon_i)$, the solutions of eq. (3.3) are approximately

$$\lambda_1=1/\tau \approx \frac{4\pi\delta}{\varepsilon_m}\left(\frac{2\sigma_i\sigma_e}{2\sigma_e+\sigma_i}+\frac{\sigma_m}{\delta}\right) \qquad (3.8\text{a})$$

and

$$\lambda_2 \approx \frac{4\pi}{2\varepsilon_e+\varepsilon_i}(2\sigma_e+\sigma_i). \qquad (3.8\text{b})$$

It should be noted that λ_2 is independent of the properties of the membrane and $1/\lambda_2$ is representative of the characteristic time of the media surrounding the membrane. In contrast, $1/\lambda_1$ is proportional to ε_m/δ and should be regarded as the characteristic time of the membrane placed in the media. Since σ_m/δ for cells and liposomes is at most of the order of σ_e, $1/\lambda_2$ is much smaller than $\tau=1/\lambda_1[(1/\lambda_2)/(1/\lambda_1)\approx O(\varepsilon_e\delta/\varepsilon_m)]$. Thus while the mode associated with the former dampens very quickly, the effects of the slow mode $1/\lambda_1$ are expected to show a visible influence on the deformation dynamics in certain favorable situations, as is discussed later.

A special solution of eq. (3.1) in the case of a static electric field $E_0(t)=E_0$ is easily obtained as

$$A_s=\frac{(\sigma_i-\sigma_e)(\sigma_m/\delta)-\sigma_i}{(\sigma_i+2\sigma_e)(\sigma_m/\delta)+2\sigma_i}E_0 \qquad (3.9\text{a})$$

and

$$D_s=\frac{-3\sigma_i(\sigma_m/\delta)}{(\sigma_i+2\sigma_e)(\sigma_m/\delta)+2\sigma_i}E_0. \qquad (3.9\text{b})$$

This solution corresponds to an asymptotic solution, namely $A_s=A(t=\infty)$ and $D_s=D(t=\infty)$.

In the remaining part of this subsection, the case of an insulating membrane $(\sigma_m=0)$ is discussed. The asymptotic values are $A_s=-(1/2)E_0$ and $D_s=0$, corresponding to the vanishing electric field inside the vesicle and to the vanishing normal component of the electric field at the external surface of the membrane. Imposing initial conditions (3.5), we obtain the complete solution from eq. (3.4). However, its approximate solution is sufficient for the usual case of $(\varepsilon_m/\delta)/\varepsilon_e \gg 1$ and is given as

$$A(t)/E_0 \approx -\frac{1}{2}+\frac{3\sigma_i}{2\sigma_i+4\sigma_e}e^{-\lambda_1 t}+C_2e^{-\lambda_2 t} \qquad (3.10\text{a})$$

$$D(t)/E_0 \approx -\frac{3\sigma_e}{\sigma_i+2\sigma_e}e^{-\lambda_1 t}+C_2e^{-\lambda_2 t}, \qquad (3.10\text{b})$$

where $C_2 \approx 3(\varepsilon_i\sigma_e-\varepsilon_e\sigma_i)/[(2\varepsilon_i+\varepsilon_e)(\sigma_i+2\sigma_e)]$.

At $t=0$, $A(0)/E_0 \approx (\varepsilon_i-\varepsilon_e)/(\varepsilon_i+2\varepsilon_e)$ and $D(0)/E_0 \approx -3\varepsilon_e/(\varepsilon_i+2\varepsilon_e)$. After a short time t_s satisfying the condition $1/\lambda_2 \ll t_s \ll 1/\lambda_1$, the fast mode vanishes and A and D attain their extrema, $A(t)/E_0 \approx (\sigma_i-\sigma_e)/(\sigma_i+2\sigma_e)$ and $D(t)/E_0 \approx -3\sigma_e/(\sigma_i+2\sigma_e)$. Afterwards, A and D approach the asymptotic values (3.9a, b) exponentially. Function D is always negative, namely the electric field inside the vesicle, E_i, is in the same direction as the applied field. It is important to note that the function A, on the other hand, behaves qualitatively differently for $\sigma_i<\sigma_e$ and for $\sigma_i<\sigma_e$. If $\sigma_i<\sigma_e$, A remains negative. But if $\sigma_i>\sigma_e$, A becomes positive during a certain initial period of time before it decays eventually to the negative value of $-E_0/2$. In Figs. 1(a), 1(b), and 1(c), we show examples of equipotential surfaces. Figure 1(a) shows the case for $\sigma_i/\sigma_e=0.2$ at the time $t=4/\lambda_2$, while Fig. 1(b) shows the case for $\sigma_i/\sigma_e=2$ at $t=4/\lambda_2$. At this time, the effect of the fast mode nearly reaches maximum. Figure 1(c) shows the case at $t=\infty$, where the potential shape does not depend on the ratio σ_i/σ_e.

The quadrupole component of the electric force is determined by inserting eqs. (3.10) into $Q(t)$ defined by eq. (3.6b). Corresponding to the values of $A(t)$ and $D(t)$ at specific times discussed above, the values of $Q(t)$ are

$$Q(t=0)=(9/2)E_0^2[(\varepsilon_i-\varepsilon_e)/(\varepsilon_i+2\varepsilon_e)]^2 \geq 0 \qquad (3.11\text{a})$$
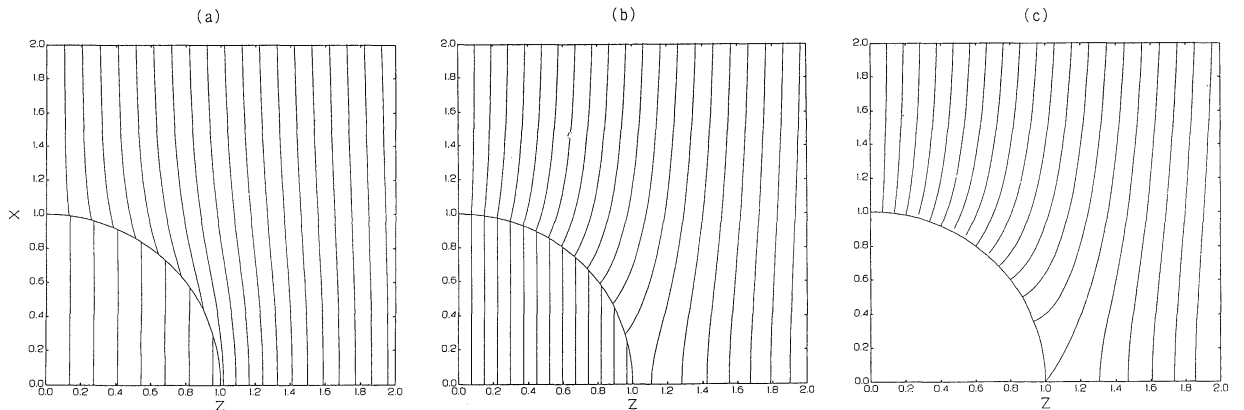

(a)   (b)   (c)

Fig. 1. Equipotential surfaces. It is assumed that $\varepsilon_i=\varepsilon_e$ and $\sigma_m=0$. Length is given in units of the radius of the vesicle: (a) for $\sigma_i/\sigma_e=0.2$ and at $t=4/\lambda_2$, (b) $\sigma_i/\sigma_e=2$ and at $t=4/\lambda_2$, (c) $t=\infty$.

$$Q(t_s) \approx (9/2)E_0^2[\sigma_i^2 - (2\sigma_i/\varepsilon_e - 1)\sigma_e^2]/(\sigma_i + 2\sigma_e)^2 \quad (3.11b)$$

for $1/\lambda_2 \ll t_s \ll 1/\lambda_1$ and

$$Q(t) \approx \frac{9}{8}E_0^2\left[1 - \frac{2\sigma_i}{\sigma_i + 2\sigma_e}e^{-\lambda_1 t} + \frac{5\sigma_i^2 - 8(\varepsilon_i/\varepsilon_e)\sigma_e^2}{(\sigma_i + 2\sigma_e)^2}\varepsilon^{-2\lambda_1 t}\right]$$

(3.11c)

for $t \gtrsim 1/\lambda_1$. The time dependences of $Q(t)$ are shown in Fig. 2 for various values of the ratio $x = \sigma_i/\sigma_e$ in the case of $\varepsilon_i = \varepsilon_e$. The abscissa indicates the values of the time in units of $\tau = 1/\lambda_1$, namely the characteristic time of the slow mode. The ordinate indicates the value of $Q(t)/E_0^2$. The bottom curve in the figure corresponds to the case of $\sigma_i/\sigma_e = 0$, and the other curves from the bottom upwards correspond to the cases of $\sigma_i/\sigma_e = 0.2, 0.5, 1, 2$, and 3, respectively. It should be noted that the quadrupole force at $t = 0$ is nearly zero, $Q(0) \approx 0$, in the usual case of $\varepsilon_i \approx \varepsilon_e$. After a very short time up to about t_s, the sign of $Q(t)$ is determined by whether σ_i/σ_e is greater or less than $(2\varepsilon_i/\varepsilon_e - 1)^{1/2} \approx 1$, as one can see from eq. (3.11b). Therefore even if the membrane is assumed to be a perfect insulator, the sign of the quadrupole force depends crucially on the ratio of the conductivities, σ_i/σ_e. As the time passes, the contribution from the fast mode vanishes quickly and the force factor $Q(t)$ develops, as is given in (3.11c). It gradually approaches the asymptotic positive value of $(9/8) E_0^2$ irrespective of σ_i and σ_e. In the case of $Q(t_s) < 0$, the sign of $Q(t)$ changes at the time $t_c = \tau \ln[\{x + (8\varepsilon_i/\varepsilon_e - 4x^2)^{1/2}\}/(x + 2)]$, with $x = \sigma_i/\sigma_e$, as is easily derived from eq. (3.11c). We see from this figure that for $\sigma_i/\sigma_e < 1$, the force factor $Q(t)$ remains negative for an appreciable amount of time, $\lesssim 0.35\tau$.

The dynamics of the vesicle is determined by eq. (3.7). Therefore if $Q(t)$ is negative, the deformation becomes oblate, $g_2 < 0$. Equation (3.11) shows that for decreasing values of σ_i/σ_e, $Q(t)$ becomes more negative and, at the same time, remains negative for a longer time (t_c large). This dependence on $x = \sigma_i/\sigma_e$ is clearly exhibited in Fig. 2. For transient oblate deformation to be observable, x should be small. In addition, one needs a sufficiently long characteristic time τ. These conditions may be satisfied by large liposomes containing a low-salt solution as the internal medium.

In the case of the step-function field, $E_0(t) = E_0(t \geq 0)$, the membrane potential in eq. (2.22) is given as

$$V \approx -(3/2)E_0 a_0[1 - \exp(-t/\tau)]\cos\theta. \quad (3.12)$$

As we have assumed an insulating membrane,, the quadrupole force given in (3.11) is valid up to the time of electroporation t_p. The time t_p is obtained by equating the membrane potential at $\theta = 0$ with the threshold value of the potential difference for electroporation V_{TP}, and is expressed as

$$t_p = -\tau \ln(1 - E_p/E_0), \quad (3.13)$$

where E_p is the critical field and $E_p = 2V_{TP}/3a_0$. For a strong pulsed field ($E_0 > E_p$), the time t_p is of the order of the characteristic time τ. Therefore the dynamical behavior of the vesicle in a strong step-function field involves not only the dynamics of the insulating membrane but also that of the conducting membrane, which has yet to be studied.

3.3 Alternating field

As is discussed in the previous subsection, qualitative features of the time development of the quadrupole force (3.11) differ markedly depending on whether the ratio σ_i/σ_e is greater or less than $(2\varepsilon_i/\varepsilon_e - 1)^{1/2}$. However, many biological cells have a rather short characteristic time, τ, of the order of 0.1 μs. Therefore, experimental determination of the quantities such as σ_i and $\varepsilon_m/4\pi a_0 \delta$ from the deformation under a step-function field would be difficult except possibly for large cells or large liposomes.

Instead of a step-function field, an alternating field can be used to perform these tasks. It will be shown that studying the steady state of the vesicle deformation induced by an alternating field yields information about the above quantities. It is elementary to obtain the steady-state solution of eq. (3.1) for the alternating field $E_0(t) = E_0 \exp(i\Omega t)$. Inserting $A(t) = E_0\mathscr{A}\exp(i\Omega t)$ and $D(t) = E_0\mathscr{D}\exp(i\Omega t)$ into eq. (3.1), we obtain the solutions for the complex coefficients $\mathscr{A}$ and $\mathscr{D}$ as

$$\binom{\mathscr{A}}{\mathscr{D}} = (i\Omega S + \Sigma)^{-1}\binom{-(i\Omega/4\pi)\varepsilon_e - \sigma_e}{(i\Omega/4\pi)(\varepsilon_m/\delta - \varepsilon_e) + \sigma_m/\delta - \sigma_e},$$

(3.14)

where the matrices S and Σ are defined in eqs. (3.2a) and (3.2b). We expect some interesting results when $1/\Omega$ is near the characteristic time of the membrane, τ given in eq. (3.8a). Since the characteristic time of the dynamical motion of a vesicle is generally much larger than τ, it is sufficient to consider only $\bar{g}_2$, which is g_2 averaged over the period of the applied field. Hence, the dynamical equation of the vesicle (3.7a) is reduced to the following simple equation,
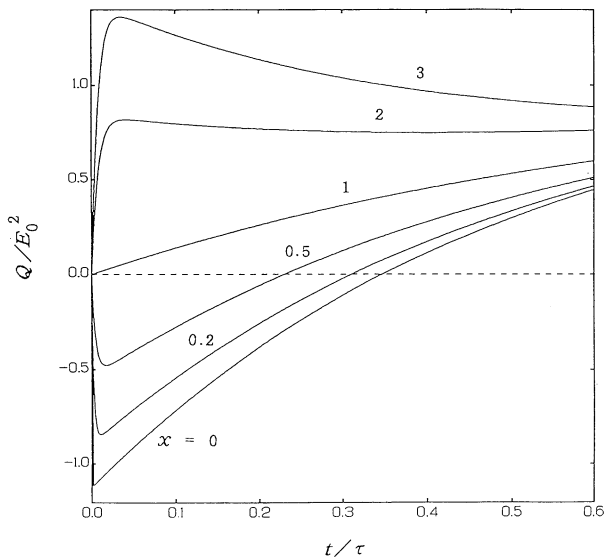

Fig. 2. Time dependence of the force function $Q(t)/E_0^2$. It is assumed that $\varepsilon_i = \varepsilon_e$ and $\sigma_m = 0$. Abscissa denotes $\lambda_1 t = t/\tau$. The curves from the bottom to the top correspond to $\sigma_i/\sigma_e = 0, 0.2, 0.5, 1, 2$, and 3, respectively.

$$\bar{g}_2 = (k/K_2)\bar{Q} = (a_0^3 \varepsilon_e E_0^2 / 864\pi\kappa)\bar{q}, \qquad (3.15)$$

where κ is the modulus of elastic curvature of Helfrich[1] and $\bar{q}$ is related to the time average of $Q(t)$ and is written as

$$\bar{q} = \frac{1}{E_0^2}\bar{Q} = \frac{1}{E_0^2}\frac{\Omega}{2\pi}\int_0^{2\pi/\Omega} Q(t)\,dt. \qquad (3.16)$$

From eqs. (3.6) and (3.16), $\bar{q}$ is shown to be

$$\bar{q} = (1/2)(1 + \text{Re}\,\mathscr{A}) + (5/4)|\mathscr{A}|^2 - (\varepsilon_i/2\varepsilon_e)|\mathscr{D}|^2. \qquad (3.17)$$

In the following, we will limit our discussion to the case of an insulating membrane $[\sigma_m = 0,$ or $\sigma_m/\delta \ll (\sigma_i$ or $\sigma_e)]$. As ε_m/δ is much greater than ε_e and ε_i, we neglect higher-order terms in eq. (3.14), insert the resulting expression into eq. (3.17) and obtain the explicit form of $\bar{q}$ as

$$\bar{q} = \frac{9}{16}\frac{1}{1+\tau^2\Omega^2}\left(1 - \frac{\Omega^2}{\Omega_c^2}\right), \qquad (3.18)$$

where

$$\frac{1}{\Omega_c^2} = \tau^2\frac{(2\varepsilon_i/\varepsilon_e - 1)\sigma_e^2 - \sigma_i^2}{(\sigma_e + \sigma_i/2)^2}. \qquad (3.18a)$$

From these equations and eq. (3.15), we observe the following.

(i) If $\varepsilon_i/\varepsilon_e < 1/2$ or $\sigma_i/\sigma_e > (2\varepsilon_i/\varepsilon_e - 1)^{1/2}$, then $1/\Omega_c^2$ is negative. Thus $\bar{q} > 0$ and $\bar{g}_2 > 0$ irrespective of Ω (prolate deformation).

(ii) If $\sigma_i/\sigma_e < (2\varepsilon_i/\varepsilon_e - 1)^{1/2}$, then $1/\Omega_c^2$ is positive. Therefore for $\Omega > \Omega_c$, $\bar{q}$ is negative and the deformation is oblate ($\bar{g}_2 < 0$). For $\Omega < \Omega_c$, the deformation is prolate ($\bar{g}_2 > 0$). We call Ω_c the critical frequency. In Fig. 3, the dependence of $1/\Omega_c$ on the ratio σ_i/σ_e is shown for $\varepsilon_i/\varepsilon_e = 0.8$, 1.0 and 1.2. When the angular frequency Ω is increased through Ω_c in case (ii), the vesicle undergoes a change in the type of deformation from a prolate shape to an oblate shape. It is worthwhile to note that the ratio
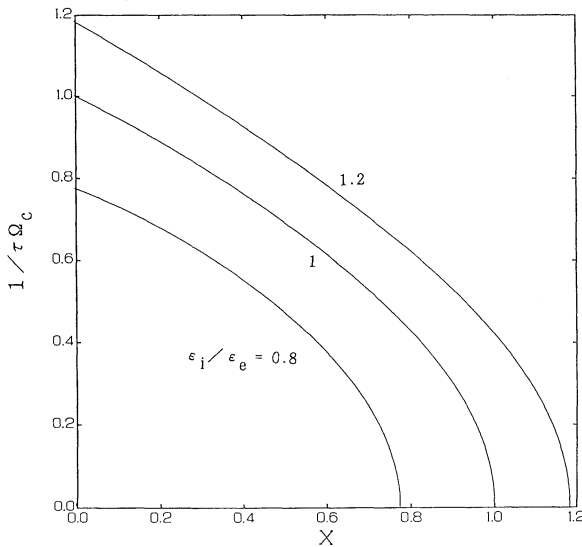

Fig. 3. The dependence of the critical frequency on the conductivity ratio. Ordinate denotes $1/\tau\Omega_c$ and abscissa $x = \sigma_i/\sigma_e$. The curves from the inside to the outside correspond to $\varepsilon_i/\varepsilon_e = 0.8$, 1 and 1.2. $\sigma_m = 0$ is assumed.

between σ_i/σ_e and $(2\varepsilon_i/\varepsilon_e - 1)^{1/2}$ is crucial in determining the type of deformation, in accord with the results for the step-function electric field treated in the previous subsection.

So far, the discussions are based on the steady-state solution, eq. (3.15), of the dynamical equation (3.7a). Additional information is obtained by analyzing the transient phase. By averaging eq. (3.7a) over an oscillating period of the applied field $2\pi/\Omega$ and by assuming that this period is short enough for the inertial term to be negligible, we obtain the time-averaged dynamical equation

$$2\Gamma\,d\tilde{g}_2/dt + K_2\tilde{g}_2 = kE_0^2\bar{q}, \qquad (3.19)$$

where

$$\tilde{g}_2(t) = \frac{\Omega}{2\pi}\int_{t-\pi/\Omega}^{t+\pi/\Omega} g_2(t')\,dt'. \qquad (3.20)$$

The solution of this equation is

$$\tilde{g}_2(t) = [1 - \exp(-t/t_r)]\bar{g}_2, \qquad (3.21)$$

where t is the time after the onset of the alternating field. The relaxation time t_r is expressed as

$$t_r = K_2/2\Gamma = 72\kappa/\gamma a_0^4, \qquad (3.21a)$$

where K_2, Γ, and γ are given in ref. 7. As is discussed there, the quantity γ is defined as the coefficient of fluid resistance which is assumed to be proportional to the membrane velocity component normal to the vesicle surface. Therefore, measurement of the relaxation time allows the estimation of γ.

3.4 Analysis of steady-state measurement

From the point of view of experimental measurements, it is preferable to describe deformations by means of the radius parallel to the applied field, $r(\theta = 0) = a_0(1 + g_2)$, and the radius perpendicular to the field $r(\theta = \pi/2) = a_0 (1 - g_2/2)$. Therefore we introduce a quantity called the elongation modulus, ξ, defined as

$$\xi = \frac{r(\theta = 0) - r(\theta = \pi/2)}{r(\theta = 0)} = \frac{3\bar{g}_2/2}{1 + \bar{g}_2} \approx 3\bar{g}_2/2. \qquad (3.22)$$

$\bar{g}_2$ in the denominator is neglected since the present theory is applicable only to small deformations ($|\xi| \ll 1$). From eqs. (3.15), (3.18) and (3.22), ξ is approximately given as a function of Ω by

$$\xi(\Omega) = \frac{1}{1 + \tau^2\Omega^2}\left(1 - \frac{\Omega^2}{\Omega_c^2}\right)\xi(0). \qquad (3.23)$$

where

$$\xi(0) \approx \varepsilon_e a_0^3 E_0^2 / 108\pi\kappa. \qquad (3.24)$$

A typical value for κ, the curvature elastic modulus of the membrane, is 5×10^{-13} erg (ref. 1; we refer to this value as κ_H). For this κ_H and $\varepsilon_e = 81$ (the dielectric constant of water), $\xi(0)$ is expressed as

$$\xi_H(0) = 1.5 \times \left(\frac{E_0}{10\,\text{V/cm}}\right)^2 \times \left(\frac{a_0}{30\,\mu\text{m}}\right)^3. \qquad (3.24a)$$

In Fig. 4, we show the dependence of $\xi(\Omega)/\xi(0)$ on Ω for various values of $x = \sigma_i/\sigma_e (= 0, 0.2, 0.4, 0.6, 0.8, 1, 1.5,$
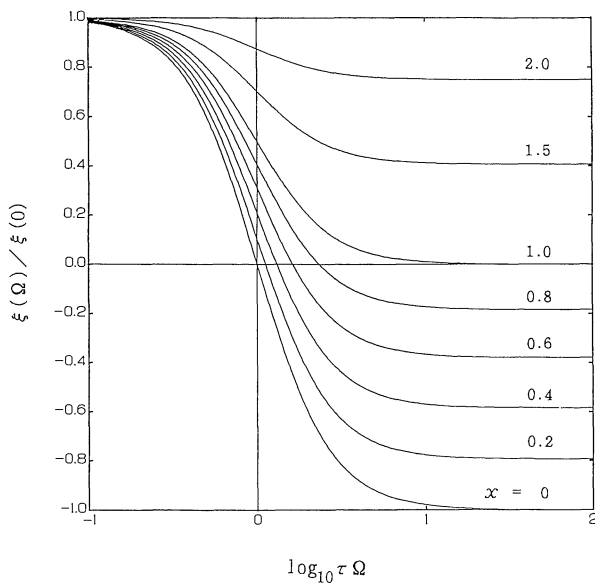
Fig. 4. The dependence of the elongation modulus $\xi(\Omega)/\xi(0)$ on $\tau\Omega$, the product of the characteristic time and the angular frequency of the applied field. Abscissa shows the values of $\log_{10}\tau\Omega$. The curves from the bottom to the top correspond to the case of $x=\sigma_i/\sigma_e=0$, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2, respectively. The curve for $x=\infty$ is identical to the top straight line, $\xi(\Omega)/\xi(0)=1.0$. $\varepsilon_i=\varepsilon_e$ and $\sigma_m=0$ are assumed.

2) by semilog plots.

A few noteworthy properties are immediately derived from eqs. (3.23) and

(1) $\xi(1/\tau)=[\xi(0)+\xi(\infty)]/2,$ (3.25a)

(2) $\xi(\Omega_c)=0,$ (3.25b)

(3) $\kappa/\kappa_H=\xi_H(0)/\xi(0)=\xi_H(\Omega)/\xi(\Omega).$ (3.25c)

Once ξ is experimentally determined as a function of Ω, the above relations may be utilized to facilitate the analysis of the data.

(1) The characteristic time of the membrane τ is estimated as the inverse of the angular frequency at the mid-point of the transition from a high $\xi[=\xi(0)]$ to a low $\xi[=\xi(\infty)]$.

(2) The critical frequency Ω_c is determined as the frequency at which no deformation takes place ($\xi=0$). From eq. (3.18a) and the knowledge of τ above, the conductivity ratio $x=\sigma_i/\sigma_e$ is determined, provided that $\varepsilon_i/\varepsilon_e$ is assumed to be known. In most cases, the relation $\varepsilon_i=\varepsilon_e$ holds and x can be estimated from Fig. 3. The value of x, combined with σ_e determined from an independent measurement, yields the value of the conductivity inside the vesicle σ_i. Furthermore, the membrane capacitance per unit area $C_m=\varepsilon_m/4\pi a_0\delta$ can be obtained from σ_e, σ_i and τ, since τ in eq. (3.8a) is reduced to $a_0C_m(1/\sigma_i+1/2\sigma_e)$ for $\sigma_m=0$.

(3) The actual value of the curvature elastic modulus κ of the membrane is determined by the use of the simple scaling relation (3.25c). Measurements of $\xi(\Omega)$ at several frequencies will give a reliable estimate of κ. It should be emphasized that κ so determined is an effective curvature elastic modulus. In the case of a cell, κ reflects the elastic properties of the cell as a whole, including those of cytoskeleton and cytoplasm.

The present theoretical calculations are based on idealized assumptions. However, we hope that the results obtained pave the way to a novel method of determining the properties of cells and a further development in the theoretical treatment of cell dynamics.

References

1) W. Helfrich: Z. Naturforsch. **29c** (1973) 693.
2) K. S. Cole: *Membranes, Ions and Impulses* (University of California Press, Berkeley, 1972).
3) K. Kinosita, Jr., I. Ashikawa, N. Saita, H. Yoshimura, H. Itoh, K. Nagayama and A. Ikegami: Biophys. J. **53** (1988) 1015.
4) M. Hibino, M. Shigemori, H. Itoh, K. Nagayama and K. Kinosita, Jr.: Biophys. J. **59** (1991) 209.
5) C. T. O'Konski and H. C. Thacher, Jr.: J. Phys. Chem. **57** (1953) 955.
6) C. T. O'Konski and F. E. Harris: J. Phys. Chem. **61** (1957) 1172.
7) H. Hyuga, K. Kinosita, Jr. and N. Wakabayashi: Jpn. J. Appl. Phys. **30** (1991) 1141.
8) H. Hyuga, K. Kinosita, Jr. and N. Wakabayashi: Jpn. J. Appl. Phys. **30** (1991) 1333.