Inner Surface Charge and Membrane Mechanical Stability

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Basing on the results obtained from linear hydrodynamic analysis was performed a study of effect of inner surface charge on the mechanical stability of a erythrocyte membrane model. Numerical values of the parameters related this cell was used to obtain the critical stability curves. According these results, the mechanical stability of membrane decreases with the inner surface charge modulus increase.

I. Introduction

There are suggestions that mathematical method of hydrodynamic stability analysis can be applied in the study of the role of some agents which modify membrane bending in biological membranes[1–5].

In recent publication[6], we have demonstrated that the linear hydrodynamic analysis in normal modes can be used to investigate the stability criteria for biological membranes. This method shows to be an efficient way of evaluating how changes in physico-chemical parameters can influence stability, and for determining which values of these parameters give the membrane the greatest resistance to perturbation, because its permits to investigate the initial phase of the wavy development process.

Many functions of the cell membrane are involved in wavy membrane profile and vesicle development. An example of this is the materials transport from the Golgi apparatus to other parts of the cell, as shown by electron microscopy. This transport is made by small vesicles which pinch from the crests of a wavy profile on the rim of the Golgi discs[7,8]. This is a spontaneous process but the formation of this kind of perturbation on membranes can also be observed during external excitation, such as a temperature increase.

It has been shown that a wavy disturbance occurs along the cell rim in human red blood cell (erythrocyte) that have been heated to temperatures near 48°C [9]. These changes culminate with fragmentation on heating to temperatures around 50°C [10,11,12]. At temperature of 49°C the stabilising influence of the skeleton protein spectrin is suddenly lost through its denaturation[1]. Spectrin is a membrane cytoplasmatic-face extrinsic protein which, at physiology conditions, has stabilising effect on the membrane. Human red blood cells develop vesicles when heated to temperatures close to the thermal transition for spectrin. There are evidences that the changes in cell outer surface charge, ionic strength of suspending medium[13] and diffusion potential[14], influence the interfacial stability of

heated erythrocytes.

The membrane spontaneous deformations induced by thermal flotation are influenced by the field strength within the membrane. Like most cellular membranes, the erythrocyte membrane is constituted of a bilayer of phospholipids, cholesterol and proteins[15]. Within bilayer, the hydrophilic groups of phospholipids are oriented at the surface of bilayer and hydrophobic groups (hydrocarbon chains) at its interior. Proteins are partially or totally embedded within one or both monolayers, or through the bilayer with portions exposed at both surfaces. Such proteins are amphiphilic and held in the bilayer through hydrophobic interactions[16,17]. So the membrane presents a distribution of surface charges and one of the best-established features of red blood cell membrane is the distribution asymmetry of phospholipids between the two lipid monolayers[18]. Most the phosphatidylserine and phosphatidylethanolamine are founded in the inner monolayer, while phosphatidylcholine and sphingomyeline are preferentially located in the outer monolayer[19]. This generates a electric asymmetry across membrane, since about 28% of total membrane lipids are phosphatidylserine which carries, at physiological pH, one negative elementary charge per molecule, while phosphatidylcholine as well as sphingomyeline do not carry any one charge[20]. The charge on outer membrane side is due to the glicoprotein layer associated to outer surface[20-22].

In present study, we investigate the effects of inner surface charge on the erythrocyte membrane stability by use of the dispersion equation from linear hydrodynamic analysis, which was developed by us in foregoing study[6]. According to this methods, the development of surface waves on the rim of the cell is free of stabilising effect of the membrane skeleton spectrin. From this point of view was considered the stability of the membrane without the cytoskeleton effect.

In that paper[6], we performed a study about the influence of the outer surface charge and ionic strength on this stability and the knowledge of the role of inner surface charge becomes important for a better statement about the effect of electrical parameters on the membrane stability. It is important to mention that the inner surface charge density cannot be measured. Estimates of this charge proceeds from calculations based on theoretical models, and realistic values are not quite clear at present.

II. Reference state

The conformation of biological membranes permits that they can be considered as a thin planar, viscoelastic liquid film; the thickness h of the film is defined as the thickness of the lipid bilayer, since this thickness ($h < 100A$) is several orders of magnitude smaller than the cell diameter (8μm) so the planar configuration is a good approximation. The membrane is charged and acts as an electric double layer immersed in the adjacent fluids[8].

The membrane adopted model was the same used in previous paper[23], in which the membrane was modelled by a dielectric fluid film of thickness h, mass density ρ and dielectric constant ϵ_f (Fig.1).

The electric potential equation was obtained by solving the Poisson-Boltzmann equation[23]. In reference state, within external aqueous phases, for a symmetric univalent electrolyte we have

$$\phi_i^0 = 2\ln\left(\frac{1+\chi_i}{1-\chi_i}\right) + \phi_{0i}^0, \tag{1}$$

$$\chi_i = thg\left(\frac{\beta}{4}\phi_{soi}\right)\exp[k_i(\pm z + h/2)]\begin{cases}(+)\text{for } i=1\\(-)\text{for } i=2\end{cases}, \tag{2}$$

$$\phi_{soi} = \phi_{si}^0 - \phi_{oi}^0, \tag{3}$$

where ϕ_i^0 is the potential along z-axis, $\beta = Ze/K_BT, K_B$ is Boltzmann constant, Ze is molar charge, k_i is related to the Debye length, ϕ_{si}^0 the surface potential on $S_i (i = 1, 2)$, S1 and S2 denote the two surfaces of the membrane, ϕ_{oi}^0 is the bulk potential in phase i. The reference state (superscript o) is assumed to be stationary.

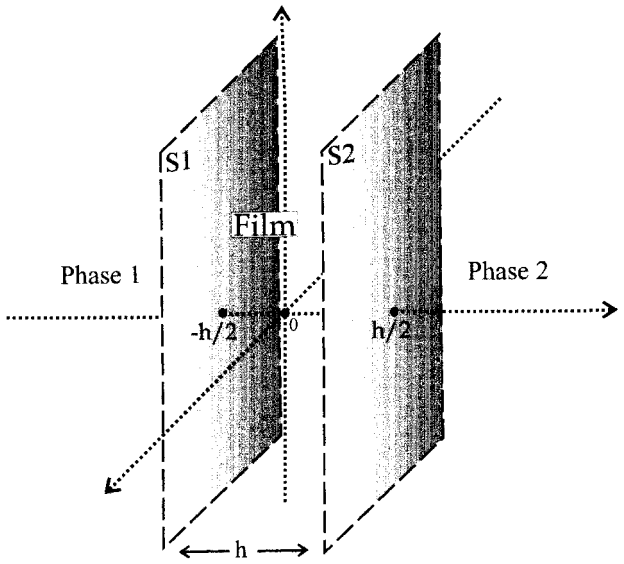

Figure 1. The membrane is represented by dielectric fluid film limited by two plane parallel infinite surfaces that separate two aqueous phases of different electrolyte concentrations and dielectric constant $\epsilon_i (i = 1, 2)$. The membrane extends from $z = -h/2$ to $z = h/2$. Phase 1 corresponds to the extracellular environment and phase 2 to cytoplasm. On both surfaces, the charges are distributed homogeneously. At both phase 1 and phase 2, the electrochemical potentials, $u^0_{1\gamma}$ and $u^0_{2\gamma}$ respectively, of each solute ion γ were considered constant. The charge density into the bilayer-fil (ρ_f) is taken to be zero $(\rho_{(+)} = \rho_{(-)} = 0)$.

III. Perturbed state

The state of convective motion of Newtonian and incompressible fluids is described by the Navier-Stokes equation

$$\rho \frac{d}{dt}\vec{v} = -\nabla \cdot \overleftrightarrow{p} + \mu \nabla^2 \vec{v} + \vec{F}_{\text{ext}} \qquad (4)$$

together with the incompressibility condition $\nabla v = 0$, being ρ the total mass density, $\vec{v}$ is the baricentric velocity vector, $\overleftrightarrow{p}$ is the pressure tensor, μ is the bulk viscosity, ∇ is the differential operator $\frac{\partial}{\partial x}\tilde{i} + \frac{\partial}{\partial y}\tilde{j} + \frac{\partial}{\partial z}\tilde{k}$, and F_{ext} is the body force vector involving here only electric effects:

$$\vec{F}_{\text{ext}} = Z\rho\vec{E} + [(\epsilon - 1)/4\pi]\vec{E}\,\text{grad}\vec{E}, \qquad (5)$$

where $Z\rho$ is the volume charge density, and $\vec{E}$ is the electric field[23]. The density and the viscosity are considered constant and uniform in each phase. The baricentric velocity is the sum of the product mass times velocity for all components of the solution in the considered volume element. Here, we do not take into account van der Waals and repulsive forces, which have been considered by some authors[3,24,25,26], since we are interested mostly in the electric effects.

The evolution of a small perturbation on the surface generates motion in the fluid. Each perturbed quantity δQ is expressed in Fourier components as [8]

$$\delta Q = \delta Q(z) \exp[iK_x x + ik_y y + \omega t], \qquad (6)$$

where $K = (K_x^2 + K_y^2)^{1/2}$ is the wavenumber and $\omega = \omega_R + i\omega_I$ is the complex frequency of the perturbation.

The solution of the Navier-Stokes equation (eq.4) gives the amplitude of the velocity field components[8]. The integration constants of the general solution in bulk phases were calculated using the following boundary conditions: continuity of velocities and their derivates; continuity of electrical potential and discontinuity of the electrical displacement; mechanical force balance and balance of surface charge[6].

The mechanical force balance was decomposed into normal and tangential momentum balance, which are represented respectively by equations 7 and 8 [6],

$$K^2\sigma^0_m\delta z + \Delta_s[(\mu/K^2)(D^2 - K^2)Dv_z - [(\omega\rho/K^2) + 2\mu]Dv_z] - \Delta_s[(\epsilon/4\pi)(DE^0_z\delta\phi_s + E^0_z\delta E_z)] = 0, \qquad (7)$$

$$K^2\delta\sigma_m - K^2\eta^0_s Dv_z + \Delta_s[\mu(D^2 + K^2)v_z] - K^2\Delta_s[(\epsilon/4\pi)E^0_z\delta\phi_s] = 0, \qquad (8)$$

where Δ_s denotes the finite difference jump through the surface, σ^0_m is the mechanical surface tension taken at the reference state and $\delta\sigma_m$ denotes the perturbation of the mechanical surface tension. D is the operator $\partial/\partial z$

and D^2 is $\partial^2/\partial z^2$, η_s^0 is the sum of the shear and dilational surface viscosities, $\delta\phi_s$ is perturbation of surface potential, δE_z is perturbation of electric field and δz is the interface deformation.

The balance of surface charge was based on calculations of an additional relation which describes the perturbation of the surface charge density for deformed surface[27], taking into account the adsorption-desorption equilibrium for the inorganic ions, and that the membrane components (for example, phospholipids) were completely adsorbed to the surface[6].

The integration constants of general solution in aqueous phases were fixed via the boundary conditions. We decomposed the general solution for the z-component of the velocity field and its derivative at the film surfaces into a symmetric and antisymmetric components, $v_{As}(z)$, $v_{Bs}(z)$, $Dv_{As}(z)$ and $Dv_{Bs}(z)$. This procedure gives us [6] a set of four linear algebraic equations (two for each interface due to the balance of normal and tangential momenta) for four independent variables. The sum and difference of these equations lead to a 4 x 4 matrix, whose elements are given by sum and difference between the coefficients. Thus, the secular determinant, leading to the dispersion relation, has the general form[6],

$$\begin{array}{c|cc|cc|c} & \mathrm{Kv_{As}} & \mathrm{DK_{As}} & \mathrm{Kv_{Bs}} & \mathrm{Dv_{Bs}} & \\ \mathrm{Tr(+)} & & & & & \\ & \cdots\cdots & \mathbf{B\,E} \cdots\cdots & \cdots\cdots & \mathbf{C\,O} \cdots\cdots & \\ \mathrm{Lo(-)} & & & & & \\ \hline \mathrm{Tr(-)} & & & & & \\ & & \mathbf{C\,O} & & \mathbf{S\,Q} & \\ \mathrm{Lo(+)} & & & & & \end{array} = 0 \qquad (9)$$

where Tr(+) and Tr(-) correspond respectively to the sum and difference between S1 and S2-transverse modes, and Lo(+) and (Lo(-) are the sum and difference between S1 and S2-longitudinal modes, respectively (the matrix elements are presented in foregoing paper[6]). The existence of nontrivial solution of this set of equations requires that the secular determinant vanishes.

The bending vibration mode (BE) in eq. 9 corresponds to superposition of v_{As} and Dv_{As} fields, the squeezing vibration mode (SQ) represents a superposition of v_{Bs} and Dv_{Bs} and CO defines coupling terms. Due to an important electric asymmetry between the two interfaces, CO$\neq$0 [8].

The dispersion relation obtained was an equation of eighth order in ω. Two limiting regimes could be considered[24]: (1) Fast Regime ($\omega \ll D_s K^2$) - for which the wave motion would be fast enough that the surface diffusion becomes negligible; and (2) Slow Regime ($w \gg D_s K^2$) - for which the motion would be sufficiently slow compared to the surface diffusion. In this regime the time is large enough to permit an ionic redistribution on the interface.

The stability criteria associated to a model were established solving the dispersion equation $\omega(K)$ from the secular determinant and analysing their roots. However the calculation of such roots becomes very complex because of the membrane asymmetry, hence simplifications were made based on some experimental data from literature.

First, we chose the slow regime condition. This procedure leaded to the disappearance of many terms of $\omega(K)$ [6], since the slow regime requires that $\omega(K) \ll D_s K^2$. It is considered a good approximation for describing the surface dynamics of lipid films, because the surface diffusion coefficient of the lipids is relatively high, $D_s \cong 1,5 \times 10^{-12}$ m^2.s^{-1} [2]. Although, the diffusion coefficients for the erythrocyte membrane are not available, we choose the slow regime since the wavenumber is typically 10^6 m^{-1} in the example[12], since the wavelengths cannot exceed the cell perimeter and the erythrocyte diameter is about 8μm [6].

Second, based on results from use of the REDUCE mathematical software, the dispersion equation for $\omega < 10\ s^{-1}$ could be written as a equation of first order in ω. From REDUCE we obtained the analytical solution of the secular determinant. This enable us to investigate the order of magnitude of each ω-coefficient. In fact, we found that the ω-coefficients decreased significantly as the ω-exponent increased. So it was reasonable to take such approach, since we were interested in wavenumbers of about 10^6m^{-1} and time coefficients of about 3s^{-1}.

According to experimental results from literature[8,13] the average times for disturbance development on the membrane of erythrocyte (from the first appearance of a wave form to the final gragmented shape) are up to about 0.3 s . The derived value for the time coefficient ω_R is thus about 3 s^{-1}.

So we had only one real root, $\omega = \omega_R$. In this case, for ω_R positive, the system was unstable and the perturbation would grow (eq.8). On the other hand, for ω_R negative, the system is stable. Among the possible wavenumbers there was a marginal or critical one, K_c, for which $\omega_R = 0$. It was associated with a marginal wavelength $\lambda_c = 2\pi/K_c$. We obtained that small wavenumbers were unstable. In unstable region, it was possible to calculate a dominant wavenumber corresponding to a maximum of the $\omega(K)$ function, for which $d\omega/dK = 0$. It is associated with a dominant wavelength λ_M. The corresponding ω_M was the fastest rate of growth of the perturbation, i.e., this dominant wavelength was the most probable wavelength. The inverse ω_M^{-1} corresponded to the characteristic time of instability τ_M [8].

IV. Results and conclusions

Table 1 shows the values of several parameters, which are related to the erythrocyte membrane, adopted here.

The results presented in Fig. 2(a) show that the decrease of inner surface charge density modulus ($|Q_{S2}|$), or decrease of Q_{S2}/Q^0_{S1} ratio, leads to increase of stability region within critical stability curve $\omega = 0$; Q_{S2}/Q^0_{S1} versus normalised critical wavenumber hK_c. This curve defines the limit between stable and unstable mode regions. From these results we can conclude that the increase of surface potential difference modulus produce membrane instability.

How it can be seen in Fig. 2(b), when $|Q_{S2}|$ decreases, ω_M and K_M also decrease. It is observed that the variations in ω_M-values are well pronounced, into studied interval, when compared to K_c and K_M-variations.

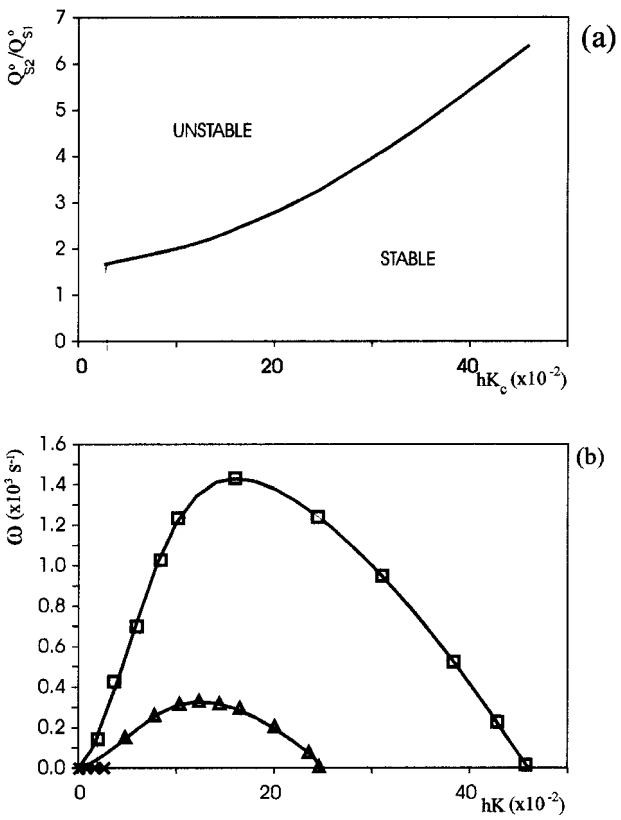

Figure 2. (a) Marginal stability curve $\omega_R = 0$; ratio Q_{S2}/Q^0_{S1} (Q^0_{S1} is reference value of the inner surface charge density - table 1) versus normalized marginal wavenumber hK_c. (b) Rate of growth ω versus normalized wavenumber hK. For: Q_{S2}/Q^0_{S1} = 6.4 (□), 5.0 (Δ), 3.3 (x) and 2.0 (○).σ^0_m = 1.3 mN/m (h=10nm).

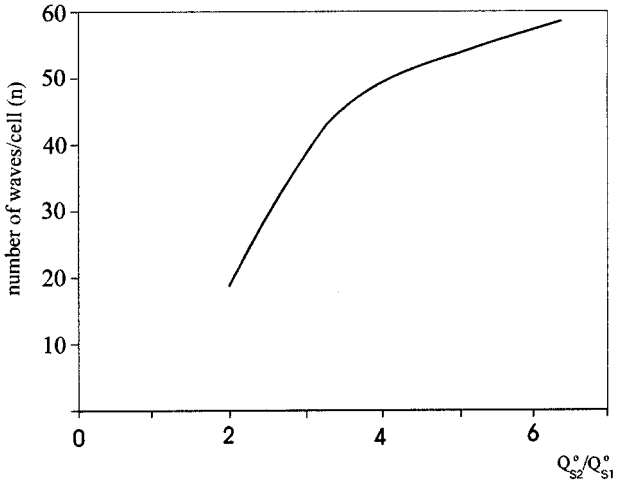

Figure 3. Number of wave per cell as function of the inner surface charge density.

Table 1

Adopted Physico-chemical parameters for the reference state

Parameter	(symbol)	Value	[Ref.]
Film viscosity	(μ)	1.00P	[24]
Bulk phase viscosities	($\Sigma_i[\mu]$)	0.02P	[24]
Shear and dilational surface viscosities	($\Sigma_i[\eta_s]$)	10^{-6}sP	[24]
Film densities	(ρ_f)	10^3 kg/m^3	[24]
Bulk phases densities	(ρ_1,ρ_2)	10^3 kg/m^3	[24]
Outer ionic strength	(F^o_1)	0.172	[28]
Membrane thickness	(h)	10nm	[8]
Transmembrane potential	($\Delta\phi^o_o$)	-12mV	[2]
Membrane diecletric constant	(ε_f)	2	[2]
Bulk phases dielectric constant	(ε_i)	81	[2]
Inner surface charge density	(Q^o_{S2})	-9x10^{-2}A.s/m^2	[20]
Outer surface charge density	(Q^o_{S1})	-1.4x10^{-2}A.s/m^2	[23]

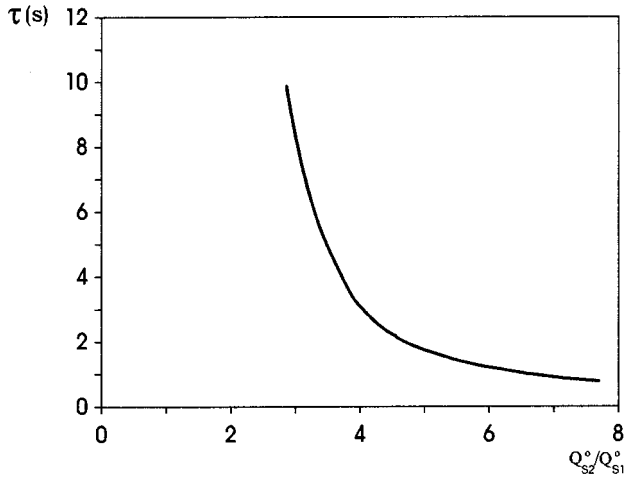

Figure 4. Characteristic time (τ_M) as function of the inner surface charge density.

Dividing the cell perimeter by λ_M, we obtained an estimate for the number of waves per cell (n). This corresponds to average number of waves that could originate synchronously and spontaneously along the cell rim by development of small perturbations. The variation of n as function of Q_{S2} is shown in Fig. 3. It can be observed that n decreases about 60% when Q_{S2}/Q^0_{S1} falls from 6.4 to 2.0. On the other hand, the characteristic time, τ_M, increases with $|Q_{S2}|$ reduction (Fig.4), and it tends to very large values for $Q_{S2}/Q^0_{S1} < 3$.

These results show that the increase of the inner surface charge modulus, which is associated to the increase in absolute value of the surface potential difference, reduces the membrane resistance to deformations. In fact, the increase of the τ_M that arises from decrease in $|Q_{S2}|$ implies on a larger time to develop instability; the membrane becomes more stable.

According to Coakley and Deeley[12], at constant

ionic strength, reduction of outer surface charge leads to marked increase in the membrane stability. The same occurs for reduction of ionic strength. These results were demonstrated by experiences with heated human erythrocyte. However, in previous study[6], we showed that the increase in erythrocyte membrane stability that follows the ionic strength reduction, according to linear analysis, is specially due to subsequent alterations in transmembrane potential, and beyond doubt the contributions of the inner electric modifications are very important to stability changes, in particular, the decrease in the negative inner surface charge tends to destabilise the membrane, when outer surface charge is kept fixed.

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