

Ion Permeation Across Model Lipid Membranes: A Kinetic Approach

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Current–voltage characteristic measurements have been utilized to investigate the ion transport properties in dimyristoylphosphatidylethanolamine [DMPE] bilayers at the tip of a patch pipet (patch-clamped pure lipid bilayers) with the aim of determining the permeation of Ca^{++} and Mg^{++} ions in the presence of KCl aqueous solutions, at two different ionic strengths (1 and 10 mM). The data have been analyzed on the basis of a modified version of the Skinner model, considering different kinetic reactions at the water–membrane interfaces and within the membrane core. Transient aqueous pores produced by thermal fluctuations facilitate the ion transport, avoiding the Born energy barrier associated with the solubility–diffusion mechanism. The pore–ion coupling, allowing ion translocation across the bilayer, is modeled as a kinetic process within the statistical rate theory. Using this approach, from the I – V characteristics measured at different temperatures below the lipid transition temperature, the surface energy Γ , that governs the stability against rupture of the bilayer, and the difference in the electrochemical potentials ($\mu^i - \mu^e$), associated with the two membrane interfaces at the inner and external medium, have been determined. Values of Γ in the range from 0.010 to 0.030 J/m², depending on the salt concentration and temperature, have been obtained. The relevance of these parameters in connection with the proposed model is briefly discussed.

Introduction

The transport of ions and small neutral molecules across plasmatic cell membranes driven by ionic concentration or electrical potential gradients (passive transport) is crucial for the function of any biological system. On the other hand, because of the complexity of the cell membrane, these processes result, at least partially, from a combination of other mechanisms, involving the molecular details and the inhomogeneity of the lipid matrix and, moreover, the presence of ion channels.

Lipid bilayer membranes represent a system in which the transport theories (limited to the passive mechanisms) are more easily tested than in cell membranes, where the protein components give rise to more highly complex systems. In contrast to biological membranes, the composition and the structure of the model lipid bilayers are generally well-established, offering advantages in studies of ion transport.

The mechanism of permeation of ions across phospholipid bilayers, despite the availability of experimental data and extensive studies in the past decades, is still not completely understood.

In passing through a bilayer, an ion encounters at least three distinct regions, i.e., two highly polar interfacial regions, influenced by the different aqueous media bathing the membrane, and a relatively disordered hydrocarbon chain region in the center of the bilayer. The contributions of these different regions to overall transport is a not yet completely resolved issue, although numerous plausible physical mechanisms have been discussed.

In the past, two alternative mechanisms have been proposed. In the first approach,¹ the ion permeation is described within a

solubility–diffusion theory based on the energy required to transfer an ion from the aqueous phase to the hydrocarbon membrane core (the Born energy).² Within this scheme, different contributions must be taken into account, including electrostatic and solvophobic contribution to the Gibbs free energy, the hydrophobic effect and, moreover, contributions deriving from the dipolar potential distribution inside the lipid bilayer. The second approach^{3,4} considers the ion permeation to be due to hydrated transient defects (transient pores) produced by thermal fluctuations, where ions can largely evade the high-energy Born barrier associated with the solubility–diffusion mechanism. The term “pore” defines a structure that facilitates the transport of ions (more generally of certain molecules) across the membrane where the diffusion coefficient is much higher than that in a pore. As pointed out by Paula et al.,⁵ permeation across pores seems to be the dominant mechanism in sufficiently thin membranes, where “water wires” spanning the hydrocarbon core can account for the relatively high ion permeability observed (exceeding by various orders of magnitude that derived from Born energy).

We have recently analyzed⁶ electrical conductivity measurements in lipid bilayer systems on the basis of a transport model^{6,7} where the pore formation was coupled with an ion, giving rise to a diffusion structure across the bilayer itself governed by kinetic reactions, in the presence of a transmembrane potential.

In this work, we have measured the response of patch-clamped pure lipid bilayers to applied voltage and ionic concentration gradients in the presence of different ionic strengths (1 and 10 mM KCl) and the observed voltage–current characteristics have been discussed on the basis of a simple

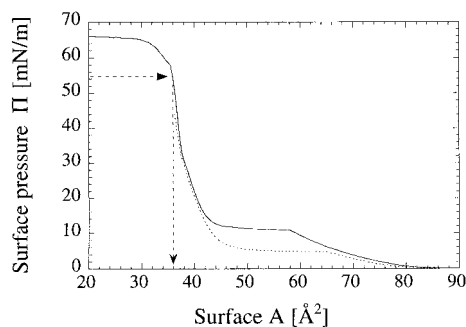


Figure 1. The surface pressure–area isotherm of DMPE monolayer at the water–air interface at two different temperatures: solid line: $T = 25\text{ }^{\circ}\text{C}$; dotted line: $T = 20\text{ }^{\circ}\text{C}$. The aqueous subphase is 1 mM KCl, 1 mM Hepes, pH = 8.

kinetic model consisting in a revised version of the above-mentioned ion–pore coupling mechanism.

This mechanism may be significantly important in understanding the basic principle of the transport process in biological membranes in order to clarify the role of passive ion translocation, when different combined effects are present.

A reasonable good agreement between predictions and experimental values supports our proposed model for ion translocation through preformed aqueous pathways across the lipid bilayer.

Experimental Section

Lipid model bilayers were formed at the tip of the patch pipets using the tip-dip technique of Coronado and LaTorre.⁸ After immersion of a glass pipet of appropriate diameter (of the order of $1\text{ }\mu\text{m}$) into an electrolyte solution, a phospholipid monolayer was formed at the air–water interface by spreading a fixed volume of a solution of the lipid investigated dissolved in chloroform, at a concentration of 1 mg/mL. The thermodynamic state of the lipid assembly at the interface was controlled by measuring the surface pressure–area isotherm by means of a Langmuir trough. A typical isotherm is shown in Figure 1, where the existence of four different phases, ranging from gas to the solid state, are evidenced. When the liquid-condensed phase is reached, progressively reducing the area of the interface, the pipet is moved into the air and back into the solution which corresponds to the formation of a lipid bilayer at the tip of the pipet. We choose a surface pressure of 55 mN/m, before the collapse pressure, where the headgroups of the phospholipid molecules are completely ordered. The formation of the seal corresponds to a marked increase of electrical resistance, passing from values of the order of hundreds of $M\Omega$ to values of some $G\Omega$ (Figure 2). The number of trials needed to form a stable seal was usually limited to one, only occasionally a second trial was necessary.

The lipid used was dimyristoylphosphatidylethanolamine [DMPE], purchased from Sigma Chem. Co. (USA) and was used without any further purification. In this lipid, the gel-liquid crystalline phase transition occurs at a temperature of about $50\text{ }^{\circ}\text{C}$.⁹ Before each measurement, the lipid dissolved in chloroform was maintained at a temperature above the main transition temperature in order to avoid any possible lipid aggregate and to obtain a monodisperse solution.

The phospholipid bilayer at the tip of the glass pipet separates two different aqueous compartments, conventionally defined as the inner medium (the aqueous phase inside the pipet) and the external medium (the aqueous phase outside the pipet).

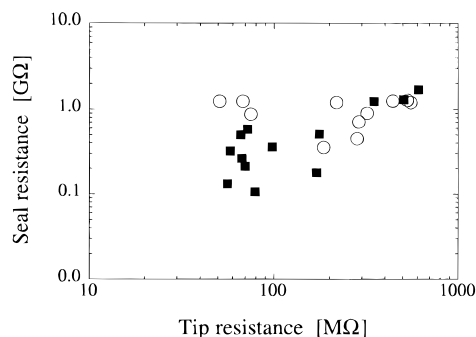


Figure 2. Dependence of the seal resistance on the open pipet resistance (pipet size). Solid symbols: CaCl_2 ; open symbols: MgCl_2 . Open pipet resistance was measured in 1 mM KCl (or 10 mM KCl).

We have investigated the ionic transport of divalent cations (Ca^{++} and Mg^{++}) through the lipid bilayer in the presence of a uni-univalent electrolyte solution (KCl) at different concentrations (1 and 10 mM). Under these conditions, the transport of Ca^{++} and Mg^{++} ions is studied in systems of different ionic strength (1 and 10 mM KCl) induced by either a potential gradient or an ionic concentration gradient (in the inner medium, the concentration of CaCl_2 or MgCl_2 has been varied from 0.5 to 5 mM, whereas in both the internal and external medium the concentration of KCl has been maintained constant to the value of 1 or 10 mM).

The electrical current–voltage curves (I – V characteristics) were measured by means of a current-to-voltage converter under voltage-clamp conditions. The inner medium is the one to which a voltage is connected to bilayer through an Ag/AgCl electrode, whereas the external medium is connected to the ground through a second Ag/AgCl electrode. The electrical voltage was applied to the electrode inside the pipet while the external electrode was grounded through the current amplifier.

As pointed out by Coronado,¹⁰ divalent cations (Ca^{++} , Mg^{++}) play an important role in the stabilizing negatively charged lipids, but have no influence in the stabilization of neutral and positively charged lipid bilayers. At the pH investigated (1 mM Hepes, pH about 8), DMPE molecules partially bear a negative charge and the presence of divalent cations act as a stabilizing factor.

Theoretical Background

The Mechanism of Pore Formation. The mechanism of pore formation in a lipid bilayer is associated with lateral thermal fluctuations of the lipid molecules giving rise to spontaneously formed hydrophobic high-dielectric defects which convert into hydrophilic pores with an appropriate reorientation of the lipid headgroups at the edge of the pore.

The formation of these pores has been seen as a two-dimensional nucleation process.¹¹ From this point of view, rupture of the bilayer and transport of ions across it are caused by the appearance of large enough holes that limit the lifetime of the whole structure.

The free energy of pore formation can be written as¹²

$$W(r) = 2\pi\gamma r - \Gamma\pi r^2 \quad (1)$$

deriving from a balance between the energy necessary to create the pore of radius r and the energy released by the pore surface, where γ is the energy per length along the circumference needed

to form the pore and Γ is the energy per area of a flat, pore-free membrane.

Equation 1 is modified by the presence of a transmembrane potential V to give

$$W(r,V)=2\pi\gamma r - \Gamma\pi r^2 - \pi r^2 aV^2 \quad (2)$$

where

$$a = \frac{\epsilon_0 (\epsilon_w - \epsilon_l)}{2d} \quad (3)$$

is the coupling coefficient of the electrical to mechanical energy transduction, with ϵ_w and ϵ_l the permittivity of the water and the lipid interior, respectively, d the thickness of the hydrocarbon bilayer, and ϵ_0 the dielectric constant of free space.

If the potential is assumed to be constant during size fluctuations driven by thermal fluctuations, the critical pore radius r_c and the barrier maximum $W(r_c,V)$ are given by

$$r_c = \frac{\gamma}{\Gamma + aV^2} \quad (4)$$

$$W(r_c, V) = \frac{\pi\gamma^2}{\Gamma + aV^2} \quad (5)$$

The average membrane lifetime against rupture is given by¹³

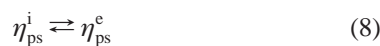
$$\tau = \frac{(K_B T)^{3/2}}{4\pi C_0 S D_p \gamma \sqrt{\Gamma + aV^2}} \exp\left(\frac{\pi\gamma^2}{K_B T(\Gamma + aV^2)}\right) \quad (6)$$

where S is the membrane area, C_0 the pore concentration, D_p the diffusion coefficient of the pore, and $K_B T$ the thermal energy.

The Permeation Kinetic Model. Recently, we have proposed¹⁴ a modified version of the Skinner model¹⁵ consisting of a diffusion process through pores, described by different kinetic processes at the water-membrane interfaces and within the membrane core.

The basic mechanism of ion diffusion and permeation across a lipid membrane involves the ion pairing with a pore at one interface (for example, the inner bathing medium), its passing through and finally its release in the external bathing medium.

The kinetic equation describing the above reactions within the Skinner model can be written as¹⁴



where C_s^i and C_s^e are the ion concentrations and η_p^i and η_p^e the pore concentrations in the internal and external medium, respectively and η_{ps}^i and η_{ps}^e represent the pore concentrations when ion-pore coupling has occurred, at the two interfaces, respectively. On the basis of the statistical rate approach proposed by Ward et al.,^{16,17} following Skinner et al.,¹⁵ the ion fluxes through the internal and external interface and through the membrane itself can be written as¹⁴

$$J_i = k_i \left(\frac{k_i C_s^i \eta_p^i \exp(\phi_p^i)}{\eta_{ps}^i \exp(\phi_p^i)} - \frac{\eta_{ps}^i \exp(\phi_p^i)}{k_i C_s^i \eta_p^i \exp(\phi_p^i)} \right) \quad (10)$$

$$J_e = k_e \left(\frac{k_e C_s^e \eta_p^e \exp(\phi_p^e)}{\eta_{ps}^e \exp(\phi_p^e)} - \frac{\eta_{ps}^e \exp(\phi_p^e)}{k_e C_s^e \eta_p^e \exp(\phi_p^e)} \right) \quad (11)$$

$$J_{ch} = k_{ch} \left(\frac{k^p \eta_{ps}^i \exp(\phi_p^i)}{\eta_{ps}^e \exp(\phi_p^e)} - \frac{\eta_{ps}^e \exp(\phi_p^e)}{k^p \eta_{ps}^i \exp(\phi_p^i)} \right) \quad (12)$$

where k_i , k_e , and k_{ch} are the equilibrium exchanges rates per unit area of the interface from the inner medium to the bilayer, from the bilayer and the external medium and across the bilayer (the membrane), respectively. ϕ_p^i and ϕ_p^e are the reduced potential at the interfaces, ϕ^i and ϕ^e are the reduced potentials in the bulk inner and outer medium, where, at each interface, the reduced potential is defined as

$$\phi^j = \frac{zF}{RT} V^j \quad (13)$$

where z is the ion valence, R the gas constant, T the absolute temperature and $F = 96\,500$ C/mol the Faraday constant. The constants k^i , k^e , and k^p are the equilibrium constants of each of the three reactions (eqs 7, 9, and 8, respectively).

With the additional assumption that

$$\eta_p^i + \eta_p^e + \eta_{ps}^i + \eta_{ps}^e = \eta_{tot} \quad (14)$$

and that, on average, the pore concentration maintains constant at each interface

$$\eta_p^i + \eta_{ps}^i = \eta_p^e + \eta_{ps}^e \quad (15)$$

in steady condition, eqs 10–12 can be rearranged to give the ionic flux (due to a single ionic species) as

$$J = 2k_{ch} \sinh(\ln X) \quad (16)$$

where

$$X = k^p \exp(\phi_p^i - \phi_p^e) \left(\frac{1 + \frac{\exp(\phi_p^e - \phi^e)}{k^e C_s^e}}{1 + \frac{\exp(\phi_p^i - \phi^i)}{k^i C_s^i}} \right) \quad (17)$$

Condition 15 implies that the pore concentrations are largely independent of the ionic strength of the electrolyte solutions bathing the bilayer and that the pore-ion coupling occurs independently of the ion concentration. In salt excess, the following approximations

$$\frac{\exp(\phi_p^e - \phi^e)}{k^e C_s^e} \ll 1$$

$$\frac{\exp(\phi_p^i - \phi^i)}{k^i C_s^i} \ll 1 \quad (18)$$

hold and the quantity $(\phi_p^i - \phi_p^e)$, representing the potential drop across the membrane interfaces, gives the dependence on the

applied transmembrane potential V through a proportionality constant α , according to

$$\phi_p^i - \phi_p^e = \frac{zF}{RT} \alpha V \quad (19)$$

Within the above model, the ionic flux for each ion involved in the transport across the bilayer can be written in the form

$$J = 2k_{ch} \sinh\left(\frac{zF}{RT} \alpha V + C\right) \quad (20)$$

where the quantity C is given by

$$C = \ln(k^p) + \left(\frac{\exp(\phi_p^e - \phi^e)}{k^e C_s^e}\right) - \left(\frac{\exp(\phi_p^i - \phi^i)}{k^i C_s^i}\right) = \ln(k^p) + \exp(-\mu^e/RT) - \exp(-\mu^i/RT) \approx \ln(k^p) + \left(\frac{\mu^i - \mu^e}{RT}\right) \quad (21)$$

with the quantities μ^e and μ^i defined as $\mu^e = RT \ln(k^e C_s^e) + zF(V^e - V_p)$ and $\mu^i = RT \ln(k^i C_s^i) + zF(V^i - V_p)$. The term $(\mu^i - \mu^e)$ represents the difference in the electrochemical potentials associated with the two membrane interfaces, at the internal and external medium, respectively. In the present case, there are three different ionic species present at the membrane interfaces, deriving from the different salts in the external and internal aqueous solution. Consequently, eq 20 must be specialized as

$$\frac{J}{2k_{ch}} = A \cdot \sinh\left(\frac{z^+ F}{RT} \alpha V + C(K^+)\right) - (A + 2B) \cdot \sinh\left(\frac{z^- F}{RT} \alpha V + C(Cl^-)\right) + B \cdot \sinh\left(\frac{z^{++} F}{RT} \alpha V + C(Ca^{++})\right) \quad (22)$$

where the prefactors A and B take into account the stoichiometric ion concentrations and $C(K^+)$, $C(Cl^-)$ and $C(Ca^{++})$ (or $C(Mg^{++})$) depend on the electrochemical potential difference of the ion in the ionic layer at the two sides of the bilayer. The total current I is obtained from eq 22 multiplying by the number density of pores effective to the ion transport, i.e., pores the diameter of which is larger than the respective ion diameter. This number n/N_{tot} is given by

$$\frac{n(r_h < r < r_c)}{N_{tot}} = H \frac{\exp\left(-\frac{\pi \gamma^2}{K_B T (\Gamma + a V^2)}\right)}{\sqrt{K_B T (\Gamma + a V^2)}} \quad (23)$$

where H is a hindrance factor which limits the pore population to those having an effective radius larger than the hydrated radius r_h of the ion and lower than the critical pore radius r_c . We have assumed the same value of the parameter H even if it should depend on the ionic species investigated. In fact, the hydrated radius of the ions^{2,18,19} are $r_h = 0.331$ nm, $r_h = 0.332$ nm, $r_h = 0.428$ nm, and $r_h = 0.412$ nm for K^+ , Cl^- , Mg^{++} , and Ca^{++} , respectively. Equations 22 and 23 provide the complete information on the ion permeation mechanism through fluctuation pores and allow a systematic comparison with the experimental results on the basis of the model parameters.

Results and Discussion

I - V Characteristics. Typical I - V characteristics of a DMPE bilayer with a $MgCl_2$ concentration difference of 1.5 mM in a bulk KCl electrolyte solution of two different ionic strengths

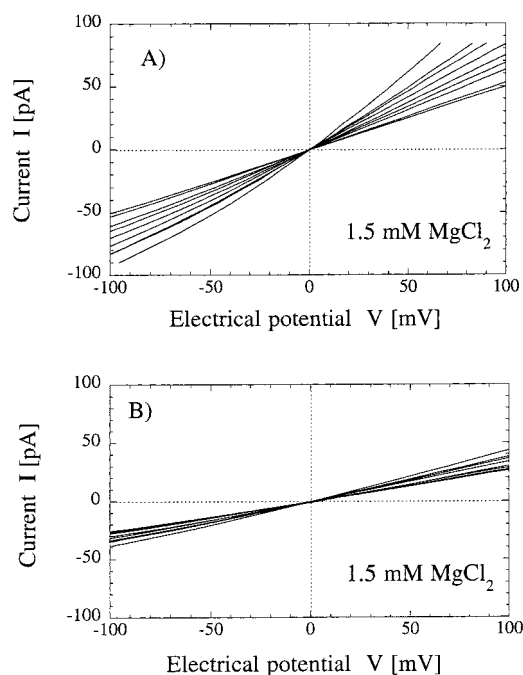


Figure 3. The I - V characteristics of DMPE bilayer in the presence of 1.5 mM $MgCl_2$ at different temperatures for two different ionic strengths: (A): 1 mM KCl; from bottom to top, the temperatures investigated are 20.0, 20.5, 21.0, 22.0, 23.0, 25.5, 27.5, 29.5, and 31.5 °C. (B): 10 mM KCl; from bottom to top, the temperatures investigated are 21.0, 22.5, 24.9, 25.5, 27.5, 28.0, 29.0, 29.5, and 30.0 °C.

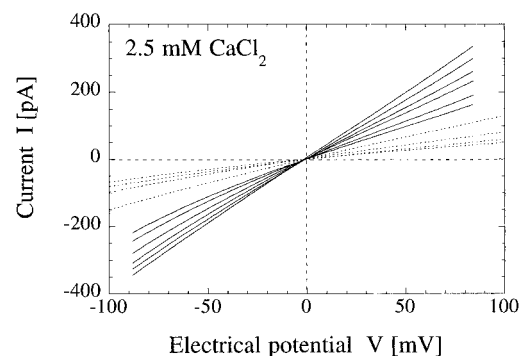


Figure 4. The I - V characteristics of DMPE bilayer in the presence of 2.5 mM $CaCl_2$ gradient at different temperatures. Solid lines: bilayer bathed by a 1 mM KCl electrolyte solution; from bottom to top, the temperatures investigated are 26.5, 27.5, 28.5, 29.5, 30.5, and 31.5 °C. Dotted lines: bilayer bathed by a 10 mM KCl electrolyte solution; from bottom to top, the temperatures investigated are 24.0, 25.0, 26.0, and 27.0 °C.

(1 and 10 mM) are shown in Figure 3. Qualitatively, all the I - V characteristics investigated are similar, showing an approximately linear behavior in the interval of small potential (ohmic behavior). As an example, the I - V characteristics for bilayers with 2.5 mM $CaCl_2$ concentration difference in the presence of 1 and 10 mM KCl ionic strength are shown in Figure 4. The applied transmembrane potential was confined to values lower than ± 100 mV to avoid electroporation effects.¹²

The analysis of the I - V characteristics within the framework of such a permeation model has been carried out on the basis of eqs 22 and 23. To compare the results of calculations with experimental data, we should specify all parameter values. According to Chernomordik and Chizmadzhev²⁰ and Zhelev and Needham,²¹ the edge energy γ has been assumed to be $\gamma = 9 \times 10^{-11}$ J/m, independent of temperature and insensitive to the

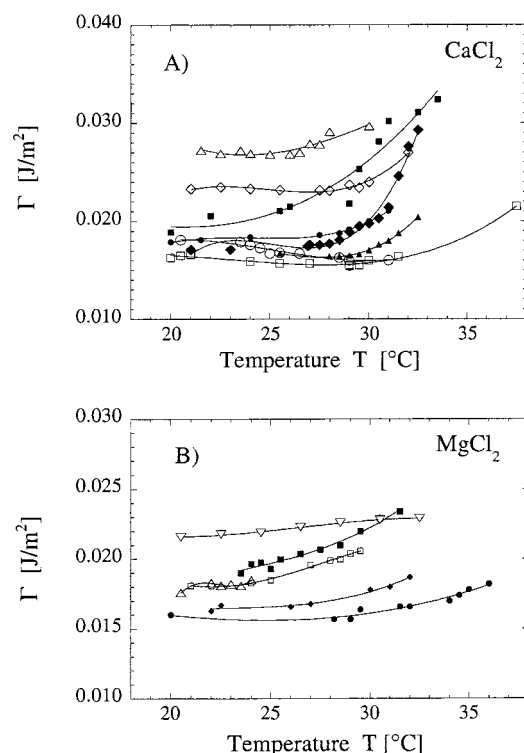


Figure 5. The surface energy Γ of the bilayer as a function of temperature. (A): Effect in the presence of CaCl₂. Solid symbols: 1 mM KCl (●); 0.5 mM CaCl₂ (■); 1 mM CaCl₂ (◆); 2.5 mM CaCl₂ (▲); 5 mM CaCl₂. Open symbols: 10 mM KCl (○); 0.5 mM CaCl₂ (□); 1 mM CaCl₂ (◇); 2.5 mM CaCl₂ (△); 5 mM CaCl₂. (B): Effect in the presence of MgCl₂. Solid symbols: 1 mM KCl (●); 0.5 mM MgCl₂ (◆); 1 mM MgCl₂ (■); 1.5 mM MgCl₂. Open symbols: 10 mM KCl (○); 0.5 mM MgCl₂ (□); 1.5 mM MgCl₂ (▽); 2.5 mM MgCl₂.

ionic environment. This parameter is the line tension (the edge energy) of the pore wall and is expected to depend on the chain length. In the case of short chains, a limiting lower value is on the order of $\gamma = 2 \times 10^{-11}$ J/m. We have assumed a value relatively higher than that, to take into account the chain length of the lipid investigated and the strong interaction between bulky chains due to the initial compression of the monolayer in a completely ordered state (recent measurements on stearylolphosphatidylcholine membrane attached to a pipet yield a value of $\gamma = 9 \times 10^{-12}$ J/m).²¹

In our model, the relevant parameters are the surface energy Γ that governs the stability against rupture of the bilayer and the parameters C_i ($i = \text{K}^+, \text{Cl}^-, \text{Ca}^{++}, \text{Mg}^{++}$), related to the difference in the electrochemical potentials of each ionic species investigated at the two interfaces during the ion–pore coupling across the membrane.

We have evaluated these parameters by means of a nonlinear least-squares minimization procedure, by fitting eqs 22 and 23 to the experimental I – V characteristics. The results are shown in Figures 5 and 6.

As can be seen, the surface tension Γ is of the order of 15–25 mJ/m² for both the systems investigated (CaCl₂ and MgCl₂), with a weak increase as the temperature is increased, approaching to the chain-melting transition temperature. These values differ from those obtained by different methods and in different experimental conditions. Surface tensions for various lipids and lipid mixtures obtained by the Laplace method (by applying a known pressure difference and measuring the change in the electrical capacitance) or by other methods cover a broad spectrum, with reported values between 0.2 and 2 mJ/m².^{22,23}

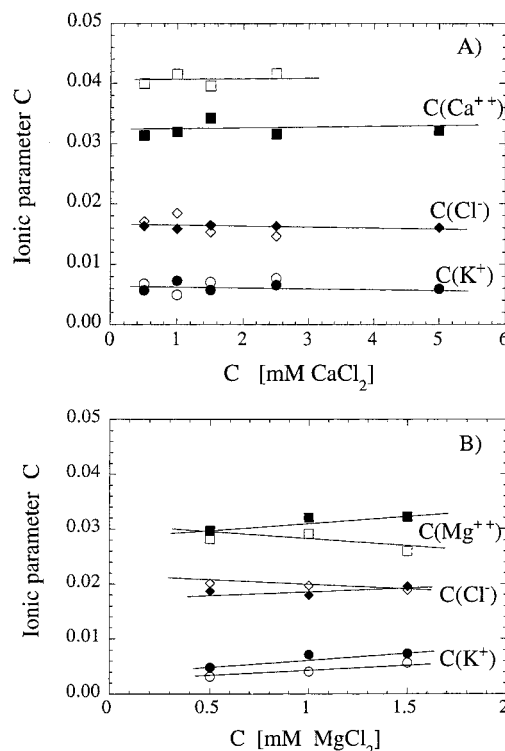


Figure 6. (A) The ionic parameters $C(i)$, ($i = \text{K}^+, \text{Cl}^-, \text{Mg}^{++}$) as a function of CaCl₂ concentration at the temperature of 22 °C in the presence of two different ionic strengths. Solid symbols: 1 mM KCl (●): K^+ ; (◆): Cl^- ; (■): Ca^{++} . Open symbols: 10 mM KCl (○): K^+ ; (◇): Cl^- ; (□): Ca^{++} . (B): The ionic parameter $C(i)$, ($i = \text{K}^+, \text{Cl}^-, \text{Mg}^{++}$) as a function of MgCl₂ concentration at the temperature of 22 °C in the presence of two different ionic strengths. Solid symbols: 1 mM KCl (●): K^+ ; (◆): Cl^- ; (■): Mg^{++} . Open symbols: 10 mM KCl (○): K^+ ; (◇): Cl^- ; (□): Mg^{++} .

depending on the type of the lipid. Probably, this is the least accurately evaluated parameter since variations in the solution ionic strength and surface charge can lead to the discrepancies reported.

Since the surface tension Γ is given by the chemical potential difference of a lipid molecule between the bulk solution and the membrane phase,²⁴ the observed values, higher than those quoted above, suggest that the membrane stability could depend on the aqueous environments and that the presence of an ionic strength in the range of 1–10 mM, as in the present case, favors a more stable structure, evidencing how a membrane (a lipid bilayer) can undergo a variety of dynamic behavior induced by fluctuation in the solvent environment. The membrane stability dependence on environmental factors has been recently described on the basis of pore growth treated as a non-Markovian stochastic process by Sung and Park.²⁵ The electrostatic aspects modifying the lipid bilayer bending induced by unequal screening layers on the two side of the bilayer derived from divalent cations on one side and monovalent cations on the other have been analyzed by Chou et al.²⁶

It is also interesting to note that these results provide, for the first time, evaluation of the surface tension from electrical conductivity measurements, as far as lipid bilayers are concerned.

The most informative data derived from the fitting of eqs 22 and 23 are the C_i dependencies shown in Figure 6. The results show that for both the systems investigated (CaCl₂ and MgCl₂), the parameters C_i are approximately independent either of the divalent salt concentration or of the ionic strength of the aqueous phase bathing the membrane. This behavior can be, at least

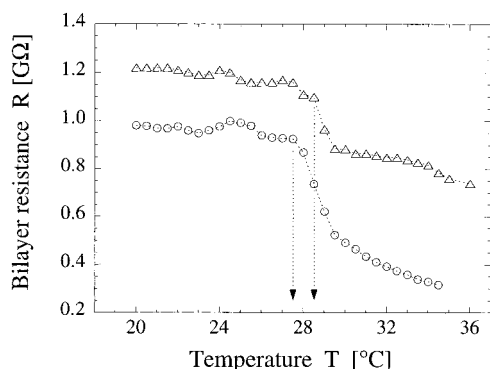


Figure 7. The resistance of the DMPE bilayer at the ionic strength of 1 mM KCl, in the presence of different gradients of divalent electrolyte solutions. (Δ): 0.5 mM MgCl_2 ; (\circ): 5 mM CaCl_2 .

qualitatively, justified on the basis of eq 21 and we will discuss in detail these dependencies in the following. Since k^p represents the equilibrium constant of the kinetic reaction governing the transport across the (bulk) membrane core, its value is expected to be close to unity and consequently the differences observed in the C_s values for K^+ , Cl^- , and Ca^{++} or Mg^{++} must be due to the terms associated with the difference in the electrochemical potentials at the two interfaces. The electrochemical potentials take into account the influence of the diffuse double layer at the interfaces on the transport mechanism, in particular on the ion-pore coupling that allows the kinetic reaction to be effective.

In the case of a symmetric membrane, in the absence of an ionic gradient across the membrane, as it happens for K^+ ions (all experiments are carried out with the same K^+ concentration at the two sides of the membrane), the electrochemical potentials associated with this ion are very close each other and the term C_{K^+} is close to zero (its deviation from this value is due to the presence in the inner medium of different ionic species (Cl^- and Ca^{++} or Mg^{++}) that partially modify the ionic structure of the interfacial layer). As far as the Cl^- ion is concerned, owing to the higher concentration in the inner medium ($C_s^i > C_s^e$), a more marked asymmetry at the two interfaces is present, resulting in a difference in the electrochemical potentials ($\mu^i > \mu^e$) and, as a consequence, $C(\text{Cl}^-) > C(\text{K}^+)$. This asymmetry is further emphasized in the case of divalent ions where, due to the higher concentration in the inner medium, a specific contribution due to the divalent character of these ions must be added, modifying the ionic structure of the diffuse layer at the aqueous-membrane interface. This reflects in a higher value of $C(\text{Ca}^{++})$ or $C(\text{Mg}^{++})$ with respect to the corresponding value of monovalent ions.

Comparison Between the Two Different Cations. Although the efficacy of divalent cations in induced pores across biological membranes is in the order $\text{Ca}^{++} > \text{Mg}^{++}$,^{27,28,29} our results do not provide strong evidence for a different behavior of these two ions, when their permeation across a bilayer is considered. This could be attributed to the fact that these ions are solvated to the same extent. As pointed out by Chapman et al.,³⁰ an approximate measure of the solvation interaction is given by the ratio of the square of the electric charge to the bare ion radius or, more precisely, by the hydration radius that, in the present case, does not differ appreciably for Ca^{++} and Mg^{++} ions.

Temperature Stability of the Bilayer. Occasionally, bilayers with a lower temperature stability, corresponding to a decrease of the membrane resistance, were also formed. Figure 7 shows the membrane resistance as a function of temperature for bilayers with different ionic gradients of MgCl_2 and CaCl_2 , at the ionic

strength of 1 mM KCl. As can be seen, the progressive reduction of the resistance is indicative of an increase of the density fluctuations, favoring the formation of hydrophilic pores.

According to the transient pore model, the fractional membrane area containing pores available to ion transport is generally small. The pore density estimated from the Boltzmann factor, considering an edge energy density of 9×10^{-11} J/m, is on the order of 10^{-5} , i.e., on the order of one pore per 10^5 lipid molecules and the total number of transient pores per clamped membrane at the tip of the pipet (of size of 1 μm) is of the order of 10. Thus, even small changes in density fluctuation, increasing the number of transient pores, are able to produce pronounced effects in the ion permeability. This effect should be particularly enforced in the transition region when the concomitant appearance of different gel and liquid-crystalline domains occurs.

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