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NON-ISOTHERMAL POTENTIAL OF PHOSPHOLIPID BILAYER FILMS

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Summary

The thermal electrical potential generated in isochemical conditions by a temperature gradient at the two sides of lipid bilayer leaflets is measured. The experimental results agree rather well with the theoretical predictions. The cephaline (from sheep brain) bilayer behaves like a film with zero charge while the phosphatidylcholine (from egg yolk) film performs like a charged membrane. The results presented suggest that the measurement of electrical thermal potential is an interesting method to investigate the electrical behaviour of bilayer membranes.

Introduction

Films of lipids have been extensively studied since their earlier description [1]. Physico-chemical measurements [2] on these films suggest that they form bilayer structures similar to those prepared by Danielli and Davson [3,4] and by Robertson [5]. These structures can be assimilated to a membrane separating two liquid phases and formed by a liquid-like hydrocarbon interior, 50 Å thick, with the polar groups at the membrane-solution interfaces. The nature and number of polar groups can determine the existence of a space charge in the interfacial region. Alternatively, the existence of zero charge films is also possible. The transport across these films either charged or with zero charge, can be treated by using the existing theories for the ion transport across liquid or solid membranes. In the case of isothermal liquid ion exchange membranes the ion transport has been described by coupling the ion diffusion (through the membrane) and the ion exchange reactions at the solution-membrane boundaries [6]. The possible contribution of ion pairs to the transport equations has been also accounted for. The non-isothermal ion transport has been described by using the thermodynamics of irreversible processes both for solid [7] and liquid ion exchange membranes [8]. In this latter case the theory covers the contribution of the mobile charged sites, of the counterions and of the uncharged species (ion pairs). The theories predict that an electrical potential is originated across the membrane as a consequence of both the chemical potential and the temperature gradients. The experimental available data on the non-isothermal electrical potential of solid ion exchange membranes in monoionic conditions [7] and those on thick liquid ion exchange membranes in monoionic and biionic conditions [12,8] confirm the theoretical predictions. In the case of monoionic systems the influence of the chemical reaction and of the presence of associated species, which play an important role in the biionic case, disappear. For solid ion exchange membranes the electrical membrane potential in non-isothermal conditions is given by [7]

$$-\Delta\psi = (2t_+ - 1)(RT/F)\ln(a''_\pm/a'_\pm) + (2t_+ - 1)(R\Delta T/F)\ln a''_\pm$$

$$+ (t_+\alpha_+ + t_-\alpha_-)\Delta T + G$$

$$G = \tau_0 RT\ln(a''_\mathrm{w}/a'_\mathrm{w}) + \tau_0 R\Delta T\ln a''_\mathrm{w} \tag{1}$$

with $\alpha_+ = \eta - (s_+/F)$; $\alpha_- = \eta - (s_-/F)$.

All quantities, but η and $t(\pm)$, refer to the external aqueous phases: phase ($'$) at temperature T and phase ($''$) at temperature $T + \Delta T$; $a_\pm$ and a_w are the mean aqueous electrolyte and water activities respectively; s_+, s_- and t_+, ,t_- are the cation and the anion entropies and membrane transport numbers respectively; η is assumed constant (see ref. 7); τ_0 is the water-reduced transport number. In the absence of electrolyte uptake and therefore with co-ion exclusion, as is the case for many liquid ion exchange membranes, the membrane is highly selective to the counterions. Under these conditions the thermal potential equation is obtained from Eqn. 1 with either $t_+ = 0$ (anionic membrane) or $t_- = 0$ (cationic membrane). Following ref. 8 the liquid membrane potential for monoionic system is given by

$$-\mathrm{e}_\mathrm{k}\Delta\psi = e_\mathrm{k}(\psi'' - \psi') = RT\ln(a''_\mathrm{k}/a'_\mathrm{k}) + R\Delta T\ln a''_\mathrm{k} + S_\mathrm{k}\Delta T$$

$$S_\mathrm{k} = S^*_\mathrm{k}/T\ ; \qquad S^*_\mathrm{k} = u^0_\mathrm{k} - \mu^0_\mathrm{k} - Ts_\mathrm{k}\ ; \qquad s_\mathrm{k} = (\partial\bar{\mu}_\mathrm{k}/\partial T) \tag{2}$$

where $\overset{\circ}{u}_\mathrm{k}$ and $\overset{\circ}{\mu}_\mathrm{k}$ are the energy transported per unit mass flux and the chemical potential of the kth ion in the membrane phase; a_k, μ^-_k and s_k are the aqueous activity, the standard chemical potential and the entropy of the kth ion respectively; e_k is given by $z_\mathrm{k}F$. Eqn. 2 refers to highly permselective membranes (t_+ or $t_- = 0$). Under the conditions of ideal permselectivity, absence of water transport ($\tau_0 = 0$), and setting $\alpha(\pm) = S^*_\mathrm{k}/z_\mathrm{k}TF$ ($z_\mathrm{k} = \pm 1$ for cationic and anionic membranes, respectively), Eqns. 1 and 2 are identical. The formal differences between the two equations are due to the different set of forces used for the fluxes equations. Therefore, in the case of monoionic permselective membrane systems there is no difference in the membrane potential equation between solid and liquid membranes despite the fact that these latter can be characterized by a mobile charged site and by the presence of uncharged mobile species (ion pairs). Eqns. 1 and 2 can be then used to analyze the electrical phenomena generated by heat and mass transport across thick or thin membranes in monoionic systems.

In order to study the electrical behaviour of phospholipid bilayer membranes it is useful to distinguish between isothermal and non-isothermal conditions.

(i) Isothermal conditions. In isothermal conditions ($\Delta T = 0$) an electrical potential can be originated by a difference in the chemical potential of some mobile species present at the two sides of the membrane. In the case of zero charged film, where the liquid-like hydrocarbon interior is conceived as the essential barrier through which ions must move unaffected by the polar groups at the membrane-solution interfaces, [9—11] the electrical potential difference has to take the form of a diffusion potential (Eqn. 1 with $\Delta T = 0$). Its value will depend on the cation and anion (t_+, t_-) transport numbers in the liquid-like hydrocarbon interior and on the contribution of the water transport. For charged films, highly permselective, the electrical potential is given by Eqn. 2 with $\Delta T = 0$.

(ii) Non-isothermal conditions. In non-isothermal conditions an electrical potential difference can be originated by a difference in temperature at the two sides of the membrane. The electrical potential value will be determined by the type of film. It will be given by Eqn. 1 for a film which has not a preferential selectivity with respect to cations or anions and by Eqn. 2 for permselective (ideally selective) films. In isochemical conditions ($a'' = a'$) Eqns. 1 and 2 become, respectively,

$$-(\Delta\psi/\Delta T) = (2t_+ - 1)(R/F)\ln a_\mathrm{k}'' + (t_+\alpha_+ + t_-\alpha_-) \tag{3}$$

$$-z_\mathrm{k}(\Delta\psi/\Delta T) = 2.302(R/F)\log a_\mathrm{k}'' + (S_\mathrm{k}^*/TF) \tag{4}$$

The transport of water disappears from Eqn. 3 since for $a_\mathrm{k}'' = a_\mathrm{k}'$ there is no difference in the water activities and therefore no osmotic pressure exists provided the effect of the temperature difference on the chemical activities on either side of the film is negligible. For permselective charged membranes Eqn. 4 predicts a slope of $2.303(R/F) = 200\ \mu\mathrm{V}/^\circ\mathrm{C}$ for the plot $-z_\mathrm{k}(\Delta\psi/\Delta T)$ vs. $\log a_\mathrm{k}''$. This result is characteristic of permselective membranes and it is a proof that the film behaves like an ion-specific electrode. This statement is absolutely correct when the contribution of the thermal diffusion in the water phases can be neglected, as is the case of NaCl or KCl electrolytes whose cation and anion transport numbers are quite the same. In other words, when in the presence of NaCl or KCl a slope of $\pm 200\ \mu\mathrm{V}/^\circ\mathrm{C}$ is obtained the film behaves as a highly selective electrode (or membrane) sensitive only to cations ($-200\ \mu\mathrm{V}/^\circ\mathrm{C}$) or anions ($+200\ \mu\mathrm{V}/^\circ\mathrm{C}$). This result has already been tested [12] for thick liquid anion membranes. The isothermal electrical potential originated by different aqueous electrolyte concentrations at the two sides of phospholipid bilayers have already been measured [9,13]. In the case of phosphatidylcholine films (from egg yolk and pure synthetic L-β,γ-diplamitoyl-α-phosphatidylcholine in *n*-decane) a well defined membrane potential (59 mV/decade) has been observed [13] only with iodide solutions. In this case the film acts as an anion membrane. With NaCl, KCl and $\mathrm{KIO_3}$ in the 10^{-2}—10^{-3} M concentration range, inconstant and non-reproducible potentials of about 30 mV/decade were obtained [13]. Further, with films of phosphatidylethanolamine (from *Bacillus megaterium* and *Escherichia coli*) in presence of NaCl and KCl the potential measurements for a tenfold increase in salt activity scatter [9] from —14 to 30 mV. From these data it is rather difficult to reach any well established conclusion on the isothermal electrochemical behaviour of the phospholipid films.

Several causes can contribute to make unclear the isothermal electrical measurements such as the possible flow of water due to different water activities at the two sides of the membrane. No information is available on the thermal electrical potential of phospholipid membranes. However, these measurements are rather important for several reasons, namely: (a) to test the theoretical predictions of Eqns. 1 and 2 for very thin membranes; (b) to verify the electrochemical behaviour of the lipid films either charged or uncharged; (c) to remove, by using isochemical conditions (Eqns. 3 and 4) transport processes due to different water activities at the two sides of the membranes; (d) to further exploit the existing models regarding the structure of the bilayer films.

In this paper the thermal potential originated across bilayer films of L-α-phosphatidylcholine from egg yolk and cephalin (animal) from sheep brain is studied in isochemical conditions with NaCl as electrolyte.

Experimental

Materials. L-α-Phosphatidylcholine from egg yolk and cephalin (animal) from sheep brain have been obtained from Sigma (commercial grade) and have been used without further purification. L-β-γ-Dipalmitoyl-α-phosphatidylcholine puriss, and dodecane purum (99%) have been supplied by Fluka. Sodium chloride, chloroform, methanol, benzene and *n*-hexane were Carlo Erba analytical grade purity reagents. Dinonylnaphthalensulfonic acid supplied by Columbia Organic Co. Inc. was purified following ref. 14. Silver/silver chloride electrodes and standard calomel electrodes (S.C.E.) were prepared following ref. 15. Care was taken to use electrodes having an asymmetry less than 0.05 mV.

Membrane preparation. Bilayer membranes of the type of Mueller et al. [16] were formed from a solution of lipid (2%, w/v) and *n*-dodecane (18%, v/v) both dissolved in 3 : 2 (v/v) chloroform/methanol solvent. The solutions were freshly prepared the same day of the measurements. The membranes so formed were usually stable for several hours.

Equipment. A Cary vibrating reed electrometer model 31V coupled with a Hewlett Packard recorder model 17501 was used to measure the potentials. To avoid stray $\Delta\psi$ values the measurements were performed into a Faraday cage. The temperature differences across the membranes were measured by means of two thermistors [17].

Thermal cell and procedure. Experimental values of the thermal $\Delta\psi$ for lipid films were obtained by using the apparatus schematically shown in Fig. 1. The cell shown in Fig. 1 can be schematized as follows:

$$\underset{T}{\mathrm{Ag}}/\underset{T}{\mathrm{AgCl}}/\underset{T}{\mathrm{NaCl(M)}}/\mathrm{Bilayer}/\underset{T+\Delta T}{\mathrm{NaCl(M)}}/\underset{T}{\mathrm{AgCl}}/\underset{T}{\mathrm{Ag}} \qquad \text{(A)}$$

The measurements were performed according to the following procedure. At the beginning of the experiments all the solutions, but that of section C′, were kept at 23°C. No difference in the temperature was measured between the two thermistors T. The electrical potential difference between the two electrodes, A, was also close to zero (within the electrodes' asymmetry). Then small

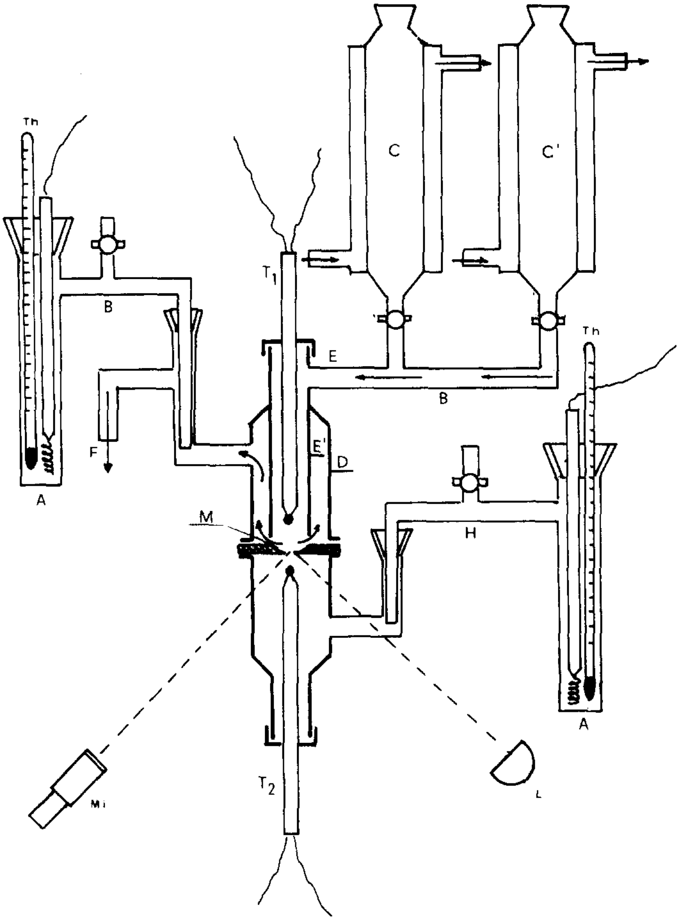

Fig. 1. Experimental apparatus used to measure the thermal potential of bilayer membranes. A, Ag/AgCl electrodes; B, NaCl solution bridges; C′ and C, NaCl solutions at temperature $T + \Delta T$ and T, respectively (the temperature is achieved by means of a thermostat); D, glass cell; E, NaCl solution inlet; F, NaCl solution outlet; G, Teflon gasket with hole of 1.9 mm diameter; M, bilayer; T, thermistors (1 mm distance between the two heads); Th, calibrated thermometers; L, light source; Mi, stereomicroscope.

volumes of solution C′, which was kept at 80°C by means of a thermostat, were flowed through the half of the cell as indicated in Fig. 1 by arrows. The mixing of solutions at different temperatures instantaneously induced a temperature difference at the two sides of the film. The temperature increase (detected with the thermistors) and the electrical potential value were measured simultaneously (the time lag between them was very small).

The values of ΔT were obtained by suitably regulating the volume of solution C′ flowing through the half cell. Particular care was taken to avoid any change in the temperature in the two electrode areas, A. The temperature in these areas was controlled with two thermometers and no temperature difference was measured between them even when a ΔT was present across the bilayer. All electrical potential values were instantaneous and therefore were considered at time $t = 0$. The formation of black films and their presence during the measurements was optically observed by means of the stereomicroscope. To allow for a correct angular disposition of the light source, L, and of the microscope, a square shaped cell with plane surfaces of optical glass had been used. Also the conductance of the whole cell has been measured and it was found of about $10^{-8}\ \Omega^{-1}$. It has to be noted that in the experimental cell of Fig. 1 a temperature gradient can be present (besides the membrane area) in the part of the NaCl solution bridge, B, which is dipped into the cell. However this effect can be neglected because the thermal diffusion potential for NaCl is small and concentration-independent [18]. In fact, experimental measurements in absence of a bilayer membrane gave values of $\Delta\psi/\Delta T$ = 20—30 μV/°C which are independent of concentration and well within the experimental error (±30 μV on each $\Delta\psi$ value). The thermal $\Delta\psi$ measurements (Fig. 3; triangles on dotted line) for the cationic ion exchange membrane, sodium dinonylnaphthalensulfonate (DNNSNa) 0.15 M in benzene, have been performed following ref. 12 by means of the cell

$$\underset{T}{\text{S.C.E.}}/\underset{T}{\text{NaCl(M)}}/\text{DNNSNa-benzene}/\underset{T+\Delta T}{\text{NaCl(M)}}\underset{T}{\text{S.C.E.}} \qquad (A')$$

Comments on the experimental procedures. With regard to the experimental procedures used in this paper two points deserve attention, namely (i) that we are really dealing with a true bilayer and not a very thin membrane; (ii) that the ΔT value measured at the distance of 0.5 mm from the bilayer surfaces, is the ΔT at the two boundaries of the bilayer film.

The direct observation of the black films during the measurements remove the first possible objection.

As far as the second question is concerned the following arguments have to be considered.

(a) In isothermal conditions ion selective electrodes fulfill the function $\Delta\psi$ vs. log a_k with a slope given by the Nernstian factor $2.303(RT/zF)$ (±59 mV at 23°C). This slope value is an upper limit (in absolute value). In fact, in the case of non-ideal selectivity lower slope values are obtained. In the non-isothermal isochemical condition the Nernstian slope of the ($\Delta\psi/\Delta T$) vs. log a function is given by $2.303(R/zF)$ (±200 μV/°C). This value can be obtained only with an ideal selective system and any deviation from it requires the contribution of other processes besides the transport of the selected ion. In the case of the bilayers studied in this paper the value of 200 μV/°C can be obtained only when the ΔT measured between the two thermistors at 1 mm distance (Fig. 1) is assumed to be the temperature drop present at the two boundaries of the bilayer. In fact, let us assume by hypothesis, that we have a linear temperature gradient between the two thermistors. Under these circumstances for a bilayer of 100 Å (10^{-6} cm) and a distance of 0.1 cm between the two thermistors it is $\Delta T_b = 10^{-5}\,\Delta T_t$ (ΔT_b and ΔT_t are the bilayer and thermistor ΔT differences). Consequently it will be

$$\Delta\psi/\Delta T_t = -\left(200 \log a_k + \frac{S}{F}\right)\mu\text{V/°C} \quad \text{or} \quad \Delta\psi/\Delta T_b = -10^5\left(200 \log a_k + \frac{S}{F}\right)\mu\text{V/°C} \qquad (5a,b)$$

Therefore, we would obtain for the thermal bilayer potential a slope of $200 \cdot 10^5$ μV/°C. This result, besides being physically unrealistic, would imply that Eqn. 4 is not satisfied and that our system does not behave as a selective electrode. This latter conclusion is, however, contradicted by the linear experimental observed dependence of $\Delta\psi/\Delta T$ vs. log a_k (Fig. 3).

(b) A more direct test of the arguments of point (a) has been obtained producing a linear temperature gradient through a cell (slightly modified with respect to the cell of Fig. 1) with no flowing liquid (static arrangement). The bilayer was then inserted into the gradient. The temperature drop across the bilayer was calculated to be approx. 10^{-6} °C but no thermal electrical potential, $\Delta\psi$, was measured. However if Eqn. 5b was to be followed, we should have obtained for $a_{NaCl} = 0.01$ M and $-S/F = 400$ μV/°C (see Fig. 3) $\Delta\psi = -\Delta T_b (200 \log a_k + S/F) \times 10^5 = 80$ μV instead of the experimental observed $\Delta\psi = (0 \pm 30)$ μV.

(c) In order to test the real temperature profile in the cell with the flowing arrangement of Fig. 1 we have replaced the bilayer with a thermistor (T_3) inserted into the hole of the teflon gasket. Following the experimental procedure as in presence of the bilayer we have obtained

(1—2)	(2—3)	(1—3)	
0.0	6.0	6.0	A expected
0.5	5.5	6.0	B experimental
0.0	12.0	12.0	C expected
2.0	10.0	12.0	D experimental

A and C represent the expected ΔT values between the i—j thermistors. B and D represent the experimental measured values. The results show that the temperature drop between the 1—2 thermistors is small (ideal value = 0), while the 80—90% of the temperature drop takes place between the thermistors 2—3. From the above results it can be concluded that our experimental device produces a discontinuous temperature drop at the plane where the bilayer will be formed. Furthermore, the drop is sharper decreasing the imposed ΔT (0.5°C instead of 0°C at $\Delta T = 6$°C; 2°C instead of 0°C at $\Delta T = 12$°C). Moreover, the temperature drop should be more pronounced in the presence of the bilayer due to the lower heat conductivity of an hydrocarbon-like layer compared with an aqueous layer of the same thickness.

From these arguments we conclude that the ΔT measured at the thermistor heads 1 mm apart corresponds effectively to the temperature drop across the bilayer.

Results and Discussion

In Fig. 2a the experimental $\Delta\psi$(mV) vs. ΔT(°C) values at different NaCl concentrations ($5 \cdot 10^{-3}$ to 0.5 M) for phosphatidylcholine (curves a, b, c, d) and cephalin (curve e) films are reported. The $\Delta\psi/\Delta T$ vs. log a_{NaCl} values for the same films are shown in Fig. 3. Isothermal electrochemical data for cephalin and phosphatidylcholine films ($\Delta\psi$(mV) vs. log a_{NaCl}) are presented in Fig. 4.

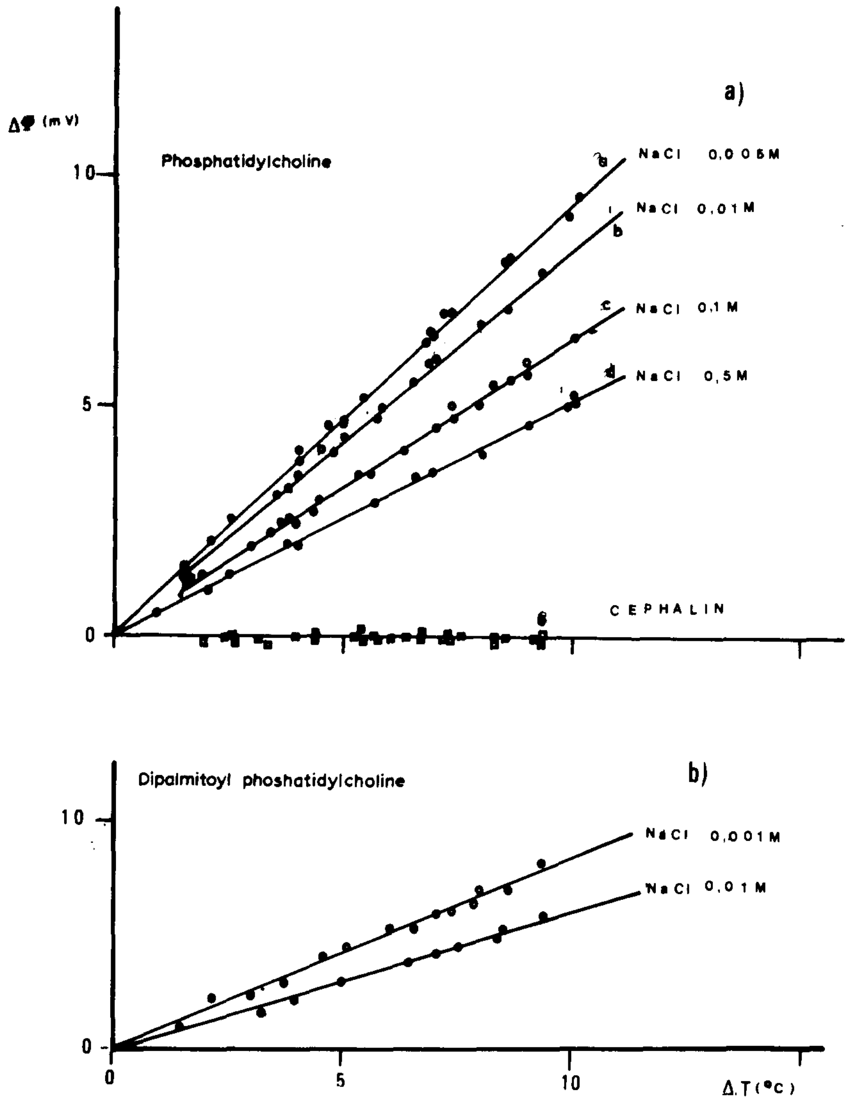

Fig. 2. a. Electrical thermal membrane potential ($\Delta\psi$ mV) vs. temperature difference (ΔT^{o}C) plots at different NaCl aqueous concentrations for phosphatidylcholine (from egg yolk) and cephalin (from sheep brain) bilayers. b. Electrical thermal membrane potential ($\Delta\psi$ mV) vs. temperature difference (ΔT^{o}C) plots for L-β,γ-dipalmitoyl-α-phosphatidylcholine (synthetic) bilayers.

Let us first consider the phosphatidylcholine case. It is interesting to observe that phosphatidylcholine films behave like positively charged permselective membranes. The potential values for phosphatidylcholine films (Fig. 3) are fitted by equation $(\Delta\psi/\Delta T) = -200 \log a_k - S/F$ (cationic membranes; Eqn. 4 with $z_k = +1$) with $-S/F = 400\ \mu V/°C$. Therefore, the phosphatidylcholine is a membrane highly selective to the cations. It is useful to remember that the value of the derivative $d(\Delta\psi/\Delta T)/d\log a_k = \pm 200\ \mu V/°C$ (+200 for anionic membranes) has been verified to be the slope of permselective thick liquid anionic membranes [12]. For comparison purposes, in Fig. 3 the data for a cationic

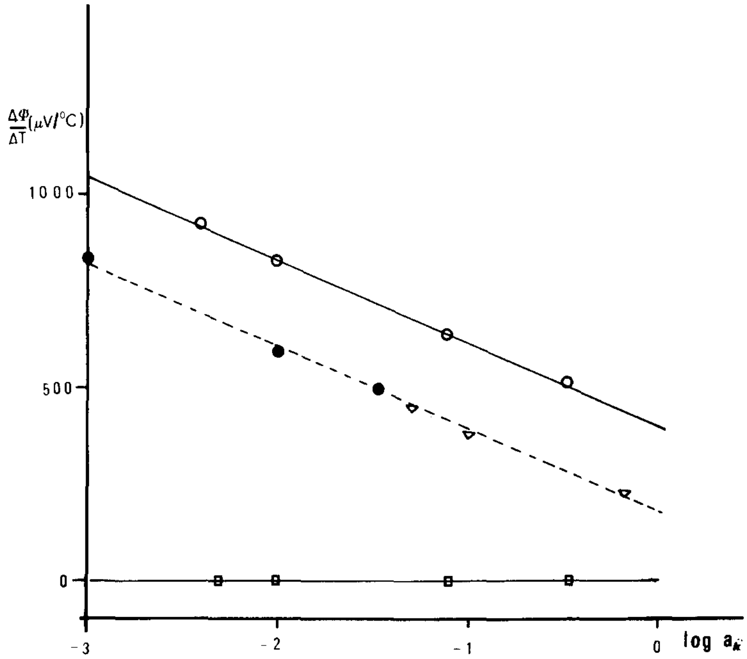

Fig. 3. Thermal coefficient of bilayer membrane potential ($\Delta\psi/\Delta T$ μV/°C) vs. logarithm of aqueous electrolyte activity (log a_{NaCl}) for: ○, phosphatidylcholine; ●, L-β,γ-dipalmitoyl-α-phosphatidylcholine; ▽, sodium dinonylnaphthalen sulfonate in benzene and □, cephalin films.

membrane (triangles on dotted line; dinonylnaphthalene sulfonic-NaCl system) are reported.

The experimental data on this system were obtained during this investigation. In order to avoid any possible mistake about the sign of the potential values both set of data (phosphatidylcholine and thick membranes) have been obtained with the same electrical arrangement (the electrode of the half cell at temperature T + ΔT inserted in the positive inlet of the electrometer). The use of NaCl as aqueous electrolyte and the experimental arrangment make any aqueous thermal diffusion process negligible and therefore the slope of —200 μV/°C is an intrinsic characteristic of the membrane. It is well known that commercial phosphatidylcholine is a mixture of different lipids and therefore the question arises if pure phosphatidylcholine (synthetic L-β,γ-dipalmitoyl-α-phosphatidylcholine) shows the same behaviour as commercial phosphatidylcholine. In Fig. 2b the $\Delta\psi$ vs. ΔT data for synthetic phosphatidylcholine bilayers at two different aqueous electrolyte concentrations are reported. These data fit Eqn. 4 well as commercial phosphatidylcholine but with a different S/F value ($-S/F$ = 180 μV/°C). Pure phosphatidylcholine bilayers are still permselective to cations and their S value shows how the mass-heat transport coupling function depends on the structure of the membrane phase as theoretically expected [8].

With cephalin films the temperature gradient does not originate any significant electrical potential (Fig. 2a). All the cephalin-film thermal potential values seem to be consistent with a membrane model in which the main ion transport

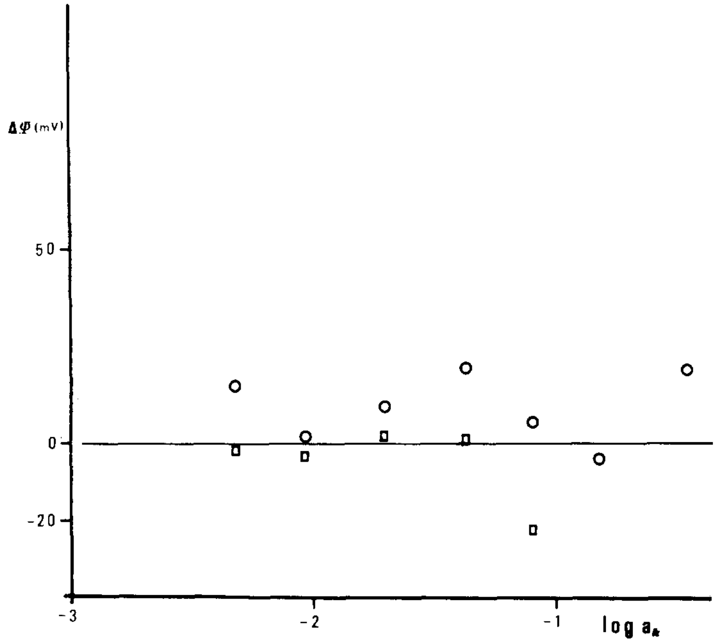

Fig. 4. Isothermal bilayer membrane potential (Δψ mV) vs. logarithm of aqueous electrolyte activity (log a_{NaCl}) for: phosphatidylcholine (○) and cephaline (□) films.

barrier is due to the interior hydrocarbon-like region. For these films, in fact, the cation and anion transport numbers (for example $t_{Na^+} = 0.55$; $t_{Cl^-} = 0.45$) [9] suggest that, as predicted by Eqn. 3, a thermal diffusion potential close to zero (within the experimental errors) is to be expected. In fact, in Eqn. 3 for NaCl, the first term on the right hand side is of the order of 20 μV/°C and moreover, even if no information is available about $(t_+\alpha_+ + t_-\alpha_-)$ values, the experimental data of Fig. 2a suggest that the terms of the sum are of the same order of magnitude but opposite in sign.

Isothermal data. Also the isothermal electrochemical data for cephalin films (Fig. 4) are reasonably coherent with a model in which diffusion is the main transport process. From Eqn. 1 with $\Delta T = 0$, by assuming as negligible the water transport and with $t_{Na^+} = 0.55$ and $t_{Cl^-} = 0.45$ as transport numbers for the electrolyte, a slope of 5.9 mV/decade is expected for the $\Delta\psi$ vs. log a_{NaCl} plot. The experimental value for the $d(\Delta\psi)/d\log a_{NaCl}$ is close to zero. A not negligible water transport contribution could reduce the calculate slope value (5.9 mV/decade) through the term $(\tau_0 \log a''_w/a'_w)$. In the case of phosphatidylcholine the isothermal electrochemical data sharply disagree with the information obtained from thermal potential measurements. In fact, these latter support a permselective charged film as a model for phosphatidylcholine bilayers. In this case a slope of 59 mV/decade at 23°C for the $\Delta\psi$ vs. log a_{NaCl} plot (Eqn. 3 with $t_\pm = 0$ and $\Delta T = 0$; water transport negligible) should be expected for isothermal membrane potentials. On the contrary, a very small (close to zero) derivative $d(\Delta\psi)/d\log a_{NaCl}$ value is obtained.

Conclusions

The experimental results obtained have some implication on the currently accepted models for phospholipid bilayers. From surface potential and force area measurements on monolayers of cephalin and phosphatidylcholine at the air-water interface [19] it has been concluded that the polar groups of the phospholipid molecules lie in a plane parallel to the surface of the monolayer and normal to the direction of the closed, packed hydrocarbon chains. The normal displacement of positive and negative charges in the phospholipid zwitterions from the plane has been calculated to be less than 0.1 Å on a time-average. This point of view has been further supported by means of electrokinetic potential measurements on phosphatidylcholine bilayer leaflet [9]. From these measurements it has been concluded that the phosphate and tetramethylammonium ions are nearly in a plane parallel to the leaflets. More generally it is thought that arrangements of phospholipids in which the positive and negative charges are not coplanar are energetically unstable unless for the binding or interdigitation of zwitterions [19]. The thermal potential measurements with cephalin films agree with these views. In fact the thermal potential values can be coherently interpreted with an electrolyte diffusion process through a film with zero net charge. A different situation arises with the phosphatidylcholine films. As already stressed, the ±200 $\mu V/^\circ C$ slope for the $d(\Delta\psi/\Delta T)/d\log a''_K$ plot derivative is a result obtainable only with a membrane selective to one ion (−200 for cation transport). Since, in the system, there is not any other process which can give rise to these values of the slopes (ion thermal diffusion in the aqueous diffusion layers can be neglected as a consequence of the use of NaCl as electrolyte) the conclusion is drawn that the measured thermal potentials are a proof that the phosphatidylcholine films behave like an ion exchange membrane highly permeselective to the cations with respect to the NaCl system.

Of course, these results imply that in presence of cations the positive and negative charges of the phosphatidylcholine zwitterions have not the same repulsion effect towards the opposite charged ions. In the presence of lanthanides [20] it has been found that the cations interact with the phosphate ester group $\mathrm{Eu} + \text{phospholipid} \rightleftharpoons \mathrm{Eu} - \text{phospholipid}$. This interaction is retained responsible for a plane bilayer arrangement type of lysophosphatidylcholine molecules in spherical micelles. Instead of staggered molecules with neighbouring phosphorus and nitrogen atoms in different planes, thus allowing for maximum electrostatic interaction between oppositely charged groups, as it is the case in absence of lanthanides, the phosphorus and nitrogen atoms are each arranged in different planes approximately perpendicular to the long chain axis. If the interaction between the phosphate group and the cation is stronger than the repulsion potential due to the positively charged nitrogen group the cation can overpass the membrane-solution positive potential and move through the bilayer. Following this point of view the Na^+ diffusion across the film acts as the potenial determining process in phosphatidylcholine films. On the contrary, no ion selectivity is shown by cephalin films. It is worth remembering that thermal potential measurements refer to the potential difference originated between the two aqueous solutions by the ion diffusion process across the film and not to the potential drop at the membrane-solution interface which has to be detected by means of other experimental methods (for example electrokinetic or interfacial potential measurements).

The results presented in this paper suggest that the use of isochemical conditions in the thermal potential measurements is a very interesting way to investigate the electrochemical behaviour of bilayer membranes. The method allows one to obtain simultaneously both selectivity (through the $\Delta\psi/\Delta T$ vs. $\log a_k$ slope) and mass-heat transport coupling (through the S function) data.

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