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# Surface changes induced by osmotic stress and its influence on the glycerol permeability in lipid bilayers

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**The penetration rate of glycerol across lipid bilayers can be assayed dispersing liposomes filled with a 0.1 M glucose solution in an isotonic or a hypertonic solution of glycerol. The kinetic of glycerol permeation is found to be different in each of those cases. Liposomes dispersed above the phase transition temperature in hypertonic solutions show an increase in the surface polarization as measured by means of merocyanine 540. Under this condition, the permeation of glycerol shows a two-step kinetic which is indicative of a non-fickean diffusion process. In contrast, liposomes dispersed in isotonic solutions of the permeant show a fickean behavior. The changes in polarization of the membrane interface are ascribed to variations in the surface potential due to the osmotic collapse and the glycerol concentration in contact with the outer surface. The permeability of polar molecules can, in consequence, be considered as a function of the surface potential of the liposome which is congruent with previous data in literature reporting that water permeability increases as a function of the zeta potential of liposomes shrunken in hypertonic solutions.**

## Introduction

Cell membrane constitutes a selective barrier separating the intra- and extra-cellular milieu. The barrier permeability is mainly achieved by the lipid bilayer which is permeable to water and polyols and impermeable to sugars and ions [1,2]. In spite of its structural complexity the bilayer is generally considered as an homogeneous phase interposed between the two aqueous phases and thick enough to neglect membrane/solution interphases [3]. For these reasons, the permeability coefficient is usually calculated on the basis of Fick's law which is only applicable to binary systems exhibiting thermodynamic ideality [4].

However, the energy barrier determining the permeation rate is a function of the physical state of the lipids, the hydration and the packing constrains [2,5,6]. Osmotic stress imposed by permeant or non permeant gradients alters the volume of large liposomes and cells due to the formation of high curvature regions in the bilayer. Moreover, the high permeant concentration in the membrane adjacences decreases the water activity and may affect the structural properties of the bilayer [7–10].

How these factors affect the permeability is unclear. An indication that the energy barrier region can be altered in relation to the osmotic state of the liposomes is given by the lower activation energies for permeation of glycerol and erythritol for liposomes in hypertonic solutions as compared to those obtained in isotonic conditions [6,12,13]. In addition, the observation that the zeta potential ($\xi$) of phospholipid liposomes is related to the liposome volume suggests that the changes in the permeability can be of electrical nature [1].

In this paper, we have analyzed the conditions under which the permeability of lipid bilayers departs from a solubility-diffusion mechanism predicted by the Fick's law and correlated with the surface changes observed by spectrophotometry and fluorometry using merocyanine 540 as membrane probe for liposomes

Abbreviations: DPPC, L-α-dipalmitoylphosphatidylcholine; DPPA, L-α-dipalmitoylphosphatidic acid; MC, merocyanine 540.

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dispersed in isotonic, hypotonic and hypertonic solutions.

In the light of these results, the kinetics of polar solute permeation in lipid membranes is discussed considering the surface changes occurring during the osmotic constrain.

## Materials and Methods

Dipalmitoylphosphatidylcholine and phosphatidic acid were obtained from Avanti Polar Lipids Inc. A thin-layer chromatography gave a single spot when exposed to $I_2$ vapours. Therefore, lipids were used without further purification.

Liposomes were prepared using the method of Bangham [14]. A chloroformic solution of the lipids was evaporated under vacuum in a rotavapor. The dry film obtained in a round bottom flask was dispersed in buffer solution above the phase transition temperature.

All the solutions were prepared in buffer Tris-HCl 10 mM (pH 7.4) and chemicals were of analytical grade. For the surface membrane studies merocyanine 540 was used as an optical probe [15,16]. After the liposomes were dispersed in the solutions of the described tonicity, aliquots of a merocyanine stock solution in buffer Tris was added. After an incubation time of 30 min the spectra were run.

*Surface properties determinations*

In order to determine the surface properties of liposomes in different osmotic states it was necessary to establish the effect of the concentration of glucose and glycerol on the merocyanine spectra in the absence of liposomes. Blank experiments were performed adding MC to the different solutions to achieve a concentration of $10^{-5}$ M. The spectra were analyzed at 45 °C. The control spectra in glucose and glycerol 1 M did not show variations with respect to buffer at this temperature. In no cases an increase in the absorbance at 570 nm was observed in the absence of lipids.

The dielectric constants of the solutions of glycerol and glucose were determined. The dielectric constant of glucose and glycerol at 0.1 M was nearly that of water at the same temperature. When the concentration was increased at 1 M the dielectric constant was slightly lower than that obtained at 0.1 M concentration. However, such reduction has no effect on the maximum wavelength corresponding to merocyanine.

The viscosity of the different solutions were measured at 45 °C. The viscosity of the solutions containing glucose were higher than those with glycerol. However, it has a negligible effect on the dimer-monomer equilibria of merocyanine according to the absorbance ratio 530/500. Therefore, it can be concluded that the range of concentrations used in this study did not affect the MC spectra at 45 °C. The changes in the spectra observed in this work can be ascribed to changes in the properties of the liposomes dispersed in the aqueous solutions.

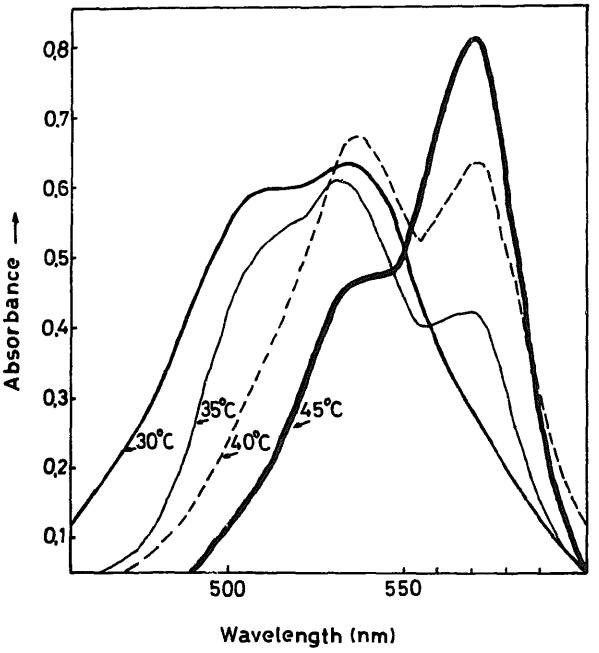


Fig. 1. Merocyanine spectra in the presence of dipalmitoylphosphatidylcholine liposomes below and above the phase transition temperature (41 °C). (a) 30 °C; (b) 35 °C; (c) 40 °C; (d) 45 °C.

Merocyanine spectra show in water a peak at 500 nm and a peak at 530 nm. When dissolved in organic (low dielectric constant) solvents it shows a peak at 570 nm. In the presence of lipids the merocyanine spectra vary according to the physical state of the lipids. In contact with dipalmitoylphosphatidylcholine liposomes in the gel state MC shows spectra similar to that obtained in water. However, when added to a dispersion of DPPC liposomes in the liquid crystalline state, it shows a peak at 570 nm as that observed with MC dissolved in non polar solvents (Fig. 1).

Changes in the fluorescence or the light absorption of this dye in response to membrane potential variations provide alternative technique in systems in which microelectrodes cannot be impaled [28].

Molecular reorientation of MC 540 in an electric field in the lipid membrane is involved in the fluorescence polarization [25]. Changes in fluorescence of MC 540 has been detected as a consequence of energization and changes in ionic strength attributed to changes in the surface potential of mitochondria [20,28].

The osmotic gradient imposed to liposomes in the liquid crystalline state (45 °C) was determined taking into account the reflection coefficient ($\sigma$) for glucose and glycerol (1 and 0.78, respectively) [17]. Therefore, the gradient imposed in the system 0.1 M glycerol/0.5 M glucose is given by $\Delta\pi = c_{glu}\sigma_{glu} - c_{glyc}\sigma_{glc}$ and in consequence the osmotic force is $\Delta C = 0.428$ M. The absolute value gives the magnitude of the gradient and

the sign the direction in which the force is acting. The possitive sign denotes an osmotic force towards the outer media, producing an eflux of water and a decrease in the liposome volume. On the contrary, swelling is produced by a negative osmotic gradient due to the influx of water. It was observed that the difference of the absorbance at 570 and 530 nm is lineal with the osmotic difference between the inner and the outer solutions of the liposomes.

The titration of liposomes in different osmotic states were carried out after dispersing in 0.5 M glucose, buffer Tris and 1 M glucose equal aliqouts of liposomes prepared in 0.5 M glucose to obtain isotonic, swollen and shrunken liposomes, respectively. After an incubation of at least 30 min to achieve the osmotic equilibria each solution was titrated with a stock solution of MC of an accurately determined concentration ($10^{-3}$ M). The concentration range in which MC varied in the cuvette was from $10^{-6}$ to $10^{-5}$ M.

All the spectra were obtained at 45°C. At this temperature DPPC liposomes show a typical spectra of liposomes in the liquid crystalline state (Fig. 1). In all cases, the results showed that at low concentrations of the dye the peak at 530 nm is lower than that at 570 nm, corresponding to the equilibrium dimer-monomer in the membrane. This is due to the fact that at this conditions all the dye is adsorbed on the membrane as a monomer. The changes of the spectra induced by osmosis were taken in these conditions.

Fluorescence polarization was determined exciting merocyanine at 365 nm and measuring the emission at 580 nm in an SLM spectrofluorometer. The sample was excited with vertically polarized light and when the analyzer was oriented parallel to the direction of the polarized light the $I_{\|}$ is detected. When the analyzer is perpendicular to the polarized light the intensity $I_{\perp}$ was obtained. The method employed consists of a T configuration in which the $I_{\|}$ and the $I_{\perp}$ are observed simultaneously through two separated channels using two systems of detection.

The anisotropy values were calculated by the expression

$$\frac{I_{\|} - I_{\perp}}{I_{\|} + 2I_{\perp}} = r$$

where $I_{\|}$ and $I_{\perp}$ the intensities of light emitted parallel and perpendicular to the excitation beam, respectively. All fluorescence measurements were corrected for lamp intensity variations and absorption of the solution. Experiments were carried out at 45°C thermostating the cuvette holder within ±0.2C°.

*Permeability assays*

The kinetic experiments were carried out in a Durrum D-110 stop-flow spectrophotometer. An aliquot of a liposome dispersion prepared in KCl or glucose 0.1 M was injected into a mixing chamber simultaneously with an isotonic or a hypertonic solution of a permeant.

Final concentration of lipids in the chamber was 0.3 mM. Typical curves obtained in an storage oscilloscope are shown in Fig. 2. Curve A is a typical curve corresponding to liposomes dispersed in hypertonic solutions of glycerol. The descending part is due to the water outflux (i.e. volume decrease). The curve B is obtained when the liposomes are dispersed in an isotonic solution of glycerol and in consequence there is no osmotic shrinkage before glycerol penetration.

The degree of liposome swelling ($\alpha$) was calculated considering the data after the arrow shown in the figure by the expression

$$\alpha = \frac{T - T_0}{T_\infty - T_0}\%$$

where $T$ is the transmittance of the dispersion at time $t$, $T_0$ the minimum of transmittance and $T_\infty$ is the transmittance at the time at which liposomes were considered at equilibrium (approx. 40 min after the minimum volume).

The transmittance was measured at 450 nm in a cuvette thermostated at 45°C ± 0.2°C. As established by the empirical Bangham's law the volume of the liposomes is inversely proportional to the absorbance at 450 nm.

$T_0$ corresponds to the minimum volume achieved by the liposomes. In curve A it is achieved by the osmotic shrinkage induced by the hypertonic gradient of glycerol and in curve B corresponds to the equilibrium value of liposomes in isotonic solution of glucose.

The minimum of transmittance ($T_0$) is obtained at approx. 500 ms or less after the injection. This process can be visualized if it is measured at a high resolution time.

In the isotonic media (Fig. 2A), no shrinkage takes place when liposomes are dispersed in 0.1 M glycerol inmediately after the injection. Transmittance increases when glycerol diffuses into the liposomes. The $T_0$ values for non shrunken liposomes were always higher than those shrunken in the hypertonic assays (see the transmittance values at the arrows). Therefore, $T_0$ denotes the transmittance at time zero for swelling in both types of assay and differences in its value represent different state of the liposome.

The difference of transmittance between $T_\infty$ and $T_0$ reflects the total amount of permeant incorporated to the liposome in the swelling process starting at $t = 0$ marked by the arrows. In the isotonic state, the solute solution diffuses into the liposome which is in osmotic equilibrium with the external medium. In the hypertonic method, the solute solution enter into liposomes

which have been previously collapsed. The differences in the surface properties of the different states corresponding to different $T_0$ values were investigated using merocyanine 540 as a membrane probe as described above.

In order to get an insight into the diffusion process in liposomes in different osmotic states, an approach used in kinetics of polymer swelling was adapted [18].

This can be accomplished plotting the degree of swelling ($\alpha$) (which is proportional to the solute incorporation) as a function of time according to

$$\alpha = K(c)t^n \quad (1)$$

where $K(c)$ is a function of $c$; the initial concentration through the sheet and $n$ is a coefficient depending on the diffusional mechanisms. The dependence of $K$ with $c$ shows the nature of the concentration dependence of the diffusion coefficient ($D$). A linear plot of $\alpha$ as a function of $t$ for $n = 0.5$ predicts a fickian process in which the diffusion coefficient is independent of the concentration. A departure of the linearity would correspond to a concentration-dependent diffusion coefficient. In these cases $n > 0.5$. The value of $n$ can be easily obtained by plotting the logarithm of $\alpha$ versus time [19].

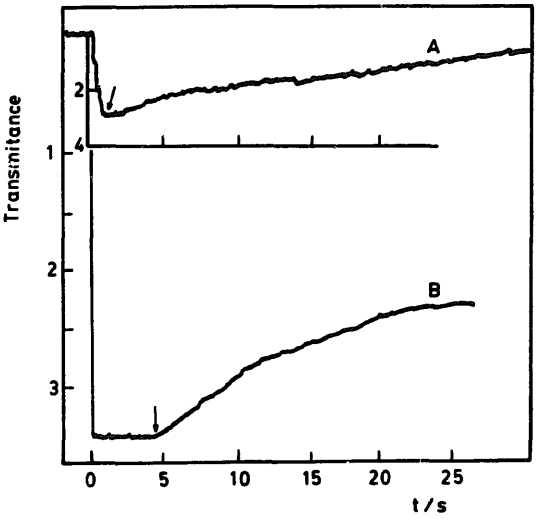


Fig. 2. Transmitance at 450 nm as a function of time of DPPC liposome prepared in 0.1 M glucose and dispersed in a hypertonic solution of 1 M glycerol (A) and in an isotonic solution of 0.1 M glycerol (B) above the transition temperature. Note the difference on the scale for transmittance. The decrease in transmittance inmediately after the injection is due to the entrance of liposomes which volume is lower than that corresponding to the previous sample. See that the transmittance value before the mixture is similar to that obtained after the liposomes are swollen at long times.

## Results

The representations of the data of Fig. 2 according to Eqn. 1 for the swelling processes starting at $t = 0$ (see arrow), are shown in Fig. 3. It is observed in part A that glycerol permeation driven by an hypertonic gradient shows a non linear behavior in comparison to that corresponding to an isotonic gradient (part B). The curve for the hypertonic condition shows two slopes: one lower and another higher than that obtained in the isotonic assay (Table I). The change in the slope at a given time period (time lag) indicates that liposomes swell more rapidly than under the isotonic conditions after the slow initial swelling rate. That is, hypertonic media alter the membrane permeability properties. The data in the hypertonic condition become linear when $\alpha$ is plotted as a function of $t^n$ for $n = 0.8$ (Fig. 4).

The different kinetics in the two conditions assayed in Fig. 2 can be caused by the osmotic collapse suffered by the liposomes due to the relatively higher water outflux in comparison to the glycerol entrance.

The membrane surface properties of DPPC liposomes above the phase transition temperature in different osmotic states are shown in Fig. 5. The 570/530 absorbance ratio of the merocyanine peaks has different values for similar concentrations of the dye depending on the osmotic state of the liposomes, which denotes that the membrane surfaces have different affinities for the dye. These curves can be represented by a Langmuir type adsorption isotherm (Fig. 5A). The double-reciprocal plot of the curves like that of Fig. 5A in which the ordinate is the inverse of the absorbance

TABLE I

*Parameters*

$t_i$, time lag; $K(c)_1$, slope before time lag; $K(c)_2$, slope after time lag; $K_{MC}$, dissociation constant of MC; $\psi_{MC}$, apparent surface potential measured by MC; *, leakage of the internal liposome solution is determined by CF, KCl or glucose. Values between brackets correspond to $K(c)$ calculated according to Eqn. 3 using the $\psi_{MC}$ determined in Fig. 8.

| Condition | Lipid composition | $t_i$ (s) | $K(c)_1$ | $K(c)_2$ | $\psi_{MC}$ | $K_{MC}$ ($10^{-5}$ M) |
|---|---|---|---|---|---|---|
| Isotonic | DPPC | 0 | 0.08 | 0.08 | 0 | 7.5 |
| Hypertonic | DPPC | 3.24 | 0.05 (0.06) | 0.25 (0.11) | +6.6 | 14.0 |
| Hypotonic | DPPC | – | * | * | −8.9 | 5.1 |
| Hypertonic | DPPC+4% PA | 7.8 | 0.11 | 0.65 | – | – |

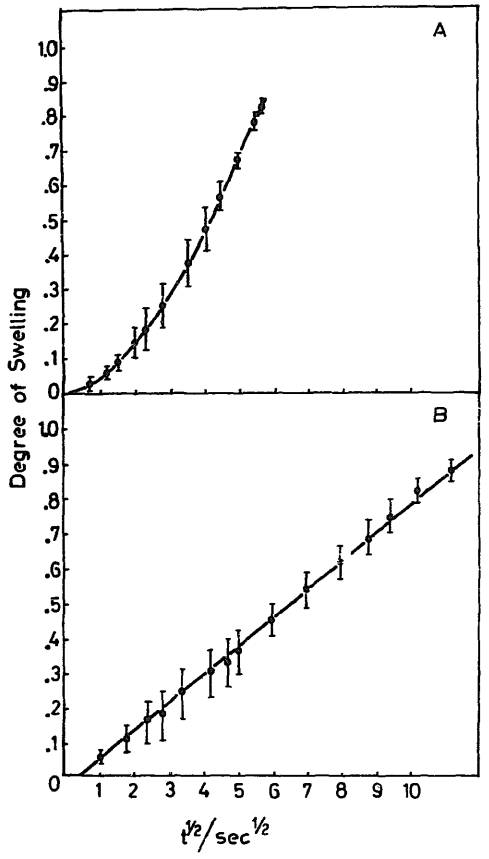


Fig. 3. Degree of swelling ($\alpha$) of DPPC liposomes as a function of $t^{1/2}$ for hypertonic (A) and isotonic (B) assays. Curves (A) and (B) are an average taken from traces as in Fig. 1 corresponding to at least four different assays. $\alpha$ is calculated as described in Materials and Methods.

ratio and the $x$-axis the inverse of the merocyanine concentration is shown in Fig. 5B. The dissociation constants obtained from this plot (Table I) indicate that the MC affinity is higher for swollen liposomes and lower for shrunken liposomes in comparison to liposomes in the isotonic condition.

It is clear that the surface properties of the liposomes change with the osmotic stress even in the absence of glycerol. However, the rate of change of the difference between the absorbances at 570 and 530 nm is higher when the osmotic gradient is settled with glycerol in comparison with glucose (Fig. 6). MC is a surface probe which is useful to detect changes at lipid membrane interfaces such as gel-liquid crystalline transitions and surface potentials [15,16,20,21]. It must be recalled from Materials and Methods that the increase in MC concentration at constant lipid concentration promotes an increase in the 530 nm peak in detriment to that at 570 nm. The increase in the absorbance at 570 nm with respect to that at 530 nm denotes an increase in the partition of the dye monomer. In the case of Fig. 6, MC and liposomes are maintained constant and the variation between the 570 nm and the 530 nm absorbance are provoked by the osmotic gradient. This indicates that osmosis is affecting the monomer partition on the membrane phase.

The Fig. 6 also indicates that glycerol increases the 570 nm peak in isotonic conditions in comparison to glucose as it has been reported previously and that this partition decreases with the osmotic stress. In addition, in contact with glycerol the membrane affinity for MC decreases more rapidly than in glucose when they are collapsed by osmosis.

The increase of the absorbance at 570 can be correlated with a decrease of the fluorescence polarization which also increases with the osmotic strength (Figs. 7A and B). Therefore, the osmotic collapse promote structural changes at the membrane interface. The increase in the surface polarization indicates a tightening in the head group region where the hydrogen bonds establish a crosslinked network contributing to the barrier properties.

As merocyanine is also sensible to the changes in surface potential [20] the adsorption of MC can be described by a Langmuir modified isotherm given by:

$$\Delta A = N(K \cdot C \cdot \exp(F\psi/RT)/[1 + K \cdot C \cdot (F\psi/RT)]) \quad (2)$$

Here, $N$ is the maximum number of binding sites on the membrane surface and $C$ is the molar concentration of unbound dye. $K$ is given by $K = (1/55.5)\exp(-\Delta G/RT)$ where $\Delta G$ represents the non electrical part of the free energy change due to binding and $\psi$ is the electrostatic potential at the membrane surface measured from that in the bulk solution. This electrostatic potential may be created by an orientation of the dipoles of water on the membrane surface which is reflected by the polarization increase shown in Fig. 7.

The data of Fig. 5 are represented in Fig. 8 as a function of the concentration corrected by a surface potential parameter according to Eqn. 2. It shows that

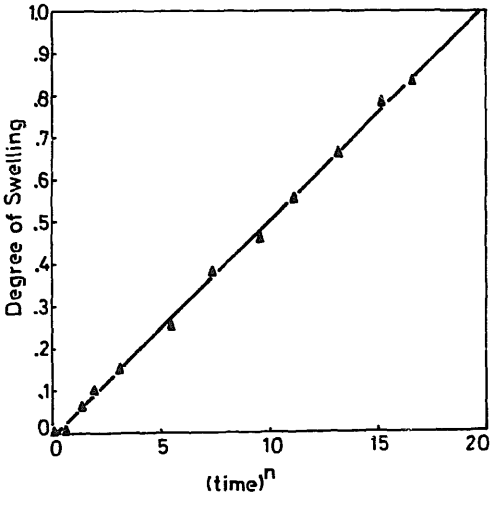


Fig. 4. Degree of swelling of liposomes after the osmotic shrinkage plotted as a function of $(time)^n$ for $n = 0.8$. For details see Materials and Methods.

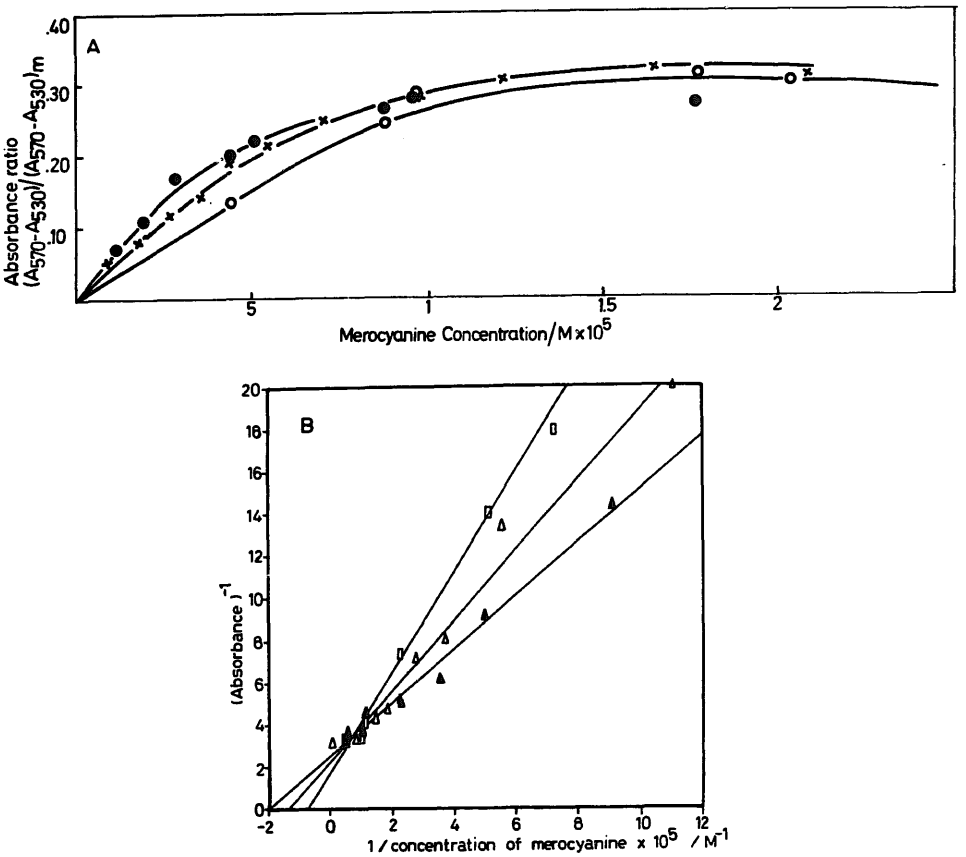


Fig. 5. Titration of liposomes in different osmotic states with merocyanine 540. (A) Liposomes prepared in 0.1 M glucose were equilibrated in 0.05 M glucose (●), 0.1 M glucose (×) and 0.5 M glucose (○) before subsequent additions of measured volumes of a $5 \cdot 10^{-3}$ M solution of merocyanine. (B) Double-reciprocal plot of the data of Fig. 3 for liposomes under hypertonic (□), isotonic (△) and hypotonic (▲) conditions.

surface potential values $\psi = -8.9$ mV and $\psi = +6.6$ mV correct the departure of the lines for shrunken and swollen liposomes, respectively, from that corresponding to liposome in isotonic equilibrium for which $\psi = 0$.

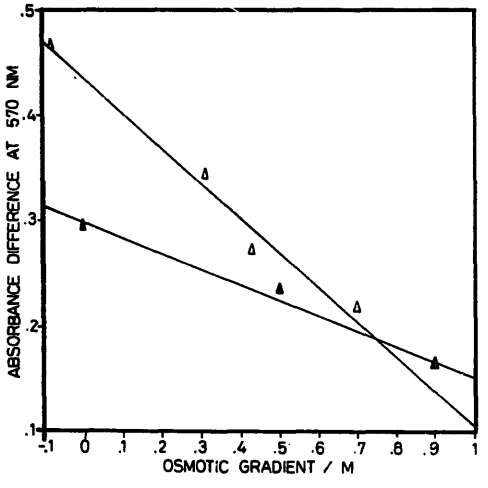


Fig. 6. Effect of the osmotic gradient on the absorbance at 570 nm of merocyanine 540 added to dispersions of DPPC liposomes in the liquid crystalline state. Liposomes were prepared in 0.1 M glycerol (△) or 0.1 M glucose (▲) and dispersed in solutions of different concentrations of glucose. Each point corresponds to average measurements obtained with different liposome preparations.

The straight line of Fig. 8 indicates that the binding of MC 540 to liposomes follows Eqn. 2 and that $N$ and $K$ stay constant in the whole range of concentration used.

The polarization of the liposome surface affects the glycerol penetration. An strong indication that the penetration of glycerol can be affected by the surface charges is given by the observation that the inclusion of 4% phosphatidic acid increase markedly the extent of the first slope in the hypertonic condition (Table I). However, caution must be taken in the comparison because the surface polarization is produced in this case by net charges instead of dipolar groups.

## Discussion

An inspection of Fig. 3 indicates that the curve under hypertonic conditions changes the magnitude of its slope at a point in which the degree of swelling is equal to that found for isotonic conditions. In this point $K(c)$ isotonic $= K'(c)$ hypertonic $= 0.08$ according to Table I.

Above and below that point $K(c)$ increases or decreases, respectively. The purpose of the following discussion is to relate the variations of $K(c)$ with the surface changes induced by osmosis.

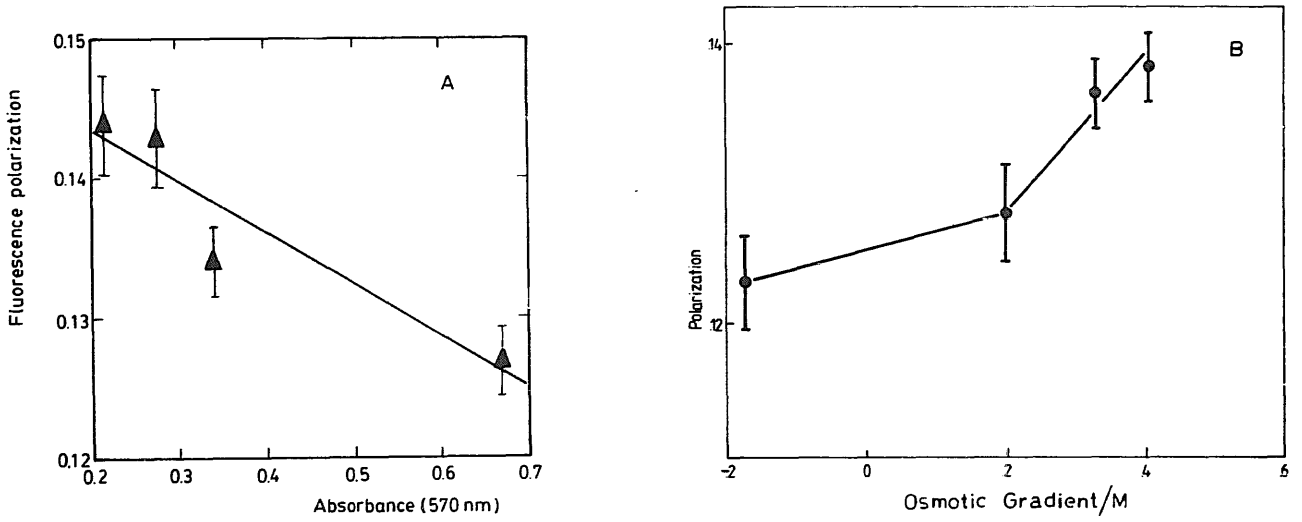


Fig. 7. Effect of the osmotic shrinkage on the surface polarization as measured with merocyanine. (A) Relation between the absorbance at 570 nm and the polarization of merocyanine for liposomes in different osmotic states. (B) Effect of the osmotic gradient on the polarization of merocyanine 540 in the presence of liposomes prepared in 0.1 M glycerol.

Shrinkage (contraction) promotes a decrease in permeability $K(c)$ together with an increase in the membrane polarization and a decrease in the monomer partition. After the lag time, an increase in swelling rate corresponds to an increase in the monomer partition and a decrease in the polarization. These oscilations in the surface properties upon shrinkage and swelling tested with merocyanine are helpful to inspect the surface state of the liposomes during the different stages of the permeation of glycerol.

Several factors can affect the adsorption of MC to lipid bilayers all of them related to the phase state of the region where the dye adsorbs. The present experiments are done above the phase transition temperature and the modification of the phase state is achieved by the osmotic stress imposed to the liposomes.

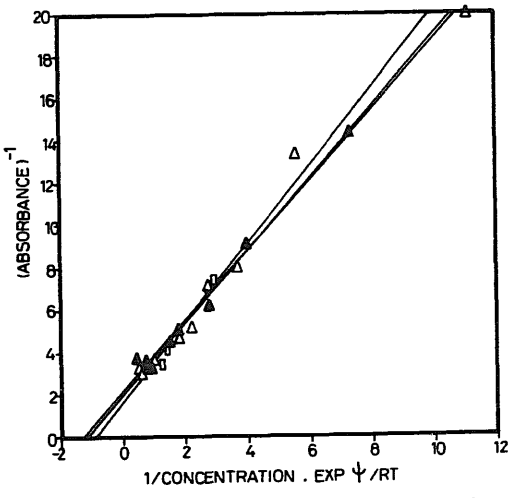


Fig. 8. Influence of the osmotic state on the surface potential Double-reciprocal plot of the data of Fig. 5 plotted as a function of the concentration corrected by the parameter $\psi = 6.6$ mV (□); $\psi = 0$ (△) and $\psi = -8.9$ mV (▲).

As glycerol is a non electrolyte with a permanent dipole moment it forms hydrogen bonds and its permeability rate can be dependent on the electrostatic changes produced at the membrane surface by the distribution of ions and dipoles. In consequence the permeability coefficient can be considered composed by an electrical and a non electrical contribution as

$$K'(c) = K(c)\, e^{-F\psi/RT} \qquad (3)$$

The $K'(c)$ value in hypertonic media calculated considering the surface potential obtained with MC in Fig. 7 is 0.062 in comparison to 0.05. In spite of a slight increase the calculated values are comparable. Hence, there is not too much differences when the shrinkage is made with glucose or with glycerol.

In the case of swelling, there are important differences if it is produced by the entrance of water caused by a gradient of impermeant molecules or by the penetration of glycerol. The $K'(c)$ value calculated using $\psi = -8.9$ mV corresponding to liposomes swollen in hypotonic solutions in the absence of glycerol (Fig. 5) is 0.11. However, the experimental value obtained from the slope after the lag time of Fig. 3, which corresponds to the swelling produced by glycerol entrance is 0.25. This denotes that the permeation of bilayers swollen in hypotonic media is much higher in the presence of glycerol.

The effect of glycerol on the osmotic response of the liposomes is also noticed when the liposomes are shrunken. An inspection of Fig. 6 indicates that the absorbance difference when the osmotic gradient is settled with glycerol is higher than that promoted by glucose. That is, the membrane with glycerol would be less polarized than in the presence of glucose. This would be in accordance with the observation that the

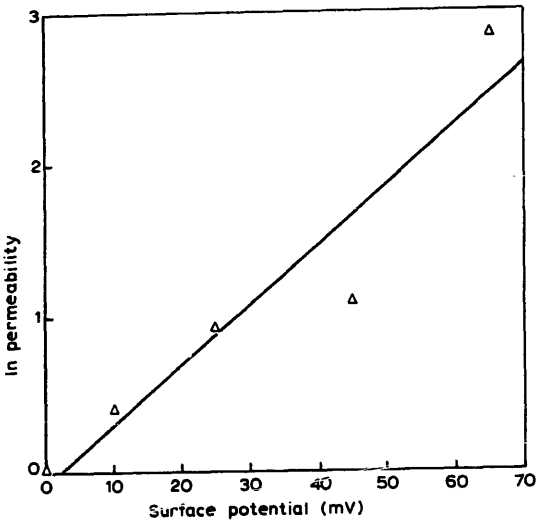


Fig. 9. Representation of the water permeability in liposomes in the fluid state as a function of the zeta potential achieved at different ionic strength according to Eqn. 4. Data taken from reference 1. The slope of the curve calculated for 45 °C is 39.4 $mV^{-1}$ in comparison to the theoretical value of 36.5 $mV^{-1}$.

presence of glycerol increases the 570 nm peak which reflects an expansion of the bilayer.

The changes in the bilayer surface affecting the permeability properties seem to be related to the distribution of charges at the interface that may generate a surface potential. A direct evidence of this contribution is given by the increase in the delay time observed when phosphatidic acid is included in the bilayer.

Another important piece of evidence demonstrating the validity of Eqn. 3 is the finding that the water permeability is a function of the zeta potential of the membrane achieved at different ionic strength [1].

In Fig. 9 the data of Bangham are plotted according to the equation

$$P = P_* \, e^{-F\psi/RT} \tag{4}$$

where $P_* = P_0 \, e^{-E/RT}$ which is the non electrical part of the permeability and $E$ the corresponding activation energy.

The slope of the plot gives 39.2 $mV^{-1}$ a value very close within the experimental error to that corresponding to the constant $F/RT$ at 45 °C. This demonstrates the generality of the Eqn. 4.

Considering the electrical contribution within the activation free energy

$$\Delta F^{\#} = E + F\psi$$

we have

$$P = P_0 \, e^{-(E+F\psi)/RT} \tag{5}$$

The activation energy for glycerol in DPPC liposomes containing 4% phosphatidic acid are 18 kcal/mol and 11.0 kcal/mol for the isotonic and the hypertonic methods, respectively [5,12,13].

The surface potential determined after the lag time for liposomes with 4% phosphatidic acid is, according to Eqn. 3 and data in Table I, −53.4 mV. Introducing this value into Eqn. 5 and taking 18 kcal/mol as the activation energy when $\psi = 0$, the activation energy calculated for the hypertonic condition is 12.7 kcal/mol which is highly satisfactory.

We have demonstrated that surface changes induced by osmosis give place to different mechanism of permeation. This might be connected to the topological changes observed in liposomes and protoplast during shrinkage induced by freezing [10]. The kinetic response, the stability and permeability properties of the vesiculations and invaginations created during cell or liposome contraction could thus be related to changes in the surface potential. It is very interesting to recall that Bangham reported a dependence of the water permeability with the zeta potential of the liposomes. Thus even in this case of neutral molecules such as water and glycerol slight structural modifications imposed by membrane collapse would give place to a distribution of the water or a reorientation of surface dipoles that can contribute to the membrane permeability barrier. In addition, electrical potentials seem to dominate the damage of membranes in freeze-thaw processes in which water permeation is a relevant phenomenon [11,28].

The studies of membrane permeability have utilized multilamellar liposomes [1] with the understanding that they behave as ideal osmometers. However, such a system has serious limitations for a complete interpretation of permeability. In this direction, this paper points to the changes in the interfacial properties which may account for differences in permeability values reported in literature. In particular, the two stage kinetics of swelling in the hypertonic cases indicates that the relative outflux of water is larger than the influx of glycerol in the first stages. This may happen by an increase of water permeation to outwards or to a delay in the penetration of glycerol. The observation that water permeability is dependant on the zeta potential would suggest that the first possibility is more probable.

Unilamellar liposomes may also be used to analyze permeability. However, caution must be taken in order to verify their osmotic behavior as a function of the size. In a more rigurous analysis, the permeation process might be visualized as a coupled process of water and solute fluxes. However, this analysis has only been applied to multilamellar liposomes and it is not completely demonstrated that the formalisms of thermodynamic of irreversible processes can be applied to membranes whose structure varies during the permeation process as shown in this paper.

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