

Accepted Manuscript

Structural and functional properties of hydration and confined water in membrane interfaces

E.A. Disalvo, F. Lairion, F. Martini, E. Tymczyszyn, M. Frías, H. Almaleck, G.J. Gordillo

PII: S0005-2736(08)00269-1
DOI: doi: [10.1016/j.bbamem.2008.08.025](https://doi.org/10.1016/j.bbamem.2008.08.025)
Reference: BBAMEM 79795

To appear in: *BBA - Biomembranes*

Received date: 15 May 2008
Revised date: 19 August 2008
Accepted date: 22 August 2008



Please cite this article as: E.A. Disalvo, F. Lairion, F. Martini, E. Tymczyszyn, M. Frías, H. Almaleck, G.J. Gordillo, Structural and functional properties of hydration and confined water in membrane interfaces, *BBA - Biomembranes* (2008), doi: [10.1016/j.bbamem.2008.08.025](https://doi.org/10.1016/j.bbamem.2008.08.025)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**STRUCTURAL AND FUNCTIONAL PROPERTIES OF HYDRATION AND
CONFINED WATER IN MEMBRANE INTERFACES.**

E. A. Disalvo¹, F. Lairion¹, F. Martini¹, E. Tymczyszyn¹, M. Frías¹, H. Almaleck¹, G. J. Gordillo²

¹Laboratorio de Fisicoquímica de Membranas Lipídicas, Cátedra de Química General e Inorgánica, Departamento de Química Analítica y Fisicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956 2º piso (1113), Buenos Aires, Argentina. eadisal@yahoo.com.ar

²Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, INQUIMAE, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2 (1428), Buenos Aires, Argentina.

Abstract.

The scope of the present review focuses on the interfacial properties of cell membranes that may establish a link between the membrane and the cytosolic components. We present evidences that the current view of the membrane as a barrier of permeability that contains an aqueous solution of macromolecules may be replaced by one in which the membrane plays a structural and functional role. Although this idea has been previously suggested, the present is the first systematic work that put into relevance the relation water-membrane in terms of thermodynamic and structural properties of the interphases that cannot be ignored in the understanding of cell function.

To pursue this aim, we introduce a new definition of interphase, in which the water is organized in different levels on the surface with different binding energies. Altogether determines the surface free energy necessary for the structural response to changes in the surrounding media. The physical chemical properties of this region are interpreted in terms of hydration water and confined water, which explain the interaction with proteins and could affect the modulation of enzyme activity.

Information provided by several methodologies indicates that the organization of the hydration states is not restricted to the membrane plane albeit to a region extending into the cytoplasm, in which polar head groups play a relevant role. In addition, dynamic properties studied by cyclic voltammetry allow one to deduce the energetics of the conformational changes of the lipid head group in relation to the head-head interactions due to the presence of carbonyls and phosphates at the interphase. These groups are, apparently, surrounded by more than one layer of water molecules: a tightly bound shell, that mostly contributes to the dipole potential, and a second one that may be displaced by proteins and osmotic stress.

Hydration water around carbonyl and phosphate groups may change by the presence of polyhydroxylated compounds or by changing the chemical groups esterified to the phosphates, mainly choline, ethanolamine or glycerol. Thus, surface membrane properties, such as the dipole potential and the surface pressure, are modulated by the water at the interphase region by changing the structure of the membrane components. An understanding of the properties of the structural water located at the hydration sites and the functional water confined around the polar head groups modulated by the hydrocarbon chains is helpful to interpret and analyze the consequences of water loss at the membranes

of dehydrated cells. In this regard, a correlation between the effects of water activity on cell growth and the lipid composition is discussed in terms of the recovery of the cell volume and their viability.

Critical analyses of the properties of water at the interface of lipid membranes merging from these results and others from the literature suggest that the interface links the membrane with the aqueous soluble proteins in a functional unit in which the cell may be considered as a complex structure stabilized by water rather than a water solution of macromolecules surrounded by a semi permeable barrier.

CONTENTS

- 1. - The membrane in an integrated cell system.**
- 2. - The interphase region in a lipid membrane.**
- 3. - Excluded volume and area per lipid.**
- 4. - Levels of hydration at the membrane interphase**
 - a. - Specific sites for hydration water*
- 5. - Effect of interphasial water on the surface potential.**
 - a. - The surface charge*
 - b. - The dipole potential*
- 6. - The dynamic properties of the interphase region**
 - a. - Surface changes by Osmotic stress*
 - b. - Water activity and head group mobility*
 - c. - Effect of electrical fields*
- 7. - Surface Defects.**
 - a. - Carbonyl arrangements*
 - b. - Exposed CH₂ regions*
 - c. - P-N alignment*
- 8. - Structural water in the hydration sites and the functional confined water.**
- 9. - Corollary: Does water at biological membrane interphases play a functional role?**
- 10. - References**

1. - The membrane in an integrated cell system

According to the current paradigm, a cell is described as a compartmentalized system in which the cell membrane is the barrier of contention of an aqueous solution of cellular material. This reductionistic approach ignores that cytoplasm is crowded with macromolecules and it is hard to understand cell function without a connection between the intracellular material and the cell membrane.

Virtually all biological macromolecules and among them molecular assemblages such as membranes, maintain a hydration shell [1]. Response to changes in the external conditions has been obtained in real time by following metabolic states in relation to bound water molecules [2, 3]. In the light of this evidence, this review focuses on the role of water at membrane surfaces as a connecting material with structural and dynamical properties imposed by the contact with lipids.

In this regard, one of the problems in understanding the lipid functionality in biological membranes is the fragmented way in which their properties have been studied. While a great emphasis has been put in the resolution of the structural properties, thermodynamic analysis has been relatively scarce. Although a thermodynamic equivalence of monolayers at collapse and fully hydrated bilayers has been proposed by modulating the surface pressure as a function of water activity [4], the relation between those structures and its surface thermodynamic response is not clear.

On the other hand, extrapolation of the results in lipid model systems, (of a limited number of synthetic components) to real membranes (a complex mixture), has resulted in simplistic models for membrane structure and, which is more important, its function. While clearly such models has been useful as a preliminary approach in the understanding of organization, polymorphism and phase states, they don't account for the complex membrane surface structure in which there is a spatial distribution of charged sites, counterions, and coions with their corresponding hydration shells. In addition, cell membrane function also should account protein-membrane interactions. Thus, the point raised that lipid structure is the key for the water surface activity should be understood in the context that membrane contains intrinsic proteins and others attached to the surfaces

constituting a much complex structure in terms of bidimensional domains and trans membrane asymmetry. Certainly, the current state of art in modelization and water structure does not allow going further than model membranes of a few components. However, the correlation of thermodynamic properties and structural arrangements presented with model membranes is a first step to account explicitly and systematically structural-function relation.

As derived from the analysis of surfaces of lipid membranes emmerging from studies in phospholipid monolayers, nuclear magnetic resonance and molecular dynamic simulation, the state of water near the interface presents peculiar thermodynamic and structural properties [4]. Under this scope, the heterogeneity in the chemical composition of the lipid membranes acquires a new projection since the different groups esterified to the phosphate in phospholipids and the consideration of the carbonyl groups as a second hydration center give a wide versatility of the surface properties due to its differences in water organization. The link between the membranes with aqueous soluble proteins in a functional unit demands a clarification of the relation between the structure of water imposed by the different lipids and lipid moieties, with its corresponding thermodynamic stability.

The stabilization in water of structures composed by those different types of lipid molecules [5-8], specifically of widely different head group structure, introduces changes in the normal liquid water network [9, 10]. It may be reinforced or it may collapse, increasing the density depending on the hydrophilic-hydrophobic character of the exposed groups. The non polar surfaces reinforce the hydrogen bonds of the low density water clusters. The collapse of this type of organization can result from the stronger water-lipid interaction in the hydrophilic portions. In both cases, a change in the surface free energy could be the origin of the driving force to aggregate nonpolar surfaces such as membrane-membrane or membrane-peptide contacts.

It is clear that lipid heterogeneity would account for different exposures of hydrophilic-hydrophobic regions according to the different groups esterified to the phosphates (choline, ethanolamine, glycerol, and inositol). Voluminous groups can extend from the phosphate group vicinity according to the balance of steric factors and hydration [11]. The membrane ensemble results in domains unevenly distributed in the plane of the surface that protrudes at different distances into the solution (Figure 1). The rectangle in Figure 1 denotes the

different membrane group-water interaction of different excess free surface energy. This free energy excess could be the key to trigger and modulate the response of membranes to different changes in the environment.

The interfacial energies in heterogeneous mixtures will be governed by the lateral interaction within the bilayer and with the aqueous solution at the different phase states of a lipid membrane [12-14]. The cohesive forces between the chains, which tend to minimize the area exposed to water, are counteracted by the repulsive forces between head groups buffered by the hydration. The net attractive forces between head groups mediated by water bridges or H bonding such as in PC's and PE's can be drastically altered by the hydration giving place to different lipid surface arrangements and interfacial area per molecule.

Changes in the composition of the solution in contact with the membrane can affect this picture. In this context, osmosis denotes a special situation. It induces changes in the water activity producing membrane curvature, as those resulting from the osmotic shrinkage of cells or liposomes, or membrane expansion as in swelling. The local changes of packing leads to the formation of defects in the membrane interface. From the surface energy point of view, defects mean more reactivity and they are favored in solid-like materials such as membranes in the gel state or in the borders of coexisting gel-fluid domains [14-16]. From the structural view, defects should probably be characterized by an organization of a few water molecules in a pocket or restricted domains between lipids, establishing equilibrium between fjords and reefs topography as described in micelles [17, 18]. Defects in the interfacial packing could be modulated in a much more complex manner when the surface is composed by groups protruding to the aqueous phase as shown in Figure 1. Details of the organization of water at the defects are not available but they have been postulated as targets where amphiphilic molecules bind and displace water [19, 20].

Therefore, the equilibrium area developed as a compromise between the packing of complex mixtures of head groups and the interactions of hydrocarbon chains depends on head groups type and size and, in consequence, water-hydrocarbon contact can be altered by changes in temperature and water content.

Drastic decrease or increase of temperature causes alterations on the water bound to membranes. The order-disorder in the hydrocarbon chains produces the expansion or

contraction of the membrane and consequently in water activity at the membrane [4]. This produces changes in the surface free energy (surface pressure) that may trigger adsorption of compounds from the solution.

Under a thermodynamic view, these coupled phenomena show the membrane as a mechano-electro-chemical system. Chemical composition in the water phase may cause area and surface potential changes and viceversa. The different rearrangements of water or water displacement can be the vinculating material. Solutes excluded from the membrane interphase may cause an osmotic stress at the surface. Other types of solutes may adsorb or penetrate into the lipid membrane interphase at different depths competing with the hydration water of the surface groups. In both cases, the hydration energy of the solute and of the lipid surface should be included in the thermodynamic balance considering in an explicit way the interfacial properties. For this, the changes of the water intimately related to the structure at different levels should be considered. The distinction between two types of solutes that may cause hydration stress: one that is excluded from the surface and another that can replace water at the surface, will be a useful tool to distinguish water at different states, in terms of the energy of binding to the lipids and hence its structural organization. The solutes replacing water would denote the hydration water, solutes excluded from the interphase and extruding water from it would denote confined water or osmotic water. In both case, they may trigger differ effects on the area per lipid and the organization of dipole and ions determining the surface potentials. The understanding of the variations produced by these hydration stresses on the different lipids and lipid mixtures can be followed by the changes in the water- lipid interaction and could be physiologically important.

2. - The interphase region in a lipid membrane

The study of the interfacial thermodynamics needs a novel visualization of the membrane interphase to introduce the relevance of water in membrane structure. The consideration of the membrane in its functional role implies that it should be able to respond to changes in the environment, as those named above causing hydration stress. To accomplish this, the evaluation of the membrane phenomena in terms of surface thermodynamics requires the lipid membrane to be visualized as a material with unique mechanical and electrical

properties inherent to its interfacial stability. In this approach, water and solutes interacting or penetrating the membrane play a role of surface active agents. In this sense, the approach of the membrane taken as an inert phase interposed between two compartments is unsatisfactory. In consequence, a model in which the dynamical properties are reflected should be provided. The dynamics of water exchange between the surface groups and the group would give place to fluctuation at local level in restricted domains of the membrane surface.

This interfacial phenomenon would take place in a 40 Å thick membrane in which the 40% of its thickness is occupied by an aqueous region [21, 22]. Within this limit, we introduce a description of the region of the lipids in the membrane in contact with the aqueous phase: the *interphase* region. In this, water is considered as a component of the membrane, organized in different levels: the hydration water and the confined water.

The first includes water tightly bound to the groups and excludes large water soluble compounds, such polyethylene glycols. The second corresponds to water that may dissolve low molecular weight compounds such sugars and aminoacids. This water has different thermodynamic properties than that in the bulk. The physical chemical properties of this region allow an explanation of the interaction of proteins from the aqueous environment and the modulation of enzyme activity by the introduction of metastable structural properties that may fluctuate between different states of comparable energies.

The *interphase* region is defined between an internal plane (the water-hydrocarbon interface) and an external plane (the slipping plane in contact with the bulk aqueous phase). A scheme of the *interphase* that will be analyzed and justified along this work is presented in the Figure 2.

The outer plane (A) is drawn tangentially to the external surface of the phosphate or bulky polar groups, although this may vary, depending on the volume of the groups esterified to the phosphate (see Figure 1). For convenience, we will define this plane as that tangential to the phosphates. This plane divides the region dragged by the membrane from the bulk aqueous phase in an electrophoretic mobility assay. The inner plane (B) divides the low dielectric constant region of the hydrocarbon phase from the polar region.

The 20 Å thick region confined between the two planes is defined as a bidimensional solution in which the polar head groups are hydrated. In this, water and hydrated moieties

are located and there are enough arguments to consider that its properties differ from the bulk water. One of them is the partial specific volume of water [4], the other dielectric properties. The hydrophilic-hydrophobic character of the exposed groups may reinforce or collapse the hydrogen bonds of the water clusters, thus affecting the density. The dielectric constant in this region is much higher than that of the hydrocarbon core (c.a. 2) but different than that corresponding to bulk water (c.a. 78). The alignment of the water dipoles may decrease the dielectric constant to about 40 in this region as predicted by molecular dynamics and fluorescent measures [23, 24].

The conceptual definition of the interphase as a bidimensional solution composed by hydrated groups and water helps to understand many of the responses of the lipid membrane to changes in the environment. One of them is the adsorption of anions and anionic groups at the Stern layer, considering the hydration shell of phosphate and the choline groups, which insert a net charge at the surface. Another is the adsorption and subsequent penetration of peptides and proteins [25-28].

An additional property derived from the alignment of the water dipoles is that the region is impenetrable by another interphase. That is, two interphases repel each other. This argues in favor of the definition of an excluded volume containing the different types of water and the polar groups contributing to the permeability barrier [21].

3. - Excluded volume and area per lipid.

When cells are exposed to changes in the osmotic pressure of the external environment, water is displaced between the inner and the outer compartment of the cell.

The first response to changes in the osmotic pressure is the variation of the cell volume. This is due to changes in the chemical potential of water inside or outside the cell that drives the flux of water across the cytoplasm membrane.

It is crucial for the cell recovery after dehydration that the water not to be extracted below a certain level [29, 30]. If this occurs the changes due to dehydration are irreversible and the cell dies. These results have many implications. The elimination of this water affects the overall structure of proteins and membranes. For protection, the cell responds to osmolarity changes by activating a metabolic pathway denominated “osmoregulation” [31]. To

accomplish this, transmembrane proteins can adopt new conformations and associations, and then modify their interactions with the lipid membrane [29, 32, 33].

To achieve the volume changes and recovery, cells must behave as osmometers [21, 34, 35]. In Figure 3, the linear relation between the volume of the cells and lipid vesicles with the inverse of NaCl concentration in the outer solution are shown. This lineal relation indicates that cells and vesicles obey the van't Hoff law for ideal osmometers. In these graphs, the extrapolation to the y axis allows calculating the non-osmotic volume. This is related to the volume of the material that cannot be reduced by osmosis. Part of this volume is related with the barrier of repulsion opposing the interpenetration of the interphase regions described in the previous section [36-39]. Vesicles prepared with lipids also show a non osmotic volume. Although much lower than the whole bacteria, it is far from zero. This volume has been shown by different methodologies to correspond to water sequestered by the membrane. Thus, part of the excluded volume found in cells corresponds to the excluded volume in the membrane phase. Taken together, the decrease of cell volume below the critical point of dehydration implies elimination of water from membrane structure. Hence, certain level of water at the membrane is essential for cell functions.

As defined previously, one of the properties of the interphase region is to be impenetrable. This is derived from the results in Figure 3 and accounts for the repulsive forces between adjacent interphases. It has been related to the partial specific volume and the dielectric properties of the interphase region by the repulsive forces due to hydration that account for the dipoles at the interface constituting the dipole potential (see below) [40]. The excluded volume of a membrane is the volume occupied by constituents that do not act as solvent for solutes of the aqueous phase, i.e. it counteracts the permeation across the membrane. It includes the molar volume occupied by the lipids and the molar volume of water immobilized by them. It has been calculated that the average thickness is 1nm, which is two to three layers of water at most. Simulation studies have shown tetrahedral ordering in the interphase, and orientation ordering extending approx. 0.7 nm into the water phase from the average [41]. The bilayer of phosphatidylcholines in the fluid state incorporates water up to a water/lipid ratio of 18-20 as found by DSC constituting the excluded volume that contributes to the bilayer thickness [42, 43].

The aim is now to discuss the contribution of the excluded volume of the membrane in terms of structural dimensions (thickness and area per lipid) to the thermodynamic behavior of the interphase region of the model in Figure 2.

The excluded volume in a fully hydrated state is directly related to the area per lipid of membranes in the different states. There are different methods to calculate the area per lipid.

One of them relies on the determination of the interbilayer distance by X ray diffraction derived from the electron density of the phosphates as a function of water content [44, 45].

This can be accomplished by two procedures. In one of them, lipids in the dry state are hydrated step by step to reach full hydration. When water exceeds that needed to fully hydrate the lipids, the interbilayer distance increases and the bilayer thickness and area shows no further changes.

In another experimental design, water from fully hydrated lipids is extruded by applying osmosis outside the compartments enclosed by the bilayers. In this case, macromolecules that are able to swell in water such as polyethylene glycol or dextran are used [37-39]. Using the very accurate thickness data obtained from X ray diffraction, and knowing the volume of the lipids, it is possible to derive the corresponding area.

Stabilization of the water-lipid interphase corresponds to the incorporation of water molecules to the lipids in order to reach a stable area per lipid. Water becomes distributed around the polar head groups, phosphate and choline, with a small penetration into the carbonyl region as derived from X ray studies [21, 27, 44-46].

However, it is difficult to separate the area calculation from other physical changes occurring in the membrane during the determination, especially when osmotic stress is employed. This is because the interphase can be altered by the changes in water activity in the bulk.

As stated by Nagle and Tristram-Nagle [22] and references therein, area values reported in the literature are scattered in a range of 50%. One correction introduced by these authors, which seems to be satisfactory, is the consideration of fluctuations. However, details on the physical meaning of these fluctuations are not given. As will be discussed below they may be related to the dynamical properties of the groups at the inner and outer planes of the membrane described in the previous section.

An alternative (and direct) method is to determine the area per lipid in a monolayer spread on an air-water surface. The principal drawback to area determination in monolayers is to adjust the lateral pressure to that corresponding to a bilayer. In this method, one has to decide at what surface pressure the area is determined. For example, at the pressure of collapse, the area is much lower than that at pressures similar to those corresponding to a bilayer. This means that interphases of monolayers and bilayers are compressible and lateral forces of compression can squeeze water from the lipids.

As the area per lipid may vary with the applied lateral pressures, which is the limit corresponding to the bilayer in equilibrium? Moreover, what distortion may induce the lateral pressure on the head group conformation, carbonyl arrangements and apolar groups' exposure according to the fluctuations discussed previously?

A strategy to tackle this problem consists in the spread of lipids in a monolayer at the air-water interface at constant area and temperature. For this, no assumptions are required, but it needs a skillful precision in its determination and the acceptance that a monolayer is energetically equivalent to a bilayer. In this regard, many papers have demonstrated that this is the case [47, 48], although several others have challenged it [49].

An answer to these questions is to work with monolayers that spontaneously accommodate at an air-water interface in the absence of any lateral forces, except that of the adjacent lipid molecules. In this method, it has been argued that the excess of lipids achieved when the monolayer is saturated forms vesicles or liposomes in the subphase underneath the monolayer [48]. In the equilibrium, the chemical potential of lipids is the same in the monolayer than in the liposomes. Therefore, the interfacial properties can be accepted to be equal.

The method consists in the determination of the area per lipid from surface pressure (π) vs. nmoles of lipids by adding aliquots of lipids in an organic solvent to the surface of water at constant area (Figure 4).

The sequential addition of lipids to an air-water surface increases the surface pressure due to the decrease in the surface tension. Saturation is reached at a surface pressure corresponding to the lipids and to the temperature denoted by the arrows in Figure 4. In the fluid state at 28°C, a lower amount of lipids is needed to achieve the saturation in

comparison to the gel state at 18°C due to the higher area per lipid. In the monolayer method, the uncertainty is ± 0.5 nmol of lipid.

From this, a careful extrapolation can give the limit area per lipid when the monolayer is not subject to any external force. The values of the area per lipid obtained with this method are $65.4 \pm 4.4 \text{ \AA}^2$ for the fluid state and 56.4 \AA^2 for the gel state [50-52].

The values derived from X ray diagrams for DMPC (30°C) are 60.6 \AA^2 with fluctuations [22] and between 59.7 - 65.2 \AA^2 when fluctuations are not considered [44, 51, 52-60].

The difference between the lower and the higher values in both methods is 5 \AA^2 , which is a distance of around one water molecule. The differences in area related to fluctuations could then be ascribed to different water organization and molar volume in the adjacencies of the polar and non polar groups of the lipids. This assumption deserves a detailed discussion of the effect of water extrusion on phosphate and carbonyl arrangements, i.e on the topology of the outer and inner plane of the interphase region of Figure 2.

4. - Levels of hydration at the membrane

In both methods used to determine the area per lipid, the membrane appears to stabilize a water arrangement which is a compromise between the orientations imposed to the water molecules by the groups of different chemical nature and the water structure of the network bulk liquid. This compromise defines the aqueous region of the interphase. Dry lipids dispersed in water form bilayers when the lipid phase reaches the saturation level. In the monolayer, the free surface of water saturates at a given amount of lipids. In both cases, the full hydration level is ascribed to the excluded volume described above that includes water molecules at different depths. For the corresponding thickness of 20 \AA , 11-12 water molecules are located out of the membrane inner plane in Figure 2.

In the curves of X ray, Bragg spacing versus hydration saturation levels near 12 water/lipid, as observed experimentally by Hristova and White [62] and marks the completion of the first hydration shell [63-64]. In monolayers at constant area, the saturation is reached for 11 water molecules per DMPC at 30 °C a value near to that found in inverted micelles [65]. This data are resumed in Table I.

Thus, water at the interphase is not constrained to the polar groups as derived from the Gaussian distribution emerging from X ray studies. Water beyond the first hydration shell may be related to the extension of water into the water-hydrocarbon interface [27].

The centers of hydration of the phospholipids are the phosphate and the carbonyl groups.

Data of FTIR in Table I reports that dried lipids exposed to water vapor show a downward shift in the asymmetric stretching frequency of the phosphate of phosphatidylcholines reaching a constant value when a ratio of six water molecules per lipid is attained [66, 67].

After full hydration, the asymmetric stretching of phosphate groups is displaced to higher values when exposed to hypertonic solution of PEG. This shift was interpreted as a consequence of the dehydration of the PO due to the osmosis [68, 69].

These two lines of evidences suggest that water volume in the lipids can be roughly identified in a similar way as that organized around an ion: a first tightly bound water and a second loosely bound shell. The first hydration shell is water excluded from the volume to dissolve solutes. Outside that region, solvent properties depend on the type of ions and its concentration. Water bound to the membrane is sequestered and unable to dissolve solutes. Thus, this region also contributes to the permeability barrier [21, 34]. Loose water might account for the dissolution of some polar solutes in the interphase region affecting the water activity and for the fluctuations in the standard deviation in the area determination.

Loose water can be displaced by osmosis. The tightly bound water can be replaced in drastic conditions by specific compounds that may mimic water [30, 70-72]. The limits between both effects will be given by the size, shape and the type of the molecule and the energy by which each water molecule is bound to specific groups.

a.- Specific sites for hydration water.

The two different types of water distinguished as tightly bound and another displaced by osmosis suggests that water molecules included in the excluded volume are not energetically equivalent. How can we identify structurally and thermodynamically the difference? Phospholipids bind approx. 0.5–3 water molecules/lipid very tightly. The energy to remove these water molecules is c.a. 40kJ/mol [73], which is equivalent to the energy of a hydrogen bond. Therefore, inherent to the different water distribution in the

structure, water bound to lipids may have different thermodynamic properties, such as, activity.

When a weighted amount of DMPC, fully hydrated in water and then dehydrated at 70°C and under vacuum, is introduced in a sealed chamber at constant temperature, the activity of water at equilibrium is $a_w = 0.480 \pm 0.01$ [74]. Therefore, some water remains attached to the lipids with the ability to exchange even after the drastic drying.

Interestingly, when the liposomes are dried with the same procedure in the presence of increasing trehalose ratio, the water activity decreases to $a_w = 0.327$, a value comparable to that found in saturated solutions of $MgCl_2 \cdot 6H_2O$, denoting a decrease of the water in contact with the lipid with the ability to be exchanged with the vapor phase [74]. This means that in the presence of trehalose less water remained attached to the lipids. In other words, trehalose molecules have been able to displace exchangeable water from some sites. In Table I, it was shown that water bound in reverse micelles were about 12-14 water molecules per PC. When a chloroform lipid solution is titrated with a solution of trehalose, the water per lipid decreased to a limit value of 7-8 water molecules for 4 trehalose/ lipid.

The lower value of 7-8 water molecules can be identified as those tightly bound to the lipid which cannot be displaced by trehalose. Interestingly this is a value very close to that found for the frequency shift of the phosphate groups when dried lipids are exposed to water vapor (Table I). It is concluded that phosphate group in the lipid membrane is a primary hydration site with c.a. six water molecules stabilized in a strong environment [66, 67, 75]. Phosphate groups are the sites of the phospholipids that may immobilize water in a tetrahedral array by attaching the molecules through hydrogen bonding with the PO groups. On the other hand, the choline group appears to have a clathrate-like shell. Considering the difference between the lower value obtained for the phosphates with that obtained with calorimetry (18-22 water molecules per lipid), we deduced, that there are 10-12 water molecules in a less tightly bound hydration shell [76-88].

The location of these 10-12 water molecules can be found around other polar groups such as the carbonyl groups and beyond the inner plane as deduced from the calculation of water per lipid in monolayers.

Carbonyl groups are also a center of hydration but with a more complicated behavior. Hübner and Blume [89] suggested that the resolvable subcomponents of the $\nu_{C=O}$ bands of

diacyl phospholipids may reflect subpopulations of hydrogen- bonded and non- hydrogen-bonded ester carbonyl groups. This suggestion has been adopted in the interpretation of the $\nu_{C=O}$ bands in several studies of diacylglycerolipid bilayers [75, 89-99]. For fully hydrated phosphatidylcholines, the high and low frequency components of the $\nu_{C=O}$ bands cannot be assigned to the individual ester carbonyl group of acyl chains esterified at the sn_1 - and sn_2 -positions of the glycerol backbone. These studies indicate that the carbonyl groups at the sn_1 and the sn_2 position in phospholipids consists in two populations: one hydrated (bound) and another non-hydrated (non bound) [89-92].

Carbonyl groups are affected to a more significant degree for lipids arrayed in the L_α phase than in the gel phase. A number of spectral features reveal the lyotropically triggered chain-melting transition as well as other structural rearrangements of PC's [93-96, 102]. In consequence, the CO groups can be arranged in one population exposed to the water phase and another facing the non polar bilayer side (Table II). In this Table it is also observed that the difference in frequencies between the two populations depends on the topological phase state of the surface. Thus, carbonyl exposure to water depends on the water-lipid ratio at each phase state. A schematic orientation of these possibilities is drawn in Figure 5 (part A).

The interesting point is that each of sn_1 and sn_2 populations split in those two states, which may be explained as a consequence of a dynamic exchange of water in the inner plane as a result of the permutation of the CO from one position to the other. The dynamic orientation of the CO across the inner plane of Figure 2 implies a fluctuation around the stable position. Fluctuations can be local and independent of each other making the surface more heterogeneous at the membrane plane.

This accounts for the diffuse limit at the water- hydrocarbon interface and the fluctuations in the water penetration [95, 96]. Considering these properties, it is reasonably to understand the variations in the values of area through the volume determination. As an aside, it suggests that carbonyl group may, in certain stages, determine the area per lipid (see Table III below).

As pointed out later by Nagle and Tristram-Nagle [22] the difficulty to quantify the structural properties is the presence of fluctuations in fully hydrated lipids.

In agreement with our considerations related to the sharpness of the planes defining the interphase region, the fluctuations account for the lack of insight on the organization at the carbonyls in the inner plane, the water penetration beyond that plane and, in consequence, the exposure of hydrophobic regions to water.

The non-osmotic water derived from the non-zero volume at high osmotic pressures (Figure 3) is probably the water sequestered by the lipid at the interphase region of Figure 2 in the first and second shells. More water than that previously estimated could be present in the hydrophobic region, i.e, beyond the inner membrane plane of the carbonyls [103]. A fraction of water is at the membrane inner plane and another underneath it in discrete sites (named indistinctively as defects, hydrophobic pockets etc.). They may be assumed as dynamic domains where carbonyl groups oscillate between different orientations and hydration states and affected by the lateral contact of the P-N dipole whereas in PC's and PE's membranes.

Knowledge on dynamic structural properties of the lipid interphase includes the mobility of those groups and the water exchange in this region and is a step toward comprehending the fluctuations and the thermodynamic interfacial instability of the bilayer. This may help us to understand the interaction of biomolecules with the membrane and provide reasoning to the mechanisms of several surface phenomena of biological relevance such as, membrane fusion, protein-membrane interaction and even permeability.

In the case of phospholipid bilayers, the hydrating water molecules are less hydrogen bonded than those in water, which is dependent on lipid head group [101,104, 105]. This gives hydration a certain level of lability that could be the thermodynamic base for functional membrane phenomena in terms of changes in the interfacial tension. This implies to distinguish osmotic water and non osmotic water at the interphase region included in the hydration layer of the head groups but with different energetic content.

As described above, the phosphate and carbony groups are considered hydration sites in phospholipids.

The phosphate group is accessible in PC's to the compounds in the water phase, as shown by the shift in the asymmetric frequency, producing an increase in the area per lipid (Table III). This mechanism is general enough to admit that it may be applied to free sugars, amino acids or those attached to peptides and proteins.

As inferred above, carbonyl groups may have a role in the determination of the area per lipid. In this Table, it is observed that the area of the ether derivatives of PC is higher than the ster PC, a fact not observed in the PE's. In addition, a different picture to that found in phosphates is obtained when the carbonyl groups are observed (Table IV). Apparently, these groups may adopt different orientation with respect to the plane. This is derived from the different distributions obtained when the lipids interact with H-forming compounds in which the spatial conformation of the OH groups differs. Trehalose, a molecule having all the OH in the equatorial position, decreases the frequency of both carbonyl group populations, indicating that it binds to all of them. Sucrose (with a 60% of equatorial OH's) and phloretin (with a phenol group) do not affect the bound and unbound carbonyl groups. Arbutin (which combines glucose with a phenol moiety) displaces the formerly hydrated population to higher frequencies and binds the non hydrated one.

Trehalose maintains the difference between the frequencies of bound and unbound carbonyl groups found in pure hydrated DMPC. Phloretin and sucrose decrease slightly that difference between the two CO populations. In contrast, arbutin produces a notable decrease [106-109].

The interaction of the different OH compounds with the phosphate and/or carbonyl groups, displaces different amounts of water. Trehalose, which interacts with phosphate and carbonyl groups of DMPC displaces 11 water molecules (Table V) [72]. This effect has been ascribed to the formation of stronger H bonds between the lipid and trehalose than with the water congruent with the FTIR results (Table III and IV).

As shown in Table III, the area per lipid is affected by trehalose when carbonyl groups are present. Therefore, part of the water replaced by trehalose could be inserted at the carbonyl level. In the same Table III, another polyhydroxylated compound, such as phloretin (Phl), affects the phosphate bands increasing the area per lipid but no effects on the carbonyl groups are detected. In this, the water displacement is much lower (Table V).

Water activity levels in the mixtures are added for comparison.

5. - Effect of water on surface potentials.

The definition of the interphase region is useful to analyze also the electrical properties derived from the organization of water, constitutive dipoles and ions in this domain. As

said, the hydration state would determine the orientation of the dipolar and charged groups at the interphase region. Two important properties inherent to water and ion distribution must be considered in this context: the surface charge potential and the dipole potential. The adsorption of anions and anionic groups at the Stern layer can be dependent on the hydration shell of phosphate, carbonyl and choline groups [25, 26]

a. The surface charge

An electrical surface potential drop can be considered in the region defined between the inner plane dividing the water-hydrocarbon phase and the external plane dividing the bulk water and the polar region (Figure 2).

The structure of this region may be described as the electrical double layer in which different arrangements can be distinguished. One is constituted by fixed charged groups on the phospholipids: i.e. the negative charges of the phosphates and the positive charges of the choline or ethanolamine in the case of PC's and PE's respectively. This can be identified as the inner Helmholtz layer. The presence of these charges induces the distribution of counterions with their respective hydration shells (Figure 2). These counterions could be large anions, such as, chlorides that specifically adsorb by non coulombic forces (chemical or van der Waals interactions) or cations that shield the electrical charges of the fixed and adsorbed anions. The first constitute the Stern layer and the second the diffuse double layer, which thickness is a function of the temperature and on the ionic strength of the aqueous solution [117-120].

The limit between this layer and the aqueous phase can be defined in electrical terms as the slipping plane, which determines the zeta potential [121]. This is usually measured by means of the determination of the electrophoretic mobility of liposomes or vesicles in aqueous media. For convenience, our main source of data is PC's, for which it has been proposed that the phosphate group is in an outer plane with respect to the choline. Thus, we take the slipping plane corresponding to the zeta potential as that near the tangent of the hydrated phosphates. However, care must be taken to extrapolate this criterion to lipids with other polar heads groups such as phosphatidyl inositol or phosphatidylserine in which the groups esterified to the phosphates can protrude and penetrate to a larger distance from the surface into the bulk water phase (see Figure 1).

b. The dipole potential

The orientation ordering extending approx. 0.7 nm into the water phase from the average gives place to the dipole potential. The hydration of the polar groups promotes the polarization of water molecules at the water-hydrocarbon interface described by the inner membrane plane in Figure 2.

The total water molecules of hydration detected by calorimetry and located by the scheme of Figure 3 in the stabilization of the area per lipid, are oriented in a solid angle with respect to the normal to the membrane plane [4]. In the Figure 2, the orientation is mainly given by the constitutive groups CO and PO. The first consists of a population that is located normal to the hydrocarbon-water interface and contributes by themselves to the dipole potential [122].

As described, hydration affects the head group orientation, as measured by the P-N vector. At low hydration, the sign of the membrane dipole potential is reversed with the membrane interior negative relative to the interlamellar region [64].

Carbonyl and phosphate groups in the polar head of the lipids are able to form hydrogen bonds along their free electron pairs. Water bound to them appears to be polarized and contribute to the dipole potential of the interface.

The amount of water bound decreased by the polyhydroxylated compounds such as trehalose, sucrose or arbutin (Table IV and V) decreases the dipole potential (Table VI) [50, 52, 68, 72, 106, 107, 122]. Figure 6 indicates that there is a direct correlation between polarized water in the polar groups and the dipole potential.

6. - The dynamic properties of the interphase region

One of the problems in the visualization of membrane properties as an interfacial phenomenon is that they are taken as static structures. For the understanding of the versatile response of the membrane to changes in the adjacencies, it is essential to take into account that the polar head groups of the lipids are not located as in a homogeneous perfect plane and that the limit between the polar and apolar regions depicted in Figure 2 is rather diffuse. The degrees of freedom of the lipid constituents admit fluctuations in the definition of the outer and the inner plane of the model introduced in the previous point. The outer plane fluctuates because the group esterified to the phosphate may have different

projections on the membrane normal according to the state of hydration. In turn, the inner plane is affected due to water penetrating to a certain extent into the glycerol region which, in the fluid state, is manifested by a high lateral mobility of the membrane components [27]. Therefore, it is expected that this complexity would give place to cross phenomena between different properties such as hydration, packing, surface potential and surface tension. Precisely, these features make it possible that the changes occurring in the adjacencies of the membrane be sensed, modulated, attenuated or amplified into signals by the introduction of metastable structural configurations that may fluctuate between different states of comparable energies. The appearance of those states at the inner and the outer planes may account for the dynamical aspects of the interphase.

In the inner plane, carbonyl groups may change its orientation with respect to the membrane plane modifying the exposure of apolar regions (CH_2) to water (Table II).

In the outer plane, the mobility of the head group would depend on the hydration of phosphate groups and the esterified groups (choline, ethanolamine or others) modulating the lipid-lipid and lipid-water contact reflected in the surface potentials (Table VI).

The degrees of freedom of carbonyls and phosphates are not independent and they have consequences on the adsorption of counter ions and ions in the Stern layer according to the distribution of water layers and dipoles described in the previous point [26].

The appearance of these singularities in the membrane surface involves the dynamical response to physicochemical agents accounting for the functionality and regulation. This is known as a non autonomous phase which accounts for the variations in surface tension due to changes in the phases connected by the interphase region [28].

To discuss these properties we have to consider the energetics of hydration and the water confined between the groups involved in the excluded volume and the area per lipid of the interphase region.

The conformational equilibrium can be altered by perturbations such as the electrical field and the osmotic stress. Then, the analysis of these properties involves fluctuations in the dynamics of the head group orientation triggered by hydration (osmotic) stress, electric fields and surface active agents in correlation to lipid-lipid lateral interactions.

Failures in the structural arrangements in the inner and the outer plane may give place to the formation of defects consisting in the exposure of apolar regions to the water phase.

a .- Surface changes by Osmotic stress

The lipid bilayer is a sensitive material for conveying information in the osmotic conditions of a cell to the regulatory machineries. Under the scope of this review and based on the model introduced in Figure 2, it is of interest to describe the effects of the osmotic stress on the physical state and molecular interactions in the bilayer influencing lipid packing and dynamics, 2-dimensional ordering and the different microdomains [1, 64, 68, 123, 124]

As shown above, hypertonic unbalance causes water outfluxes affecting the volume of the cells or liposomes reaching the excluded volume as a limit of approach of bilayers when water in the interlamellar space is extruded. The well-known reviews of Rand and Parsegian [36] emphasized bilayer interactions with structural data on lipid bilayers denoting the presence of repulsive hydration forces at the membrane interphase.

The presence of water affects the lipid-lipid interaction and it appears to be nearly the same as in excess water when, approximately, ten water molecules/PC is reached, as judged by T_m and lipid rotational mobility [102]. Interestingly this is the number of molecules that appear to stabilize underneath the inner membrane plane as inferred from monolayers and micelle experiments and near the hard core for phosphates obtained with FTIR (Table I).

On the other hand, hypotonic swelling produces the stretching of the membrane against the cohesion forces.

Water moving out (into) the interbilayer region into (from) the bulk water is accompanied by an increase (a decrease) in the head groups packing. The pattern of order in the head groups has been interpreted as a ‘quasicritical’ fluctuations near T_m comparable to that occurring around the main phase transition. Such fluctuations show a correlation between reduced ordering and increased hydration observed near T_m , which is consistent with the prediction of an osmotic model [110].

The osmotic stress produced between the interphase region and the bulk solution by the asymmetric distribution of solutes would affect the distribution of the carbonyl groups. Data in Table VII show that there are qualitative changes in the carbonyl groups when arbutin is only outside in comparison to when arbutin is evenly distributed between the inner and the outer solutions. In this case, the frequency of both carbonyl population's shifts to lower values. These results suggest that the interaction of some OH compounds

may be affected by the hydration level of the bilayer. In other words, the displacement of water can enhance the interaction of OH compounds with the groups.

This partial dehydration can enhance OH solute intercalation. Although what kind of water is displaced is not a straightforward issue, structural changes would be expected by water extrusion below the limit of ten water molecules.

The occurrence of osmotic stress enhancing solute interaction or changes in the molecular packing does not require an osmotic gradient across the membrane. Also in the absence of a transmembrane osmotic gradient, the osmotic strength produced by dextran or polyethyleneglycol extrudes water from the bilayer [68, 69]. This can be understood assuming that osmosis can be produced as a consequence of the difference in water chemical potential between the interphase region and the bulk. The removal of water from the hydration shell of the lipids diminishes the effective size of the headgroup, compressing the membrane [69]. The energetics of the conformational changes of the lipid head group related to the head-head and head-water interactions will be in accordance with the energy of binding of the water molecules to the groups. Hence, structural arrangement such as, alignment with respect to the membrane plane, free volume of the groups and packing density may occur as a result.

b. - Water activity and head group mobility.

NMR studies have indicated that water molecules associated with the lipid headgroups are in exchange with bulk water [125, 126]. This exchange was already put into relevance when the water activity in lipids was discussed (Table V).

The widths of the P–N vector distributions and the choline segment relaxation response to hydration indicate that the headgroup is capable of undergoing a substantial range of motion. An increased alignment of the average lipid headgroup with the bilayer surface was observed lowering the water content. In this context and in relation to the discussion in the next point, this reorientation affects the surface dipole potential which shows a reversal in sign in going from 11.4 to 5.4 waters/lipid, due to the depletion of the headgroup hydration shell [104, 105, 127, 128].

In this regard, it is interesting to denote that the dipole potential of lipid monolayers is increased in the presence of high molar concentrations of sucrose in parallel to the dehydration of the surface groups [68, 71].

c.- Effect of electrical fields

It is known from deuterium magnetic resonance NMR studies, that the surface charge density of a phospholipid membrane is correlated with the P-N dipole conformation. In this sense, the orientation attained by the polar head groups of lipid molecules works as a molecular electrometer [115, 116]. At constant temperature, the P-N dipole orientation can be affected by an external electrical field. This idea has been applied in the context of a model [117] for the interpretation of the electrochemical response obtained in cyclic voltammetry experiments, performed on DMPC and DMPE monolayers adsorbed onto mercury electrodes. This experimental system has been employed from the 60's for basic studies of lipid monolayers and several applications have been performed on DOPC monolayers [117-120].

When a potential sweep is applied across a phospholipid monolayer adsorbed on Hg, the presence of a capacitive peak at about - 0.80 V against Ag|AgCl is observed [131]. The peak potential (E_p) corresponding to DMPE is farther from the zero charge potential than that corresponding to DMPC, taken as a reference the value determined for DOPC ($E_{pzc} \cong 0.070$ V) [129]. This would be indicative of a higher energy involved in the process, which is congruent with a higher lateral interaction between polar head groups in DMPE than in DMPC. As it is known, the polar heads of DMPE have the capability of establishing hydrogen bonds between the H_3N^+ group of one molecule and the PO group of an adjacent molecule [131-133].

As observed for both phospholipids in Figure 7 and Table VIII, the capacitance of the system in the state previous to the peak, is lower than the corresponding value obtained after the peak. This indicates a change in the thickness or of the dielectric constant of the monolayer. However, these changes are not enough to explain the magnitude of the capacitance increase. Therefore, these alterations should be concomitant to an increase in the permeation of the monolayer to water and ions.

These results could be interpreted as a consequence of the rotation of the P-N dipole group, around the chemical bond between it and the glycerol backbone. The electrical field would reorient the groups against its hydration shell.

As described in Table II, the exposure of the carbonyl groups is probably due to the curvature of the bilayer at different phase states which are related with changes in the hydration state. Further studies should be done to clarify these arguments.

Thus, the carbonyl and phosphate groups at the interphase present dynamical properties related with the interaction with the water phase. In this condition, these groups are, apparently, surrounded by more than one layer of water molecules. The first one is tightly bound by H bonds to the phosphates or stabilized as clathrates around the choline. A second layer is loosely bounded.

In conclusion, the changes in hydration causes a reorientation of the charged groups and the effect of the electrical field affects water rearrangement being osmotic and electric properties two interdependent phenomena.

7. - Surface Defects.

In the previous points, the organization at the interphase and the possibility of orientation of constitutive groups (CO and P-N) in relation to hydration was discussed. This picture was described for a membrane equilibrated in water in the absence of external forces. The problem to derive interphase thermodynamic properties can be focused by identifying how water interacts with the different lipid groups and how structural changes result from them. In principle, water faced to polar surfaces will be qualitatively different from that faced to non-polar surfaces at the membrane interphase. Thus, differences in the excess free energy of the surface at each region would be expected.

As pointed out, area determination is crucial for the understanding of membrane behavior. One reason is because its magnitude depends on the average stabilization of surface groups such as carbonyl and phosphates. The fluctuations of these groups around an equilibrium value are a consequence of the liquid (soft) character of the material. Although long-range forces operate to maintain a bidimensional coherent structure, at short range (molecular scale at few neighbors) the organization can be modified from one point to another. When the fluctuation does not follow a common oscillation (harmonic movement) defects in

terms of dynamic ones can be formed. The defect is then, a spontaneous local arrangement with a limited lifetime which can be paralleled to the fluctuations in the inner plane limit. Furthermore, the fluctuations imply the exposure of the membrane surface to the water phase affecting water structure in different extents and orientations. Some of them would correspond to a strengthening of the lattice array in pure water and some would promote its disruption. Hence, the coexistence of groups of different polarities determines the organization of water in the different arrangements. Therefore, fitting of those arrangements in the lateral network may account for appearance of structural defects in the surface.

The induction of defects can be achieved by the coupling of area/thickness due to a thermotropic phase transition in excess of water; or, at constant temperature, by affecting the water activity at the interphase region by osmotic stress.

Both factors may produce defects at the interphase by different arrangements as described in Figure 5: the arrangements of carbonyl groups (A), the alignment of the phosphocholine group (P-N group) with respect to the membrane plane (B) and the exposure of hydrophobic moieties (C).

a. - Carbonyl arrangements

The preferred interfacial area per molecule is modulated by the hydrophobic interaction and the repulsive forces between head groups. The cohesive forces between the chains tend to minimize the exposed area to water, while the head group repulsion result in its expansion.

In this context, carbonyl groups in phosphatidylcholines have an important role in area determination (Table III). As derived from monolayer studies in ether and ester lipids the area per lipid in DMPC gel state is 56.3 \AA^2 and for diether of the same length (ditetracylPC) in the same state is 67.7 \AA^2 . However, for ester and ether derivatives of PE the area is 56.3 \AA^2 in both cases. This suggests that CO (and therefore its hydration) becomes important when strong lateral interactions such as those between P-N bindings are absent.

It is known that the surface topography in phosphatidylcholine membranes varies below and above the pretransition and above the main transition temperature. The planar

arrangement below the pretransition evolves into a ripple (corrugated) phase above it [12, 13].

The formation of the corrugated phase profile in the pretransition is mainly dependent on the interfacial energies governed by the lateral interaction within the bilayer. Recent results suggests that carbonyl groups are involved in this process [109]

An increase in temperature introduces more disorder in the hydrocarbon chains while the higher water concentration enhances the lateral repulsion within the head groups. They results in the enhancement of water-hydrocarbon contact area, an effect strongly disfavored by the hydrophobic characteristic of the chains. A physical packing frustration may develop as a compromise between the equilibrium packing area in the head group region and in the hydrocarbon chains when hydration changes.

The packing frustration leads to different arrangements of carbonyl groups. In Table II, the relative frequency values of the two carbonyl population are reported for ripple ($P_{\beta'}$), planar gel ($L_{\beta'}$), and fluid phase (L_{α}). It may be observed that in the ripple phase the difference between the frequencies of the bound and unbound carbonyl groups is 18 cm^{-1} . However, at 10°C at which DMPC is in the planar gel phase (no ripples) the difference decreases to 8 cm^{-1} for which the separation between the two populations cannot be identified without error.

As pointed out above, water is unevenly distributed at the inner membrane plane of Figure 2, penetrating into the carbonyl region. It is interesting in this regard to recall that in solids, the frequency of the carbonyl band is centered at 1740.0 cm^{-1} . In fully hydrated lipids, two components are observed; one population is centered at 1742 cm^{-1} and another at a much lower frequency [89-93, 109]. This suggests that, at equilibrium, a dynamic conversion between the two populations takes place maintaining a fraction of the total carbonyl population towards the water and another segregated from it. The lateral packing determines the magnitude of these fractions. Packing frustrations produced by the spontaneous curvature could result in a displacement from one fraction to another and defects in which the exposure of the carbonyl groups could be magnified.

b. - Exposed CH_2 regions.

The titration of liposomes with hydrophobic probes denotes that a given amount of apolar moieties are accessible from the water phase. Merocyanine 540 shows a 570 nm peak for membranes in the fluid state that disappears when they are cooled down below the phase transition temperature to the gel phase. Similar results were obtained with ANS [111]

The exposure of apolar regions to the aqueous phase means an excess of surface free energy [103] that could be functional to the insertion and adsorption of different types of molecules. In first place, this accounts for a water organization that may drive hydrophobic interactions with aminoacids or peptide residues. On the other hand, this water organization causes an interfacial surface tension different than that when the polar head groups are packed as rigid hydrated spheres. The coexistence of the hydrated polar head groups with regions in which hydrophobic groups are exposed, results in a surface of heterogeneous polarity that may be identified as defects (Figure 5, part C).

The defects of hydrophobic polarity explain why amphipathic molecules such as lysophosphatidylcholines can insert as molecular harpoons or the activity of surface enzymes, such as phospholipase, is enhanced. In both cases, the hydration state of the interphase affects the interaction and the hydrolysis, respectively. In the first case, the lysoPC penetrates into the bilayer by interacting with the carbonyl groups. Interestingly, OH bonding compounds that bind to carbonyl groups inhibit the penetration of such harpoons molecules [99, 112] and the enzymatic activity of phospholipase [20, 113].

c. - P-N alignment.

Haines and Leivovitch [114] have pointed out the importance of the head polar dipole on the formation of kinks along the membrane affecting the water permeability.

The P-N dipole of the head group in a PC or a PE bilayer may have different positions between a direction normal or parallel to the surface membrane. As pointed out by NMR studies the P-N dipole lays on the membrane surface and penetrates slightly into the hydrophobic plane in the case of PC's. This group behaves as an electrometer and, in consequence, it may reorient by the application of an electrical field across the membrane interphase (115, 116) (Figure 5, part B). The displacement is counteracted by the strength of the interaction of the polar groups with water and with the lateral lipids.

Thus for PC's and for mixtures PC's-PE's, it is likely that an additional reason for the roughness of the surface is the heterogeneous orientation of the P-N groups. The slight penetration of the choline group into the hydrocarbon plane is another possible source of defects in the membrane surface.

FTIR results also point out the different exposure to water of the phosphate group depending on the area available for the head group. Although phase transition does not produce significant changes in the phosphate hydration, the presence of carbonyl group has important effects.

As reported [51, 52], the area per lipid in DMPC is 56 \AA^2 and in the etherPC is larger (64 \AA^2). In the first case, the phosphate band is centered at 1229 cm^{-1} and in the etherPC at 1225 cm^{-1} , which indicates that the increase in area due to the absence of carbonyl groups promotes a higher hydration of the phosphates. These results suggest the cooperative properties of interfacial groups such as carbonyl and phosphates due to hydration.

8. - Structural water in the hydration sites and the functional confined water.

Analysis of the excluded volume suggests a question: is it constituted by only one kind of immobilized water and what is its activity in thermodynamic terms?.

The definition of the interphase region allows an understanding of the behavior of the membrane assuming that the interphase is a bidimensional solution of hydrated head groups (134). The hydration water is that found to be displaced by OH compounds described in the previous section that amount 7-11 water molecules.

Under the concept of this model the osmotic pressure can extrude water from this region. This water would be beyond the hydration water molecules i.e the difference between these molecules and the total amount of 18-20 found by DSC.

The dissolution of some solutes in the interphase region in the water beyond the hydration shell of the groups promotes a decrease in the chemical potential of water. In consequence an entrance of water from the solution is equivalent to an increase in the lateral surface pressure [135].

The results allow distinguishing between the two kinds of water at the interphase region one corresponding to non osmotic and another to osmotic water. These different possible

arrangements assumed by the water molecules around the phosphate and carbonyl groups are responsible for the chemical and biological behavior of these systems.

For convenience, we will distinguish the non osmotic water as the hydration water in the polar groups and as confined water that with osmotic activity.

The finding that peptide penetration within phospholipid monolayers depends on the surface pressure of phospholipid molecules opens several questions on the properties of the interphase region. Increasing the lipid pressure, the DPPC monolayer becomes almost insensitive to peptide penetration at a pressure of 27-30 mN/m. This limit defines the “cut off” above which no interaction of peptides with the monolayer is detected.

The “cut off” surface pressure for melittin penetration was found to be 45 mN/m with PC and 60 mN/m with phosphatidylglycerol (PG). Other studies showed that the penetrating peptide adsorption, 16 amino-acid sequence extracted from *Antennapedia* homeodomain, was maximal for DPPC pressure of 10-15 mN/m [136, 137]. In our model, the limit “cut off” is related to the absence of confined water.

What makes that below the “cut off”; the addition of proteins at the subphase produces an increase in the surface pressure?

The initial value of the surface pressure will be a function of the water molecules affected by the lipids: i. e., number of lipids and degree of hydration of the lipid specie. A given amount of specific lipid molecules spread at the air-water surface would give a value of interfacial tension (Figure 8) [138].

To produce a significant change by protein adsorption the surface pressure must be below 40mN/m for PC's.

The “cut off” pressure for different PC's is, in average, around 40mN/m. This is significantly lower than the surface pressure of a saturated monolayer of PC which averages around 46.6 - 48.0 mN/m. That is, the pressure at which the protein does not penetrate is considerable lower than the pressure at which the head groups are packed. (Table IX). This indicates that the excess free energy required in order to protein to adsorb is not related to the structural limit at which the polar head groups are in contact through the hard-core hydration shell. In other words, water thermodynamically active would be beyond the hydration shell.

For lipids having strong lateral interactions such as PE's, the corresponding surface pressure at saturation is very similar to that for PC's (45mN/m). However, the cut off pressure is 30.6mN/m, much lower than for PC's of similar acyl chain. Therefore, the excess of surface energy is lower than in PCs and therefore a much lower interaction of the protease for the same surface pressure is observed in comparison to PC's

Summarizing, at a similar surface pressure, the changes produced in PC's are larger than in PE's. This means that the propensity of the interfacial change is larger when the hydration of the lipids is higher and the lateral interactions are weaker.

For a given cut off, the slopes of the curves depend on the acyl chain length (Figure 8). The slope of the $\Delta\Pi$ vs Π curve increases as DPPC < DMPC < DOPC < DPhPC [138]. That is, for a given hydration shell, the interaction varies with the unsaturation or branching of the acyl chains. This can be interpreted as a consequence that for unsaturated lipids more confined water is available at the interphase.

When lipids are spread on the surface of an air-water solution a decrease in the surface tension is observed. However, the decrease in surface tension does not drop to zero after the monolayer is formed unless it is at its highest packing, i.e the cut off. That is, when the hydration shells of the polar groups enter into contact.

Thus, the interaction of the protease with the membrane is controlled in large extent by the specific ability of the different lipid components to interact with the water phase. In particular, the arrangements around the carbonyl and phosphate groups are responsible to change the force profile of the interface being this described roughly as hydrophobic or hydrophilic character [139]. This imposes energetic and entropic restrictions to water that affect protein interaction.

Sequestering of water by the lipids in the formation of a monolayer imposes a different organization for which the water activity at the interphase region is decreased [140]. This could be related to the formation of clustering or tetrahedral arrays around the phosphate groups or the clathrate forms induced by hydrophobic regions and cholines, respectively.

Immobilized hydration water and confined water determines the surface free energy. The high free energy of the interphase region is responsible of the surface tension.

The connection between the surface pressure and the thermodynamic activity of water is described by [135, 140]

$$\mu = RT \ln a_i + A \pi = RT \ln a_w$$

$$\pi = (RT/A) \ln a_w/a_i$$

Replacing 1 for pure water and 0.268 for a_w and a_i , as that found in trehalose solutions respectively, (see Table V) we find that the surface pressure is around 10 mN/m. This is the change observed at low surface pressures in Figure 7.

The relation between hydration and confined water determines the water activity that the protein needs to interact with the membrane.

9. - Corollary: Does water at biological membrane interphases play a functional role?

The reductionistic approach that considers the membrane as a simple permeability barrier ignores the correlation between the cytoplasmic crowded macromolecules and the different states of the membrane caused by different water stresses.

The cell function is inherent to the fluctuations in local arrangements due to water exchange and this works as a connection between the intracellular material and the cell membrane. A fraction of water at the membrane level, possible at the inner plane and another underneath it in discrete sites (named indistinctively as defects, hydrophobic pockets etc.), may be assumed as dynamic domains where carbonyl groups oscillate between different orientations. In addition, hydration states affect the lateral contact of the P-N dipole in a qualitatively different magnitude in PC's and PE's membranes affecting the surface charge potential [146]

This means that the propagation and amplification of the surface effect depends on the lipid constituents [147, 148].

The organization of water at the defects is not available but they have been postulated as targets where amphiphilic molecules bind and displace water [19, 20]. The insertion of different types of molecules that may mimic water shed some light on the possible structure of these defects. Recent molecular dynamic studies showed that in fully hydrated

DMPC bilayers, the headgroups are extensively charge paired and hydrogen bonded with water molecules, forming a network structure in the headgroup region [141,142]. Even in a crystal of DMPC dihydrate, phosphate groups from two monolayers are linked together by a highly ordered water ribbon, and two adjacent phosphate groups in the same monolayer are bridged by a water molecule [143]. Thus, we would expect that the ordering of the water and the headgroups are strongly coupled.

This could be one of the several links proposed to master the functionality and its physiological state of living cells. By this it is understood that cells have a stationary value in the amount of water for which the maximum efficiency in its macromolecular structure is expressed. One expression of the physiological state of living cells is given by the protein-membrane interactions. Its understanding requires a detailed knowledge of the structure and the dynamical properties of the chemical groups, namely the sites of hydration at the membrane surface, extensively discussed in this review and the influence of water on protein dynamics [144]. The synergism of their respective hydrations could be modulated by the lipid species (in terms of head group and fatty acid chains) usually found in biological membranes [146]. Surface pressure (osmotic pressure) displaces water not severely bound; electrical polarization of the interphase groups may produce water exchange and H-bonding or apolar groups may replace water tightly bound to the exposed centers. These effects change the dipole potential, the area of the lipid interphase, the local defects and the ion distribution at the double layer [149].

The concept structure-function relationship is traditionally employed to frame an overwhelming amount of information obtained by a diversity of methodologies. These ones are able in certain stages to give details of structure at molecular level.

In order to establish a dialogue with the cell, signals derived from the metabolic process or as a response to environmental changes have to be interpreted. Welch and Berry [145] suggested that a proton networks is involved in regulating enzyme reactions within the cell, reactions that are predominantly of a redox nature. Proton currents may well flow throughout the extracellular matrix, and linked into the interior of every single cell through proton channels.

Volume and cell membrane potential are probably the most evident signal from which cells denotes the environmental changes. As seen in this review, both are directly or indirectly

determined by the organization for water at membrane level and within this at the interphase [149].

In physical terms, signal can be interpreted as the thermodynamic response of a system in a time dependent (irreversible) or a time independent (reversible) way. None of these extremes appear to be operative in a biological system. Therefore, fluctuations around metastable positions may be a reasonable answer. The vitality of a cell should be related to the control on these metastable states in the metabolism. These states, for many reasons appear related to hydration states. The equilibrium swelling of the lipids is relevant at the molecular level to modulate the phase state of the lipid membrane and its surface properties. The partial enthalpies of the process of hydration vary with the water content and this with the phase state of the membrane and the types of solutes interacting with it [147]. The thermodynamic picture is related to the surface thermodynamics [150]. The interpretation of the energetic changes needs to include the degree of freedom of the molecular species involved in the process [147]. In this review we extensively have shown this in terms of water and fluctuations of the surface groups of membrane constituents. Although still limited in the number of species considered, this illustrates the importance of giving interpretation to the molecular mechanisms governing cell response.

The prefix "meta" means beyond, behind, the end to which the actions or desires are directed. Bolism states by organism reactions. Metastability accounts for states that could further evolve to others.

Altogether metabolism means change or movement towards new metastable states.

The description of the membrane interphase as a non autonomous region appears as paradigm in the cell response giving relevance to the essential component of water.

10. REFERENCES

- [1] P. K. J. Kinnunen, Lipid bilayers as osmotic response elements, *Cell. Physiol. and Biochem.* 10 (2000) 243-250.
- [2] Clegg, J. S. (1983) Reversible dehydration and the aqueous compartments of cell. In: G Benga (ed.) *Water transport in biological membranes*. CRC Press, Boca Raton, FL
- [3] Crowe J. H., Clegg, J. S. (1973) *Anhydrobiosis*. Dowden, Hutchinson & Ross, Strousberg, PA
- [4] H. Brockman, Dipole potentials of lipid membranes, *Chem. Phys. Lipids.* 73 (1994) 57.
- [5] V. P. Tychinskii, Dynamic phase microscopy: is a "dialogue" with the cell possible? *Phys. Usp.* 50 (2007) 513-528.
- [6] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, in *Molecular Biology of the Cell*, Garland Science, New York, 2000.
- [7] R. Lipowsky, E. Sackmann, *Structure and Dynamics of Membranes (Handbook of Biological Physics)*, Elsevier, Amsterdam, 1995.
- [8] W. Rawicz, B. A. Smith, T. J. McIntosh, S. A. Simon and E. A. Evans, Elasticity, Strength, and Water Permeability of Bilayers that Contain Raft Microdomain-Forming Lipids, *Biophysical J.* March 3, 2008, doi:10.1529.
- [9] M. F. Chaplin, A proposal for structuring of water. *Biophys. Chem.* 83 (1999) 211- 221.
- [10] H. Binder, Water near lipid membranes as seen by infrared spectroscopy, *European Biophysics Journal* 36 (4-5) (2007) 265-279.
- [11] D. Carrier, P. T. T. Wong, Effect of dehydration and hydrostatic pressure on phosphatidylinositol biayers: infrared spectroscopic study, *Chem Phys. Lipids* 83, (1996) 141-152
- [12] T. Heimburg, A model for the Lipid Pretransition: Coupling of Ripple Formation with the Chain-Melting Transition, *Biophys. J.* 78 (2000)1154–1165.
- [13] T. Le Bihan and M. Pezolet, Study of the structure and phase behavior of dipalmitoylphosphatidylcholine by infrared spectroscopy: characterization of the pretransition and subtransition, *Chem. Phys. Lipids* 94 (1998) 13–33.

- [14] S. Banerjee, Exploring the ripple phase of biomembranes, *Physica A.* 308 (2002) 89-100.
- [15] O. G. Mouritsen, Theoretical models of phospholipid phase transitions, *Chem. Physics Lipids* 57 (1991) 179-194.
- [16] O. G. Mouritsen, K. Jørgensen, T. Hønger, in *Permeability and Stability of Lipid Bilayers*, (Disalvo-Simon eds), (1995) 37-160.
- [17] C. Tanford, *The hydrophobic effect*, Wiley, New York (1973) 16-21.
- [18] S. Channareddy, N. Janes, Direct Determination of Hydration in the Interdigitated and Ripple Phases of Dihexadecylphosphatidylcholine: Hydration of a Hydrophobic Cavity at the Membrane/Water Interface, *Biophysical Journal* 77 (4) (1999) 2046-2050.
- [19] Diaz, S., F. Amalfa, A.C. Biondi de Lopez, and E.A. Disalvo, 1999. Effect of water polarized at the carbonyl groups of phosphatidylcholines on the dipole potential of lipid bilayers, *Langmuir* 15:5179-5182
- [20] G. A. Senisterra, E. A. Disalvo, J. J. Gagliardino, Osmotic dependence of lysophosphatidylcholine lytic action on liposomes in the gel state, *Biochim. Biophys. Acta* 94 (1988) 264-270.
- [21] E. A. Disalvo, J. de Gier, Contribution of aqueous interphases to the permeability barrier of lipid bilayer for non-electrolytes, *Chem. Phys. Lipids* 32 (1983) 39-47.
- [22] J. Nagle and S. Tristam-Nagle, Structure of Lipid Bilayers, *Biochim. Biophys. Acta* 1469 (2000) 159-195.
- [23] D. L. Bernik, E. A. Disalvo, Gel state surface properties of phosphatidylcholine liposomes as measured with merocyanine 540, *Biochim. Biophys. Acta* 1146 (1993) 169-177
- [24] G. A. Yegiazaryan, A. H. Poghosyan, A. A. Shahinyan, The water molecules orientation around the dipalmitoylphosphatidylcholine head group: a molecular dynamics study, *Physica A.* 362 (2006) 197-203.
- [25] Clarke, R. J. The dipole potential of phospholipid membranes and methods for its detection, (2001) *Advances Colloid Interface Science*, 89-90: 263-281.
- [26] Lairion, F. and Disalvo, E. A. Effect of Dipole Potential Variations on The Surface Charge Potential of Lipid Membranes (2008) *Journal of Physical Chemistry A*

- [27] S. A. Simon and T. J. McIntosh, Depth of water penetration into lipid bilayers, *Methods in enzymology* 127 (1986) 511-521.
- [28] J. T. Davies, E. K. Rideal, in *Interfacial Phenomena*, Academic Press, New York, 1961.
- [29] M. Potts, Desiccation tolerance of prokaryotes, *Microbiol. Rev.* 58 (1994) 755–805.
- [30] E. E. Tymczyszyn, A. Gomez-Zavaglia, E. A. Disalvo, Effect of sugars and growth media on the dehydration of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *J. Appl. Microbiol.* 102 (2007) 845–851.
- [31] J. M. Wood, Osmosensing by bacteria: Signals and membrane-Based Sensors, *Microbiol. Mol. Biol. Rev.* 63 (1999) 230–262.
- [32] P. M. Texeira, H. Castro, C. Mohácsi-Farkas, R. J. Kirby, Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress, *Appl. Microbiol.* 83 (1997) 219–226.
- [33] J. T. Trevors, Fluorescent probes for bacterial cytoplasmic membrane research, *J. Biochem. Biophys. Methods* 57 (2003) 87–103.
- [34] E. A. Disalvo, Permeation of water and polar solutes in lipid bilayers, *Advances Colloid Interface. Sci.* 29 (1988) 141-170.
- [35] R. N. McElhaney, J. de Gier, E. C. M. van der Neut-Kok, The effect of alterations in fatty acid composition and cholesterol content on the nonelectrolyte permeability of *Acholeplasma laidlawii* B cells and derived liposomes, *Biochim. Biophys. Acta* 298 (1973) 500-512.
- [36] R. P. Rand, V. A. Parsegian, Hydration forces between phospholipids bilayers *Biochim Biophys Acta* 988 (1989) 351–376.
- [37] V. A. Parsegian, N. Fuller, R. P. Rand, Measured Work of Deformation and Repulsion of Lecithin Bilayers , *Proc. Natl. Acad. Sci. USA* 76 (1979) 2750–2754.
- [38] D. M. Le Neveu, R. P. Rand, V. A. Parsegian, Measurement of forces between lecithin bilayers *Nature* 259 (1976) 601–603.
- [39] S. Leikin, V. A. Parsegian, D. C. Rau, R. P. Rand, Hydration forces, *Annu Rev Phys Chem.* 44 (1993) 369-395.
- [40] S. A. Simon and T. J. McIntosh, Magnitude of the solvation pressure depends on dipole potential, *Proc. Natl. Acad. Sci. U.S.A.* 86 (1989) 9263-9267.
- [41] S. Bhide, M. Berkowitz, Structure and dynamics of water at the interface with phospholipid bilayers, *The Journal of Chemical Physics* 123 (2005) 224702 1.16.

- [42] A. Watts, Biophysics of the membrane interface, *Biochem. Soc. Trans.*, 23 (4) (1995) 959-965.
- [43] A. S. Ulrich, A. Watts, Molecular response of the lipid headgroup to bilayer hydration monitored by ²H-NMR, *Biophysical Journal* 66 (5) (1994) 1441-1449.
- [44] J. F. Nagle, R. Zhang, S. Tristram-Nagle, W. -S. Sun, H. I. Petrache, R. M. Suter, X-ray structure determination of fully hydrated L alpha phase dipalmitoylphosphatidylcholine bilayers *Biophys. J.* 70 (1996) 1419-1431.
- [45] S. H. White and M. C. Wiener, Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of X-ray and neutron diffraction data. III. Complete structure, *Biophys. J.*, 61 (2I) (1992) 434-447.
- [46] S. H. White and M. C. Wiener in "Permeability and Stability of Lipid Bilayers". (Disalvo, Simon, Eds). CRC. Press, Boca Raton, FL. (1995) pp. 1-20.
- [47] M. C. Luzardo, G. Peltzer, E. Disalvo, Surface potential of lipid interfaces formed by mixtures of phosphatidylcholine of different chain lengths *Langmuir* 14 (20) (1998) 5858-5861.
- [48] R. C. Mac Donald, S. A. Simon, Lipid Monolayer States and Their Relationships to Bilayers, *Proc. Natl. Acad. Sci. USA* 84 (1987) 4089-4093.
- [49] P.A. Janmey¹, and P. K. J. Kinnunen, Biophysical properties of lipids and dynamic membranes, *Trends in Cell Biology* 16 (10) (2006) 538-546
- [50] F. Lairion, E. A. Disalvo, Effect of phloretin on the potential of phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol monolayers, *Langmuir* 20 (2004) 9151-9155.
- [51] F. Lairion, E. A. Disalvo, Effect of arbutin on the dipole potential and area per lipid of ester and ether phosphatidylcholine and phosphatidylethanolamine monolayers, *Biochimica Biophysica Acta, Biomembranes* 1768 (2007) 450-456.
- [52] F. Lairion, E. A. Disalvo, Effect of trehalose on the contributions to the dipole potential of lipid monolayers, *Chemistry and Physics of lipids* 150 (2) (2007) 117-124
- [53] W.-J. Sun, R. M. Suter, M. A. Knewton, C. R. Worthington, S. Tristram-Nagle, R. Zang, J. F. Nagle, Order and Disorder in Fully Hydrated Unoriented Bilayers of Gel Phase DPPC, *Phys. Rev. E* 49 (1994) 4665-4676.
- [54] R. J. Pace, S. I. Chang, Molecular motions in lipid bilayers. II. Magnetic resonance of multilamellar and vesicle systems, *J. Chem Phys.* 76 (1982) 4217-4227.

- [55] G. Buldt, H. U. Gally, J. Seelig, G. Zaccai, Neutron diffraction studies on phosphatidylcholine model membranes. I. Head group conformation, *J. Mol. Biol.* 134 (1979) 673-691.
- [56] H. Schindler, J. Seelig, Deuterium order parameters in relation to thermodynamic properties of a phospholipid bilayer. A statistical mechanical interpretation, *Biochemistry* 14 (1975) 2283-2287.
- [57] B. A. Lewis, D. M. Engelman, Lipid bilayer thickness varies linearly with acyl chain length in fluid phosphatidylcholine vesicles, *J. Mol. Chem.* 166 (1983) 211-217.
- [58] M. J. Janiak, D. M. Small, G. G. Shipley, Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin, *J. Biol. Chem.* 254 (1979) 6068- 6078.
- [59] L. R. DeYoung, K. A. Dill, Solute Partitioning into Lipid Bilayer Membranes, *Biochemistry* 27 (1988) 5281-5289.
- [60] L. J. Lis, M. McAlister, N. Fuller, R. P. Rand, V. A. Parsegian, Interactions between neutral phospholipid bilayer membranes, *Biophys. J.* 37 (1982) 657-666.
- [61] R. L. Thurmond, S. W. Dodd, M. F. Brown, Molecular Areas of Phospholipids as Determined By ²H NMR Spectroscopy: Comparison of Phosphatidylethanolamines and Phosphatidylcholines, *Biophys. J.* 59 (1991) 108-113.
- [62] K. Hristova and S. H. White. Determination of the hydrocarbon core structure of fluid DOPC bilayers by x-ray diffraction using specific bromination of the double-bonds: effect of hydration, *Biophys. J.* 74 (1998) 2419–2433.
- [63] C. Selle, W. Pohle, Fourier Transform Infrared Spectroscopy as a probe for the study of the hydration of lipid self-assemblies. II. Water binding versus phase transitions, *Biospectroscopy* 4 (1998) 281– 294.
- [64] R. J. Mashl, H. L. Scott, S. Subramaniam and Eric Jakobsson, Molecular Simulation of Dioleoylphosphatidylcholine Lipid Bilayers at Differing Levels of Hydration, *Biophysical Journal* 81 (2001) 3005–3015.
- [65] F. Lairion, R. Filler, E. A. Disalvo, Reversed micelles as model systems to study the interfacial properties of lipid bilayers, *Colloids and Surface B: Biointerfaces* 25 (4) (2002) 369-371.
- [66] J. L. Arrondo, F. M. Goñi, J. M. Macarulla, Infrared spectroscopy of phosphatidylcholines in aqueous suspension a study of the phosphate group vibrations, *Biochimica et Biophysica Acta* 794 (1) (1984) 165-168.
- [67] F. M. Goñi and J. L. Arrondo, A study of phospholipid phosphate groups in model membranes by Fourier-transform infrared-spectroscopy, *Faraday Discussions of the Chemical Society* 81(1986) 117-126.

- [68] E. A. Disalvo, F. Lairion, S. Díaz, and J. Arroyo in "Recent research developments in biophysical chemistry" Physical chemistry of lipid interfaces: State of hydration, topological and electrical properties, Carlos A. Condat, Ana Baruzzi, Ed. Research Signpost (2002) 181-197.
- [69] J. Y. Lehtonen and P. K. Kinnunen, Poly(ethylene glycol)-induced and temperature-dependent phase separation in fluid binary phospholipid membranes, *Biophysical Journal* 68 (1995)525-535.
- [70] J. H. Crowe, F. A. Hoekstra, L. M. Crowe, Anhydrobiosis, *Annu. Rev. Physiol.* 54 (1992) 579-599.
- [71] M. C. Luzardo, F. Amalfa, A. Núñez, S. Díaz, A. C. Biondi de López, E. A. Disalvo, Effect of trehalose and sucrose on the hydration and dipole potential of lipid bilayers, *Biophys. J.* 78 (2000) 2452-2458.
- [72] J. H. Crowe, L. M. Crowe, J. F. Carpenter, C. A. Wistrom, Stabilization of dry phospholipid bilayers and proteins by sugars, *Biochem. J.* 242 (1987) 1-10.
- [73] G. L. Jendrasiak, The hydration of phospholipids and its biological significance, *Journal of Nutritional Biochemistry* 7 (11) (1996) 599-609.
- [74] Y. Marcus, Ion properties, Marcel Dekker, Inc., New York, 1997.
- [75] W. Pohle, C. Selle, H. Fritzsche, M. Bohl, Comparative FTIR spectroscopic study upon the hydration of lecithins and cephalins, *J. Mol. Struct.* 408/409 (1997) 273-277.
- [76] H. E. Alper, D. Bassolino-Klimas, T. R. Stouch, The limiting behaviour of water hydrating a phospholipid monolayer: A computer simulation study, *The Journal of Chemical Physics* 99 (7) (1993) 5547-5559.
- [77] G. L. Jendrasiak and R. L. Smith, The effect of the choline head group on phospholipid hydration, *Chem. Phys. Lipids* 131(2) (2004)183-195.
- [78] R. P. Taylor, C. H. Huang, A. V. Broccoli and L. Leake, Nuclear magnetic resonance studies of amphiphile hydration. Effects of cholesterol on phosphatidyl choline hydration, *Archives Biochem. Biophys* 183 (1) (1977) 83-89.
- [79] L. Ter-Minassian-Saraga, G. Madelmont, Cholesterol-induced modulation of membrane hydration studies by thermal analysis, *FEBS Letters* 137 (1) (1982) 137-140.
- [80] G. Cevc, Polymorphism of the bilayer membranes in the ordered phase and the molecular origin of the lipid pretransition and rippled lamellae, *Biochim. Biophys. Acta.* 1062 (1991) 59-69.

- [81] Y. -K. Shin, D. E. Budil and J. H. Freed, Thermodynamics and dynamics of phosphatidylcholine-cholesterol mixed model membranes in the liquid crystalline state: effects of water, *Biophysical Journal* 65 (3) (1993) 1283-1294.
- [82] S. J. Slater, C. Ho, F. J. Taddeo, M. B., Kelly, C. D. Stubbs, Contribution of hydrogen bonding to lipid-lipid interactions in membranes and the role of lipid order: effects of cholesterol, increased phospholipid unsaturation, and ethanol, *Biochemistry* 32 (14) (1993) 3714-3721.
- [83] K. Ariga, Y. Okahata, Hydration Behavior of Phospholipid Langmuir-Blodgett (LB) Films Deposited on a Quartz-Crystal Microbalance Depending on Temperatures in Water, *Langmuir* 10 (7) (1994) 2272-2276.
- [84] C. Ho, S. J. Slater, C. Stubbs, Hydration and Order in Lipid Bilayers, *Biochemistry* 3(1995) 6188- 6195.
- [85] T. J. McIntosh, Hydration properties of lamellar and non-lamellar phases of phosphatidylcholine and phosphatidylethanolamine, *Chemistry and Physics of Lipids* 81 (2) (1996) 117-131.
- [86] C.-H. Hsieh, S. -C. Sue, P. -C. Lyu, W. -G. Wu, Membrane packing geometry of diphtanoylphosphatidylcholine is highly sensitive to hydration: phospholipid polymorphism induced by molecular rearrangement in the headgroup region, *Biophysical Journal* 73 (2) (1997) 870-877.
- [87] D. Bach, I. R. Miller, Hydration of phospholipid bilayers in the presence and absence of cholesterol *Biochim. Biophys. Acta - Biomembranes* 1368 (2) (1998) 216-224.
- [88] S. Channareddy, N. Janes, Direct Determination of Hydration in the Interdigitated and Ripple Phases of Dihexadecylphosphatidylcholine: Hydration of a Hydrophobic Cavity at the Membrane/Water Interface, *Biophysical Journal* 77 (4) (1999) 2046-2050.
- [89] W. Hübner, A. Blume, Interactions at the lipid–water interface, *Chem. Phys. Lipids* 96 (1–2) (1998) 99–123.
- [90] R. N. A. H. Lewis, R. N. McElhaney, M. A. Monck and P. R. Cullis, Studies of highly asymmetric mixed-chain diacyl phosphatidylcholines that forms mixed-interdigitated gel phases: Fourier transform infrared and ^2H -NMR spectroscopic studies of hydrocarbon chain conformation and orientational order in the liquid-crystalline state, *Biophys. J.* 67 (1994) 197-207.
- [91] Lewis R. N.A.H., Pohle W. and McElhaney R.N. 1996, The Interfasial Structure of Phospholipid Bilayers: Differential Scanning Calorimetry and Fourier Transform Infrared Spectroscopic Studies of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoryl-choline and Its Dialkyl and Acyl-Alkyl Analogs, *Biophys. J.*: 70:2736-2746

- [92] R. N. A. H. Lewis, R. N. McElhaney, W. Pohle and H. H. Mantsch, The components of the carbonyl stretching band in the infrared spectra of hydrated 1,2-diacylglycerolipid bilayers: a re-evaluation, *Biophys. J.* 67 (1994) 2367-2375.
- [93] W. Pohle, C. Selle, W. Rettig, U. Heiser, B. Dobner, S. Wartewig. (2001) Phase transitions and hydrogen bonding in a bipolar phosphocholine evidenced by calorimetry and vibrational spectroscopy. *Arch. Biochem. Biophys.* 396 (2): 151-161.
- [94] W. Pohle, D.R. Gauger, H. Fritzsche, B. Rattay, C. Selle, H. Binder, H. Bohlig. (2001) FTIR-spectroscopic characterization of phosphocholine-headgroup model compounds. *J. Mol. Struct.* 563: 463-467.
- [95] W. Pohle, C. Selle, H. Fritzsche, H. Binder. (1998) Fourier transform infrared spectroscopy as a probe for the study of the hydration of lipid self-assemblies. I. Methodology and general phenomena. *Biospectroscopy* 4 (4): 267-280.
- [96] Gordon L. Jendrasiak and Ralph L. Smith, The effect of the choline head group on phospholipid hydration, *Chemistry and Physics of Lipids*, (2001) 113,(1-2)5 55-66
- [97] Pohle, W., D. R. Gauger, H. Fritzsche, B. Rattay, C. Selle, H. Binder and H. Böhlig FTIR-spectroscopic characterization of phosphocholine-headgroup model compounds *J. Mol. Struct.* (2001), 563/564, 463-467.
- [98] Binder, H., Kohlstrunk, B., Heerklotz, H.H. 1999, A humidity titration calorimetry technique to study the thermodynamics of hydration, *Chem Phys Lett* 304:329–335
- [99] Binder, H., Peinel, G. 1985, Behaviour of water at membrane surfaces—a molecular dynamics study, *J Mol Struct* 123:155–163
- [100] Binder, H. 2003, The molecular architecture of lipid membranes - New insights from hydration-tuning infrared linear dichroism spectroscopy, *Applied Spectroscopy Reviews* 38 (1), 15-69
- [101] H. Binder, B. Kohlstrunk, H. H. Heerklotz, Hydration and lyotropic melting of amphiphilic molecules—a thermodynamic study using humidity titration calorimetry, *J Colloid Interface Sci* 220 (1999) 235–249.
- [102] R. N. A. H. Lewis and R. N. McElhaney, in “Infrared Spectroscopy of Biomolecules”, Eds H.H. Mantsch and D. Chapman, Wiley-Liss, NY, 159–202 (1996).
- [103] H. Heerklotz, R. M. Epand, The Enthalpy of Acyl Chain Packing and the Apparent Water-Accessible Apolar Surface Area of Phospholipids, *Biophys J.* 80(1) (2001) 78.
- [104] D. A. Pink, S. McNeil, B. Quinn, M. J. Zuckermann, A model of hydrogen bond formation in phosphatidylethanolamine bilayers, *Biochim. Biophys. Acta.* 1368 (1998) 289–305.

- [105] H. U. Gally, W. Niederberger, J. Seelig, Conformation and motion of the choline head group in bilayers of dipalmitoyl-3-sn-phosphatidylcholine, *Biochemistry*. 14(16) (1975) 3647–3652.
- [106] S. B. Díaz, F. Amalfa, A. C. Biondi de López, E. A. Disalvo, Effect of water polarized at the carbonyl groups of phosphatidylcholines on the dipole potential of lipid bilayers, *Langmuir* 15 (15) (1999) 5179- 5182.
- [107] S. B. Díaz, F. Lairion, J. Arroyo, A. C. Biondi de Lopez, E. A. Disalvo, Contribution of phosphate groups to the dipole potential of dimyristoylphosphatidylcholine membranes, *Langmuir* 17 (3) (2001) 852-855.
- [108] M. A. Frías, S. B. Díaz, N. M. Ale, A. Ben Altabef and E. Disalvo, FTIR analysis of the interaction of arbutin with dimyristoyl phosphatidylcholine in anhydrous and hydrated states, *Biochimica et Biophysica Acta* 1758 (2006) 1823–1829.
- [109] M. A. Frías, A. Nicastro, N. Casado, A. Gennaro, S. B. Díaz, E. A. Disalvo, Arbutin blocks defects in the ripple phase of DMPC bilayers by changing carbonyl organization, *Chemistry and Physics of Lipids* 147 (2007) 22- 29.
- [110] G. Mingtao, H. Jack Freed, Hydration, Structure, and Molecular Interactions in the Headgroup Region of Dioleoylphosphatidylcholine Bilayers: An Electron Spin Resonance Study, *Biophysical Journal* 85(6) (2003) 4023–4040.
- [111] J. Yguerabide and M. C. Foster, Fluorescence spectroscopy of biological membranes. In: E. Grell, Editor, *Membrane Spectroscopy*, Springer, New York, (1981) 199–269.
- [112] E. A. Disalvo, L. I. Viera, L. S. Bakas, G. A. Senisterra, Lysophospholipids as Natural Molecular Harpoons Sensing Defects at Lipid Membranes, *J. Coll. Interf. Sci.* 178 (1996) 417-425.
- [113] A. E. Oliver, L. M. Crowe, P. S. de Araujo, E. Fisk, J. H. Crowe, Arbutin inhibits PLA₂ in partially hydrated model systems, *Biochim Biophys Acta* 1302(1) (1996) 69-78.
- [114] T. H. Haines and L. S. Leivovitch, in *Permeability and Stability of Lipids Bilayers*, (Disalvo and Simon eds), C.R.C. Press, Florida 1995, 123-136.
- [115] B. Bechinger and J. Seelig, Interaction of electric dipoles with phospholipid head groups. A ²H and ³¹P NMR study of phloretin analogues in phosphatidylcholine membranes, *Biochemistry* 30 (1991) 3923-3929.
- [116] A. Seelig, P. R. Allegrini, J. Seelig, Partitioning of local anesthetics into membranes: surface charge effects monitored by the phospholipid head-group. *Biochim. Biophys. Acta*, 939 (2) (1988) 267.

- [117] I. R. Miller, D. Bach, Structure and membrane properties of lecithin monolayers at the polarized mercury/water interface, *J. Colloid Interface Sci.* 29 (1969), 250.
- [118] D. Bach, I. R. Miller, The inhibition of oxygen reduction by adsorbed monolayers of phospholipids, proteins and synthetic polybases, *Electrochim. Acta* 15 (1970) 533.
- [119] M R Moncelli, L Becucci, A Nelson, and R Guidelli , Electrochemical modeling of electron and proton transfer to ubiquinone-10 in a self-assembled phospholipid monolayer, *Biophys. J.* 70 (1996) 2716-2726.
- [120] D. Bach, I. R. Miller, F. Robert, Transport of ions across lipoprotein monolayers adsorbed at the polarized mercury/water interface, *Chem. Phys. Lipids*, 4 (1970) 269.
- [121] J. Lyklema in *Physical Chemistry of Biological Interfaces* (A. Baszkin and W. Norde, eds) Marce Dekker, New York, 2000, 1-47.
- [122] K. Gawrisch, D. Ruston, J. Zimmerberg, V. A. Parsegian, R. P. Rand, N. Fuller, Membrane dipole potentials, hydration forces and the ordering of water at the membrane surfaces, *Biophys. J.* 61 (1992) 1213-1223.
- [123] J. Y. A. Lehtonen, P. K. J. Kinnunen, Changes in the lipid Dynamics of liposomal Membranes Induced by Pole(Ethylene Glycol) : Free volume alterations receaed by Inter- and Intra molecular escier-foring Phospholipids analogs, *Biophysical J.* 66 (1994) 1981-1990.
- [124] T. Söderlund, J-M. I. Alakostela, A. L. Pakkanen, and P. K. J. Kinnunen. Comparison of the Effects of Surface Tension and Osmotic Pressure on the Interfacial Hydration of a Fluid Phospholipid Bilayer, *Biophysical J.* 85 (2003) 2333-2341.
- [125] T. Lazrak, A. Milon, G. Wolff, A. M. Albrecht, M. Miele, G. Ourisson and Y. Nakatani, Comparison of the effects of inserted C40- and C so-terminally dihydroxylated carotenoids on the mechanical properties of various phospholipid vesicles, *Biochim. Biophys. Acta.* 903 (1987)132-141. See also Erratum, *Biochim. Biophys. Acta.* 22 (1988) 427.
- [126] J. M. Pope and B. A. Cornell, A pulsed NMR study of lipids, bound water and sodium ions in macroscopically oriented lecithin/water lyotropic liquid crystal model membrane systems, *Chem. Phys. Lipids.* 24 (1979) 27-43.
- [127] G. W. Hunter, T. C. Squier, Phospholipid acyl chain rotational dynamics are independent of headgroup structure in unilamellar vesicles containing binary mixtures of dioleoyl-phosphatidylcholine and dioleoyl-phosphatidylethanolamine, *Biochim. Biophys. Acta* 1415(1) (1998) 63-76.

- [128] K. H. Cheng, M. Ruonala, J. Virtanen and P. Somerharju, Evidence for superlattice arrangements in fluid phosphatidylcholine/phosphatidylethanolamine bilayers, *Biophys J.* 73(4) (1997) 1967-1976.
- [129] A. Nelson, N. Auffret, Phospholipid monolayers of di-oleoyl lecithin at the mercury/water interface, *J. Electroanal. Chem.*, 244 (1988) 99
- [130] M. Kodama, T. Miyata, Effect of the head group of phospholipids on the acyl-chain packing and structure of their assemblies as revealed by microcalorimetry and electron microscopy, *Colloids and Surfaces A*, 109 (1996) 283.
- [131] S. H. Almaleck, F. Lairion, E. A. Disalvo and G. J. Gordillo, Lipid monolayers on Hg as a valid experimental model for lipid membranes under electrical fields, *Chemistry and Physics of Lipids* 139 (2006) 150-156.
- [132] H. Aoki, M. Kodama, The behavior of water molecules in semi-crystalline bilayer structure of phosphatidylethanolamine, *Journal of Thermal Analysis and Calorimetry*, 49(2) (1997) 839.
- [133] S. Leekumjorn, A. Sum. Molecular Simulation Study of Structural and Dynamic Properties of Mixed DPPC/DPPE, *Bilayers. Biophys. J.*, 90 (2006) 3951.
- [134] R. Defay, I. Prigogine, *Surface Tension and Adsorption*, John Wiley & Sons, New York (1966).
- [135] S. Damodaran, Water activity at interfaces and its role in regulation of interfacial enzymes: A hypothesis, *Colloids and Surfaces B: Biointerfaces* 11 (5) (1998) 231-237.
- [136] G. Schwarz and S. E. Taylor, Polymorphism and Interactions of a Viral Fusion Peptide in a Compressed Lipid Monolayer, *Biophysical J.* 76 (1999) 3167-3175
- [137]. T. J. McIntosh, A. Vidal, S. A. Simon, The energetics of peptide-lipid interactions: Modulation by interfacial dipoles and cholesterol” in *Peptide-Lipid interactions* (Simon-McIntosh, eds.) Academic Press. Chapter 11, 309-338 (2002).
- [138] M. F. Martini and E.A. Disalvo, Superficially Active Water in Lipid Membranes and its Influence on the Interaction of an aqueous soluble Protease, *Biochim. Biophys. Acta.* 1768 (10) (2007) 2541-2548.
- [139] M. F. Martini, E. A. Disalvo, Effect of polar head groups on the activity of aspartyl protease adsorbed to lipid membranes, *Chemistry and Physics of Lipids* 122 (1-2) (2003) 177-183.

- [140] C. S. Rao and S. Damodaran, Surface pressure dependence of phospholipase A2 activity in lipid monolayers is linked to interfacial water activity, *Colloids and Surface B. Biointerfaces* 34 (2004) 197-204.
- [141] M. Pasenkiewicz-Gierula, Y. Takaoka, H. Miyagawa, K. Kitamura, A. Kusumi, Hydrogen bonding of water to phosphatidylcholine in the membrane as studied by a molecular dynamics simulation: location, geometry, and lipid-lipid bridging via hydrogen-bonded water, *J. Phys. Chem. A* 101 (1997) 3677–3691.
- [142] M. Pasenkiewicz-Gierula, Y. Takaoka, H. Miyagawa, K. Kitamura, A. Kusumi, Charge pairing of headgroups in phosphatidylcholine membranes: a molecular dynamics simulation study, *Biophys. J.* 76 (1999) 1228–1240.
- [143] H. Hauser, I. Pascher, and S. Sundell, Preferred Conformation and Dynamics of the Glycerol Backbone in Phospholipids. An NMR and X-ray Single-Crystal Analysis, *Biochemistry*, 27 (1988) 9166-9174
- [144] L. Zhou and S. A. Siegelbaum, Effects of Surface Water on Protein Dynamics Studied by a Novel Coarse-Grained Normal, *Biophys. J.*, 94 (2008) 3461-3474.
- [145] G.R. Welch and M.N. Berry, Long-range energy continua in the living cell: protochemical considerations. In: H. Fröhlich and F. Kremer, Editors, *Coherent Excitations in Biological Systems*, Springer, Heidelberg (1983), pp. 115–140.
- [146] H. Binder, B. Kohlstrunk, and W. Pohle Thermodynamic and Kinetic Aspects of Lyotropic Solvation-Induced Transitions in Phosphatidylcholine and Phosphatidylethanolamine Assemblies Revealed by Humidity Titration Calorimetry 2000, *J. Phys. Chem. B*, 104, 12049-12055.
- [147] H. Wennestrom and E. Spaar, Thermodynamics of membrane hydration, *Pure Appl. Chem.* 75 (7), (2003) 905-912.
- [148] Kalani J. Seu, Lee R. Cambrea, R. Michael Everly and Jennifer S. Hovis, Influence of Lipid Chemistry on Membrane Fluidity: Tail and Headgroup Interactions (2006) *Biophysical J.* 91, 3727–3735
- [149] Collins K. D., Washabaugh M.W. The Hofmeister effect and the behaviour of water at interfaces (1985) *Quart Rev Biophys* 18:323–422
- [150] T. Soderlund, J.-M. I. Alakoskela, A. L. Pakkanen, and P. K. J. Kinnunen, Comparison of the Effects of Surface Tension and Osmotic Pressure on the Interfacial Hydration of a Fluid Phospholipid Bilayer (2003) *Biophysical J.* 85:2333-2341

LEGENDS TO THE FIGURES

FIGURE 1

PROTRUSION OF THE GROUPS ESTERIFIED TO THE PHOSPHATE IN THE SURFACE OF THE MEMBRANE

FIGURE 2

THE INTERPHASE OF A LIPID MEMBRANE

The interphase region confined between two planes: A) an inner plane containing the carbonyls (- - -) and B) An outer plane tangent to the external phosphates (....). The region between the two planes confines the hydrated groups and water.

FIGURE 3

OSMOMETER BEHAVIOR OF CELLS AND LIPID VESICLES

Volume % of cells (■) and of lipid vesicles (□) exposed to increasing concentrations of NaCl in the outer media. Final volumes are plotted as a function of the inverse of NaCl concentration to show the behavior of cells and lipid vesicles obeying the Boyle-van't Hoff equation.

FIGURE 4

DETERMINATION OF THE AREA PER LIPID IN MONOLAYERS

Saturation values of DMPC monolayers spread on an air-water interphase at 18°C (□) and 28°C (■). Arrows indicate the saturation value at which areas are calculated (see text and refs. 50-52).

FIGURE 5

DEFECTS IN THE LIPID SURFACE

Schematic representation of the orientation of the C=O at the water-membrane interface (A) , phosphocholine or ethanolamine (P-N) alignment (B) and CH₂ exposure (C).

FIGURE 6

RELATION BETWEEN THE DECREASE IN DIPOLE POTENTIAL WITH THE WATER DISPLACEMENT BY TREHALOSE AND PHLORETIN IN DIFFERENT LIPIDS.

(■) dipole potential (♦) water activity a_w

FIGURE 7

VOLTAMETRIC RESPONSE OF DMPE IN THE CENTRAL REGION OF POTENTIAL RANGE.

Temperature = 23 °C. Sweep rate = 1 V s⁻¹. Electrode area = 0.018 cm²

FIGURE 8

CHANGE IN INTERFACIAL TENSION OF MONOLAYER WHEN A PROTEIN IS INJECTED AT THE SUBPHASE AS A FUNCTION OF ITS INITIAL VALUE

Changes of the surface pressure of monolayers of phosphatidylcholine with different acyl chains, due to the protease, at 25°C. (●) DPhPC; (■) DOPC; (▲) DMPC; (♦) DPPC; (△) DMPE. Final enzyme concentration in the subphase was 1.57 μM. Each point was measured by triplicate. Each point and its repetitions were independent measures.

ABBREVIATIONS AND ACRONYMS

DMPC 1, 2-Dimyristoyl-sn-Glycero-3-Phosphocholine
 DPPC 1, 2 Dipalmitoyl - sn- 3-Phosphocholine
 DPPE 1, 2 Dipalmitoyl - sn- 3- Phosphoethanolamine
 DMPE 1, 2-Dimyristoyl-sn-Glycero-3-Phosphoethanolamine
 Di (ether) PC, di tetradecyl phosphatodylcholine
 DMPG Dimyristoyl Phosphatidylglycerol
 DOPC 1, 2 Dioleoyl - sn- 3- Phosphocholine
 DPhPC 1, 2 Diphytanoyl - sn- 3- Phosphocholine
 PEG, polyethylene glycol.
 PC Phosphatidylcholine
 PE Phosphatidylethanolamine
 L_{α} Lamellar Liquid-crystalline
 L_{β} gel planar phase
 P_{β} gel corrugated phase
 P-N phosphocholine or phosphoethanolamine dipole as described in Figure 5.
 $\nu_{C=O}$ Carbonyl Stretching
 ν_{CH_2} Methylene Asymmetric Stretching
 $\nu_{PO_2^-}$ Phosphate Asymmetric Stretching
 LDW Low Density Water
 HDW High Density Water
 FTIR Fourier Transform Infrared Espectroscopy
 DSC Differential Scanning Calorimetry
 a_w Water activity
 NMR Nuclear Magnetic Resonance
 E_{pzc} Zero charge potential
 π : Surface pressure (mN/m)
 T_c , T_m T_m , hydrocarbon chain-melting phase transition temperature.

FIGURE 1

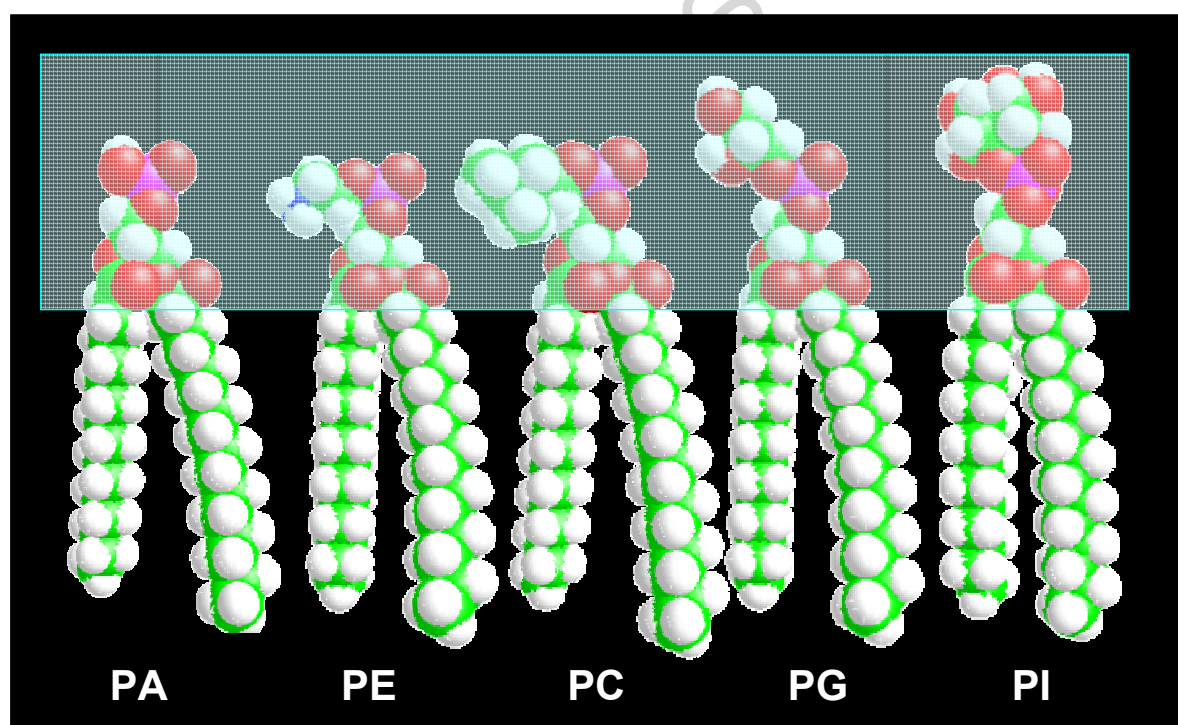


FIGURE 2

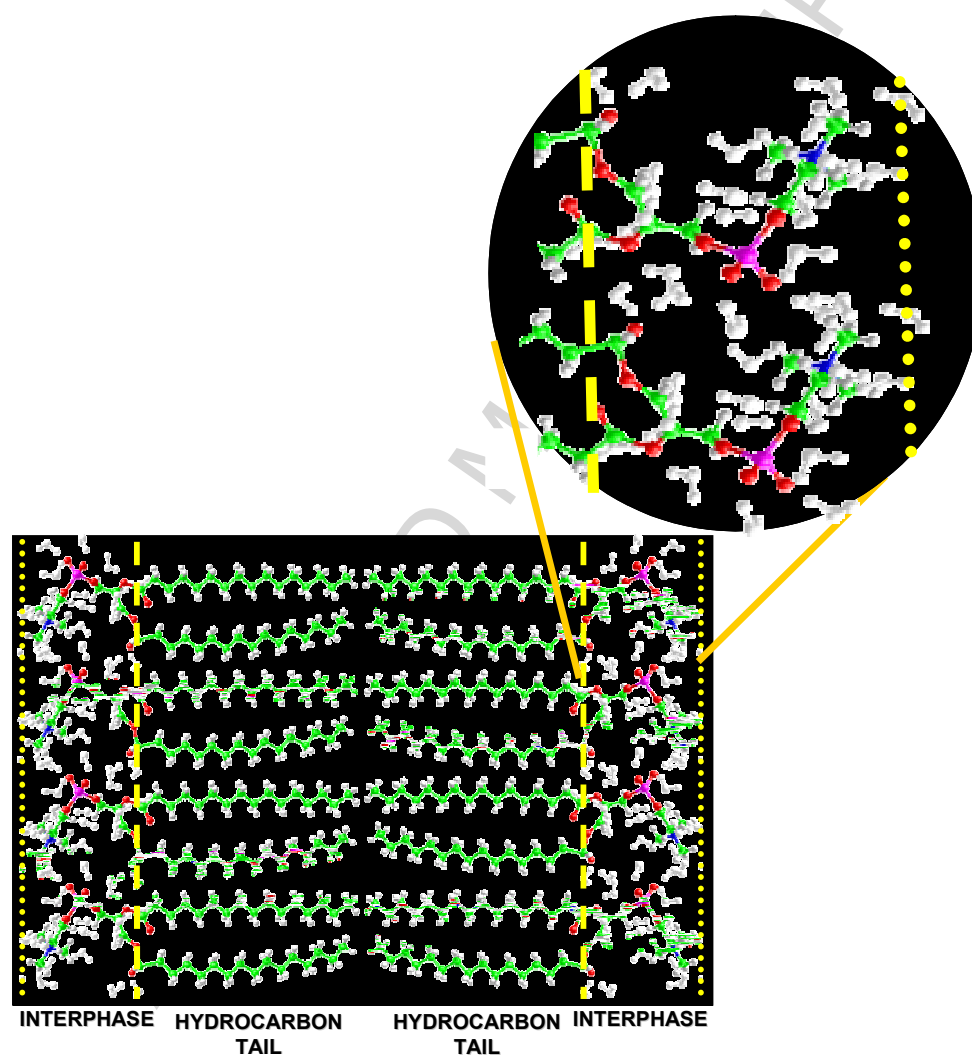


FIGURE 3

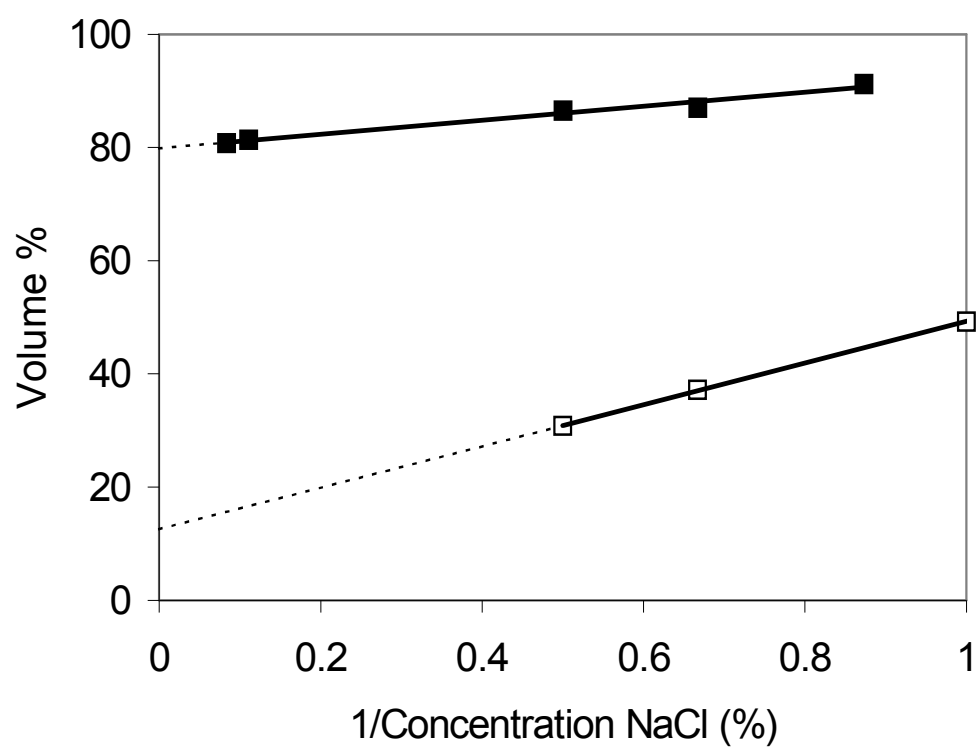


FIGURE 4

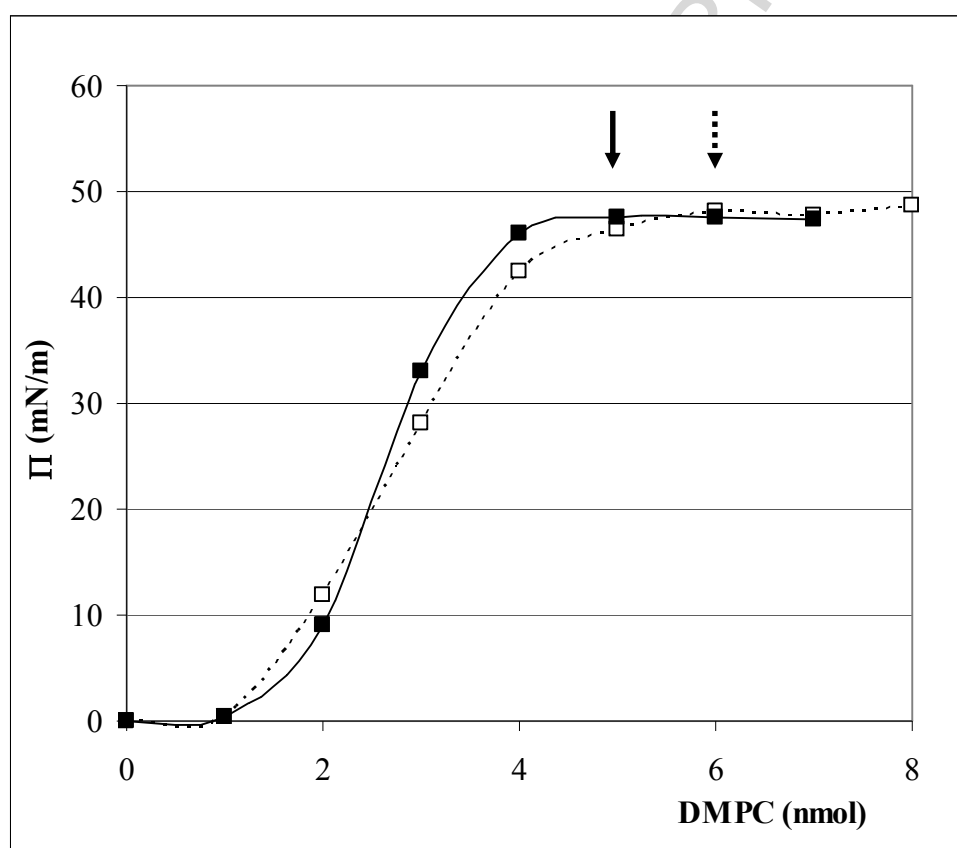


FIGURE 5

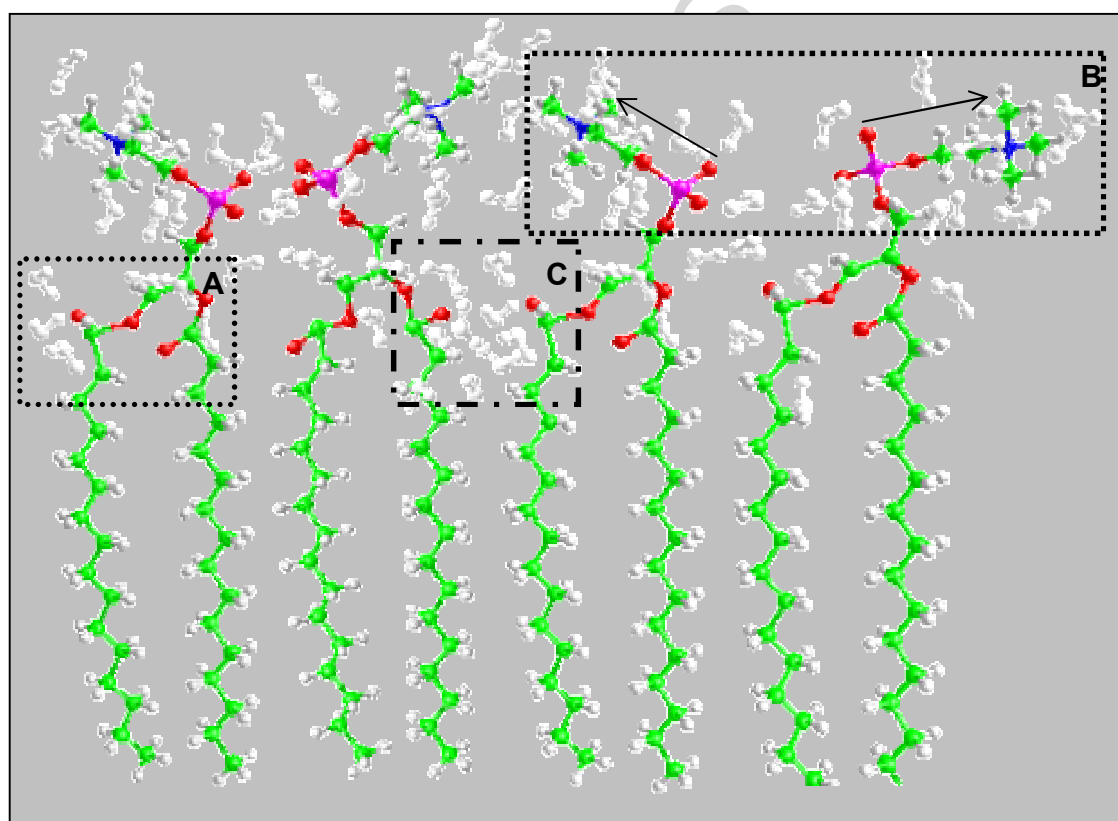


FIGURE 6

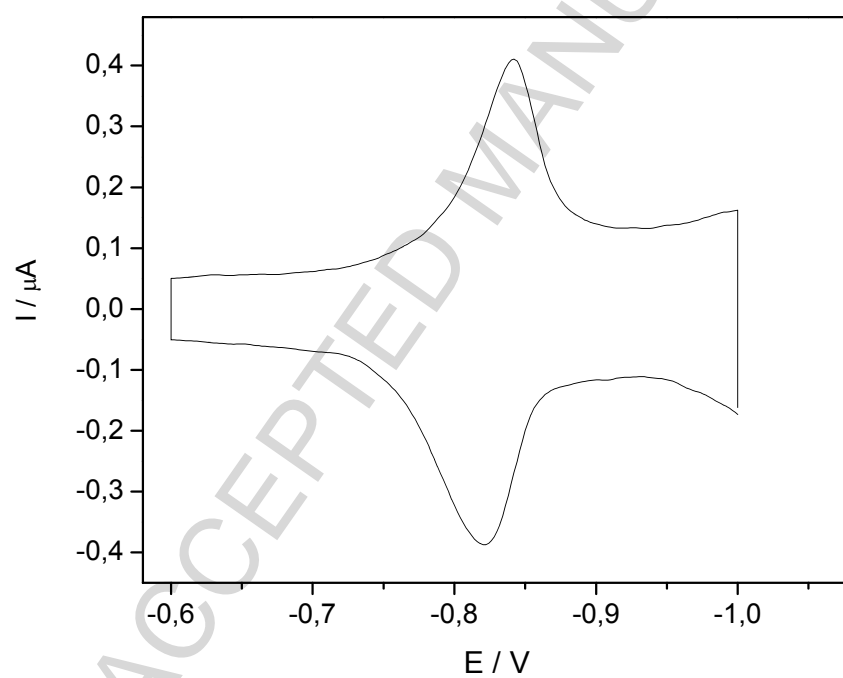


FIGURE 7

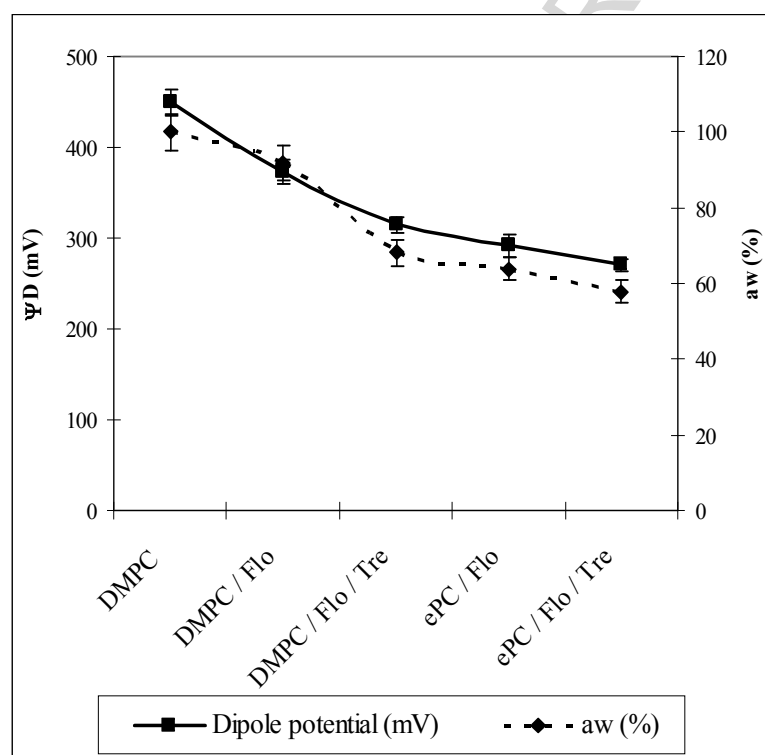


FIGURA 8

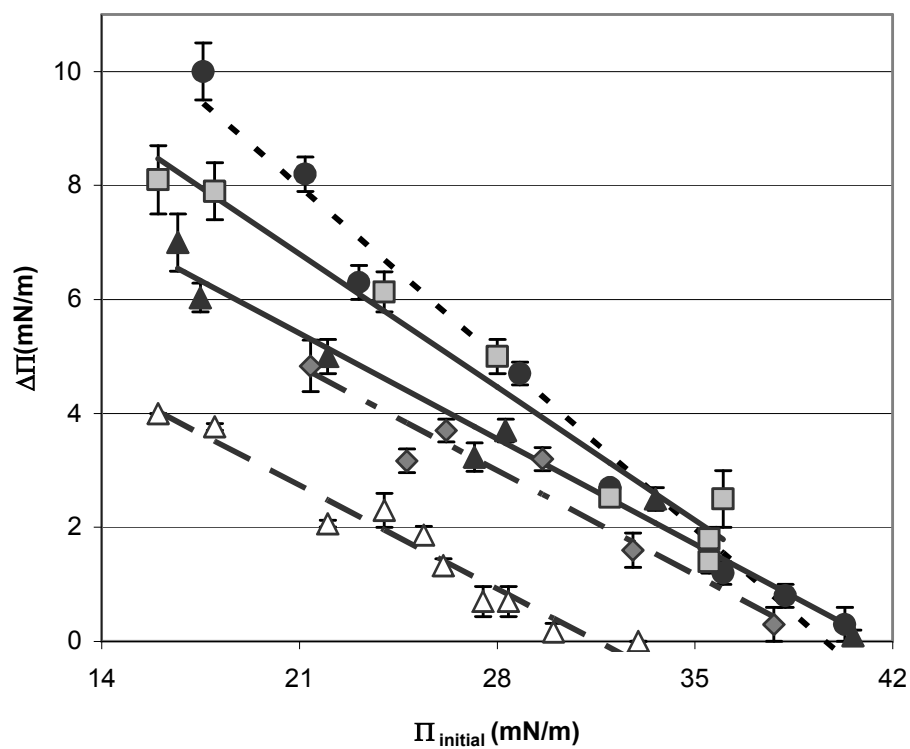


TABLE I
WATER /LIPID IN PC'S AND PE'S AS DETERMINED BY THE REVERSE
MICELLE FORMATION AND BY THE LIMIT OF SATURATION IN
MONOLAYERS.

	Water per lipid at the break of <i>Bragg spacing</i> [64, 64]	Water per lipid in monolayers [50, 52]	Water per lipid in micelles [65]	Water at phosphate (FTIR) (66,67)
DMPC	12	11	12-14	6
DMPE	ND	9	4	ND

TABLE II

FREQUENCY VALUES OF THE CARBONYL GROUP POPULATION FOR
DMPC MEMBRANES IN DIFFERENT PHASE STATES

DMPC	$\tilde{\nu}_p / \text{cm}^{-1}$ st C=O (<i>Unbounded</i>) [108-109]	$\tilde{\nu}_p / \text{cm}^{-1}$ st C=O (<i>Bounded</i>)	Difference between bound and unbound frequencies $\Delta\tilde{\nu} / \text{cm}^{-1}$
Fluid phase (L_α) (30°C)	1738,0	1721,5	15,5
Ripple phase (P_β) (18°C)	1742,0	1724,0	18
Planar gel phase (L_β) (10°C)	1738,4	1730,7	7,7
<i>Solid</i> DMPC	1740,0	1740	-

TABLE III

FREQUENCY SHIFT OF THE ASYMMETRIC STRETCHING BAND OF PO_2^- GROUPS IN PC AND ETHER-PC, s IN THE PRESENCE OF POLYHYDROXILATED COMPOUNDS (T= 18°C) IN P_β' PHASE

	$\tilde{\nu}_p / \text{cm}^{-1}$ st antisym PO_2^-	$\Delta\tilde{\nu} / \text{cm}^{-1}$	Area per lipid (\AA^2)
Pure DMPC (28°C)	1229,5	0	65.4 ± 4.4
Pure DMPC (18°C)	1228.2	0	56.28 ± 2.5
PC/arbutin (0,10M)	1212,7	-16,8	84.4 ± 2.3
PC/trehalose (0,10M)(28°C)	1207,0	-22,5	112.6 ± 18.7
PC/phloretin (1:1)	1200,0	-29,5	84.5 ± 10.6
Pure di (ether) PC (18°C)	1225.5	0	67.7 ± 1.3
Pure di (ether) PC (28°C)	1225.5	0	84.68± 1.3
Di (ether)PC/trehalosa (28°C)	ND	ND	84.68± 1.5

TABLE IV
EFFECT OF OH COMPOUNDS ON THE POPULATION OF CARBONYL
GROUPS OF PC'S. (T= 18°C) [106-108]

	$\tilde{\nu}_p / \text{cm}^{-1}$ st C=O (<i>Unbound</i>)	Displacement of <i>Unbound</i> frequency from pure lipid $\Delta\tilde{\nu} / \text{cm}^{-1}$	$\tilde{\nu}_p / \text{cm}^{-1}$ st C=O (<i>Bound</i>)	Displacement of <i>bound</i> frequency for pure lipid $\Delta\tilde{\nu} / \text{cm}^{-1}$	Difference between bound and unbound frequencies $\Delta\tilde{\nu} / \text{cm}^{-1}$
Pure DMPC	1742,0	0,0	1724,0	0,0	18
PC/trehalose (0,10M)	1734,2	-7,8	1715,0	-9,0	19
PC/sucrose (0,10M)	1743,5	+1,4	1728,1	+4,1	15
PC/phloretin (1:1)	1740,0	+2,0	1725,0	+1,0	15
PC/arbutin (0,10M)	1737,3	-4,7	1728,7	+4,7	9

TABLE V
WATER DISPLACEMENT FOR HYDRATION SITES BY DIFFERENT OH
COMPOUNDS [68]

	a_w	Water molecules remained in the hydration shell
DMPC	0,480 +- 0,011	18
DMPC:Phl (1:0,3)	0,441 +- 0,015	16,5
DMPC:Phl (1:1)	0,317 +- 0,015	12
DMPC + Tre 0,15 M	0,265 +- 0,006	7
Ether-PC:Phl (1:0,3)	0,307 +- 0,011	11,5
Ether-PC:Phl (1:0,3) + Tre 0,15 M	0,278 +- 0,010	7

TABLE VI
DIPOLE POTENTIAL OF DMPC AND DMPE WITH AND WITHOUT
CARBONYL GROUPS IN THE PRESENCE OF TREHALOSE

	(I) Dipole potential (mV) (without trehalose)	(II) Dipole potential (mV) (with 0.15M trehalose)	Difference (I-II)
DMPC	449.1	391.7	60
Di(ETER) PC	360.0	325.7	35
DMPE	525.5	464.5	60
Di(ETER) PE	362.8	369.8	8

TABLE VII
EFFECT OF THE UNEVEN DISTRIBUTION OF OH COMPOUNDS ON THE
POPULATION OF CARBONYL GROUPS OF PC'S.

	DMPC	DMPC/Arbutin (0,10M) sym (isotonic) [108,109]	$\Delta\tilde{\nu}/\text{cm}^{-1}$	DMPC/Arbutin (0,10M) asym (hypertonic) [108,109]	$\Delta\tilde{\nu}/\text{cm}^{-1}$
$\tilde{\nu}_p/\text{cm}^{-1}$ st antisym PO_2^-	1229,5	1212,7	-16,8	1211,4	-18,1
$\tilde{\nu}_p/\text{cm}^{-1}$ st sym PO_2^-	1086,3	1085,2	-1,1	1083,2	-3,1
$\tilde{\nu}_p/\text{cm}^{-1}$ C=O (unbounded)	1742,0	1737,3	-4,7	1730,6	-11.4
$\tilde{\nu}_p/\text{cm}^{-1}$ C=O (bounded)	1724,0	1728,7	4,7	1717,5	-6.5

TABLE VIII
POTENTIAL PEAKS AND CAPACITANCE VARIATIONS IN DMPC AND
DMPE MONOLAYERS ADSORBED ON MERCURY

LIPID	E_p (V)	Capacitance before peak ($\mu\text{F cm}^{-2}$)	Capacitance after peak ($\mu\text{F cm}^{-2}$)	Difference ($\mu\text{F cm}^{-2}$)
DMPC	-0.72	2.2	8.2	6.0
DMPE	-0.84	2.2	7.1	4.9

TABLE IX
CUT OFF AND SATURATION AREA FOR DIFFERENT MONOLAYER (138)

Type of Lipid	$\Pi_{\text{cut off}} \pm \text{sd}$ (mN/m)	$\Pi_{\text{saturation}} \pm \text{sd}$ (mN/m)	$\Delta\Pi$ (mN/m) ($\Pi_{\text{saturation}} - \Pi_{\text{cut off}}$)
DPhPC	39.6 \pm 0.4	48.0 \pm 0.7	8.4 \pm 1.1
DOPC	41.4 \pm 0.3	47.2 \pm 0.9	5.8 \pm 1.2
DMPC	41.5 \pm 0.5	47.8 \pm 0.8	6.3 \pm 1.3
DPPC	39.5 \pm 0.9	46.6 \pm 0.6	7.1 \pm 1.5
DMPE	30.6 \pm 0.1	45.0 \pm 0.5	14.4 \pm 0.6
D(ether)PC	31.9 \pm 0.3	48.0 \pm 0.7	16.1 \pm 1.0
D(ether)PE	29.4 \pm 0.6	44.5 \pm 0.5	15.1 \pm 1.1