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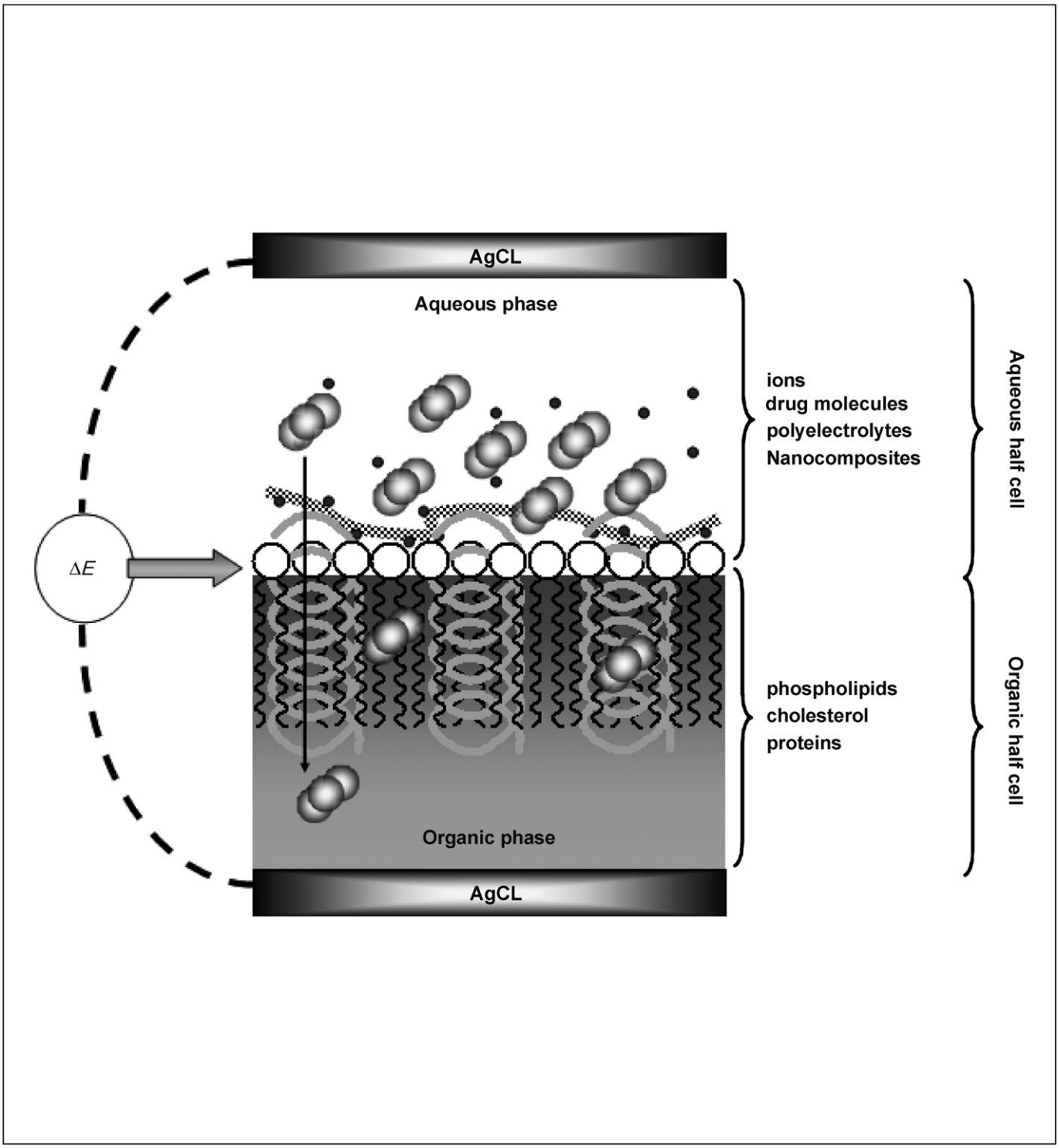
Electrochemical Properties of Phospholipid Monolayers at Liquid–Liquid Interfaces

Hélder A. Santos,*[a] Vladimir García-Morales,[b] and Carlos M. Pereira[c]



Biomembrane models built at the interface between two immiscible electrolytes (ITIES) are useful systems to study phenomena of biological relevance by means of their electrochemical processes. The unique properties of ITIES allow one either to control or measure the potential difference across the biomimetic membranes. Herein we focus on phospholipid monolayers adsorbed at liquid–liquid interfaces, and besides discussing recent developments on the subject, we describe electrochemical techniques that can be used to get insight on the interfacial processes and electrostatic properties of phospholipid membranes at the ITIES. In particular, we examine the electrochemical and physicochemical properties of (modified) phospholipid monolayers and their interaction with other biologically relevant compounds. The use of liquid–liquid electrochemistry as a powerful tool to characterize drug properties is outlined. Although this review is not a survey of all the work in the field, it provides a comprehensive referencing to current research.

1. Introduction

Effective understanding of interactions at the cellular level requires deep understanding of the interactions of the individual components that compose the cell membranes, since these are the first barriers, for example, for the transport of exogenous chemicals (the majority of common drugs) across the biological membranes.

The membranes in living cells play a central role in both the structure and function of all cells,[1] e.g. they regulate recognition events and solute permeability. Therefore, simple models of membrane functions are of great importance to understand some of the mechanisms taking place at the cellular level,[2] such as ion/drug transfer, drug delivery, and membrane activity.[3–8] Despite their different functions, all biomembranes have a basic phospholipid bilayer structure.[1,2] The lipid molecules are arranged in a continuous bimolecular layer with a thickness of approximately 50 Å.[1,9] Cell membranes are mainly composed of lipids and proteins. The phospholipids constitute more than half of the total lipid mass in most cell membranes, and they can be divided into the following most common classes (Figure 1 a): phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylserines (PSs), phosphatidylinositols (PIs), phosphatidic acids (PAs), and phosphatidylglcerols (PGs).[1,10] Phospholipids are commonly characterised by the structural features of their head groups (chemical composition, charge), their polar backbone (glycerol, etc.) and their hydrocarbon chains (number of chains, number of carbons/chain, chain saturation). Another component of the cell membrane is cholesterol. It modifies the structure and dynamic properties of the membrane by changing the packing properties within the bilayer,[11] depending on the phase state and phospholipid composition of the bilayers, as well as the cholesterol concentration. Carbohydrates are also found in many membranes, covalently bound either to proteins as constituents of glycoproteins or to lipids as constituents of glycolipids. The proteins embedded in the lipid bilayer, lipids themselves, carbohydrates and cholesterol form a characteristic pattern with locally enriched lipids floating as domains on the membranes.

Biological models aim to provide a convenient model system for studying molecular interactions in the lipid backbone of biomembranes, for example, between phospholipids, and between phospholipids, proteins, carbohydrates, enzymes or drug molecules.[7,8,12–15] As a result of their unique properties, biomembrane models with variable complexities and can

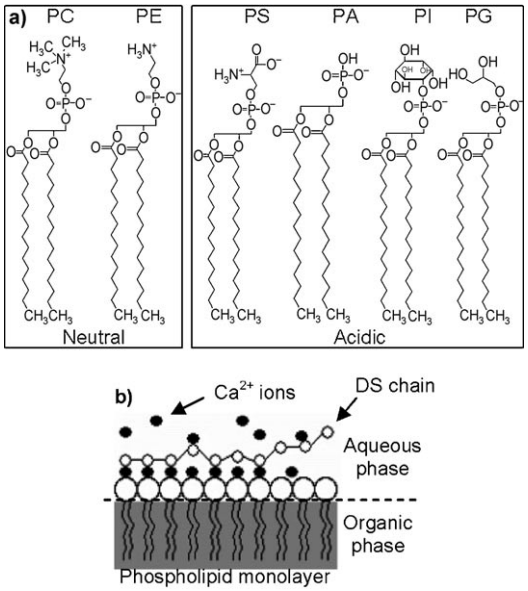


Figure 1. a) Chemical structure of different types of glycerophospholipids. b) Schematic of the ITIES interface with a phospholipid monolayer deposited in an organic phase and a dextran sulfate (DS) chain in Ca^{2+}-containing aqueous phase.

be formed at liquid–liquid interfaces and are well-suited to elucidate structural details of the bilayers membrane and to mimic its functions.[2–4] In this context, lipid monolayers at liquid–liquid interfaces have been the basis for numerous technical applications in fields such as biochemistry, electrochemistry, chemistry and biology, and have attracted particular interest for studies of the charge transfer, electroanalysis, drug delivery and membrane activity.[3,16–18] The study of electrochemical processes at the interface between two immiscible electro-

[a] *Dr. H. A. Santos*
Division of Pharmaceutical Technology
Faculty of Pharmacy, University of Helsinki
P.O. Box 56 Viikinkaari 5 E, 00014 (Finland)
Fax: (+358) 191 59 160
E-mail: helder.santos@helsinki.fi

[b] *Dr. V. García-Morales*
Physik-Department E19
Technische Universität München
James-Franck-Str. 1, 85748 Garching (Germany)

[c] *Prof. C. M. Pereira*
Departamento de Química
Faculdade de Ciências, Universidade do Porto
Rua do Campo Alegre 687, 4169-007 Porto (Portugal)

lytes (ITIES) has become a very active area of research in contemporary bio-electrochemistry. From an electrochemical point of view an interface is defined as a boundary between two distinct phases. The ITIES is an interface arising between two immiscible electrolyte solutions. An electrolyte, a medium with ionic conductivity and mobile charge carriers, introduces additional properties to the system of two immiscible phases not observed at a water–oil interface. In this way, the two phases are conductive and the charge between the two phases can achieve equilibrium through ion transport at the interface. With a proper setup, the interface between the two liquids can be made into a boundary that behaves, for electroanalytical purposes, like an electrode. The observed current flow is therefore governed by the transport of charged species across the interface.

Increasing interest in the properties of phospholipid monolayers at the polarised ITIES[19–25] were invoked by the possibility to investigate the stability and ion permeability of the monolayer as a function of the well-defined interfacial potential difference (electric field).[16,17] In the literature, phospholipid monolayers are regarded as a suitable model system for one half of the lipid backbone of biomembranes.[27–31] Traditionally, monolayers at the air–water and water–non-polar interfaces were mainly studied by the Langmuir balance technique.[4,32] Unlike a Langmuir monolayer, the phospholipid monolayer at ITIES is in a thermodynamic equilibrium with the bulk phospholipid dissolved in the liquid (usually organic) phase. By spreading a monolayer on a water–oil interface, the molecule interactions can be followed over a larger range of molecular separations due to weaker cohesion among the hydrocarbon chain of lipid molecules.[22,23] In the study of ion/drug transfer across a lipid monolayer, electrochemical methodology becomes more convenient than Langmuir techniques.[14,19,22,23,33–35] When a potential difference is applied to the ITIES, the charge is separated across the ITIES through the formation of electrical double-layers,[3] which are affected by the presence of phospholipid monolayers[14,15,36] that in turn affect the ion transfer rate. In Figure 1b is depicted an interfacial interaction at the ITIES via calcium ions between a phospholipid monolayer deposited in an organic phase and a polysaccharide chain (dextran sulfate, DS) deposited in an aqueous phase.[14] Interfacial tension[5,19,22,24,37–39] and capacitance[21,25,26,37] measurements are widely used to study the physiochemical properties of the phospholipid monolayers at the ITIES. These techniques are useful to elucidate the monolayer stability's strong dependence on the potential difference across the aqueous and the organic phases, on the pH of the aqueous phase, as well as on the nature of the aqueous cation, both affecting the ionic state of the phospholipid.

The literature regarding the electrochemistry at liquid–liquid interfaces is vast, and it is beyond the scope of this review to overview all aspects of this topic. Instead, herein we focus on recent progress on the electrochemical and physicochemical characterization of phospholipid-modified monolayers adsorbed at the ITIES. In particular, we describe the use of phospholipid monolayers as a biomembrane model to study specific biological interactions and how electrochemical methodology (experimental and theoretical) can be employed to provide information on ion/drug transfer through the adsorbed monolayers at the ITIES. Finally, we demonstrate liquid–liquid electrochemistry as a powerful tool to characterize molecules potentially relevant in biology and pharmaceutical sciences.

The discussion above highlights some of the advantages of using liquid–liquid interfaces for studying biological phenomena compared to other approaches. For example, while an indispensable asset for fundamental studies of lipid monolayers and an important tool in the construction of supported model membranes, the air–water interface is difficult to implement in a practically applicable biomembrane model when compared to liquid–liquid interfaces. The nature of the air–water interface renders the monolayer immovable and prevents permeation studies of non-gaseous compounds. Electrochemical studies of lipid monolayers can also be performed on Hg drop electrodes. However, these systems usually hamper the use of non-electrochemical characterisation methods, which, combined with the toxic nature of mercury and the preclusion of ion transfer studies across the lipid monolayers, are important limi-

Dr. Hélder A. Santos obtained his PhD in Physical Chemistry and Electrochemistry from Helsinki University of Technology in 2007 under the supervision of Prof. K. Kontturi and Dr. L. Murtomäki. He is currently an Academy of Finland post-doctoral researcher in Prof. Jouni Hirvonen's group at the University of Helsinki. His current work is focused on pharmaceutical and physicochemical characterization of nano- and micro-particles.

Dr. Vladimir García-Morales obtained his PhD in Physics from University of Valencia in 2005 under the supervision of Prof. J. A. Manzanares and Prof. J. Pellicer. He is currently a post-doctoral researcher in Prof. K. Krischer's group at the Technische Universität München. His research work focuses on nanothermodynamics and the theoretical modelling of nonlinear electrochemical systems.

Prof. Carlos M. Pereira obtained his PhD in Chemistry from University of Porto in 1997 under the supervision of Prof. Fernando Silva. He is currently Assistant Professor. His research work focuses on analytical applications of liquid–liquid interfaces, and electrochemical characterization of surfaces modified by nanoparticles and composites films and ionic-liquid–electrode interfaces.

tations for this system. Liposomes typically used to study membrane structure and function have the disadvantage that only one side of the membrane is available when compared to liquid–liquid interfaces, which limits their usefulness for electrochemical approaches. Unsupported lipid membranes also hinder their use as model membranes in biological studies because they are extremely fragile and are an unstable construction over time. This limits their use for prolonged studies, and for practical applications such as biosensors and electronic devices. Furthermore, electrified liquid–liquid interfaces present the advantage of simultaneous and minute control of the experimental parameters of both sides of the lipid layer. In the following sections we present and discuss further advantages of modified liquid–liquid interfaces as a biomimetic system and how they can be useful to understand biological phenomena.

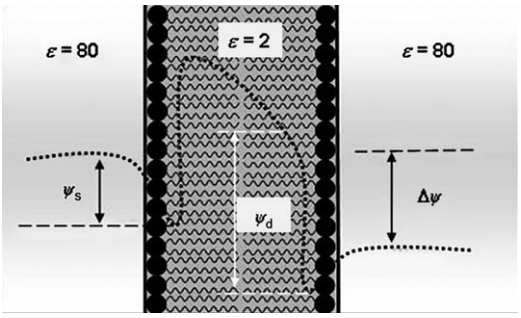


Figure 2. Electrical potential profile across a phospholipid bilayer showing the contribution of the surface potential (ψ_s) originated from the charged headgroups of the lipids and the concentration of ionic species at the membrane-solution interface. The dipole potential (ψ_d) results from the alignment of the lipids and water dipoles between the aqueous phases and the hydrocarbon region of the membrane, and the transmembrane potential difference ($\Delta\psi$) represents the overall difference in potential between the two bulk phases separated by the membrane. Adapted from ref. [44].

2. Model Membranes

The assembly of opposed phospholipid monolayers in a lipid bilayer is considered to be weak,[40] which is in agreement with the fact that phase transitions in liposomes can take place in one leaflet independent of the other.[4,32–34,36,41] Model phospholipid monolayers are a simple, controllable and well-defined system to study interactions in the lipid backbone of biomembranes, as well as other biological reactions in two dimensions.[8,15,12,42,43] Furthermore, the phase transitions[4,32] of phospholipid monolayers and bilayers are in a corresponding state when the surface pressure of the monolayer is 30–35 mN m^{-1}.[4] The state of a lipid monolayer also determines its electric properties.[41] The transverse structure of the bilayer causes the charged and dipolar lipid groups to be relatively fixed with respect to their orientation and location from the bilayer centre.[44] Consequently, these charges and dipoles are only partially compensated by water dipoles and solution electrolytes, and a complex electric profile is generated over the membrane (Figure 2).

Lipid monolayers at the ITIES are formed upon contact of an aqueous and an organic solution of poor mutual miscibility.[19] Since electrochemical techniques are sensitive to the mass transfer of charged ions across the liquid–liquid interface, complex chemical reactions, unique to biological systems, can be designed and valuable information about such systems can be gained. The advantages of the liquid–liquid interfaces compared with other systems are their smooth, dynamic and defect-free structure, their non-reactive nature and the possibility to follow ion or drug transfer from one phase to another. This is accomplished by using an organic solvent which allows sufficient dissociation of organic salts into free ions and is sufficiently non-polar to prevent significant partitioning of aqueous electrolytes. There are two ways to control the potential across the ITIES: 1) by dissolving a single common ion in both aqueous and organic phases (non-polarisable interface); and 2) with an external electric circuit, when very hydrophilic and very hydrophobic electrolytes are present in aqueous and organic phases, respectively (polarisable interface). The variation of the electrical potential between the two liquids is closely related to the distribution of the ionic and dipolar components across the ITIES. In general, there is an excess electrical charge on one side of the interface, which, due to the electroneutrality condition, has to be compensated for by an excess of opposite charge on the other side. Such a charge separation is usually referred to as the formation of an electrical double-layer.[45,46] Measurements at the ITIES are usually carried out in a three- or four-electrode electrochemical cell, where the interfacial potential difference is controlled or measured with the help of two reference electrodes and the current is supplied by means of two auxilary electrodes, one of which is placed in the aqueous phase and the second one in the organic phase (Scheme 1).

The electrochemical response from the system can be obtained by applying an electrical potential across the interface. The Galvani potential drop across the polarisable interface, $\Delta_o^w\phi$ (between the aqueous and organic phases, $\Delta_o^w\phi = \phi_w - \phi_o$), can be calculated by subtracting the potential of the reference phase from the measured cell potential, *E*. The energy required for transferring ions from one phase to the other is described by the standard transfer potential of the ion, $\Delta_o^w\phi_i^0$, defined as the difference of the solvation energies of the ion in the respective phases according to Equation (1):

			σ	
Ag|AgCl	X^+ Cl^-	X^+ Y^-, adsorbed phospholipids	M^+ Cl^-, drug	AgCl|Ag'
reference electrode	reference phase, *w'*	organic phase, *o*	aqueous phase, *w*	reference electrode

Scheme 1. Typical experimental setup of an electrochemical cell at the ITIES in the presence of a phospholipid monolayer. XCl, XY and MCl denote the base electrolytes in the reference, organic and aqueous phases, respectively. The σ indicates the polarisable interface under study, and w′ is the reference phase.

$$\Delta_o^w \phi_i^0 = \frac{\Delta G_{i,tr}^{w\to o}}{z_i F} = \frac{\mu_i^{0,o} - \mu_i^{0,w}}{z_i F} \quad (1)$$

where $\Delta G_{i,tr}^{w\to o}$ is the Gibbs free energy of transfer of species *i*, $\mu_i^{0,o}$ and $\mu_i^{0,w}$ are the standard chemical potentials of ion *i* in the organic and aqueous phases, respectively, z_i is the ion charge of the ionic species *i*, and *F* is the Faraday constant. Much information about the ITIES is gained by application of techniques that involve measurements of the macroscopic properties, such as interfacial tension and differential capacitance.[19,22,23] The analysis of these properties in terms of various microscopic models has us allowed to reach conclusions about the distribution of ions and molecules at the ITIES.

Electrochemical techniques, such as cyclic voltammetry, ac voltammetry, and electrochemical impedance spectroscopy (EIS),[45] are sensitive enough to characterize and detect interfacial processes at the ITIES. Information on the physicochemical properties of the lipid monolayer adsorbed at the ITIES, such as the effect of the monolayer state on the potential distribution across the monolayer, phase transition of the adsorbed phospholipid monolayer, and on ion, drug or electron transfer across it, can then be studied.[22,23] The electrostatic properties of the interface can be addressed by means of plots of the interfacial capacitance curves vs the potential drop applied (Figure 3). The impedance data is usually interpreted in terms of the Randles-type equivalent circuit, that is, the parallel combination of a capacitor and a Warburg impedance,[45] with the solution resistance in series.[3,45,46]

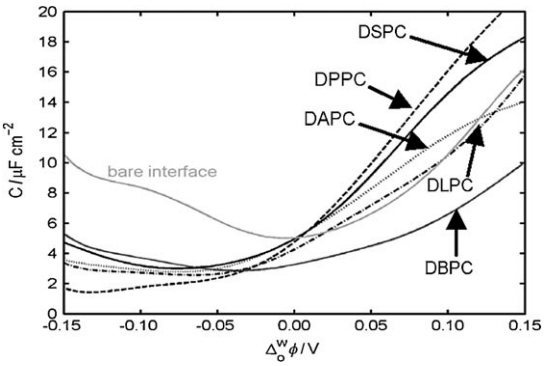


Figure 3. Experimental capacitance curves for several phospholipid monolayers adsorbed at the water-1,2-dichloroethane (DCE) interface. Phospholipids: dilauryl-PC (DLPC, C10); dipalmitoyl-PC (DPPC, C16); distearoyl-PC (DSPC, C18); diarachidoyl-PC (DAPC, C:20); and dibehenoyl-PC (DBPC, C:22). Adapted from ref. [6]; copyright © (2007) Elsevier Science B.V.

Most of the published work on lipid monolayers adsorbed at the ITIES rely on impedance techniques to study the behaviour of a number of phospholipids adsorbed at planar water–oil interfaces.[6–8,15,16,20–26] Surface tension techniques are also commonly employed, but herein we mainly focus on the capacitance data in the following sections. Based on the features of the capacitance–potential curves (Figure 3), it is possible to distinguish the adsorption behaviour of phospholipid monolayers at liquid–liquid interfaces, which can then be categorized into groups according to their saturated chains of carbons and minimum capacitances. For example, the shapes of capacitance curves of the long-chain PCs ($C > 22$) were flatter, exhibiting minimum capacitances over potential regions of 100 mV, compared to the parabolic-shaped capacitance curves of the short-chain lipids ($C < 22$).[4,6,22,23,36,32]

3. Theoretical Modelling of Phospholipid Monolayers at Liquid–Liquid Interfaces

As discussed in the previous section, capacitance measurements provide valuable qualitative and quantitative insight on lipid monolayers deposited at the liquid–liquid interfaces and their interactions with charged ions and nanostructures in solution as described below. A well-established model for the electrostatic potential profile in each liquid phase and the interface is given by the solution of the Poisson–Boltzmann (PB) equation, which means that the diffuse double-layer follows the Gouy–Chapman theory.[3,14,27,45,47] In the case of a 1:1 electrolyte, PB theory has recently been shown to provide an excellent account of the concentrations of ionic species obtained through molecular dynamics simulations in the aqueous phase close to lipid bilayers.[48] PB theory also allows the capacitance to be calculated and it was satisfactorily employed in a number of works[6,8,14,15,49–52] to yield qualitative valuable information of the experimentally observed capacitance curves.

It is well-known that a phospholipid monolayer adsorbed at the ITIES induces changes in the electrical structure of the interface.[36] The effect of the monolayer on the rate of ion transfer can be described assuming a sharp interface. In addition, it was observed that the presence of the phospholipids decreased the applied potential. The potential profile within the hydrocarbon region was almost linear and, although continuous at the interface, it reached a peak leading to a discontinuity of the electric field as a result of the net surface charge at the interface.[6,36] Surface potentials of a lipid bilayer and monolayer of similar composition are expected to be identical when the mean molecular area of the lipids is the same. Likewise, the magnitude of the dipole potential depends on the lateral packing of the monolayer. Values between 270 mV and 411 mV were measured for air–water monolayers of 30–40 mN m^{-1},[13] similar to dipole potentials observed for lipid bilayers.[53]

A sketch of the hydrocarbon region (hc), the aqueous (w) and organic (o) bulk phases with relative permittivities, ε_i (where i = hc, w, o), and the salt ions represented by their concentrations, $c_{i,k}^b$ (*k* labels the ionic species in solution) are shown in Figure 4.

In the theoretical modelling, the boundary value problem to solve the PB equation consists essentially of three different regions: I) the organic phase ($x < -d$), II) the hydrocarbon region ($-d < x < 0$), and III) the aqueous phase ($x > 0$). The phospholipid headgroups are considered to be parallel to the interface and are located at the plane $x = 0$. Defining the ITIES as the plane where the phospholipid positive headgroups are located (Figure 4), it is shown that the potential distributions in the region $0 < x < -d$ are nonlinear due to the presence of aqueous ions.[36] The surface charge due to the bound cations is

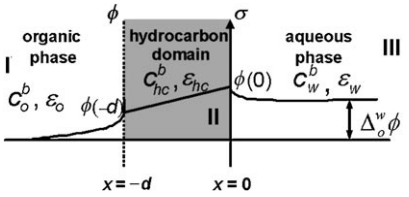


Figure 4. Three-layer model used to describe the potential distribution across the interfacial region. Adapted from ref. [49].

given by $\sigma = \alpha e/A$ (e is the elementary charge, α a dimensionless quantity and A is the phospholipid mean molecular area in the monolayer). The electric potential distribution in the region I is described by the PB Equation (2):

$$\frac{d^2\phi}{dx^2} = -\frac{e}{\varepsilon_i \varepsilon_0} \sum_i K_{i,k} z_k c^b_{i,k} \exp\left\{-\frac{e z_k [(\phi(x) - \phi_i)]}{k_B T}\right\} \quad (2)$$

where $K_{i,k} = c^b_{i,k}/c^b_o$ for (i = hc) and $K_{i,k} = 1$ for (i = o, w) represent the partition coefficient of ionic species k in region i, ε_0 is the vacuum permittivity, k_B the Boltzmann constant, and T the temperature. The boundary value problem is closed by specifying the boundary conditions at $\pm\infty$ and the continuity of the electric displacement vector at the interface, as described in Equations (3a) and (3b), respectively:

$$\left.\begin{aligned} &\phi_o = \phi_{hc} = \phi(x \to -\infty) = 0 \quad \phi_w = \phi(x \to +\infty) = \Delta^w_o\phi \\ &\left(\frac{d\phi}{dx}\right)_{x\to\pm\infty} = 0 \end{aligned}\right\} \quad (3a)$$

$$\left.\begin{aligned} &\varepsilon_o \left(\frac{d\phi}{dx}\right)_{x=-d^-} = \varepsilon_{hc} \left(\frac{d\phi}{dx}\right)_{x=-d^+} \\ &\varepsilon_w \left(\frac{d\phi}{dx}\right)_{x=0^+} = \varepsilon_{hc} \left(\frac{d\phi}{dx}\right)_{x=0^-} + \sigma \end{aligned}\right\} \quad (3b)$$

The ion size is neglected or it is considered that the plane of adsorption coincides with the plane of the phospholipid headgroups. The potential distribution $\phi(x)$ is obtained by solving the system Equation (2) subject to conditions of Equations (3a) and (3b). The interfacial capacitance is defined as in Equation (4):

$$C = \frac{\partial Q}{\partial \Delta^w_o \phi} \quad (4)$$

where Q is the surface charge density separated across the ITIES, which, since the system is electroneutral, can be evaluated by Equation (5)

$$Q = -\int_{-\infty}^{0} \rho dx = \varepsilon_0 \int_{-\infty}^{-d} \varepsilon_o \frac{d^2\phi}{dx^2} dx + \varepsilon_0 \int_{-d}^{0} \varepsilon_{hc} \frac{d^2\phi}{dx^2} dx \quad (5)$$

The interfacial capacitance is then calculated from Equation (4) by numerical differentiation. The features observed from the experimental capacitance curves can then be satisfactorily explained.[8,14,15] They are dependent mainly on the thickness of the hydrocarbon region d, the dimensionless surface charge density at the interface α, and the partition coefficient of the hydrocarbon region K_{hc}. The parameter d affects the capacitance values, as it is expected from the expression of the geometrical capacitance of this layer, $C_{geom} = A\varepsilon_o\varepsilon_{hc}/d$. An increase in this parameter lead to a decrease of the overall capacitance.[14,49] The dimensionless surface charge density, α, horizontally shifts the capacitance curves. Positive α values displace the capacitance minimum to negative potentials and the contrary holds for negative α. Finally, the curvature of the capacitance curves increases with increasing the partition coefficient K_{hc}. Recently it was demonstrated that polyelectrolyte and nanostructure multilayers[50,52] can additionally be adsorbed at the lipid monolayer creating an additional region within the aqueous phase ($0 < x < d_m$). Here d_m denotes the location of the multilayer–aqueous-phase interface which can bear a surface density charge σ_m. In this refined model, the lipid monolayer and the multilayer form an ion-free layer where the potential drop was assumed to be linear. The excess charge of the layer, σ, was located in a plane d at the aqueous interface. This qualitative model reproduced the capacitance curves measured at the multilayer-covered liquid–liquid interface.[50,51] The magnitude of the capacitance and the shape of the curve was governed by the dielectric permittivity and the thickness of the multilayer, while the surface charge at the interface determined the location of the capacitance minimum. All above equations were valid, but an additional boundary condition for the multilayer–water interface was introduced according to Equation (6):

$$\varepsilon_w \left(\frac{d\phi}{dx}\right)_{x=d_m} = \varepsilon_m \left(\frac{d\phi}{dx}\right)_{x=d_m^-} + \sigma_m \quad (6)$$

where ε_m is the permittivity within the multilayer and σ_m the surface charge density at the multilayer–water interface.

A major limitation of the abovementioned theory is the overestimation of the interfacial capacitance, especially for asymmetrical electrolytes and in the case of ion-pair formation at the interface.[54–56] Also, in the presence of electrolytes, the results are satisfactorily described by the model only at low concentrations. This is due to the fact that the model predicts very high concentration of ions at the interfaces with high potentials. Adsorption of hydrophobic and hydrophilic ions at the non-polarizable ITIES was recently analyzed with three distinct models: the Gouy–Chapman model, ions as hard spheres, and ion-pair formation at the interface.[54] Only the third model accounting for the interfacial ion-pairing as the main origin of adsorption [analyzed using the amphiphilic isotherm (Markin–Volkov isotherm)][55] showed a good agreement between ion-pairing theory and experimental values. Therefore, for a correct evaluation of many parameters such as interfacial tension, surface excesses, adsorption site area, and attraction or repulsion between adsorbed ions, an ion-pair formation model at the ITIES is preferred.[54,55] This takes into account the possibility of substitution of both solvent molecules and possible aggregation at the oil–water interface.

4. Electrochemical Applications of Phospholipid-Modified Monolayers

The interfacial amount of lipid is limited when phospholipid monolayers are adsorbed from the organic phase. The adsorption equilibrium is reached slowly, in 1.5–2 h[22,23] and lipids are unavoidably present in the organic phase. Since it is known that the lipid monolayer had a great effect on the rate of charge transfer,[1,22,23] efforts were made in order to better monitor those effects.

4.1. Combination of Langmuir and Electrochemical Techniques

For simultaneous control of both the surface pressure and the potential drop across of a monolayer at water–oil interfaces, the Langmuir technique was combined with electrochemical control over the interface.[14,33,34] This system also allowed the monitoring of the transfer rate of two probe ions, such cationic propranolol and anionic picrate, in the presence of the lipid monolayer.[33]

While this method allowed the surface pressure of the lipid monolayer to be controlled, it suffered from some limitations. For example, large interfacial area hampered the electrochemical measurements due to uneven potential distribution and instability of the monolayer due to its dissolution to the bulk organic phase. Under these conditions the use of ac voltammetry or EIS is restricted, invalidating the acquisition of quantitative information on the interfacial capacitance and membrane activity (the tendency of a compound to interact with a biological membrane) of various probe ions. This hurdle was overcome by combining the Langmuir–Blodgett technique with an immobilised liquid–liquid interface,[25,43,49] which consisted of a gelled organic phase. In this way the quality and reproducibility of data at the ITIES, as well as the monolayer stability and the control of lipid packing of monolayers at the ITIES were improved. After compression of the lipid monolayer at the air–water interface to a desired surface pressure, the monolayers were transferred to an electrochemical cell [containing *o*-nitrophenyloctylether (*o*-NPOE) gelled organic phase] by dipping the cell through the monolayer, into the aqueous phase (Figure 5). In this case, the solid substrate composed by a gelled organic solvent served simultaneously as a polymer support for the lipid monolayer and an electrochemical half-cell.

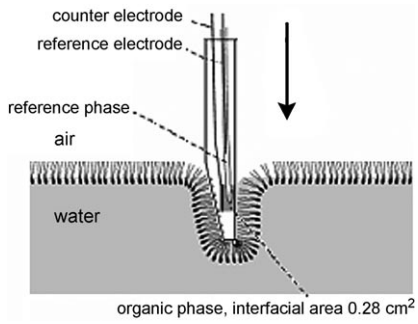


Figure 5. Electrochemical half-cell used in electrochemical measurements and the Langmuir–Blodgett deposition procedure to produce monolayers at the liquid–liquid interface. Modified from ref. [25].

The complexity of the liquid–liquid interfacial system can be increased by adding other model membrane components to the lipid monolayers, such as proteins or cholesterol, or to include hydrophilic compounds in the aqueous phase, such as polysaccharides and enzymes. This way an evaluation of their effect on the ion/drug transfer across the biological barriers can be accessed. Cholesterol, for example, modifies the structure and dynamic properties of the membrane by changing the packing properties within the bilayers (depending on the phase state and phospholipid composition of the bilayers, as well as the cholesterol concentration).[11,72,57,58] On the other hand, carbohydrates increase the hydrophilic character of lipids and proteins, helping to stabilise the conformation of many membrane proteins.[1] The effect of the cholesterol and lipid composition on the properties of the phospholipid monolayers at the liquid–liquid interface investigated by EIS[43] demonstrated that a condensed phase monolayer was formed for DSPC and DSPC/cholesterol (1:2 mol:mol) monolayers deposited at 50–60 $mN\,m^{-1}$ (capacitance values 2–3 $\mu F\,cm^{-2}$). The monolayer was more fluid with incorporation of phospholipids with shorter or unsaturated chains (DPPC, DOPC), and this was observed by an increase in the capacitance values.

On the other hand, the interfacial tension of an egg lecithin–cholesterol system measured in a Langmuir trough at the air–water interface at room temperature (22 °C) showed a 1:1 complex between PC and cholesterol at 18 $mN\,m^{-1}$ (Figure 6).[57] The hydrophobic layer–air interface dominated in the PC–cholesterol monolayer. Together with a theoretical model, and in the case of a lipid monolayer, it was shown that when the molar fraction of cholesterol is in the range of 0.35 to 0.70, there was both a complex and lipids not forming the complex. The two hydrophobic leaflets of lipid bilayer resulted in the difference between the stability constant of complex in the egg lecithin–cholesterol monolayer ($K_s = 2.56 \times 10^6\ m^2\,mol^{-1}$) and in the bilayer ($2.661 \times 10^7\ m^2\,mol^{-1}$).

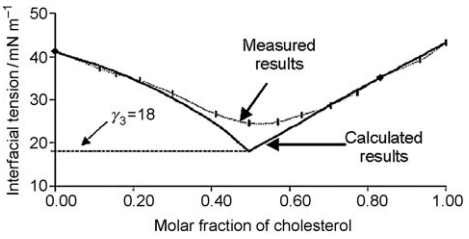


Figure 6. Interfacial tension of lipid–cholesterol compounds. Adapted from ref. [57]; copyright © (2002) Elsevier Science B.V.

Furthermore, by changing the phospholipid and cholesterol composition, and despite strong differences in the composition of both, the influence of cholesterol was very similar in all the cases,[58] which indicated that cholesterol had a symmetrical distribution between the inner and outer leaflets of the lipid membrane. Nevertheless, in a natural membrane, the

inner leaflet seems to be packed more densely than the outer leaflet.

4.2. Interactions between Phospholipid Monolayers and Biologically Relevant Compounds

The biomembrane mimic can also be improved by modifying the phospholipid monolayer and the physicochemical properties of bio-relevant compounds can be studied. In living systems, communication and interaction between cells and their environment is provided by, among others, membrane proteins. Antibiotics and peptides, such as gramicidin A (gA), are also a class of compounds that have had particular attention of the analytical work at the liquid–liquid and the solid–liquid interfaces.[8,42,59–63] The typical function of an antibiotic involves facilitated transport of ions (even though it is actually the ions that facilitate the transport of the antibiotics) across a biological membrane. Therefore, antibiotics with appropriate modification will be transported across the ITIES. Similarly, transmembranes provide pathways for the movement of charged particles across cell membranes, and mediate the interactions between the cell and its environment. For example, synthetic peptides can act as very efficient drug carriers with a very rapid internalisation process.[64,65]

EIS and cyclic voltammetry of monolayer-covered mercury electrodes have shown to be a feasible model membrane to study pore forming compounds.[66,67] Ionophore antibiotic A23187 was observed to increase the permeability of the monolayer to Cu^{2+} and Cd^{2+} ions, by complexing the metal ion in the monolayer. However, most reports have mainly focused on the electron transfer mechanism of thallium(I) across the those lipid-modified monolayers.[60–63]

The liquid–liquid interfaces can also be used to study oligonucleotide internalization[59] into lipid monolayers and for probing the behaviour of drugs in the vicinity of model cell membranes.[7,8] The membrane activity between lipid monolayers and oligonucleotides at liquid–liquid interfaces was shown to be improved by means of the surfactant cetylpyridinium chloride (CP).[59] For example, bare oligonucleotides (phosphoromonothioates and phosphodiesters) and complexed oligonucleotides (ODN1) adsorbed poorly on the lipid monolayers, while the introduction of CP at the interface increased the adsorption efficiency, as well as the oligonucleotide partition coefficient in the lipid membrane. When CP was added to the aqueous phase, it bound to the oligonucleotide, enhancing the interaction between the lipid monolayers and the oligonucleotide (Figure 7). The surface charge of the lipid monolayer decreased as a function of increased CP concentration, due to the adsorption of the CP–ODN1 complex at the interface.[8,59]

Another example is the use of the two structurally similar and electrically identical hydrophilic decapeptides of pharmaceutical relevance, leutinsing hormone-releasing hormone (LHRH) and nafarelin (a synthetic analogue of the gonadotropin-releasing hormone). They were able to migrate from the aqueous to an organic phase containing adsorbed phospholipid monolayers, due to adsorption of the peptides at the interface.[42] Although these peptides differed in just one amino

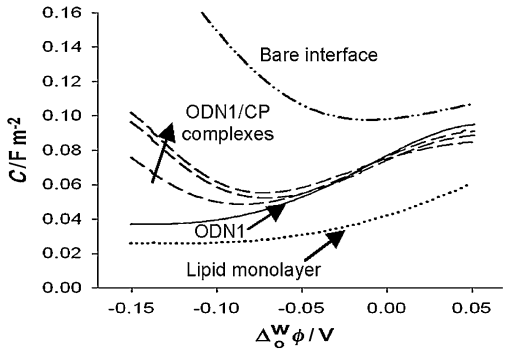


Figure 7. Effect of ODN1 (5′-CCC CAT TCT AGC AGC CCG GG-3′) and complexed oligonucleotides (CP/oligonucleotide ratios of 0.24, 0.71 and 0.96 from bottom to top) on the capacitance curves of a DSPC lipid monolayer. Adapted from ref. [59]; copyright © (2006) Elsevier Science B.V.

acid, the surface concentration of adsorbed nafarelin was found to be four times larger than that of LHRH at the ITIES. ITIES has also been shown to be useful to investigate physicochemical properties of other antibiotic-like compounds, such as valinomycin, which forms very selective complex with potassium, and β-lactam and their derivatives.[68–72]

Recently, the interaction between the peptides angiotensin III (AngIII) and Leu–enkephalin (LeuEnk), and an adsorbed monolayer of DPPC at the water-1,2-DCE interface showed that the complex formation involved both Coulombic and van der Waals interactions.[73] For example, the complexation constant of cationic AngIII measured by cyclic voltammetry (Figure 8a) was found to be $5.2\times10^{4}\,\text{M}^{-1}$. The peptide–lipid complex trans-

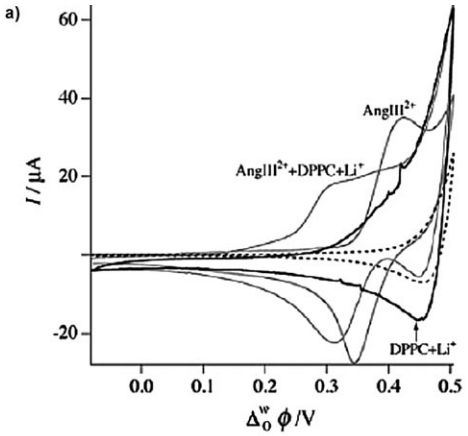


Figure 8. a) Cyclic Voltammograms for AngIII (100 μM) transfer in the absence and in the presence of DPPC (10 μM) at a scan rate of 50 mV s^{-1}. b) Illustrative mechanism for the LeuEnk transfer interactions with Li^{+} and DPPC monolayer at the water-1,2-DCE interface. The adsorption of the phospholipid at the interface from the organic phase (*o*) is denoted as (*ads*). Adapted from ref. [73]; copyright © (2008) American Chemical Society.

fer to the organic phase resulted in an adsorptive prepeak (diffusion-controlled) transfer in the voltammetry, and the lipid acted as an ionophore to facilitate the Li^+ transfer.[36,74,75] The desorptive peak from the interface to the organic phase was attributed to the formation of peptide-encapsulating micelles in the organic phase (reverse micelles). In the case of neutral LeuEnk, the interaction mechanism was firstly induced by Coulombic interactions (either by the lipid adsorbed at the interface or by the cations presented in solution), and then by hydrophobic interactions with the lipid. The partition of LeuEnk and complex formation is shown in Figure 8b. The interaction was attributed to an interface-controlled process rather than a diffusion-controlled one.

4.3. Self-Assembly of Multilayers onto Lipid Monolayers

The electrostatic adsorption of alternating layers of oppositely charged polyions on a charged substrate may be used to produce ultrathin polyelectrolyte multilayers (PEMs) with controlled properties at liquid–liquid interfaces.[50–52] Such layer-by-layer deposition of polyanions and polycations is a relatively straightforward and simple procedure. The method enables film properties such as thickness and composition to be easily controlled simply by adjusting the number of layers and the material of each deposited layer.

Recently, the experimental approaches to study phospholipid monolayers at the ITIES formed at the water-*o*-NPOE interface were applied to study drug release. This was achieved by the layer-by-layer self-assembly of oppositely charged PEMs anchored to a monolayer of cationic lipid previously deposited at the interface.[51] For interpretation of the ac voltammetry results, the electrostatic model described in Section 3 accounted for the presence of the PEM at the interface. No decrease in the transfer rate of tetraethylammonium cation was observed until the deposition of the seventh polyelectrolyte layer, whereas a positively charged last layer poly(allylamine hydrochloride) [PAH] caused retardation of the ion transfer rate already upon the introduction of the fourth layer. Using this system, tacrine (used in the treatment of Alzheimer's disease) transfer across the PEM indicated an effect of the shape and charge delocalisation of the transferring ion on the apparent rate constant.[43] In a similar approach, phospholipid-modified monolayers with DS attached were self-assembled layer-by-layer to PAH and water-soluble gold nanoparticles (AuNPs) of sizes of 1.7 and 3 nm (Figure 9).[50–52] Different capacitance responses were observed by changing the size of the AuNPs and the number of nanocomposite layers. The outmost layer determined the multilayer charge.[50,51] Similarly, phospholipid-modified monolayers with DS/ruthenium nanoparticle (average size diameter of 40 nm) complexes showed a reduction of the surface charge density at the interface upon adsorption of the composite nanoclusters to the lipid monolayer.[52] The presence of Ca^{2+} bridges between the DS, the cationic ruthenium nanoparticles and the phospholipid monolayers were found crucial for the behaviour of these systems.[14,50,51]

All the abovementioned systems were successfully used to study the behaviour and characteristics of specific drug molecules added to the interface. This kind of methodologies could be used in the future to investigate the transfer of a range of drugs across polyelectrolyte/nanoparticulate multilayer system and in assessing candidate polyelectrolyte ions or nanoparticles and multilayer formation conditions for use in drug-delivery devices.

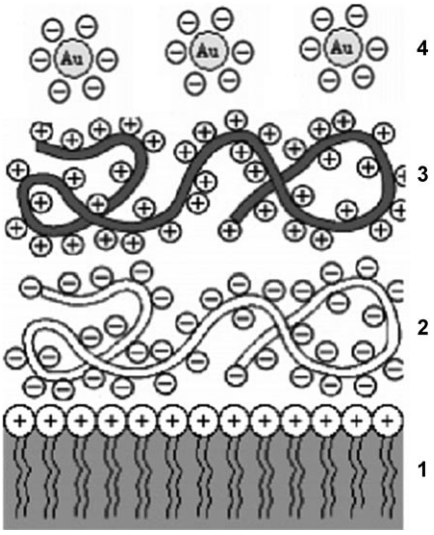


Figure 9. Interfacial composite nanostructure composed by lipid monolayer (1) + biopolymer, DS (2) + polyelectrolyte, PAH (3) + AuNPs (4) deposited at the liquid–liquid interface.

5. Ion/Drug Adsorption and Transport across Modified Liquid–Liquid Interfaces

Liquid–liquid electrochemistry provides a fast, convenient and accurate means to determine ionic partition coefficients (measure of the ability of a drug to permeate through cellular membranes by passive diffusion).[7,25,76] However these measurements are usually carried out with a bare interface (i.e. in the absence of lipid monolayers). The first studies of ion transfer across monolayers of pure lipids at ITIES showed that the long hydrophobic tails, low temperatures, large size of the transferring ion, closely packed structure of PC monolayers, and monolayers in a condensed state decreased the ion transfer rate across liquid–liquid interfaces.[3,19,22,23] On the contrary, monolayers in an expanded state appeared to be completely transparent to the transfer of small ions as a result of the more hydrated state of the hydrocarbon region of the monolayers.[22,23] Experimental evidence has suggested double-layer effects resulting from specific adsorption of ions to the monolayer headgroups and/or a restructuring of the interfacial solvent molecules as a plausible explanation for the increased rate constants.[22]

The interaction of alkali and alkaline-earth cations with DBPC monolayer at the water-1,2-DCE interface, showed that cations adsorbed at the polar headgroup of the phospholipids.[74] Enhancement or blockage of the transfer process depended on the nature and cation concentration. The enhancement of ion transfer across phospholipid monolayers was a result of the

double-layer effects arising from the orientation of the zwitterionic headgroups of PC molecules.[36] This was demonstrated by applying a theoretical model based on the electrical double-layer correction to the Bulter–Volmer equation,[45] coupled with the solution of the PB equation across the interfacial region, as described in Section 3. Recently, the thermodynamical analysis of the presence of a PC monolayer at the ITIES was extensively described.[5,77] The theoretical models accounted for the adsorption of the phospholipid both as a zwitterionic and a cation formed by the aqueous cation associated with the zwitterionic PC form, and also accounted for the aqueous cation transfer facilitated by the lipid. The results plotted as electrocapillary curves (interfacial tension vs applied potential) are shown in Figure 10. The effect of the applied potential was

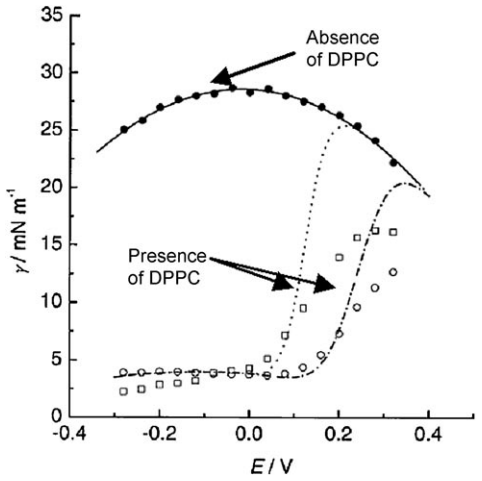


Figure 10. Electrocapillary curves for the water-1,2-DCE interface of the experimental and theoretical values in the absence and presence of 10 mM of DPPC in the organic phase. Lines represent the fittings to the theory and conditions described in ref. [5]. Adapted from ref. [5]; copyright © (2003) Elsevier Science B.V.

a result of the stable domains for the adsorbed lipid: a stable adsorbed layer of the zwitterionic PC was formed at negative potentials, while the binding of the aqueous cation to the adsorbed PC led to its desorption at more positive potentials.[5,77]

Cyclic voltammetry was also employed to evaluate the selectivity of complex formation of PC adsorbed monolayer at the water-1,2-DCE interface with aqueous ions.[75] The lipid monolayers adsorbed at the interface showed a remarkable complex formation and selectivity with alkali metal ions, NH_4^+, and alkylammonium ions, but not with $(CH_3)_4N^+$ or anions such as SO_4^{2-}, CH_3COO^-, Cl^-, Br^-, NO_3^-, I^- or ClO_4^-. The importance of this strong complex formation between basic amino acids, such as, for example, arginine cation, and lipid monolayers is fundamental to understand the strong binding of basic polypeptides to the lipid bilayers. For example, it was found that the strength of the complex formation between cations and lipids was as follows: Arginine$^+ > Li^+$; $Na^+ > K^+ > NH_4^+ > CH_3NH_3^+ > (CH_3)_2NH_2^+ > Cs^+ > (CH3)_3NH^+$.

Recently, the adsorption and ion pairing interactions of DPPC on water-1,2-DCE interface were driven from the interpretation of cyclic voltammograms, which showed to be rather a complex mechanism, and was described as a sum of sequential events, shown by Equation (7):[78,79]

$$L_o^{\pm} \rightleftharpoons L_{abs}^{\pm} + H_w^+ \rightleftharpoons HL_{ads}^+ \rightleftharpoons HL_o^+ + A_o^- \rightarrow HLA_o \rightarrow HA_o + L_o^{\pm} \quad (7)$$

where $L_o^{\pm}$ is the zwitterionic form of DPPC and HL^+ is its protonated form. The five steps indicated that the process depended both on the potential difference on the interface, as well as on the pH value. In addition clear capacitance currents stemming from the adsorption/desoprtion of charged species (protonated phospholipid molecules) at the liquid–liquid interfaces were identified. In the absence of multivalent ions, a lipid monolayer was formed, but in the presence of cerium(IV) the slow ion transport observed was due to the formation of DPPC multilayers. Introduction of flunitrazepam (a benzodiazepine compound widely administrated as anxiolytic drug) on DSPC and DSPE monolayers adsorbed at the water-1,2-DCE interface strongly influenced the physical state of the monolayer, which in turn depended on the aqueous phase composition.[80]

5.1. Membrane Activity: Drug Partition

To access the structural integrity of the model membranes and their ability to block or allow charge/ion transfer, admittance measurements[7,8,25,43] were employed at an immobilised liquid–liquid interface in order to study the apparent capacitance curves obtained from the analysis of a Randles equivalent circuit.[3,46] The experimental data together with a theoretical model based on the Bulter–Volmer description of ion transfer kinetics give further insight on the specific adsorption of the transferring ion/drug on the aqueous and organic sides of the interface. Therefore, detailed information on the kinetics and mechanistic of the drug transfer event are obtained.[49] For example, the drug molecules tacrine and propranolol were shown to preferentially interact with the headgroup region of a deposited DSPC monolayer, while metoprolol preferred the hydrocarbon tails of the lipid.[12,25] Adding cholesterol to the lipid monolayer the tendency of drug molecules to interact with phospholipids was reduced.[43] The interaction between aminoacridine-derivative molecules, such as tacrine, and the phospholipid monolayer evaluated through admittance data plotted as a function of the inverse square root of the angular frequency of the ac excitation. Comparison of the admittance curves for different drug molecules showed different adsorption behaviour to phospholipids monolayers between drugs.[7,43] For example, in the case of tacrine the location of the imaginary admittance maximum was the same at all measured potentials. This indicated that tacrine adsorbed both on the lipid headgroups and the hydrocarbon chains region of the monolayer.

5.2. Ion Transport across Bilayer Lipid Membranes

In parallel to lipid monolayers at the ITIES it is important to mention that the ion transport from one aqueous phase to another across a bilayer lipid membrane (BLM) has also been helpful to provide additional information and to interpret

mechanisms of ion transport across biomembranes.[75,81–86] These black lipid membranes are usually formed by spreading a lipid solution (or a mixture of lipid with other cell membrane components such as proteins, cholesterol, etc.) in a small (Ø 1 mm) hole of a wall separating two aqueous compartments by brushing.[75,82–86] Evaporation or diffusion of the lipid solvent leads to thinning of the film to its final bilayer state. Electrochemical techniques can then be applied over the formed BLM. For example, analysis of cyclic voltammograms of the ion transfer across a BLM suggested that the membrane transport is mainly determined by the complementary ion transfer reactions at two water–membrane interfaces only when both the membrane and the two aqueous phases contain sufficient electrolytes.[82] In addition, the amount of K^+ and Na^+ transferred across an water–BLM interface with the aid of the electron transfer was shown to be controlled by the standard Galvani potential differences for the ion and electron transfer and the concentration ratio of redox couples in both aqueous and organic solutions.[83] This is very useful information because the understanding of the individual reactions occurring at two water–membrane interfaces is essential to elucidate the ion transport coupled with the electron transport in biological membranes.

More recently, Shirai and co-workers demonstrated the facilitated transport of hydrophobic cations (such as dipicrylaminate or tetraphenylborate) from one aqueous phase to another across a BLM in the presence of valinomycin ionophore (a glycopeptide antibiotic).[84] An example of cyclic voltammograms for ion transfer across the BLM is shown in Figure 11 a. The results suggested that an ionophore like valinomycin may act as a carrier of cation transport across the BLM. Alkali metal ions and counter anions such as Cl^-, Br^- and ClO_4^- were also found to transfer across the BLMs at the same time. In the presence of gA, ion transport across a BLM was found to be facilitated.[85,86] Using cyclic voltammetry it was shown that K^+, F^-, Cl^- and Br^- were distributed from aqueous phases into the gA dimers in the BLM and were transferred across the BLMs at the same time.[85] When other anions such as ClO_4^- or I^- were used they distributed into the BLM with K^+ irrespective of the presence of gA.

Following the abovementioned studies, a new mechanism for ion transport across BLM in the presence of gA was proposed by Kubota and co-workers.[86] They showed that the magnitude of the single-channel current at a given membrane potential depended on both cationic and anionic species (Figure 11 b). The magnitude decreased with an increase in the diameter of the anion when the diameter of the anion was larger than that of the gA channel. The results indicated that the facilitated ion transport by gA consists of ion transport across the lipid bilayer site and that through the channel pore. This clearly shows the selective property of gA on ion transport across BLM.

All these results obtained in the presence of BLMs seem to corroborate very well with those obtained when using phospholipid monolayers, which demonstrates that phospholipid monolayers can be indeed a very good biomimetic system of biomembranes.

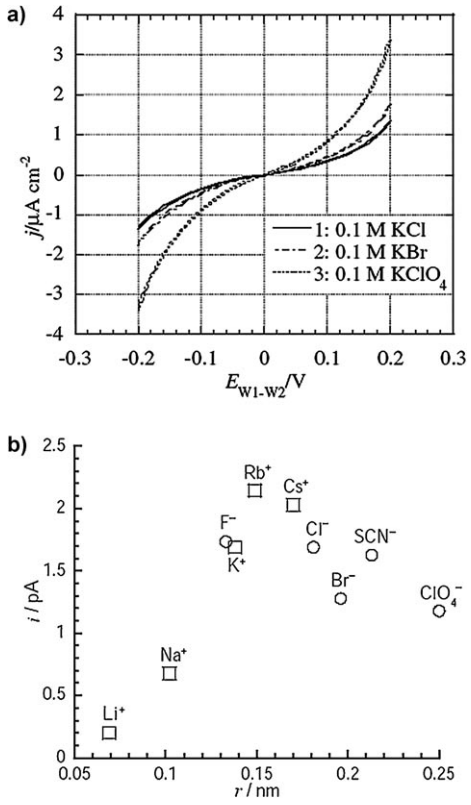


Figure 11. a) Cyclic voltammograms for the ion transfer across a BLM-containing valinomycin ($c = 3 \times 10^{-5}$ M). Potential scan rate: 0.01 V s^{-1}. Temperature: 298 ± 1 K. Electrolyte solutions are presented in the Figure. Reprinted from ref. [84]; copyright © (2004) Elsevier Science B.V. b) Dependence of single-channel currents at +120 mV on the ionic radii of ions. Concentration of gA in the BLM-forming *n*-decane solution was 10^{-7} M. Reprinted from ref. [86]; copyright © (2009) The Japan Society for Analytical Chemistry.

6. Other Techniques Used in the Study of Phospholipids at Liquid–Liquid Interfaces

Although the experimental data about phospholipid monolayers at ITIES are detailed and comprehensive, due to the possibility of controlling their electric potential and to understand the effect of potential difference across the lipid layers on membrane structure and permeability, direct information about the monolayer structure is complemented by other techniques. Second-harmonic generation (SHG), X-ray diffraction, fluorescence, scanning electrochemical spectroscopy (SECM) among other optical techniques, or even the use of computer simulations had greatly contributed to further understand the lipid monolayers and bilayers systems.[87–94] It is beyond our scope to describe all the techniques herein, but we briefly introduce to the reader some of the abovementioned techniques that have extensively contributed to elucidate the phenomena of lipid monolayers adsorbed at the ITIES.

The study of such interfaces by macroscopic measurements such as surface tension or capacitance, while yielding significant information on the interfacial properties, cannot yield microscopic or molecular details. The non-linear optical techniques of SHG and sum frequency generation have provided

some of the most detailed studies of the structure of the liquid–liquid interface at the molecular level.[95–101] Fluorescence microscopy of labelled adsorbed phospholipids allows to monitor the changes in monolayer morphology during the phase transition at water–non-polar liquid interfaces.[102] Structural changes that accompany the transition can also be followed by infrared and Raman spectroscopy techniques.[103–105] Vibrational sum frequency spectroscopy has provided direct information about the orientation and the degree of order among the acyl chains of the adsorbed phospholipid,[106,107] as well as about induced changes in water structure at a water–oil interface.[108] Confocal fluorescence correlation spectroscopy has also been used to measure and to compare the lateral diffusion coefficients of phospholipids in the supported bilayer and monolayer at the ITIES.[109] Diffusion coefficients of phospholipids on liquid–liquid interfaces have been demonstrated by molecular dynamics and Monte Carlo simulations.[110,111]

Quasi-elastic laser scattering (QELS) has been demonstrated to be a versatile tool for in situ monitoring of the frequency of thermally induced capillary waves at the liquid–liquid junction.[112,113] QELS is not sensitive to specific molecules, but to the interfacial tension of the molecular junction. QELS measurements coupled with mathematical models can provide information on capillary waves.[112–116] Figure 12a shows that there is a linear relationship between the frequency linewidth Δf vs $k^{7/4}$ (wavenumbers) for various DPPC concentrations. From QELS data a valuable insight into the dynamics of the water–oil interfaces in the presence of phospholipids can be obtained, and the theory of capillary waves for liquid–liquid interfaces acn be verified as well. A detailed analysis of the effect of the capillary wavenumber on the capillary wave frequency suggested that the dynamic behaviour of ITIES was consistent with the theoretical predictions for a sharp liquid–liquid interface over a certain range of the DPPC concentrations.[77]

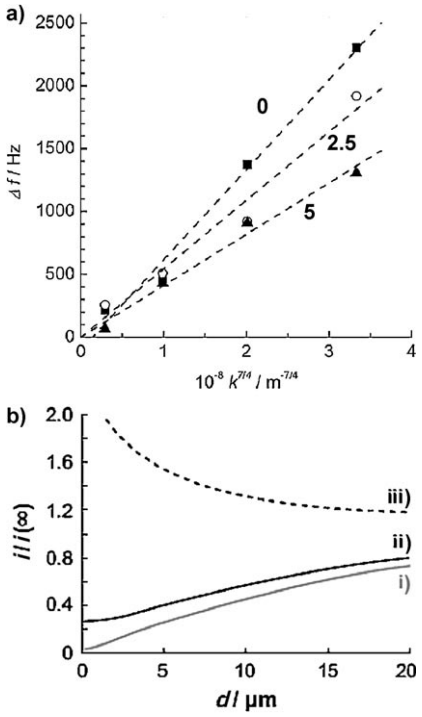


Figure 12. a) Spectrum linewith Δf vs $k^{7/4}$ for a water-1,2-DCE interface in the presence of DPPC at the concentrations (in μM) showed in the figure. Adapted from ref. [77]; copyright © (2005) Royal Chemistry Society. b) Typical approach curves for the measurement of lateral proton diffusion. The solid experimental curves are for the reduction of $H_2PO_4^-$ at a UME approaching i) a native water-air interface and ii) a DPPS monolayer. The dashed curve (iii) represent the theoretical simulation for the system for the conditions described in ref. [117]; copyright © (2002) American Chemical Society.

SECM is another technique that can monitor processes at the ITIES by employing an ultramicroelectrode (UME), also known as a tip, and scan in close proximity to a surface of interest. The electrochemical response of the tip (or of the substrate in response to the tip) provides quantitative information about the interfacial region.[94] Typically, a UME tip is usually placed in the upper liquid phase (e.g. an organic solvent) containing one form of the redox species (e.g. the reduced form).[94,117–120]

Lateral proton diffusion coefficients can be investigated by steady-state approach curves (Figure 12b),[121] as surface diffusion contributes primarily to the long-time SECM current response.[122] Examples are monolayers comprising either DPPS or DPPC at a range of surface pressures.[117] In the presence of $H_2PO_4^-$ in the aqueous solution, typical approach curves for both a native water–air interface and a DPPC monolayer (Figure 12b) showed a current response due to the diffusion of $H_2PO_4^-$ through solution. A significant increase in current is predicted as the probe approaches the monolayer.

7. Conclusions and Outlook

As a result of their unique properties, and although considered to be a simple model of biomembranes, phospholipid monolayers at the electrified liquid–liquid interfaces can accommodate variable complexity and functionalities aimed to elucidate structural details of the bilayer membranes and to mimic its functions. Furthermore, phospholipid-modified monolayers provide an excellent framework to gain understanding on ion/drug and electron transfer kinetics in biological membranes. Adsorption of ions and molecules, charge effects on molecule lipophilicities and hydrogen bonding characteristics of the compounds relevant to drug delivery and pharmacokinetics, are some other interesting properties that can be addressed using these model systems. Complex model membranes based on phospholipid monolayers can be built at the ITIES using a modular approach. Electrochemical measurements can be combined with different physical setups and different techniques to reveal the membrane activity of a wide variety of therapeutic ion/drug molecules. Nanostructures, such as nanoparticles and polyelectrolyte multilayers can also be adsorbed to phospholipid monolayers. These composite systems are potentially interesting for future applications in the field of controlled drug delivery and biosensing.

Besides discussing recent developments on the subject, we described examples of electrochemical techniques and theoretical models that can be used to get insight on the interfacial processes and electrostatic properties of biological model

membranes at the ITIES. Furthermore, we shortly described how processes occurring at the ITIES systems can be used to provide greater understanding of both chemical and biological phenomena. The increasing number of experiments conducted at liquid–liquid interfaces shows that electrochemical methodologies at such boundaries can be an efficient and versatile tool to probe ions and drug molecules of pharmaceutical relevance. Thanks to the improved theoretical understanding of interfacial structure, the transfer process of solvated ions from one phase to the other becomes clearer. Deep knowledge on the structure, dynamics and molecular interactions of bio-complex molecules adsorbed at liquid–liquid interfaces can be achieved by combining electrochemical setups with different other techniques, as well as computational methodologies. Furthermore, the contents of this review indeed showed the great versatility and advantage of performing studies at the ITIES when compared to other approaches. Although this review was not a survey of all the work in the field of the topics discussed above, we think it may provide comprehensive referencing and detailed information on the topics presented to current research.

Acknowledgements

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Keywords: electrochemistry · immiscible electrolytes · interfaces · monolayers · phospholipids

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