

Adsorption of the phospholipid DPPE at polarisable water|1,2-DCE interface: Interfacial tension measurements

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Abstract— We report the use of a custom-built quasi-elastic light scattering (QELS) setup to monitor the interfacial tension changes during a phospholipid *dipalmitoyl phosphatidyl ethanolamine* (DPPE) adsorption at the polarisable water|1,2-DCE (W-DCE) interface. The electrocapillary curves for the DPPE on the basis of the experimental data clearly evidence a strong amphiphilic character of the phospholipid.

Index Terms— Dipalmitoyl phosphatidyl ethanolamine (DPPE), Interfacial tension, Liquid-liquid interface, quasi-elastic light scattering (QELS).

I. INTRODUCTION

Phospholipids are the major components of cellular membranes and are characterized by dual structure comprising a polar head and a long hydrocarbon tail. Due to its apparent low reactivity, the lipidic component of the cellular membrane was usually regarded as an inert matrix that contains the receptors with which chemical messengers can interact and induce a response from the interior of the cell. However, this perspective has been modified over the years, regarding the cellular membranes not as a passive barrier and/or matrix for receptors but more like an active interface that can even catalyse the messenger-receptor binding [1].

Some of the most common and widely accepted mimetic cellular membranes are vesicle suspensions and immobilized bilayers or monolayers for which the potential difference across the layers cannot be controlled or is neglected in most of the cases. Another interesting approach is based on adsorbed monolayers at polarised liquid-liquid interfaces, where the control of the interfacial polarization plays an important experimental variable [2].

Liquid/liquid interfaces is formed between two solvents of low (ideally zero) mutual miscibility, each an electrolyte for electrochemical applications. Normally, one of these solvents is water, and the other is a polar organic solvent with a relatively high dielectric permittivity, such as nitrobenzene (NB) or 1,2-dichloroethane (1,2-DCE). Like the electrode/electrolyte interface, the liquid/liquid interface can also be either polarisable or nonpolarisable, depending on permeability to charged species distributed in either or both phases. If the interface is impermeable to charges (ions or electrons) it is called polarisable, otherwise it is called nonpolarisable or reversible [3]-[4].

Adsorption of compounds of biological significance at the

L/L interface is useful to understand the biological cell membrane phenomena. Indeed, it has been shown back to the eighties that the adsorption of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) at the Interface between two immiscible electrolyte solutions (ITIES) can be modulated by varying either the externally applied potential or the pH of the aqueous phase [5]-[6].

Experiments on optical, neutron reflectivity as well as molecular dynamics simulations show that ITIES is highly corrugated. The corrugation arises from capillary waves. The determination of electrocapillary data requires in situ measurement of interfacial tension in order to determine the influence of the applied electrical potential over the surface pressure. Thus, interfacial tension measurements as a function of the potential difference between the two phases, called electrocapillary measurements, have been used to characterize the adsorbed monolayer.

Many techniques have been tested over the years such as drop shape analysis [2]-[7], drop-weight determination [8], quasi-elastic light scattering [9] (QELS) and Wilhelmy plate [10] based methods. Among all these methods QELS is the only non-invasive method that does not require time-consuming procedures and gives reproducible and reliable results.

In this paper, we monitored the interfacial tension changes during DPPE adsorption at the polarisable water-DCE interface. Electrocapillary curves has been obtained for the phospholipid on the basis of the experimental data from the QELS measurements.

II. MATERIALS AND METHODS

A. Interfacial Tension Measurements

For the interfacial tension measurements, QELS setup was used. A schematic drawing of the experimental setup is shown in Figure 1. It consists of a laser ray from a 4 mW He-Ne laser at 632.8 nm (Uniphase Model- 1101) that is directed perpendicularly to the liquid-liquid interface, where it is quasi-elastically scattered by the capillary wave at the interface. The liquid|liquid interface is constructed in a cylindrical quartz glass cell with a cross section of 15.9 cm². The glass has an optically flat bottom, which is indispensable to maintaining good reproducibility of the experimental results. The surface of the aqueous phase is covered with an optical glass window to minimize the light scattering from the air|water interface. After passing through the interface, the ray is diffracted by a diffraction grating, so that the scattered and diffracted beams are optically mixed. The diffracted beams of different orders can be selected by moving a pinhole in front of the photodiode used as the detector. In the present study, the third-order diffraction spot was selected. The recorded

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signal is then analyzed by a fast Fourier analyzer (FFT, Stanford Research Systems SR 770) and a typical power spectrum is obtained.

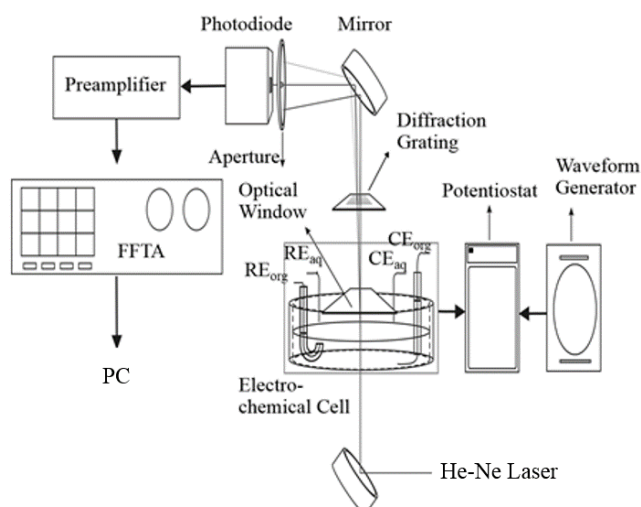


Figure 1: Schematic representation of the experimental set-up of QELS.

B. Polarisation of the Interface

The interface was polarised by means of two reference electrodes RE_{aq} and RE_{org} (Ag/AgCl salt) and the current is collected via the two counter electrodes CE_{aq} and CE_{org} (Pt wires). Representation of the electrochemical cell used for QELS measurements is given in the figure 2.

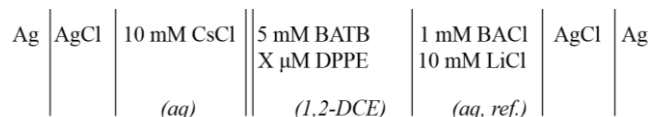


Figure 2: Electrochemical cell employed for QELS measurements. The double vertical bar indicates the ITIES under consideration.

Reagents

Dipalmitoyl phosphatidylethanolamine (DPPE) was obtained from Sigma (St. Louis, MO). Phospholipid solutions were diluted to the required concentration through adding concentrated peptide solutions to the organic phase including electrolyte.

Synthesis of organic supporting electrolytes. BATB (Bis (triphenyl phosphor anylidene) ammonium tetrakis (penta fluoro phenyl) borate) was used as supporting electrolyte in the organic phase. BATB is prepared by metathesis of BACl (bis(triphenylphosphoranyldiene) ammoniumchloride; Fluka, Switzerland) and LiTB (lithium tetrakis (pentafluorophenyl) borate; Boulder Scientific). TEATB is produced from TEACl (tetraethylammonium chloride; Fluka, Switzerland) and LiTB. In both cases equimolar amounts are dissolved separately in the possible mixture of methanol:water. Both solutions are gently mixed together and the resulting precipitate is filtered and washed thoroughly with water and dried under-vacuum. The salt is then further recrystallised in acetone. The crystals obtained are then dried under vacuum before use.

The organic phase solvent used was DCE (1,2-DCE, 99%).

Water (H₂O) was deionised by Milli-Q water system (Bedford, MA). The organic and aqueous solvents were mutually saturated prior to each experiment. All the other solvents and reagents are of analytical grade and are used without further purification, unless otherwise stated.

Preparation of the reference electrode Ag/AgCl. Reference electrode Ag|AgCl was prepared by connecting a newly polished silver wire and a platinum wire to the positive and negative terminals, respectively, of a battery with a voltage output of 1.5 V. An aqueous solution of NaCl plus a small amount of HCl plus a small amount of H₂SO₄ were employed as the electrolytic solution, respectively. The passage of current in the circuit produced a layer of the insoluble silver salt on the silver wire, which is accompanied by the electrolytic reduction of proton at the platinum wire.

III. RESULTS AND DISCUSSION

One of the typical power spectrums obtained by the QELS technique is given in figure 3. The spectrum has a peak at frequency of 5937 Hz, which can be related to the surface tension γ in terms of the equation below namely Lamb's equation.

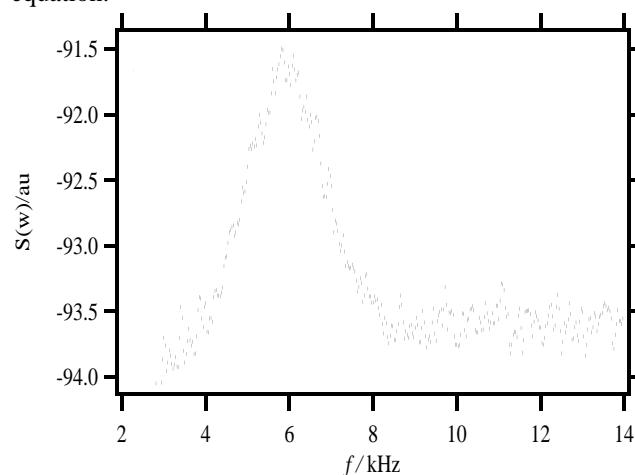


Figure 3: A power spectrum at open circuit potential corresponding to the third-order spot of QELS at a W-DCE interface without melittin during the potential scanning. The recorded signal is analyzed by a fast Fourier analyzer. The solid line represent the fitting to a Lorentzian function of the experimental data.

Depending on the potential bias applied to ITIES the frequency value of the capillary wave shifts, which corresponds to the changes of the surface tension of the W-DCE interface.

The calibration of this QELS equipment has already been performed and the power spectra found for different diffraction orders are in excellent agreement with those reported previously. Taking into account the maximum of the frequency peaks and assuming that the linewidth is much smaller than the peak frequency (which is our case) as well as in the limit of small liquid viscosities [11], the interfacial tension can be obtained according to the Lamb's equation given below [12].

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{\gamma_i k^3}{\rho^w + \rho^o}} \quad (1)$$

Where k is the wave number of the capillary wave, γ is the surface tension, ρ^r ($r = w, o$) represents the density of the corresponding liquid and f_0 is the peak frequency of the capillary wave. After being Fourier transformed, the power spectrum is approximately Lorentzian in form and is characterized by a peak frequency identified here as f_0 . We estimated the interfacial tensions γ according to the equation. For the configuration employed all measurements, the value of $k=505.15 \text{ cm}^{-1}$ was estimated as 505.15 by taking the interfacial tension of the neat W-DCE interface as 28.5 mN m^{-1} [13]. This value was used in all calculations. The dependence of on the potential bias across the interface allows constructing electrocapillary curves. Thus, after varying the potential from one end of the potential window to the other in small steps (e.g. 30 mV) an electrocapillary curve can be constructed by QELS method.

After introducing the phospholipid DPPE, it is expected that the molecules of the phospholipids in the organic phase spontaneously form a monolayer at the W-DCE interface. The orientation of the monolayer is so that the hydrophobic tail remains in the organic phase, while the hydrophilic head group is immersed in water.

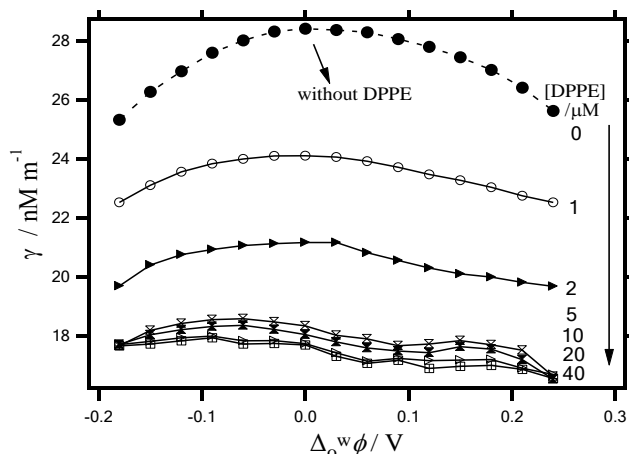


Figure 4: Electrocapillary curves obtained at the interface formed between water containing CsCl 10 mM and 1,2-DCE containing BATB 5 mM and DPPE at concentrations of 0, 1, 2, 5, 10, 20 and 40 μM in the electrochemical cell.

Figure 3 shows the electrocapillary curves for the W-DCE interface in the absence and in the presence of DPPE in the organic phase DCE. The top electrocapillary curve (signed as without DPPE) is that of the bare polarised interface between 10 mM NaCl and 10 mM PBS in water and an organic salt 5 mM BATB in the organic phase. All the other curves correspond to those obtained in presence of the peptide at fixed pH value. The top electrocapillary curves obtained in the absence of DPPE shows that the surface charge density is directly proportional to potential difference applied to the interface. By addition of the phospholipid the electrocapillary curves changes as can be seen in figure 3.

The potential scale displayed in figure 4 is the Galvani potential difference across the W-DCE interface, which depends strongly on the nature and on the chemical composition of the electrodes, which rapidly alters with time. Hence, the values of $\Delta_o^w \phi$ refer directly to the electrochemical cell used, and represent then a totally arbitrary scale. In order

to compare the results obtained in different experiments, it is of course necessary to transpose them to an absolute scale. This is achieved by referencing each measurement with the transfer of an ion whose formal transfer potential is already known.

Formal potential of transfer of tetramethylammonium cation (TMA^+) at the W-DCE interface is estimated as 0.160 V using an extra-thermodynamic assumption such as tetraphenylarsonium tetraphenylborate (TATB) scale, it is used as reference throughout this study. This value has been used extensively as the internal reference [14]-[15].

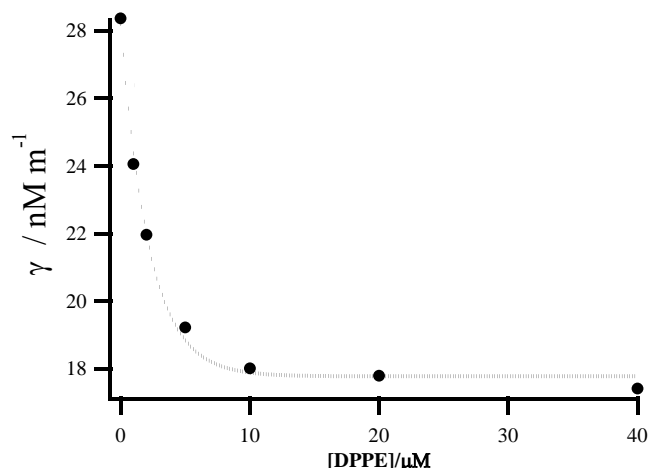


Figure 5. The relation between surface tension and DPPE concentrations at the interface at a fixed potential difference (0.05 V).

Increasing the concentration of the phospholipid was observed to have a marked influence on this process. As can be seen from the figure 5, the interfacial tension, γ , of the water-DCE interface decreases with increase of the concentration of the phospholipid at the interface. Basically the graph may be roughly divided into two regions. In the first region the slope decreases with bulk concentration of the phospholipid. In the other region the saturation of the interface with DPPE is established. The adsorption of the phospholipid does not take place in an appreciable extent beyond this point (at about $10 \mu\text{M}$), as seen in figure 5.

In conclusion, quasi-elastic light scattering (QELS) method was used in a convenient manner to investigate the adsorption behavior of DPPE at the water-DCE interface in the concentration range from 0 to $40 \mu\text{M}$. On the basis of the experimental data from the QELS measurements the corresponding electrocapillary curves show the dependence of the interfacial tension function on to the potential. Furthermore, the electrocapillary curves clearly show that the phospholipid DPPE is strongly adsorbed at the W-DCE interface.

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