Experimental report

06/11/2019

Proposal:	**9-13-836**	**Council:**	4/2019
Title:	Electrochemically Induced Lipid Vesicle Fusion at the Liquid-Liquid interface		
Research area:	Soft condensed matter		

This proposal is a new proposal

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Experimental team:	Andrea TUMMINO Giovanna FRAGNETO Ernesto SCOPPOLA Robert DRYFE
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Samples:

H2O
D2O
1,2-Distearoyl-sn-Glycero-3-Phosphocholine (DSPC)
1-palmitoyl-2-oleoylphosphatidylcholine
1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS)
deuterated dichlorobenzene
1,2-dioleoyl-3-trimethylammonium-propane

Instrument	Requested days	Allocated days	From	To
FIGARO	7	7	23/09/2019	30/09/2019

Abstract:

The aim of this experiment is to investigate the structure and composition of floating lipid bilayers formed by vesicle fusion at the interface between two immiscible electrolyte solutions (ITIES). The approach is based on the electrostatic interaction between a previously adsorbed cationic lipid monolayer at the water/1,2-dichlorobenzene (DCB) interface with oppositely charged lipid vesicles, injected in the aqueous phase. Recent neutron reflectometry (NR) experiments performed on FIGARO at the ILL by Fragneto and co-workers have shown that such approach to form floating lipid bilayers at the water/perfluorooctane and water/d26-dodecane is promising although bilayer coverage and presence of not fused vesicles at the interface may become an issue. Finally, the vesicle fusion into lipid bilayers will be triggered by applying an electrostatic field to the ITIES.

Experimetal report for proposal 9-13-836

Abstract

The aim of this experiment is to investigate the structure of the interface between two immiscible electrolyte solutions (ITIES), when Na^+ and Cl^- ions are transferred from the aqueous to the organic phase, and back, while performing *in situ* neutron reflectometry (NR) measurements. This study has been extended towards the effects of a lipid layer formed by bulk adsorption from a 0.02 mg/ml POPC:POPG mixture (70:30 molar ratio) in n-dodecane:2-octanone solvent mixture (60:40 v/v), containing 1 mM Bis(triphenylphosphoranylidene)ammonium-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (BTPPATFPB) as organic supporting electrolyte.

Results

The effects of the organic phase composition on the electrochemical response during a typical CV measurement are shown in figure 1. In detail, the response of the solvent mixture is compared to that of DCB. The increased current intensity at high potential (1.3 V for DCB and 0.45 V for the solvent mixture) is related to Na^+ transfer, while that of Cl^- ions occurs at lower potential (around 0 V for DCB and 0.1 V for the solvent mixture). Due to the high neutron absorption of chlorine in the wavelength range 6–20 Å (same as that used on FIGARO), the structure of the DCB interface could not be investigated. Therefore, we performed NR on water/solvent mixture interface only.

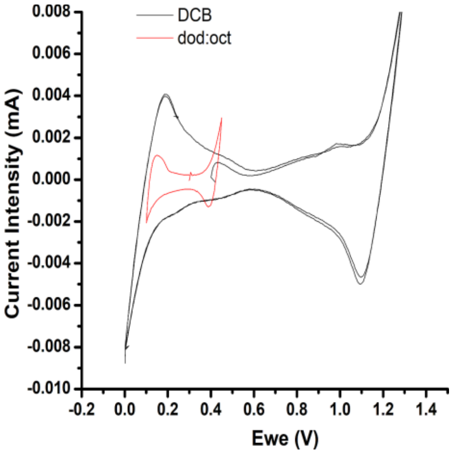

Figure 1 – Cyclic voltammograms of 10 mM NaCl/1 mM BTPPATFPB in DCB (black curve) and the solvent mixture (red curve).

NR reflectivity profile for the bare interface, before and after the addition of organic supporting electrolyte are shown in figure 2 for three different interfacial states: no potential applied (keeping the potentiostat off), 0.3 V (close to Na^+ transfer), and 0.15 V (close to Cl^- transfer). The fitted parameters (thickness, t and roughness, σ) are reported in table 1. The NR curves of the bare interface after the addition of the organic supporting electrolyte show a change in the low-q region, which can be attributed to an adsorption layer of the electrolyte itself. There are negligible effects of the electric field on the structure of the layer.

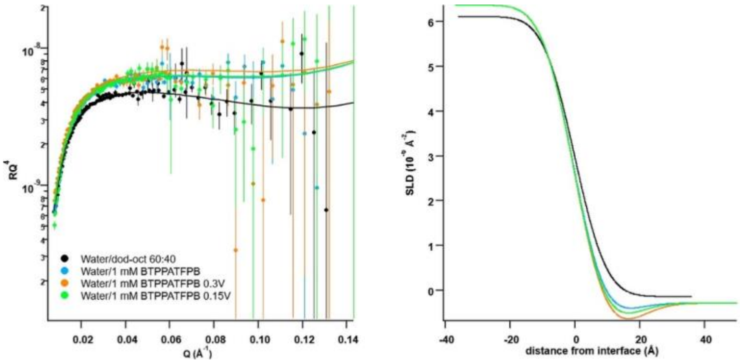

Figure 2 – left: NR profiles of the interface between 10 mM NaCl aqueous phase with the solvent mixture in absence of BTPPATFPB (black circles), after the addition of BTPPATFPB (light blue circles), after applying a potential of 0.3 V (orange circles), and 0.15 V (green circles); **right**: corresponding SLD profiles, displayed with matching colors.

Table 1 – fitted parameters for the bare interface and after the addition of BTPPATFPB for three different interfacial states.

interface	**t (Å)**	**σ (Å)**
water/solvent mixture	-	8 ± 1
Water/BTPPATFPB	12 ± 3	8 ± 1
Water/BTPPATFPB 0.3V	18 ± 1	8 ± 1
Water/BTPPATFPB 0.15V	15 ± 3	8 ± 1

NR investigation was repeated after the addition of POPC:POPG mixture (70:30 molar ratio) for three different interfacial states: no potential across the interface (lipid + boron off); consecutively switching the applied potential between 0.3 V (1st–3rd lipids + boron 0.3 V), and 0.15 V (1st–3rd lipids + boron 0.15 V). These data are shown in figure 3, and the results are summarized in table 2. It is important to keep in mind that the data collected in the mid and high q region of the NR curves all fall in the background of the measurements.

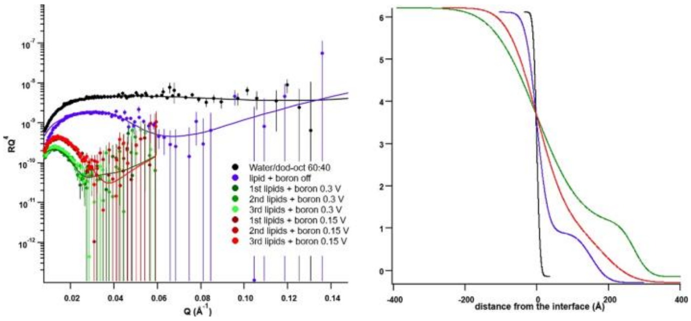

Figure 3 – left: NR profiles of the interface between 10 mM NaCl aqueous phase with the solvent mixture in absence of BTPPATFPB (black circles), after the addition of BTPPATFPB + lipids (violet circles), after applying a potential of 0.3 V (orange circles), and 0.15 V (green circles); **right**: corresponding SLD profiles, displayed with matching colors.

The interfacial roughness as well as the layer thickness increase. If from the one hand we cannot give a detailed description of the interfacial structure, on the other hand we can exclude the presence of a monolayer, as none of these curves could be resolved with an adsorbed lipid monolayer at the interface. This effect becomes even more marked when a potential is applied at the interface (red and green shaded circles). The interfacial properties can be reversibly tuned by switching the potential at values far from that of ion transfer. In fact, the reflectivity profiles at 0.3 V and 0.15 V are different from each other, but all those collected at the same potential overlap with each other.

Table 2 – fitted parameters for the bare interface and after the addition of BTPPATFPB and POPC:POG mixture for three different interfacial states.

interface	t (Å)	σ (Å)
Water/dodecane	-	8 ± 1
Lipid+boron OFF	153 ± 2	35 ± 10
Lipid+boron 0.3V	273 ± 6	60 ± 30
Lipid+boron 0.15V	183 ± 2	60 ± 30

The effects of ion transfer was investigated as well. The data are shown in figure 4, and the results summarized in table 3.

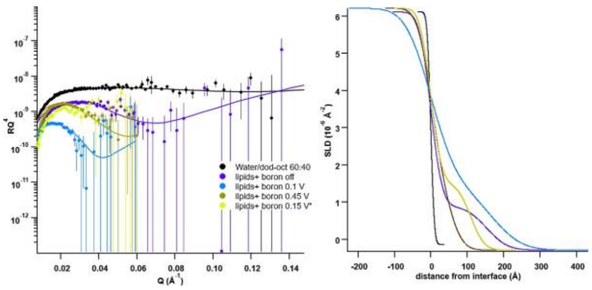

Figure 4 – left: NR profiles of the interface between 10 mM NaCl aqueous phase with the solvent mixture in absence of BTPPATFPB (black circles), after the addition of BTPPATFPB + lipids (violet circles), after applying a potential of 0.3 V (orange circles), and 0.15 V (green circles); **right**: corresponding SLD profiles, displayed with matching colors.

The reflectivity profiles, as well as fitted parameters, for the 0.1 V interface (light blue circles) do not change much from that collected at 0.15 V (close to Cl^- transfer). One could expect even higher adsorption at high potential (0.45 V), when cations cross the interface. Surprisingly, NR measurements show the opposite behavior, and now the data can be fitted with an adsorbed layer with reduced thickness. This confirms that the adsorbed lipids are destabilized when Na^+ ions are transferred, and that they are transferred together with the cations into the bulk organic phase. Moreover, the desorption of lipids from the interface is irreversible in the experimental time scale. If now the interface is kept at 0.15 V (golden points, labelled as 0.15 V"), the reflectivity profiles do not reach the same intensity as in figure 3.

Table 3 – fitted parameters for the bare interface and after the addition of BTPPATFPB and POPC:POG mixture at potentials corresponding to that of ion transfer.

interface	t (Å)	σ (Å)
Lipid+boron OFF	153 ± 2	35 ± 10
Lipid+boron 0.45V	55 ± …	30 ± …
Lipid+boron 0.1V	167 ± 2	60 ± …