Experimental report

28/08/2017

Proposal: 9-13-683 **Council:** 10/2016

Title: Interactions of drug carriers - lipidic nanoparticles, known as cubosomes with model lipid membranes at the air-water interface.

Research area: Chemistry

This proposal is a new proposal

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Samples: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine
deuterated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine
cubosomes

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	2	2	27/01/2017	29/01/2017

Abstract:

Lipidic cubic nanoparticles (LCNP) are known as convenient biocompatible drug delivery systems, hence understanding their mode of interaction with lipidic membranes is of special interest. The interactions of monoolein-based LCNP with model lipidic membranes self-assembled at the air-water interface of a Langmuir trough has been investigated in our labs. Our results indicate that at high surface pressures the lipid layer is densely packed and that LCNP interactions lead to the exchange of the lipid molecules with the monolayer, whereas at low surface pressures the monolayer is less densely packed which allows the incorporation of LCNP material into the monolayer. Here we propose to monitor lipid exchange/delivery as well as the binding of LCNP in situ at a fluid interface for the first time in a short initial study using neutron reflectometry and isotopic contrast substitution on FIGARO. These results can significantly enhance our understanding of the molecular interactions between model membranes and LCNP as alternative, biocompatible drug delivery systems. This first study will pave the way for future work on factors effecting the release of anti-cancer drugs of interest to us.

Final Report on FIGARO experiment #9-13-683

Interactions of Cubosomes with Model Lipid Membranes at the Air-Water Interface

INTRODUCTION

Lipidic cubic nanoparticles, cubosomes (LCNP) are studied extensively as therapeutic and diagnostic agents and delivery systems due to their biocompatibility, ease of drug encapsulation and targeting. The main advantages of the LCNP formulation approach are: minimization of side-effects of the toxic drug as compared to direct delivery of the drug alone and improved efficacy of low doses of drugs. We have shown recently that monoolein based LCNPs are efficient carriers of the anti-cancer drug doxorubicin, and that drug release profiles at pH of normal cells and cancer cells reveal faster drug removal in the latter case (pH 5.4).

AIMS

The aim of this project was to examine the interaction of LCNP with model biological membranes constituted of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). LCNP interactions with lipid monolayers formed at the air-water interface have not been studied with neutron reflectometry before. These results can significantly enhance our understanding of the molecular interactions between model membranes and LCNP as alternative, biocompatible drug delivery systems, as the neutrons can give us in situ information about lipid exchange.

PRELIMINARY RESULTS

In our recent studies we have used for the first time the Langmuir technique to study interactions of LCNP with model membranes. When injected in the subphase of a Langmuir trough, LCNP approach the air-water interface and spread forming a mixed monolayer. Based on the changes of isotherms recorded for different ratios of components, a non-interacting two-component monolayer is formed. Also, LCNP are shown to disintegrate in contact with a DPPC monolayer, and the penetration of lipid molecules from the disintegrated LCNP into the monolayer depends on its initial extent of molecular packing (i.e. starting surface pressure).

Figure 1 shows some surface pressure kinetic data of the interactions. Our work in general has revealed that: 1) LCNP spreads at the air-water interface in the presence of less organized lipid layers, 2) Changes in shape and area per molecule indicate that it is not the intact LCNP but spread components that are in the mixed layer with DPPC, 3) BAM images (not shown) reflect the changes in thickness of the layer: mixed monolayer structure is retained to high pressures, 4) Less condensed layers facilitate incorporation of the LCNP material into the gaps while highly organized and packed layers keep the LCNP intact and outside the monolayer. Even so, direct information about the exchange of material at the interface remains a mystery.

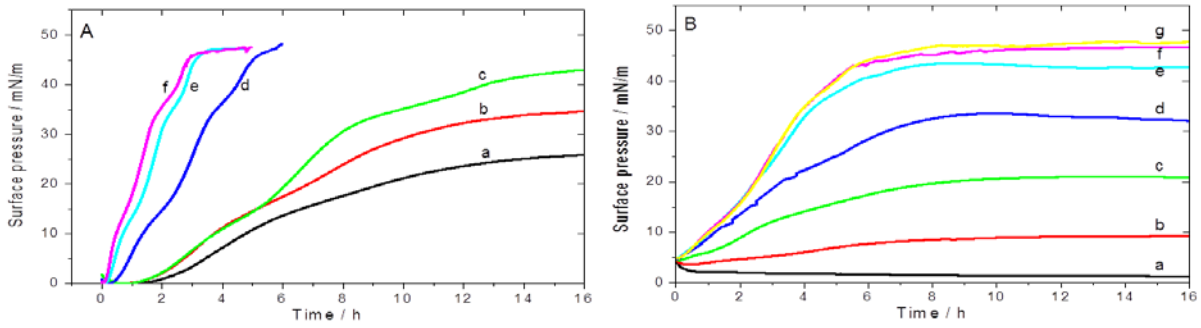

Figure 1. Time dependence of the surface pressure: (A) air−solution interface over subphases containing: (a) 0.25; (b) 0.35; (c) 0.45; (d) 1.00; (e) 2.05; (f) 3.00 mg_{GMO} dm^{-3} LCNP; (B) DPPC monolayer compressed to 5 mN/m over subphases containing: (a) 0.0; (b) 0.10; (c) 0.20; (d) 0.25; (e) 0.30; (f) 0.40; (g) 0.45 mg_{GMO} dm^{-3} LCNP.

FIGARO EXPERIMENT: PLAN

A short initial experiment was granted 2 days of beam time on the FIGARO reflectometer, which took place in February 2017. The experimental plan involved characterization of the interactions

of LCNP with DPPC monolayers. Reference data in 4 isotopic contrasts at 2 surface pressures (5 and 35 mN/m) were first recorded of pure DPPC. The interaction of LCNP injected into the subphase of a Langmuir trough in 4 isotopic contrasts (with h- and d-lipid monolayers and in D_2O and ACMW; scattering length density of zero) was then conducted at the same 2 starting surface pressures. Lipid exchange and the binding of LCNP in situ at the air-water interface was monitored by following the interactions with h- and d-DPPC monolayers only in ACMW.

FIGARO EXPERIMENT: STRUCTURAL RESULTS

First, positive results were achieved concerning the data recorded on pure DPPC, as shown in Figure 2. As it happens, different models are used to fit monolayer data in the literature. However, we have developed a robust method involving a 2-layer model of chains and hydrated head groups, a volume fraction of 1 for the chains layer, a physically-consistent number of chains and head groups, and capillary wave roughness values consistent with the surface pressure applied to all the layers. Such a model was applied successfully at 5 mN/m. However, the data at 35 mN/m required compression of the chains by 15%. This compression surprised us initially, but later we found supporting references for this change in density across the phase transition. These data have interested many colleagues and are currently being written up for a short communication on a robust method to model neutron reflectivity data of surfactant and lipid monolayers at the air-water interface. This will form the first publication resulting from this collaboration.

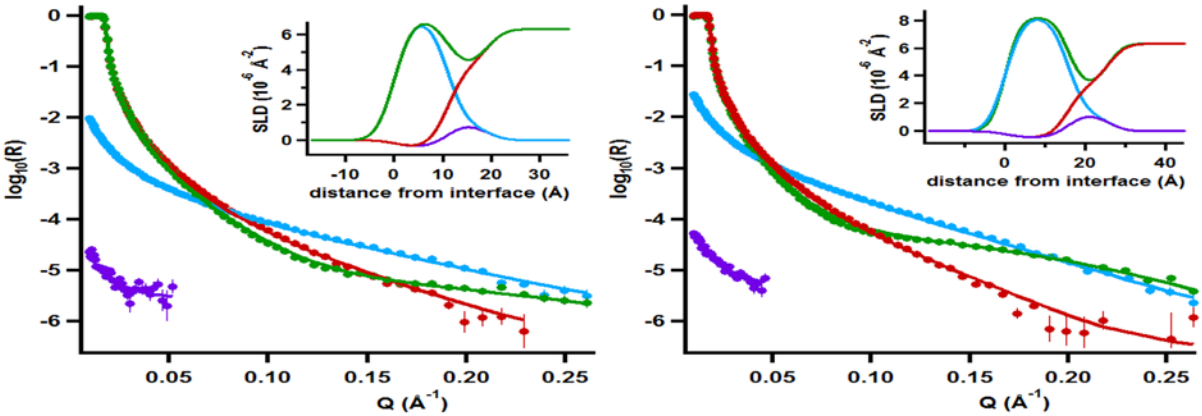

Figure 2. Neutron reflectivity profiles and model fits of DPPC monolayers at (left) 5 mN/m and (right) 35 mN/m. The isotopic contrasts are: (light blue) dDPPC/ACMW, (green) dDPPC/D_2O, (purple) hDPPC/ACMW and (red) hDPPC/D_2O. Scattering length density profiles are in the insets.

FIGARO EXPERIMENT: KINETIC INTERACTION RESULTS

Unfortunately, two difficulties were encountered during the rest of experiment. First, the surface pressure behavior of the interaction of LCNP with d-DPPC monolayers in ACMW was different to that in the other three isotopic contrasts (see left panel of Figure 3). This is particularly hard to explain because the slow kinetics observed were only apparent for the single combination involving d-DPPC and AMCW, and the LCNP formulation injected into the subphase was the same in each case. Note that the slow kinetics for this particular isotopic contrast was reproduced twice more, and the underlying reason has still not been determined. This phenomenon can be rationalized only in terms of isotopic-specific effects and it will need further work to get to the origin of the problem. Despite that, the data obtained from the other contrasts could be modeled and they demonstrate a) the removal of d-lipid from the monolayer, b) the addition of lipid from LCNP into the monolayer and c) the binding of polymer to the lipid head groups.

The second difficulty was that the neutron reflectivity results involving this contrast were not reproducible (see right panel of Figure 3). The first measurement showed a decrease in scattering excess (i.e. loss of d-DPPC) from the interface with time. This was the first direct proof of loss of lipid out of the monolayer as a result of the interaction. However, the two repeats showed first a loss then a rise in the scattering excess, and then only a rise in the scattering excess. This implied that the LCNP formulation was not stable with time, so DLS measurements were performed in the

PSCM and no significant difference in the particle size distribution was observed. However, it could not be eliminated that the free lipid component changes as a result of decomposition. Such an increase in scattering excess observed must have resulted from inclusion of a large amount of (hydrogenous) lipid from LCNP into the monolayer. But as the other isotopic contrasts did not exhibit the same behavior, the data could not be co-refined to elucidate the issue.

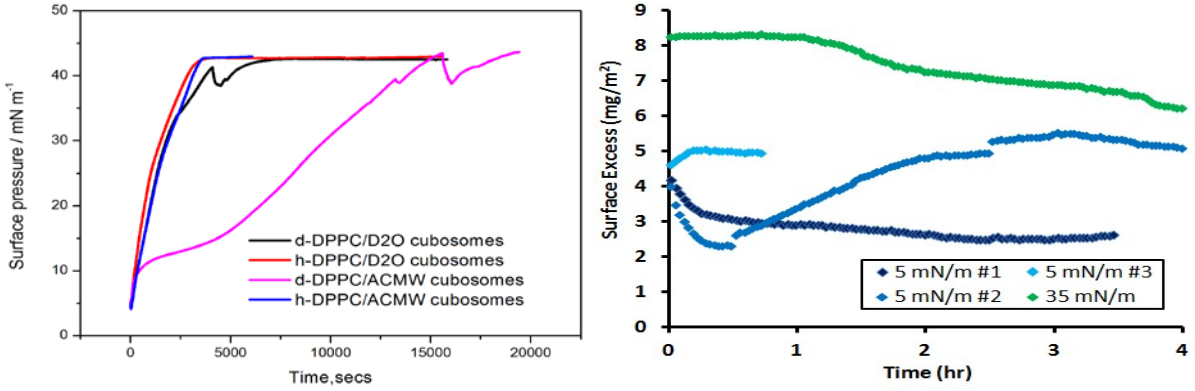

Figure 3. (Left) Surface pressure kinetics of the interaction of LCNP with DPPC monolayers. The inset describes the different isotopic contrasts. (Right) Surface excess kinetics of the interaction involving the contrast d-DPPC/ACMW where the data were modeled on the basic approximation that the scattering is dominated by the deuterated lipid.

SUBSEQUENT LABORATORY EXPERIMENTS

In order to progress with this project, we have worked to solve the two experimental difficulties experienced on FIGARO by implementing two refinements to our approach: one involving the protocols applied and one involving the system studied. First, we have developed a new protocol to spread lipid on pre-mixed LCNP solutions. The left panel of Figure 4 shows promising results from this approach where the surface pressure isotherms are sufficiently similar with respect to the isotopic contrast of the lipid monolayer. Second, from results in another project we found that interactions of the anti-cancer drug doxorubicin were much stronger with the charged lipid DMPS than DMPC (see report for #8-02-797). Therefore, we have extended the work to the interactions of a more stable type of LCNP containing phytantriol rather than monoolein and DMPS monolayers, as shown in the right panel of Figure 4. We consider these preliminary data also to be satisfactory, and they pave the way for future work involving doxorubicin release.

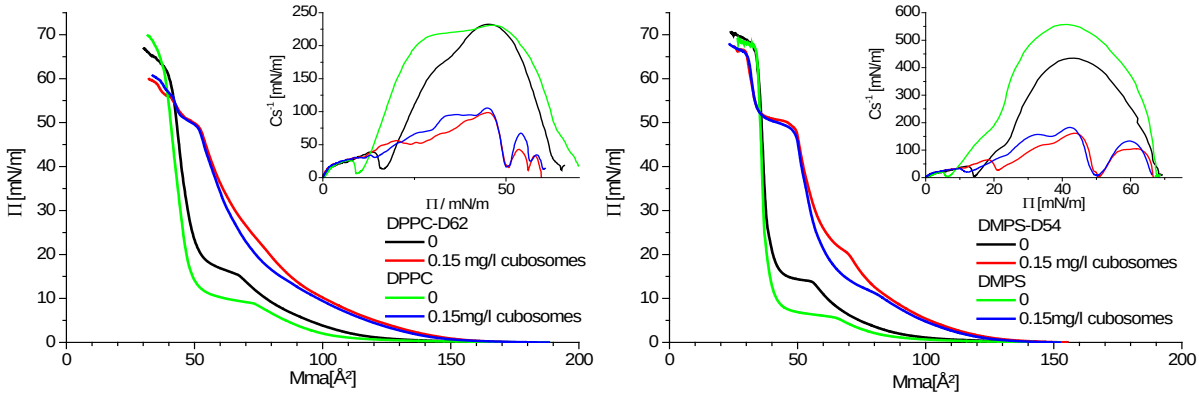

Figure 4. Surface pressure isotherms of both hydrogeneous and deuterated (left) DPPC and (right) DMPS monolayers spread on LCNP solutions on water subphase. The insets show the dependence of the reciprocal of compression modulus versus surface pressure.

We conclude that while this initial short experiment on FIGARO did not work particularly well as a result of unexpected isotope-specific effects that complicated the data analysis, we have worked to formulate new protocols that do work on samples with different isotopic contrasts, and we have applied them to an even more appropriate system with high potential for future work. We are therefore in an appropriate position to submit a continuation proposal in September 2017.