Experimental Report

30/05/2013

Proposal:	**8-03-770**	**Council:**	4/2012

Title: Characterising the Structure Change of Phospholipid Aggregates in Liquid Hydrocarbons - with Particular Focus on Reverse Vesicles

This proposal is a new proposal

Researh Area: Chemistry

Main proposer: **SKIPPER Neal T.**

Experimental Team: NORMAN Lucy
SKIPPER Neal T.

Local Contact: MARTEL Anne

Samples:
Deuterated hexane, with 20mM of lecithins and 3.5mM of NaCl.
Deuterated pentane, with 20mM of lecithins and 3.5mM of NaCl.
Deuterated butane, with 20mM of lecithins and 3.5mM of NaCl.
Deuterated propane, with 20mM of lecithins and 3.5mM of NaCl.
Deuterated ethane, with 20mM of lecithins and 3.5mM of NaCl.

Instrument	Req. Days	All. Days	From	To
D11	3	1	26/11/2012	27/11/2012

Abstract:

The research I will be pursuing at the ILL is to evaluate the structural evolution of self organised aggregates in hydrocarbon liquids at low temperatures, with a view to creating stable reverse vesicles. Reverse vesicles are bilayer structures which encapsulate nonpolar liquids within a nonpolar environment. The bilayer of surfactants in reverse vesicles are structured in the opposite way to normal vesicles – with the lipid groups lining the external surface confining the polar heads to the interior of the bilayer. This structure might be stable or evolve into other aggregate types, e.g. reverse micelles, when reductions in temperature are performed. An application for reverse vesicles includes use in drug delivery systems within the pharmaceutical industry and also has astrobiological interest as cell membranes for theoretical hydrocarbon-based biota inhabiting the methane/ethane lakes and seas on Titan (Saturn's largest satellite). The surfactants will be a mixture of long and short lecithins, with NaCl as a stabiliser. In order to achieve this we will use D11, using deuterated hydrocarbon solvents, and FISH software for analysis of the SANS data.

Preliminary Experimental Report for Experiment 8-03-770

Title of Proposal		Characterising the Structure Change of Phospholipid Aggregates in Liquid Hydrocarbons - with Particular Focus on Reverse Vesicles	
Principal Investigator		Professor Neal Skipper	
Principal Investigator Establishment		University College London	
Experiment Team		Miss Lucy Norman, Professor Neal Skipper, Dr Andrew Dominic Fortes.	
Local contact		Anne Martel	
Date of Experiment	26/11/2012 to 27/11/2012	**Beamline**	D11

Abstract

The objective of this work was to determine, for the first time, the change of aggregation properties of phospholipid surfactants in low molecular weight hydrocarbon liquids at progressively lower temperatures. We used small-angle neutron scattering to characterise reverse vesicle structures in order to understand their potential to function as cell membranes for hydrocarbon-based biota on Titan. This is a rare example of an experimental astrobiological study which may form the basis for future astrobiological work; such as focusing science packages for robotic missions to Titan with the intention of detecting predicted lipid biosignatures.

Experiment Details

A 20mM concentration of the surfactants 1,2-dibutyryl-sn-glycero-3-phosphocholine (C_4-lecithin, see Fig.2b) 99% pure and L-α-phosphatidylcholine (lecithin, see Fig.2a) 95% pure (incorporating 3% lyso lecithin), as well as 3.5mM of NaCl, was initially prepared in methanol. The methanol was evaporated and the dried amphiphilic compound was added to a deuterated hydrocarbon solvent that was then sonicated.

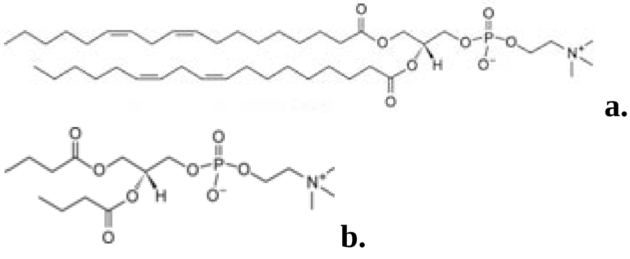

Figure 2. *Structure of main surfactants used in this study – **a.** Lecithin and **b.** C4-lecithin.*

In the 24 hours we were allocated on the D11 beamline we studied three key surfactant-rich deuterated solvent systems – d-heptane, d-hexane and d-pentane. We used Hellma Quartz SUPRASIL Cuvettes (110-QS Light Path 1mm 110-1-40 350µl volume) with a standard orange cryostat (range 1.5 - 300K).
Energy / wavelength range: 12A
Range of momentum transfer: 10%
Resolution in energy or wavelength: $8x10^{-4}$A to $0.5A^{-1}$
Resolution in momentum transfer: 10%

Results

We studied three key surfactant-rich deuterated solvent systems – d-heptane, d-hexane and d-pentane. The d-heptane systems were studied at various temperatures: 290K, 270K, 250K, 230K, 210K, 190K. D-hexane was studied at 270K and 265K and d-pentane was studied at 270K and 280K. Below (Fig 1 and 2) shows some of these results which are currently being analysed using SANSView.

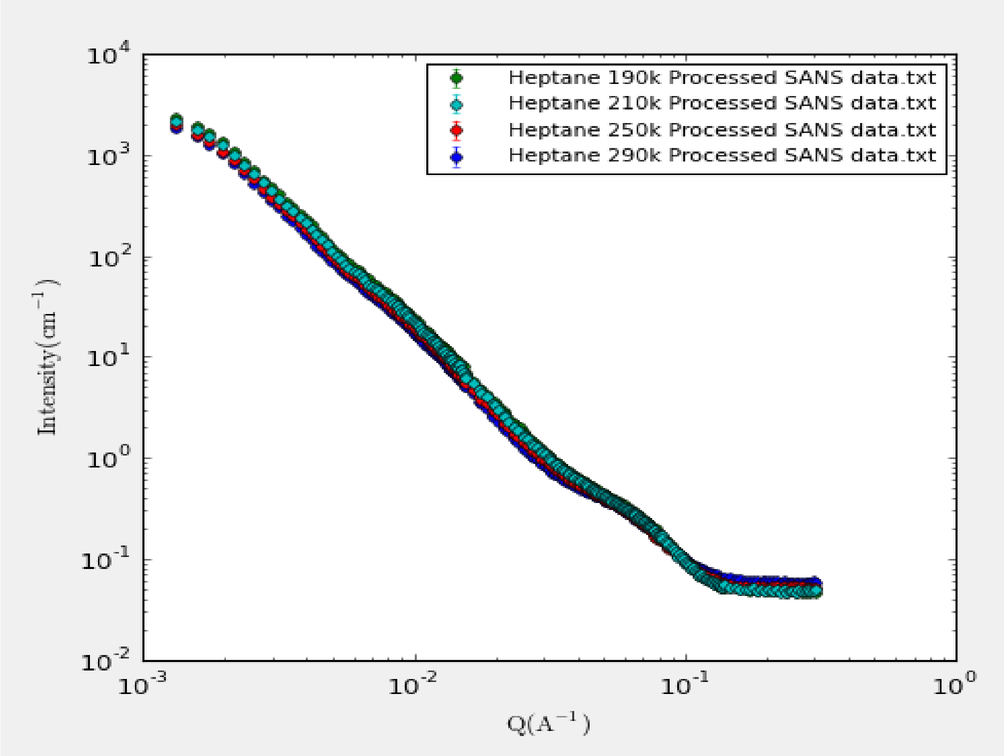

Fig.1. Scatter of d-heptane systems at different temperatures.

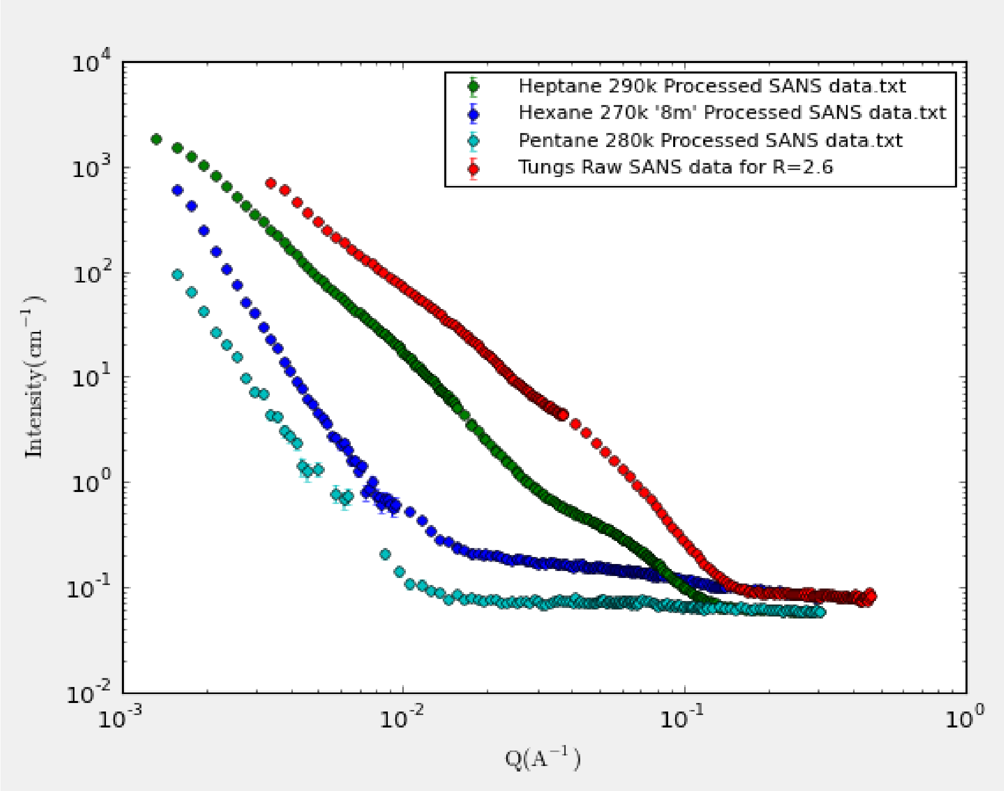

Fig.2. Compares the scatters of the three solvents we used around room temperature with that of the cyclohexane system studied by Tung *et al.* (2009).

Conclusion and Future Work

As shown in Figure 1 temperature depression does not affect the self-assembly of the phospholipids in d-heptane. Figure 2 indicates that lowering the molecular weight of the alkane solvent has significant influences on the self assembly of the phopholipids. Therefore we conclude that SANS is an appropriate analytical method to study self assembly in hydrocarbon solvents.

We are considering application for further beamtime to study different amphiphilic compounds in hydrocarbon solvents to determine their self assembly.