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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	Experiment title: Experiment title: Time Resolved Studies of the Lyotropic Phase Transitions of the Inverse Bicontinuous Cubic Phases	**Experiment number**: CH-1636
Beamline: ID02	**Date of experiment**: from: 29th April to:2nd May	**Date of report**: 1/03/2005
Shifts: 9	**Local contact(s)**: Dr Stephanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Professor Richard Templer*/Professor John Seddon*/ Dr Oscar Ces*/Xavier Mulet*/Charlotte Conn* (Dept of Chemistry, Imperial College, London), Professor Roland Winter/Julia Kraineva* (Dept of Chemistry, University of Dortmund, Germany), Dr Adam Squires* (The Cavendish Laboratory, University of Cambridge).

Report:

The aim of these experiments was to investigate the mechanisms and kinetics of transitions between various lyotropic liquid-crystalline phases formed by monoelaidin (ME) / water, dioleoylphophatidylethanolamine (DOPE)/water and 1-monoolein (MO)/water systems, including the nature of any intermediates formed. Using the pressure-jump technique we have been able to begin to probe the mechanisms of lamellar to non-lamellar (inverse hexagonal and bicontinuous cubic) phases and the interconversion of the bicontinuous cubic phases. In total, using SAXS we were able to monitor the complete time evolution of over 70 pressure jumps including: 14 for DOPE/16 wt%H_2O ($L_\alpha \leftrightarrow H_{II}$), 4 for DOPE /14 wt%$H_2O$ ($L_\alpha \rightarrow H_{II}$), 36 for MO/30 wt%$H_2O$ ($Q_{II}^D \leftrightarrow Q_{II}^G$), 10 for ME ($L_\alpha \leftrightarrow Q_{II}$) and 8 for MO/17 wt% H_2O($L_\alpha \leftrightarrow Q_{II}$). Given the large number of jumps that were undertaken over the course of our time at the ESRF it is not possible to discuss every data set in detail and we have therefore discussed one or two individual runs that give a general overview of our results.

ME L_α ->(Q_{II}^P+Q_{II}^D) Transition: A Structural Analysis

Many cellular processes including endo- and exocytosis, fat digestion and membrane budding involve changes in membrane topology. The inverse bicontinuous cubic phases may act as intermediates in these processes and it is likely that the mechanism of formation of the cubic phase from a corresponding fluid lamellar has much in common with the mechanism of cell membrane fusion and fission. The results obtained have indicated several fascinating features which agree with measurements made by us during temperature-jump experiments at Imperial College and lend credence to our proposed theory for membrane fusion processes. These include the decrease in lamellar lattice parameter on transforming to the cubic phase(Figure 1b), the appearance of a broad low-angle peak immediately afterwards and the formation of an initially highly swollen cubic phase which decreases to an equilibrium value(Figure 1c). It is thought that the decrease in lamellar lattice parameter reflects a geometrically constrained thinning of the bilayer associated with the transition to the cubic phase. The broad intermediate and the presence of a highly swollen cubic phase can be explained by the concerted movement of water on transforming from the lamellar to the much more highly hydrated cubic phase. A number of features not seen on temperature-jump experiments have also been noted, including the coexistence of the lamellar phase with highly swollen Q_{II}^P and Q_{II}^D phases, and the direct formation of the Q_{II}^D phase from the lamellar phase if a very large pressure-jump is employed.

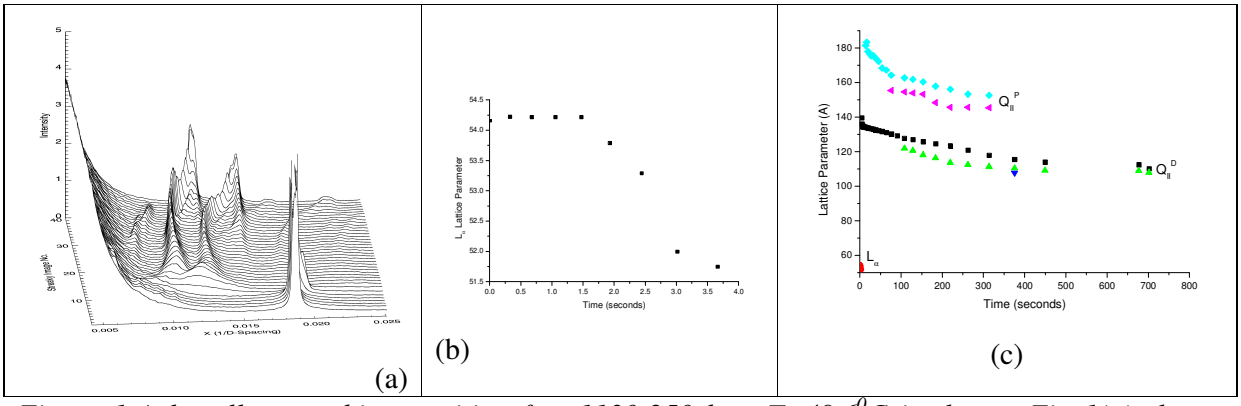

Figure 1:A lamellar-to-cubic transition from1130-250 bar, T=49.6^{0}C is shown. Fig 1(a) shows a typical 'stacked' plot. The evolution of the lamellar phase lattice parameter is shown in jump 1(b) and that of all lattice parameters in Figure 1(c).

Kinetic Analysis of the Q_{II}^D - Q_{II}^G transition in the MO/30wt% H_2O system

The kinetics of the Q_{II}^D - Q_{II}^G transition in the MO/30wt% H_2O system was investigated as a function of pressure-jump amplitude.

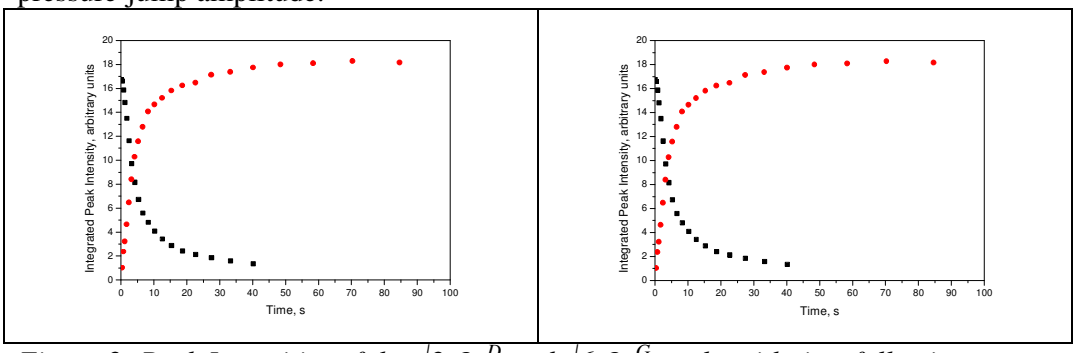

Figure 2: Peak Intensities of the √2 Q_{II}^D and √6 Q_{II}^G peaks with time following a pressure jump from (a)60-580 bar, (b)70-640 bar

Figure 2 shows the changing peak intensity of the √2 Q_{II}^D and √6 Q_{II}^G peaks with time following each of the pressure-jumps. An initial very short delay of <0.5 seconds is noted when only negligible changes in intensity are observed. The data following this initial decay was fitted using an exponential curve of the form

$$I_t = I_\infty + A\exp(\frac{-x}{t})$$

I_t denotes the intensity of the peak at time t. The decay is parameterised using the three constants I_∞, A and t. The first-order rate constant (k) is given by 1/t.

Δp / bar	k / s^{-1} Q_{II}^D	k / s^{-1} Q_{II}^G
520	0.19±0.01	0.18±0.01
570	0.26±0.01	0.26±0.01
700	0.38±0.02	0.35±0.01

Table 1: Effect of pressure-jump amplitude (Δp) on the rate constant (k).

The rate of disappearance of the Q_{II}^D phase is in good agreement with the rate of appearance of the Q_{II}^G phase. An increase of pressure-jump amplitude is clearly associated with an increase in the rate of the phase transition. The results shown in table 1 are again representative of the much larger data set that we were able to acquire during the nine shifts. These measurements are now allowing us for the first time to determine the activation energy per lipid molecule for these complex and collective processes. With the successful development of this methodology we plan to compare the activation barriers between different lipid systems in the future and draw conclusions about molecular effects on the transition state.