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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:** Time Resolved Pressure-Jump Studiesof Lyotropic Phase Transitions ofthe Inverse Bicontinuous Cubic Phases	**Experiment number**: SC-1838
Beamline: ID02	**Date of experiment**: from: 9th Sept to: 12th Sept 2005	**Date of report**: 28/02/08
Shifts: 9	**Local contact(s)**: Stephanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Prior to session 1838, our pressure-jump experiments looking at lamellar-inverse bicontinuous cubic phase transitions in the mixed lipid water system monoelaidin/H_2O had demonstrated that this transition takes place via the formation of hemi-fusion diaphragm intermediates which eventually rupture to form fusion pores, closely mimicking the process of cellular fusion in biological systems. Additionally we had observed that the rate of transition was found to be strongly dependent upon the difference between the final pressure of the system and the pressure at the phase transition boundary.

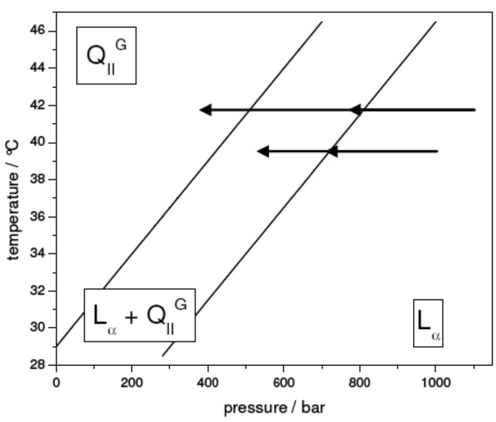

Figure 1 The p-T phase diagram for ME 30wt% H_2O

These studies had to date focussed upon the kinetic and structural aspects of phase transitions in excess water lipid mixtures. During SC-1838 we wished to expand these studies to fixed hydration and other monoglyceride systems including 1-monoolein and monomyristolein samples as such studies can provide valuable insights into the mechanism of fusion pore formation by allowing us to disentangle the effects of water transport.

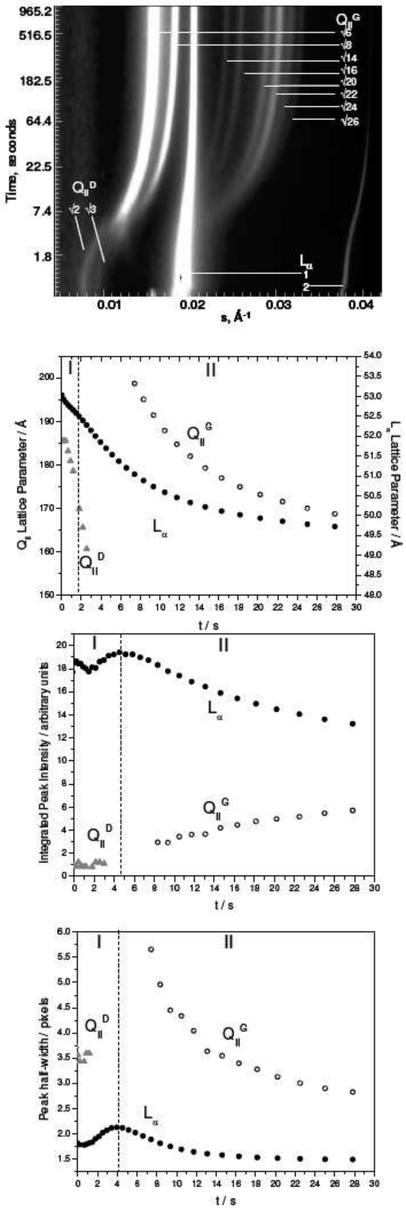

Figure 2 ‘Stacked’ plots following pressure-jumps from 1090 to 690 bar for ME / 30 wt% water, along with the lattice parameter and intensity and half-width of the L_α first order peak and Q_{II}^{G} 211 (√6) peak.

During our experiments at the ESRF we were able to successfully construct the equilibrium p-T phase diagrams for the 1-monoolein/H_20, monoelaidin/H_20 (example in figure 1), and monomyristolein/H_20 systems at fixed binary ratios. These allowed us to interpret the ensuing results of the lyotropic phase transformations under study and build upon our previous measurements. We subsequently monitored the complete time evolution of the phase transitions of 1-monoolein, monoelaidin and monomyristolein systems (both excess water and fixed hydration samples) as a function of pressure jump amplitude. Highlights from our results include the observation that for e.g monoelaidin the transition pathways between the fluid lamellar and inverse bicontinuous cubic phases (figure 2) bears obvious structural differences to that under excess water conditions including the absence of intermediate phases of crystallographic symmetry. However several structural features of the transition are deduced. These include the co-existence of a swollen Q_{II}^{D} cubic phase with the L_α phase under equilibrium conditions (figure 3), a characteristic decrease in lattice parameter of the L_α phase and a well-defined linear trend of the rate of this decrease on the jump-amplitude (figure 4). An increased level of disorder in the fluid lamellar phase throughout the transformation has been noted which we can now explain with reference to the effects of limited hydration on stalk formation. Measurements on monoolein demonstrated that stalk formation has a less pronounced effect on bilayer spacing for this system and that this is linked to the differences in the micromechanical properties of the system.

As for ME under excess water conditions, a swollen Q_{II}^{D} phase is observed to co-exist with the fluid lamellar phase in ME / 30 wt% H20 (figure 2). We have previously suggested that under excess water conditions this phase could act as a water storage vehicle relieving high curvature at the centre of the vesicular L_α domains. It is interesting that such a phase may adopt a similar role even under limited hydration conditions. The transformation itself bears obvious differences to the L_α –Q_{II}^{D} transition for the same lipid. In contrast to the complex structural route involving the presence of a number of intermediate swollen cubic phases (previously discovered during ESRF experiments) observed in excess water, no intermediate phases of crystallographic symmetry are observed during the L_α–Q_{II}^{G} transition under limited hydration conditions. Instead the disappearance of the L_α phase is accompanied by the appearance of diffuse scatter in the diffraction pattern from which peaks corresponding to the √6 and √8 reflections of the Q_{II}^{G} phase grow.

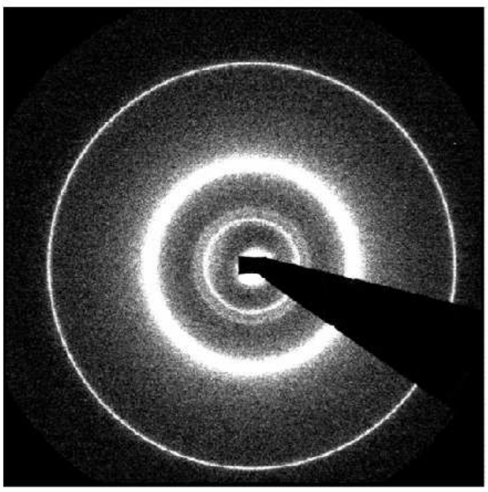

Figure 3 Highly swollen Q_{II}^{D} intermediate phase (42 °C, 1200 bar). ME / 30 wt% H_2O.

Figure 3 depicts an example of the highly swollen structures we observed to co-exist with the L_α phase. Diffraction peaks corresponding to this phase were initially broad but become more resolved with successive jumps eventually being resolved as a high lattice parameter Q_{II}^D phase. Although only two peaks were detectable for this phase, the intensity ratio and indexing were consistent with previous observations. The diffraction rings are fairly smooth indicating the presence of numerous, randomly oriented small domains. This is consistent with the theory proposed under excess water conditions that such a phase may exist at the centre of the onion vesicles where it acts to relieve high curvature elastic stress.

Immediately following pressure jump these swollen cubic intermediates display a sharp linear decrease in lattice parameter as expected following a jump to lower pressure. Despite peaks corresponding to this phase being weak it has been possible to extract intensity and halfwidth data. Using in-house technologies these intermediates may have remained undetected. The intensity and half-width are observed to remain generally constant right up until the disappearance of the phase. This is indicative of an abrupt annihilation of this phase. We noted that the lifetime of the phase is inversely proportional to the size of the pressure jump. In order for this sealed cubic phase to shrink whilst maintaining its symmetry, it must be able to burst holes in its outer layer in order to release water. This water may be used to facilitate the transition.

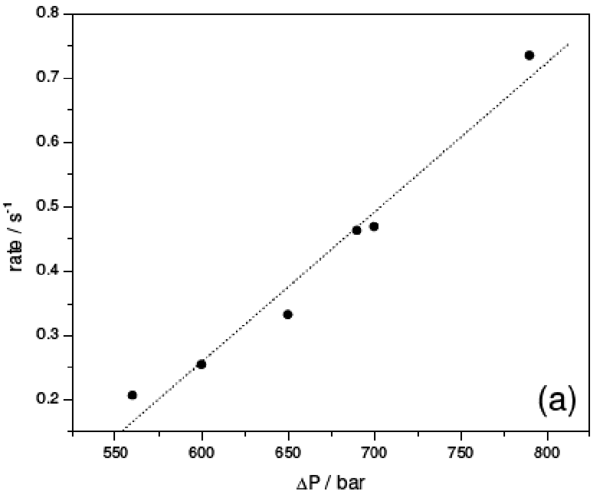

Figure 4 The rate of decrease of the L_α lattice parameter during as a function of jump-amplitude during lamellar to inverse bicontinuous cubic phase transitions in an ME / 30wt% H_2O system.

This work alongside that of SC-1962 contributed to two publications in Langmuir:

Shearman, G.C. et al, **Calculations of and evidence for chain packing stress in inverse lyotropic bicontinuous cubic phases**, LANGMUIR, 2007, Vol: 23, Pages: 7276 - 7285, ISSN: 0743-7463

Conn, C.E. et al, **A Pressure-Jump Time-Resolved X-ray Diffraction Study of Cubic-Cubic Transition Kinetics in Monoolein** (2008), Langmuir, *in press*