Standard Project

Experimental Report template

Proposal title: SAXS/WAXS study of self-assembled sophorolipids: effect of unsaturation and number of sugar groups		**Proposal number: SC- 4050**
Beamline: ID-02	**Date(s) of experiment:** from: 13/05/2015 to: 15/05/2015	**Date of report:** 08/06/2015
Shifts: 6	**Local contact(s):** Sylvain Prévost	*Date of submission:*

Objective & expected results:
The goal of this proposal is to combine SAXS/WAXS to understand the formation of self-assembled forms of various acidic sophorolipids, SLs, under dilute conditions. To do so, we operate a systematic comparisons between compounds with slight structural variation. We investigate: 1/ the role of the unsaturation, C=C, in the aliphatic C18 chain (C18:X, X= 0, 1, 2, 3) and 2/ the number of sugars (1 or 2 for C18:0 and C18:1) in the hydrophilic headgroup. SAXS will be used to study the morphology and size of SLs aggregates (from micelles to fibers) while WAXS is used to study the molecular packing within the crystalline fibrillar assemblies. Considering that the self-assembly is strongly influenced by the pH, we explored the whole pH range between 12 and 3.

Results and the conclusions of the study (main part):

The experiments have been done at 12.46 KeV and 1 m distance. AuBenh was used to calibrate the q-range.
Sophorolipids solutions were used at 0.5 w% in water. Initially, the compounds are introduced in water at pH< 12. pH is then adjusted by mean of HCl at 0.1 M. In total, 5 mL of solution have been prepared for each sample.
The experimental setup consists in a flow-through system using a peristaltic pump and a 1 mm diameter silicon tube carrying the solution from the beaker through a polycarbonate 2 mm inner diameter capillary. pH was adjusted externally by adding, drop by drop, the HCl solution. pH is monitored live using a KCl microelectrode and for each value of pH, the corresponding SAXS/WAXS spectrum is recorded. The time between two drops is about 45 s. the solution is allowed to stir during the whole period of time of the experiment.
The total experiment duration is about 45 min per sample.

We have analyzed 8 main compounds (Figure 1):

Oleic acid sophorolipid, *C18:1-cis* (monounsaturated, *cis* conformation)
Stearic acid sophorolipid, *C18:0* (saturated)
Oleic acid glucolipid *G-C18:1-cis* (monounsaturated, *cis* conformation)
Stearic acid glucolipid *G-C18:0* (saturated)
Elaidic acid sophorolipids *C18:1-trans* (monounsaturated, *trans* conformation)
Linoleic acid sophorolipids *C18:2* (double unsaturated)
Linolenic acid sophorolipids *C18:3* (triple unsaturated)
Branched acid C22 sophorolipids *Branched C22* (saturated) (not shown)

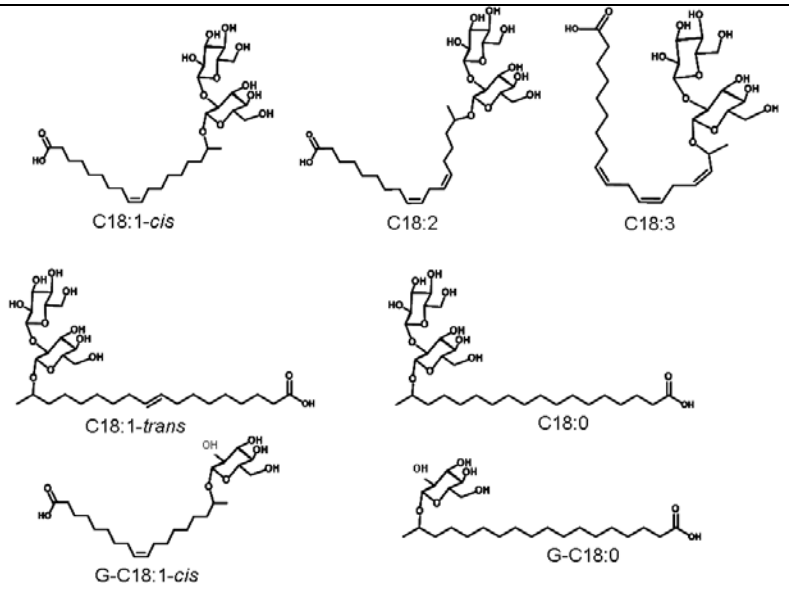

Figure 1

The typical result issued of one experiment performed on the C18:1-*cis* compound is shown in Figure 2. About 35 spectra have been recorded for this compound in the 11.6 < pH < 3 range. At high pH two regions can be distinguished: below q= 0.2 nm^{-1} identifying large aggregates providing a fairly intense scattering signal, and above q= 0.2 nm^{-1} identifying a micellar signal, still to be analyzed in detail. By lowering the pH one can observe a loss in the scattering at q= 0.2 nm^{-1}, an increase in the signal below q= 1 nm^{-1} and a pronounced signal above q= 1 nm^{-1}. These two features suggest the formation of micellar objects with narrow size distribution while the first one indicates the loss of large-scale objects.

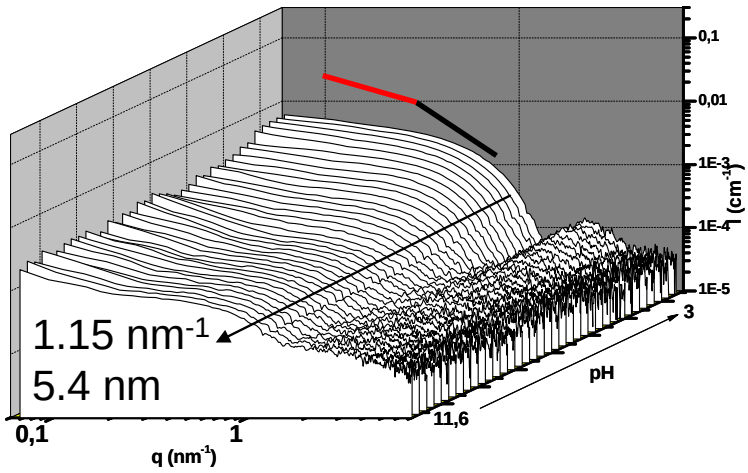

Figure 2

The previous observations can be summarized in Figure 3, where the evolution of the slope below and above q= 0.18 nm^{-1} is reported. Below q= 0.18 nm^{-1}, the slope value varies between 1 and -1.5 in the pH range below 9. Above q= 0.18 nm^{-1}, on the contrary, the slope is negligible. Above the transition pH between 8 and 7, a unique slope value, the value of which indicates the presence of micellar aggregates, is recorded instead and it does not vary until pH= 3.

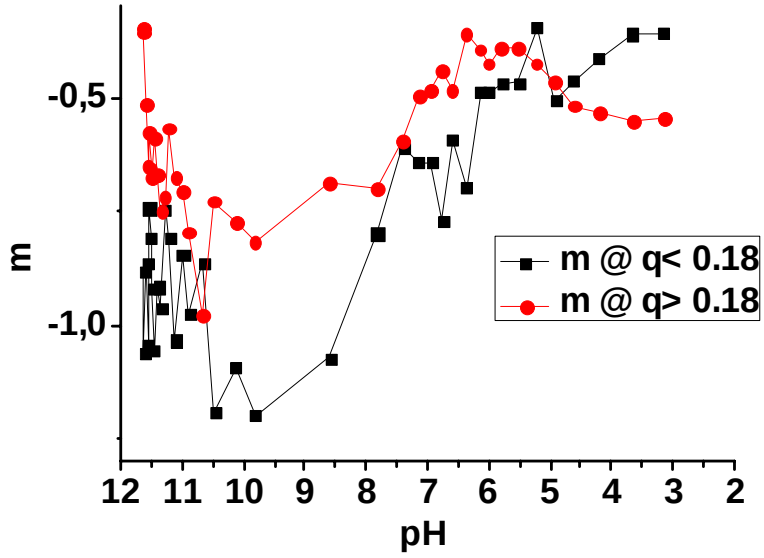

Figure 3

Similar experiments have been performed for all other sophorolipid systems mentioned above. In Figure 4, we report the comparison between sophorolipids (C18:1-cis and C18:0), glucolipids (C18:1-cis and C18:0) and the branched C22 sophorolipid at about pH 11.5. At a first glance, all samples except the branched SL and the G-C18:0 SL form polydisperse micellar systems. In all cases, an intense scattering signal at q< 0.3 nm^{-1} characterizes the spectra. This is the sign indicating the presence of large objects. Deeper analysis is under going.

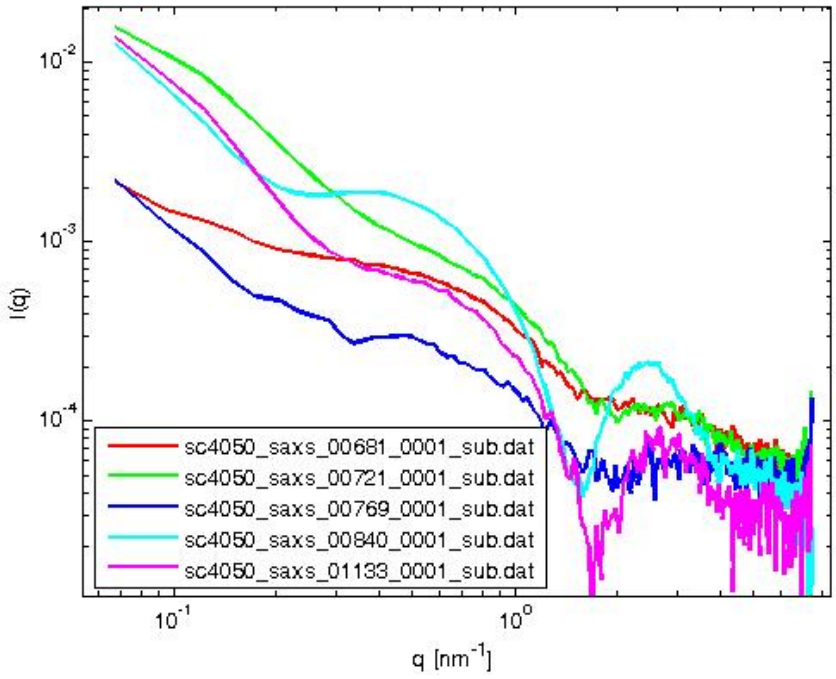

Figure 4 : Exp N. 681= C18:1-cis ; Exp N. 721= C18:0 ; Exp N. 769= G-C18:1-cis ; Exp N. 840= Sl Branched ; Exp N. 1133= G-C18:0. All samples are measured at pH= 11.5

Data treatment

All samples have been treated at the beamline soon after acquisition. The CCD images have been integrated and correction for absolute calibration was done. Background (capillary + water) has been regularly measured and subtracted from the data. When possible, three measurements per sample have been recorded and averaged. The acquisition time lies between 0.3 s and 1 s,

according to the sample, eventual beam damage and signal-to-noise ratio. In few cases, saturation of the detector at low-q was noticed.

Justification and comments about the use of beam time:

The use of the beamline was necessary becasue of the low volume fraction employed (0.5 w%) and the large number of spectra acquired per sample as a function of pH. The low-acquisition times per experiment allows to explore a large amount of pH values in a reasonable time. We employed a 1 m distance for all systems and we did not have enough time to treat the data and select those who needed larger distances (30 m). Nevertheless, some samples definately require to be explored at longer sample-to-detector distances as the signal still increases at very low-q values, as shown in Figure 4.

Problems during beamtime:

We did not experience any trouble during the beamtime. Our work was very well supported by the local contact before and during the experiment. Communication was fine and at a first glance we estimate that experiments have been done in the best conditions possible. The beamline was stable during throughout the experiment and we were provided with all the necessary tools to arrange our setup (e.g., polycarbonate capillary...).