MX NON-BAG BioSAXS

Experimental Report template

Proposal title: SAXS characterization of sophorolipid microbial biosurfactant chemo-derivatives		**Proposal number:** MX 1821
Beamline: BM29 - BioSAXS	**Date(s) of experiment:** from: 28/10/2016 to: 30/10/2016	**Date of report:** 28/01/2017
Shifts: 6	**Local contact(s):** Martha Brennich	*Date of submission: 28/01/2917*

Objective & expected results:

Sophorolipids are one of the most interesting classes of biosurfactants because of their large scale production by microbial synthesis, biodegradability and low toxicity. Typical applications are antimicrobial, dermatological, immunoregulatory, spermicidal, antiviral as well as additives in cleaning and cosmetic products. However, the range of molecular structures available is limited and chemical modification is needed to efficiently target broader fields of application. In this proposal, we study a wide range of new sophorolipid chemical derivatives (about 70 different molecules) containing quaternary ammonium and amine oxides groups as well as symmetrical bolaform derivatives. Considering the novelty of these compounds, little is known about their properties. We then want to use SAXS as a characterization tool of the self-assembly properties of these compounds in water at various concentrations and understand their behaviour as a function of their engineered chemical structure.

This work has the objective of correlating the self-assembly properties (micelles, vesicles, fibers...) of new forms of sophorolipids in water to their engineered chemical structures. We want to explore, for each sample, several concentrations from diluted (0.1 wt%) to mildly-concentrated regimes (10 wt%). The products are divided into three categories: ammonium salt sophorolipid derivatives, acetylated and deacetylated; amine oxide sophorolipid derivatives, acetylated and deacetylated and a wide series of bolaform sophorolipids.

Results and conclusion

The experiments have been done at 12.5 KeV and AuBenh was used to calibrate the q-range. Water was used as solvent as well as calibrator of the intensity (0.016 cm^{-1}). Since the intensity by default at the beamline is calibrated in Dalton, all our data have been multiplied by the factor $8x10^{-4}$ to have values in cm^{-1}. We used the standard sample automatic environment available at BM29 and described in detail here:
http://www.esrf.eu/home/UsersAndScience/Experiments/MX/About_our_beamlines/bm29/beamline-setup/sample-changer.html
We used either PCR sample holders or 96 well-plates and about 100 μL of each sample.
We studied a large number of engineered sophorolipids: 1) a set of eighteen quaternary ammonium sophorolipids; 2) a set of fourteen sophorolipid amine oxides; 3) a set of sixteen bolaform sophorolipid amines; 4) a set of sixteen bolaform quaternary ammonium sophorolipids; 5) three additional bolaform sophorolipids synthesized using a microbial pathway without any chemical step involved were also studied.

Preliminary analysis of the data so far has shown that only long chain quaternary ammonium derivatives have micelles-forming properties, while short chain quaternary ammonium modification have poor self-assembly respense. Bolaform sophorolipids form both micelles, the structure of which is invariant from the concentration, and in some cases, nanotubes, although

the latter must be confirmed through other techniques.

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. Ten measurements per sample have been recorded and averaged and background was collected before and after sample measurement. Files were analyzed one by one to eliminate corrupted data (e.g., empty capillary) and then averaged. The acquisition time lies was set to 1 s. Few cases of beam damage were observed and when that occurred, data were collected again manually at different parts of the injected solution. In summary, this beamline is perfect for the analysis of colloids in solution.

Justification and comments about the use of beam time:

The use of the beamline was necessary becasue of the high load of samples we had to analyze. Although the beamline is optimized for proteins, our micellar solution were pperfectrly adapted to the beamline characteristics, in particular its q-range. The automatic sample changer, the small volumes and software were crucial to record a large amount data in two days. The standard setup of the beamline make users save a lot of time which often derive from changing from one sample environment to another according to the previous user. The local stuff was also very helpful. The independent procedure to apply for beamtime is also very helpful.

Problems during beamtime:

We did not experience any trouble during the beamtime. Nevertheless, despite the big advantage of the automatic sample changer, one still needs to control all data simultaneously as injection problems (e.g., viscous solutions, poor self-cleaning procedure) can always occur.