Experimental report

16/03/2022

Proposal: 8-02-899 **Council:** 10/2019

Title: Probing CER EOS arrangement in thelong lamellae of skin lipids

Research area: Chemistry

This proposal is a new proposal

Main proposer: Emelie NILSSON

Experimental team: Johan ENGBLOM
Emelie NILSSON
Enamul MOJUMDAR
Bruno DEME
Chris GARVEY

Local contacts: Bruno DEME

Samples: ceramide EOS: C66H125NO5

Instrument	Requested days	Allocated days	From	To
D16	5	5	17/03/2021	22/03/2021

Abstract:

The lipid matrix in the skin's outer layer - the stratum corneum (SC) forms an effective barrier and comprised of ceramides (CERs), cholesterol and free fatty acids. These lipids form crystalline lamellar phases with repeating pattern of approx. 6 and 12 nm and referred to as short and long periodicity phases - SPP and LPP, respectively. In previous studies performed at ILL D16, we successfully probed molecular localization of various lipids in SPP with sufficient H2O/D2O contrast. In subsequent studies on the LPP system, the sufficient contrast allowed us to further determine molecular location of CER EOS linoleate tail in the LPP. However, due to limitation of deuteration, a full characterization of CER EOS was not possible. As we now have possibilities to have CER EOS deuterated in different regions of the molecule, we embark upon a journey with the aim of resolving CER EOS arrangement in the LPP unit cell, by taking model membrane approach and with CERs extracted from the native skin.

Report on Experiment 8-02-899
17-23 March 2021

The skin barrier is crucial for our survival and is primarily located on the outermost horny layer of the skin – the stratum corneum (SC). The intercellular lipid matrix in the SC serves as the main barrier and is mainly composed of ceramides (CERs), cholesterol (CHOL) and free fatty acids (FFAs). Together, these lipids form two lamellar structures with periodicities (d-spacing) of 60 Å (short periodicity phase - SPP) and 130 Å (long periodicity phase - LPP). The ceramide EOS has a prominent role in the formation of the LPP, but is difficult to synthesize due to its long structure with 2 unsaturated hydrocarbon tails. The pandemic unfortunately prevented us from receiving deuterated CER EOS, and we had to adapt the investigated system from the proposal. In this experiment we therefore investigated the localization of a small active pharmaceutical ingredient, *i.e.* salicylic acid (SA), using a protonated CER EOS never used for neutron experiments.

We made samples containing 1:1:1:0.5 mol ratios of FFA:CER:CHOL:SA, a mixture of CER EOS and CER NS was used to mimic the ceramide mixture in the skin lipid lamellae. We prepared 4 different samples, one reference sample without SA and 3 samples with either h-SA, d-SA or h/d-SA (1:1 mol:mol). All samples were measured at 32 °C and 97 %RH, and we used 100 % D_2O, 50% D_2O and 8 % D_2O contrasts. In order to capture the desired q-range a sample rotation from -0.5-12 degrees (omega) was used (Figure 1), collecting 251 scans/sample. Several samples were also measured for 30-60 min at a fixed omega, before measurement, used to verify that sample hydration, and contrast, have been reached. One sample was also measured with in-situ equilibration to verify that the desired hydration/contrast was reached within the expected waiting time.

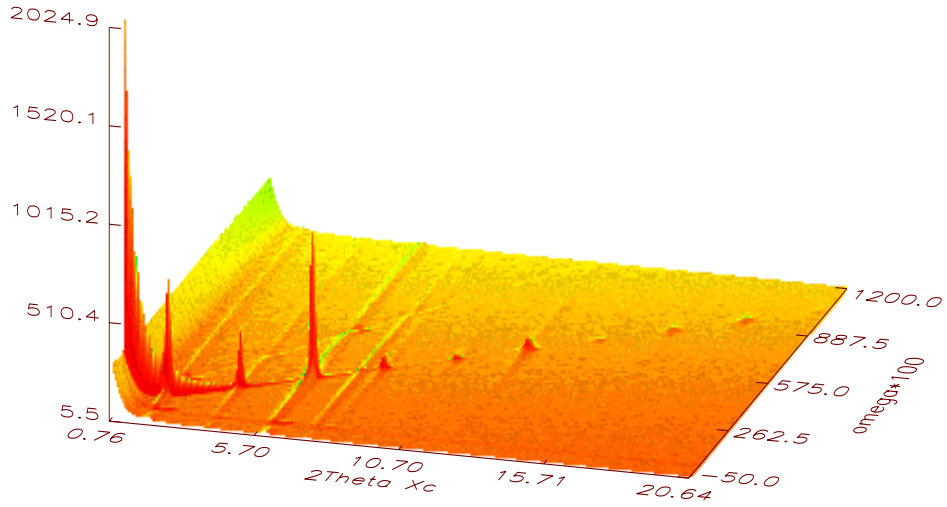

Figure 1: Lipids + d-SA with 100% D2O, clearly showing 9 reflections of the lamellae and a relatively low mosaicity. Additionally, phase separated crystalline cholesterol can be observed at angles just below the fourth reflection.

Initial data analysis reveals that the lamellar d-spacing for all samples are between 126-128 Å, as expected and that the reference sample (without SA) has a slightly shorter repeat distance. This was also observed prior to the measurements, when characterising the samples using an in-house GISAXS setup.

The data analysis reveals 9 distinguishable Bragg peaks for each sample (Figure 2). After background subtraction the integrated peak intensities reveal a linear dependence to the contrast (Figure 3), allowing for the phase sign to be determined for all samples. The initial analysis shows promise to be able to determine an accurate structure factor and subsequent scattering length density profiles for the different samples, revealing the positioning of the salicylic acid in the lipid lamellae.

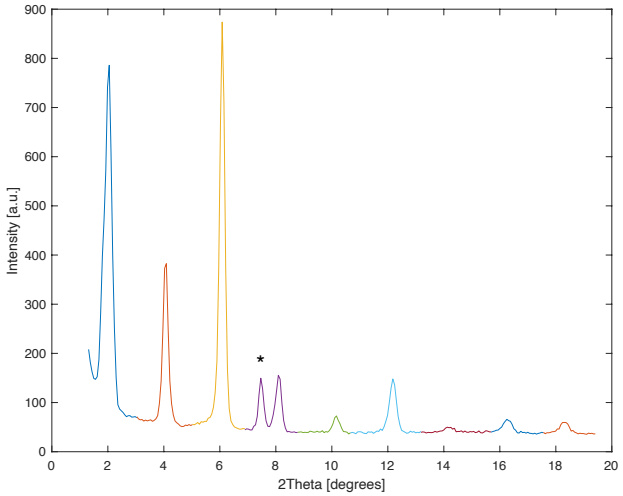

Figure 2: The extracted Bragg peaks for all 9 reflections in the reference lipid sample at 100% D_2O (5 scans are combined, omega ± 1 degree, per reflection). The data shows that the third reflection is the strongest, followed by the first, second and so forth. Phase separated crystalline cholesterol (*) is measurable in all samples, as expected but does not overlap with the intensities of the fourth reflection.

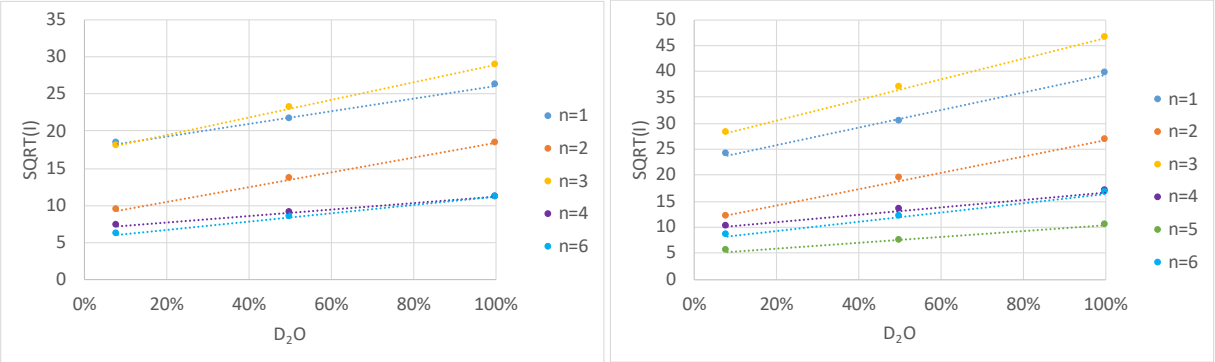

Figure 3: The square root of the intensity from the integrated Bragg reflections as a function of the contrast. Left: refence lipid sample without SA and right: Lipid sample with deuterated SA. For the data it becomes noticeable that the third reflection is the strongest reflection for all samples.