



**DUTCH-BELGIAN BEAMLINE  
AT ESRF**

**EUROPEAN  
SYNCHROTRON  
RADIATION FACILITY**




## **Experiment Report Form**

### **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.

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	<b>Experiment title:</b> Synthetic lipids form lipid organisation mimicking the phase behaviour in the horny layer of the skin	<b>Experiment number:</b> <b>26-02-417</b>
<b>Beamline:</b> BM26B	<b>Date(s) of experiment:</b> From: 06-07-2008  To: 11-07-2008	<b>Date of report:</b>  30- 07 -2008
<b>Shifts:</b> 9	<b>Local contact(s):</b> W. Bras	
<b>Names and affiliations of applicants (* indicates experimentalists):</b>  <b>J.A. Bouwstra, G.S. Gooris, D. Groen, D. de Sousa Neto</b>		

**Report: (max. 2 pages)**

### **Experimental conditions :**

We performed measurements during a 3-days session in July 2008. The beam conditions (beam intensity and beam alignment) were excellent, and we were able to use the wide-angle detector, which gave excellent diffraction patterns. However, the small angle X-ray detector condition was not optimal (the same situation as our previous beam time in February), which limited the conclusions that can be drawn from these measurements, especially concerning electron density calculations. However, the measurements can be used to draw general conclusions concerning the selection of future samples and the direction of the research in future. However, we certainly have to repeat a selected number of measurements. We were extremely happy about the wide angle X-ray data!

### **Background experiments:**

The skin barrier for diffusion of substances is located in the horny layer, the outermost layer of the skin. The horny layer consists of dead cells embedded in lipid lamellar regions. The lipid lamellar regions are crucial for the skin barrier function. The lipid composition and organisation in the horny layer is exceptional. Mainly free fatty acids, cholesterol and ceramides (9 subclasses) are present forming two crystalline lamellar phases with repeat distances of 6 and 13 nm. We have available synthetic CER (5 subclasses) that in mixtures with CHOL and FFA mimic the lipid organization in the horny layer.

The aim of the present project was to study the lipid organisation of synthetic CER, cholesterol and FFA. In addition we measured the lipid organization in the horny layer isolated from human skin equivalents. Finally some finalizing studies were performed using vernix caseosa.

**The main goal in our research is to study the structure and orientation of model lipid lamellae (parallel to a porous membrane, or randomly oriented on mica).**

1. The lipid organization of vernix caseosa has been studied and finalized.
2. We studied the lipid organization of CHOL:CER:FFA mixtures in which CER1 level was increased from 15% (normally present) to 30%. We also studied the CER:CHOL:FFA variation. I appeared that at equimolar ratios, the first order SPP is on exactly the same position as the 2<sup>nd</sup> order of the LPP. When reducing the FFA levels, the SPP repeat distance is reduced. When reducing CHOL levels in the mixture,

there is no change in the lipid organization.

3. By using oriented lipid lamellae on a porous membrane, the phase behaviour of lipid mixtures mimicking the compositions in the horny layer of diseased skin is being studied. Changes in composition are i) variation in CER composition: substitution of the CER1-linoleate chain by CER1-oleate or stearate, ii) replacement of long chain FFA by shorter chain FFA, iii) addition of cholesterol sulphate and iii) variation in CHOL:CER:FFA ratio. Studies were carried out successfully. We will combine these studies with FTIR measurements, as well as an assessment of the barrier function in permeation studies.
4. A lipid mixture with only CER1, CHOL and a variation in FFA composition was examined. Measurements were performed successfully. In the future, after additional (FTIR) measurements, the molecular organisation might be determined. This will facilitate understanding the more complex model that mimics closer the lipid organisation of the horny layer.
5. We performed the first measurements using the horny layer isolated from reconstructed skin models. We will continue on this subject in the next beam session.