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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:** Phase behaviour of isolated and synthetic ceramides	**Experiment number**: 26-02-182
Beamline:	**Date of experiment**: From: 30-11-2003 to: 02-12-2003	**Date of report**: 12-09-2004
Shifts: 8	**Local contact(s)**: Wim Bras	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

J.A. Bouwstra, G.S. Gooris, M. de Jager, Leiden University, LACDR. Leiden

Report: We performed measurements during a 3-days session in novenber/december 2003. The beam conditions (beam intensity and beam alignment) were excellent. We were able to perform all the scheduled experiments, including a number of measurements, in which the lipid phase behaviour was studied as function of temperature.

The skin barrier is located in the lamellar regions in the outermost layer of the skin, the horny layer or stratum corneum. The lipids consist of ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA). In SC from the lipids are organized in two lamellar phases with periodicities (d) of 6 and 13 nm (1-2). In stratum corneum at least 6 different ceramides are present referred to as CER1, 2,3,4,5 and CER6. The ceramides differ from each other by the headgroup architecture, i.e. the number of OH groups in the head group regions. CER1 has an exceptional structure, namely an ω-hydroxy fatty acid of approximately 33 C atoms towards which a linoleate chain is chemically linked (referred to as CER1-oleate). The characteristic phase in the horny layer is a 13 nm crystalline lamellar phase. In previous studies we observed that CER1 is crucial for the 13 nm lamellar phase. We have studied:

1. Effect of thermal history on the lipid organization. Mixtures prepared from CER, CHOL and FFA were studied. The CER mixture consists of syntheticCER1, 2,3,4,5 and 6, costume synthesized for this project. The formation of the 13 nm phase was studied using heating and cooling cycles. The formation of the 13 nm phase always started at around 60ºC, independent of the thermal history of the samples. Furthermore, it appeared that the presence of a hexagonal lateral packing is a prerequisite for the formation of the 13 nm lamellar phase. All experiments were performed with a fixed composition of synthetic ceramides.
2. Orientation of lipids parallel to a porous membrane to achieve the same lamellar orientation and lipid organization as in stratum corneum. In previous studies we achieved already a parallel orientation of the lamellae. However, the lipid organization was not similar to that in stratum corneum. The lipids are casted on the porous membrane using the paint-brush method. We performed all measurement with a fixed composition of *synthetic ceramide mixtures* (CER1,2,3,4 and 6) mixed with CHOL and FFA. To select the ideal procedure, we casted the lipids using different equilibration temperatures and various organic solvents. We also used different amounts of lipids. Wee measured samples in the central part of the lipid membrane and in the boundary regions. In addition we measured the samples in hydrated and in dry state. Both, the temperature and the solvent appeared to be very crucial. In a small number of samples we achieved for the first time lipid lamellae oriented parallel to the porous membrane in the 13 nm lamellar phase. The orientation and phase behaviour was correct throughout the whole membrane. We will optimize the casting method further. In future the optimized conditions will be used to cast also other lipid mixtures prepared from different ceramide compositions on the porous membrane. In leiden with the oriented membranes we also study the diffusivity across the samples in Leiden. The combined

experiments provide crucial information about the relation between lipid organisation and skin barrier. Only by combination of these studies barrier function of skin can be related to lipid organization.

The above experiments are part of an STW grant (4654).

3. The effect of exogenous substances on stratum corneum lipid phase behaviour. We studied the effect of substances on the lipid organization. We used two classes of substances. Substances that increase the permeability and substances that reduce the permeability. We observed that the permeation promoters “destroy” the 13 nm lamellar phase, while permeation inhibitors promote the formation of the 13 nm lamellar phase.