ESRF	**Experiment title:** On line examination of lipid organisation in the skin during electric current supply	**Experiment number:** LS708
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Report:

Introduction:

The major drawback in transdermal drug delivery is the slow penetration of drugs through the skin. Recent studies revealed that the main barrier is located in the intercellular lipid regions in the upperlayer of the skin. The intercellular lipid organisation has been determined by small- and wide-angle x-ray diffraction. The studies revealed a complex_ phase behavior. At least two crystalline lamellar phases with repeat distances (d) of approximately 13 and 6 nm are present.

One of the most advanced bio-physical methods to increase drug transport via the skin is iontophoresis. Iontophoresis increases drug transport using the driving force of an applied field. In an in vitro iontophoresis experiment the skin is clamped in a diffusion cell with the donor and acceptor phase filled with a buffer. The working electrodes responsible for the current supply are placed on both sides of the skin. Until now no information is available on the structural changes **during** current supply. This information is of great interest especially because always an initial drop in resistance have been observed after switching on the current.

In order to be able to measure the lipid organisation in the skin we decided to develop a sample cell in which an electrical field can be applied on the skin and during which the changes in lipid and protein organisation can be monitored by small angle X-ray diffraction. In this report the first results are given.

Optimalization the experimental conditions

weak scattering of the sample in a buffer environment: In the previous experiments at Daresbury synchrotron (station 8.2) the absorption of x-rays by water and the weak scattering from the skin were major problems, that could not be solved by an optimal orientation of the skin with respect to the beam. Therefore no satisfying signal/noise ratios could be achieved. However, by using a small cross section of the beam combined with an optimal orientation of the skin, the weak scattering of the skin appeared to be of no problem. We obtained a satisfying signal noise ratio with a measuring time of 5 minutes, which is an important step forward for performing the actual experiments on line.

swelling of the skin: When starting the iontophoresis experiment the skin is slightly stretched and fixed in the sample holder with its surface orientated parallel to the beam. However, during iontophoresis, water is taken up by the skin which results in swelling and bending of the skin. This event will change the orientation of the skin and thus weaken the diffraction pattern. For this reason we had to use a supporting membrane. Mesh appeared to be the optimal candidate. An optimal scattering was obtained when during the experiments the supporting membrane was located just beneath the position of the beam, since the supporting membrane causes background scattering.

Actual iontophoresis experiment on line:

Constant current was applied using the following schedule: a current density of 0.5 mA/cm^2 during 1 hour, followed by a current density 1.5 mA/cm^2 during 0.5 hours and finally a current densities of 5 mA/cm^2 during 0.5 hours. During current supply scattered x-rays was continuously monitored. The x-rays for each diffraction curve were collected for 5 minutes. The measurements were carried out in triplicate.

Result of the on-line iontophoresis experiments:

The diffraction curve obtained from hydrated SC consists of a strong peak with a mean position ($Q=1.0\ nm^{-1}$) and a shoulder ($Q=1.4\ nm^{-1}$) on the right hand side and a weak diffraction peak at $Q=1.8\ nm^{-1}$. Q is defined as $4\pi\sin\theta/\lambda$, θ being the scattering angle and λ being the wave length of the x-rays. These peaks were attributed to two lamellar phases with periodicities of approximately 6.4 and 13.4 nm. During current supply ($0.5\ mA/cm^{2)}$ the intensity of the diffraction peaks attributed to the lipids decreased during the first 5 minutes indicating a slight disordering of the lipid lamellae compared to untreated SC. No further changes in the diffraction curve were observed during an additional period of 55 min. Increasing the current density to 1.5 mA/cm^2 did not reduce the intensity of the diffraction peaks. However, when the current density was increased to 5.0 mA/cm^2 a gradual decrease in peak intensity was observed.

conclusions:

*Using the above described experimental set-up it is for the first time possible to examine the lipid organisation during current supply.

*After switching on the current a disordering in the SC lamellar organisation was observed, which seems to correlate with a drop in the resistance of the SC due to ion and possibly water uptake in the SC.

*Only when the current density was increased to 5 mA/cm^2 the bulk ordering of the lamellae in SC was further reduced.