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Analysis of effects of Adwatis water on stratum corneum

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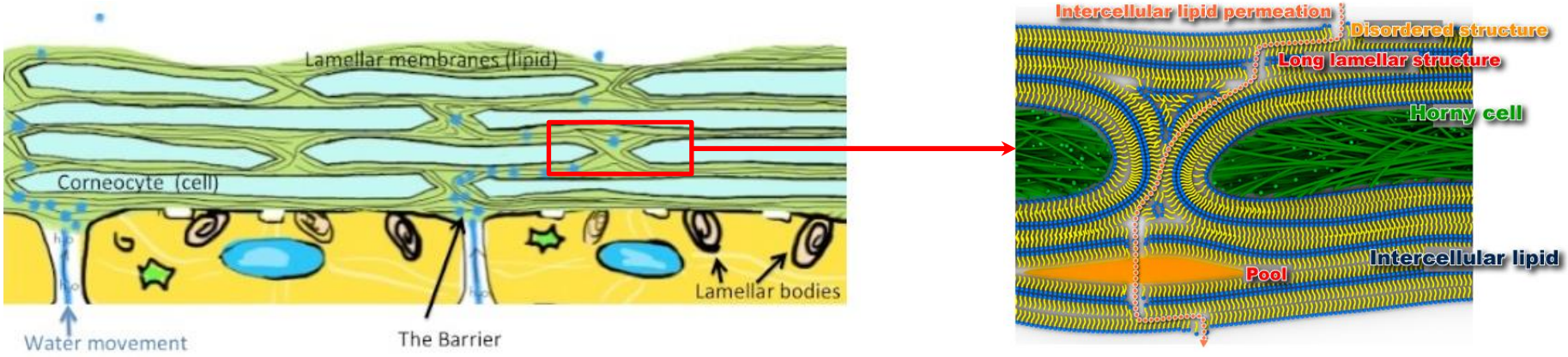
X-ray diffraction analysis

Basics about intercellular lipids in stratum corneum

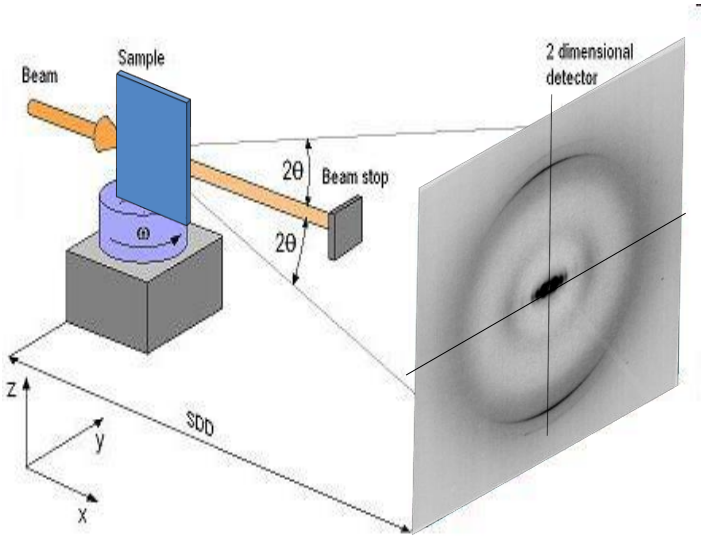
The outermost layer of epidermis, the stratum corneum (SC), consists of keratin-rich corneocytes surrounded by lipid matrix. Despite the fact that they only represent around 10% of the total mass of the SC, the intercellular lipids play a major role in the barrier function of the skin. For example, lipid removal leads to a loss of the water diffusion barrier.

The barrier function results from the complex and specific architecture of the inter-corneocyte lipid matrix composed of a mixture of ceramides, cholesterol, and long-chain fatty acids. Ultrastructural studies, using electron microscopy and diffraction techniques, have revealed that these lipids are organized in stacked bilayers that are predominantly parallel to the skin surface. Part of the lipids is covalently bound to the corneocytes whereas another part represents the “free lipids” removable by solvent extraction.

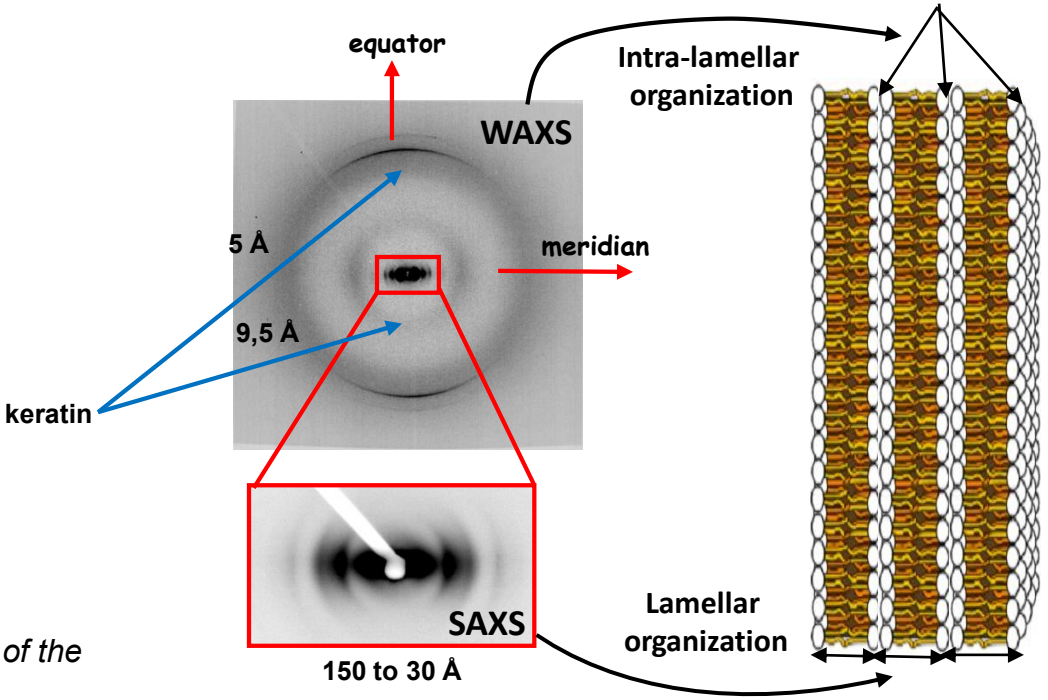


Basics about X-ray diffraction by stratum corneum

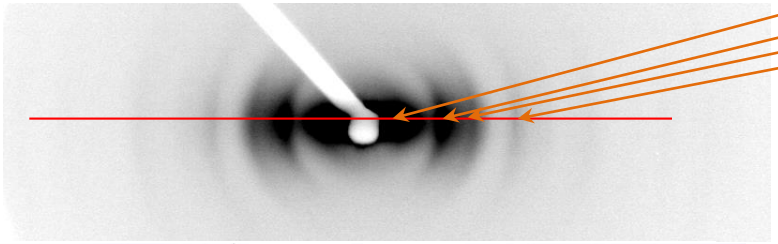
X-ray diffraction is a powerful technique to study the organization of lipids and proteins in the skin, hair, nail and more particularly in the stratum corneum.
The first analyses of the lipids of the stratum corneum (SC) were carried out in the 1950s [G. Swanbeck, J. Ultrastruct. Res. 3, 51-57 (1959)]. At the time they were practiced on samples of SC wound on itself with a millimetric spatial resolution. They have made it possible to demonstrate the fact that intercorneocytic lipids are partially crystallized.
It was not until the 1990s that the analysis was carried out more finely thanks to the use of synchrotron X-rays to work on a few sheets of stacked SC. The diffraction patterns obtained with the X-ray beam parallel to the SC sheet show in the center of the pattern (SAXS zone) anisotropic signals corresponding to an organization of the lipids with the plane of the lamellae parallel to the plane of the SC. The co-existence within the lamellae of amorphous and crystallized is deduced from the signals in the outer part of the pattern (WAXS zone). Two crystalline organizations (hexagonal and orthorhombic) were highlighted [J.-C. Garson, et al. J. Invest Dermatol. 96, 43-49 (1991) -J.A. Bouwstra, et al. Int. J. Pharmaceut. 84, 205-216 (1992)]. Diffraction signals from keratin filaments are also present on patterns.

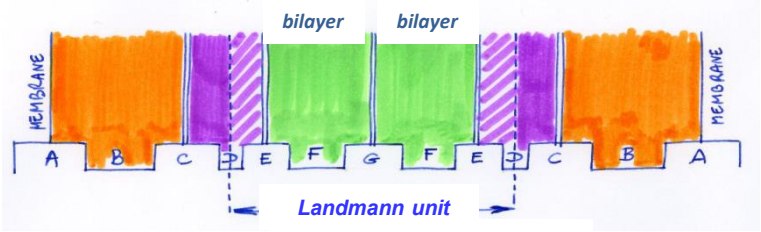


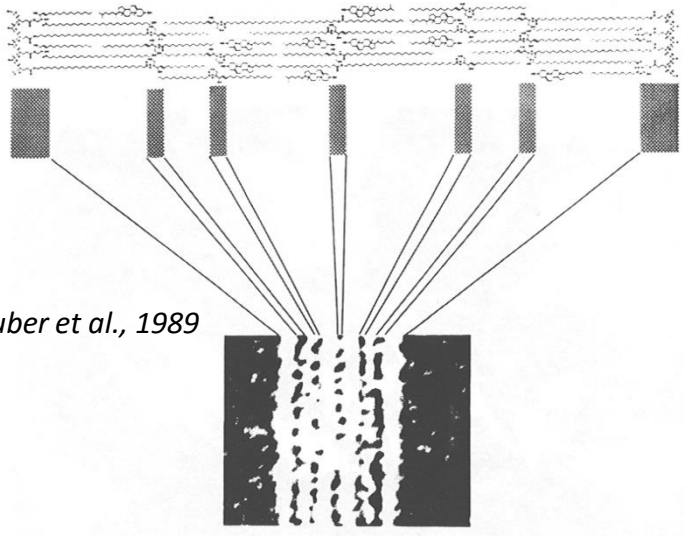
X-ray beam parallel to SC plane, i.e. to the plane of the series of stacked lipid layers



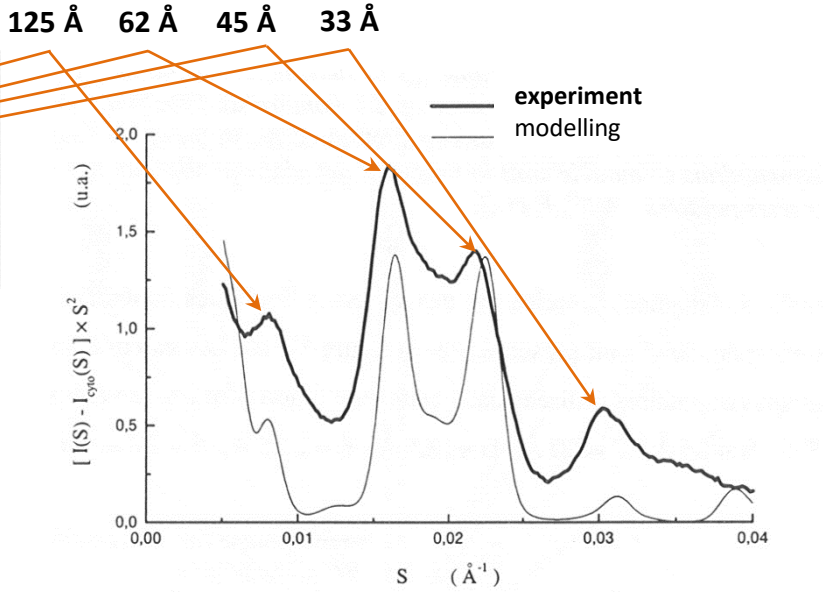
SAXS: intercellular lipid stacking







Swartzendruber et al., 1989

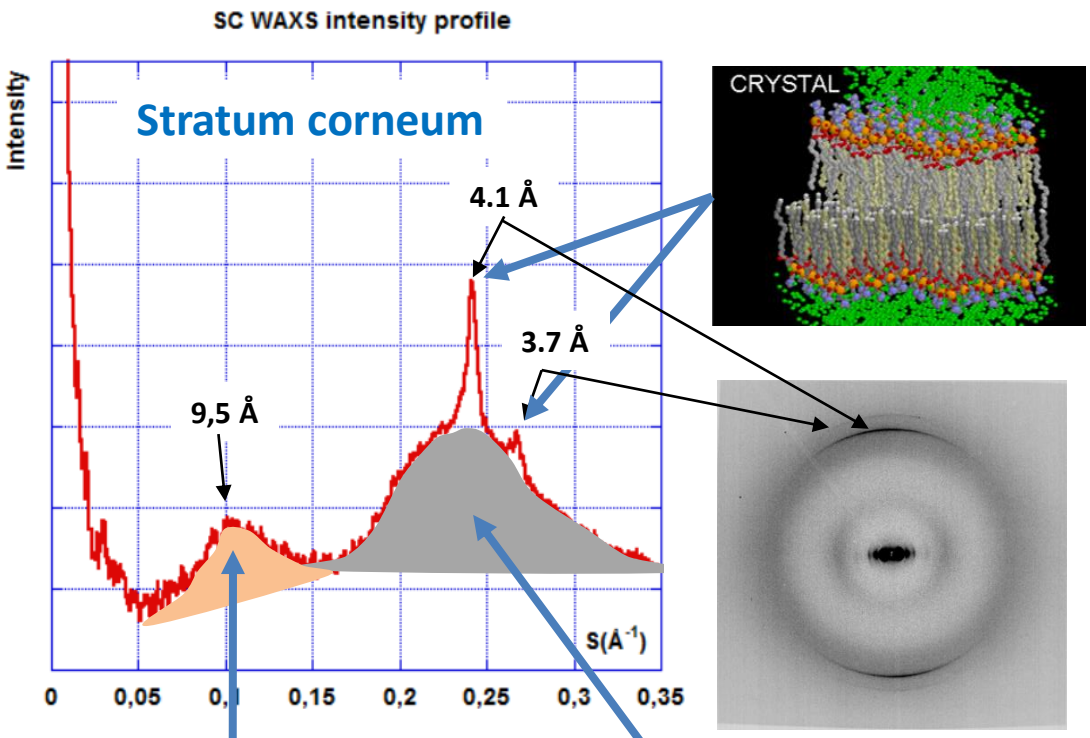


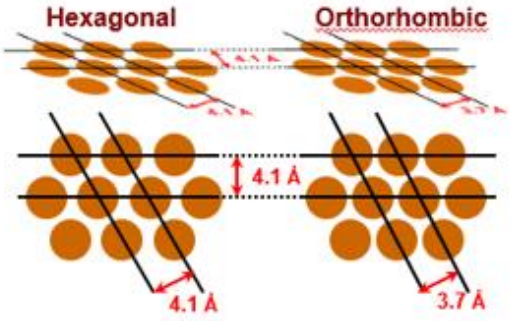
C. Mérigoux PhD University Paris-Sud 11 (1995)

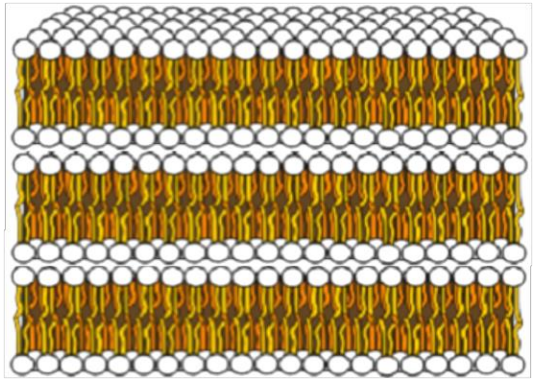
Various models of the lipid lamellae stacking have been proposed. They all consists in a complex stacking of lipids, like in the model of Swartzendruber et al. which is characterized by the repeat of a few layers unit (Landmann units) inserted between bound layers to the corneocyte membranes.

The resulting X-ray diffraction pattern shows a series of peaks at about 125 Å, 62 Å, 45 Å and 33 Å, corresponding roughly to the first harmonics of the Landmann unit thickness (125 Å).

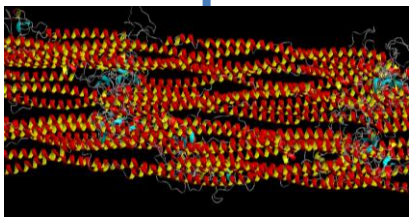
WAXS signal: crystalline organizations of lipids inside layers

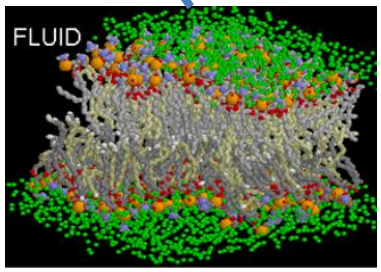






keratin





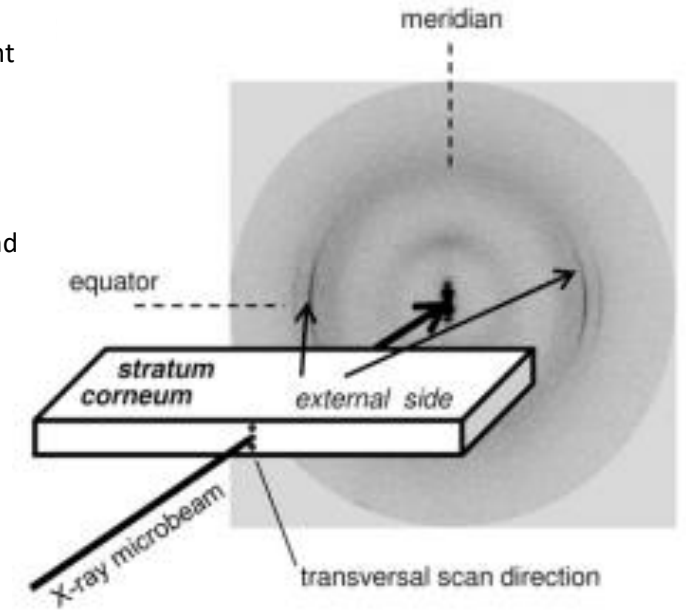
The broad band arises from the fluid part of the intercellular lipids while the sharp peaks arise from the orthorhombic and hexagonal crystalline organizations. The most intense peak at 4.1 Å is due to the two organizations while the peak at 3.7 Å is characteristic of the orthorhombic organization.
The broad peak at 9.5 Å is produced by the keratin intermediate filaments.

- **Sample preparation**

SC pieces of 1cm² were deposited on a polymeric support having the advantage of being adherent (to avoid the movement of the SC during the spreading of the product) while allowing a detachment without any damage of the SC after treatment.
The outer side of the SC pieces was sprayed three times with Adwatis water

- either without drying between the sprays (S100, batch G161010) diluted at 30% (SC1)
- or after air drying between the sprays, sprays (S100,30% dilution batch G161102) (SC2 and SC3)

Data collection was performed 5 hours after treatment.



Micron-scale assessment of molecular lipid organization in human stratum corneum using microprobe X-ray diffraction[☒]

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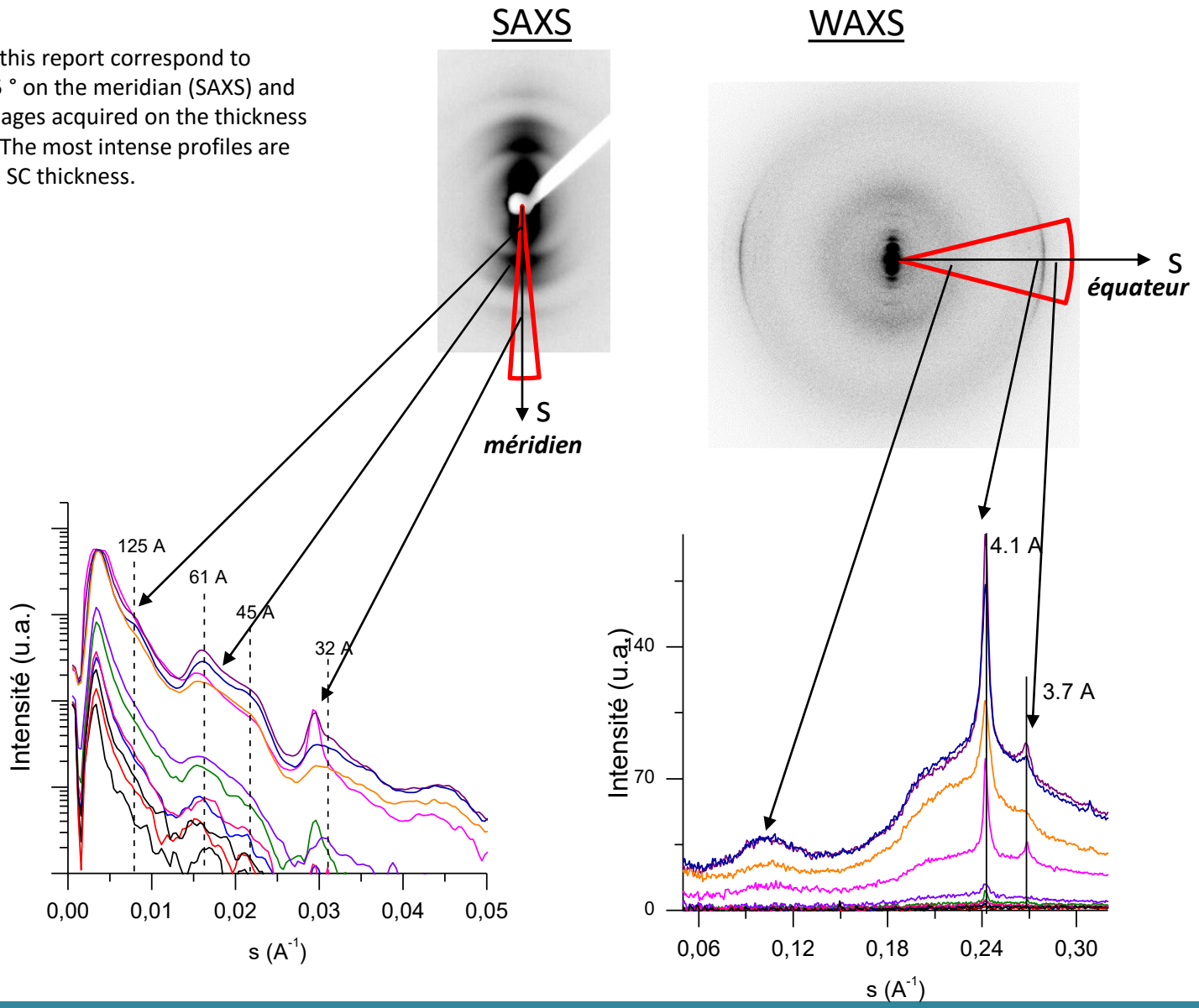
- **Diffraction**
  - beamline ID13 at the l'ESRF
  - Wavelength: 0.9537 Å,
  - mode 16 bunches
  - Beam size 3 (h) x 1.7 (v) µm²,
  - Sample-detector distance: 130 mm
  - Detector FRELON (pixel 50 x 50 µm²)
  - Vertical scanning (perpendicular to the sample place) with a 2 µm step size
  - 3 scannings per sample at different positions,
  - Exposure time per point: 0.2 second,
  - T= 22°C, HR 40%

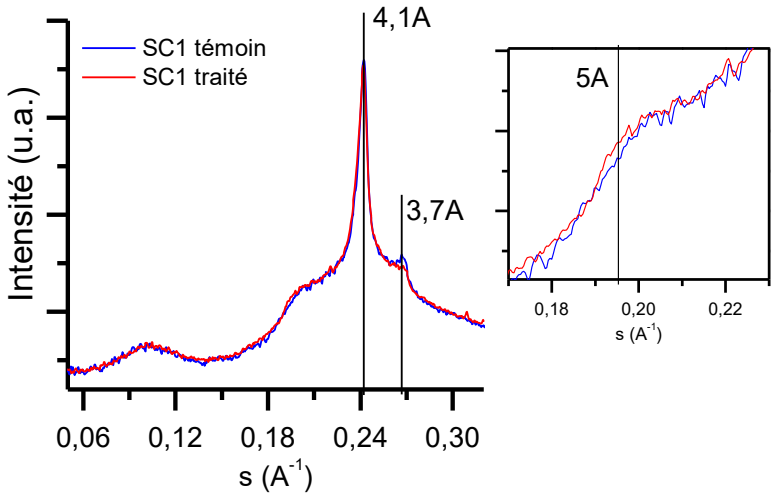
- **Analysis**

The .edf format X-ray patterns were analyzed using the ESRF FIT2D software and compared with each other.
The intensity profiles presented in this report correspond to angular profiles integrated on +/- 5 ° on the meridian and +/- 10 ° on the equator.

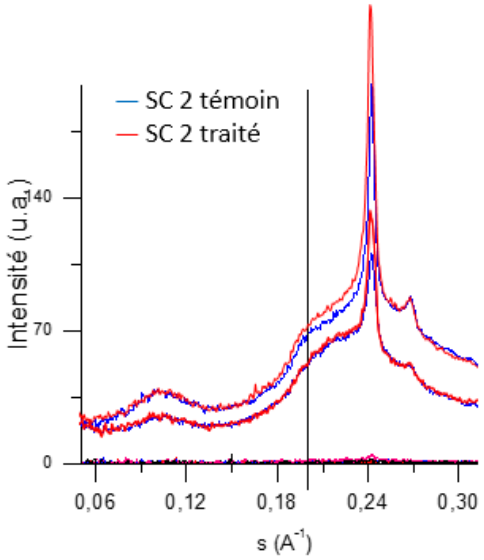
The intensity profiles presented in this report correspond to angular profiles integrated on +/- 5 ° on the meridian (SAXS) and +/- 10 ° on the equator (WAXS). Images acquired on the thickness of the SC are shown in the graphs. The most intense profiles are those acquired at the center of the SC thickness.

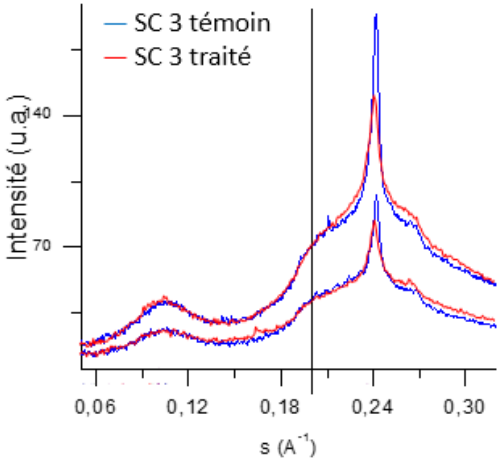


Results: SC1, SC2 and SC3



The SAXS and WAXS profiles before and after treatment with Adwatis water can be considered as identical for the three stratum corneum smples. No significant change could be detected. For SC1, a small intensity increase around 0.19 $Å^{-1}$ was observed after treatment, however this observation was not reproduced for SC2 and SC3. Therefore we can't consider this slight difference, which would have been indicative of an effect on the keratin structure, as significant.





Conclusion: This X-ray diffraction analysis clearly proves that Adwatis water does not alter the lipid and protein structures of the stratum corneum.
Adwatis water can thus be considered as a neutral product respecting the structural integrity of the stratum corneum.

SEM-FEG observations

Experimental conditions

The SEM-FEG technique is a mode of scanning electron microscopy at low voltage, which drastically reduces the effect of radiation damages, therefore suitable for the observation of tissues.

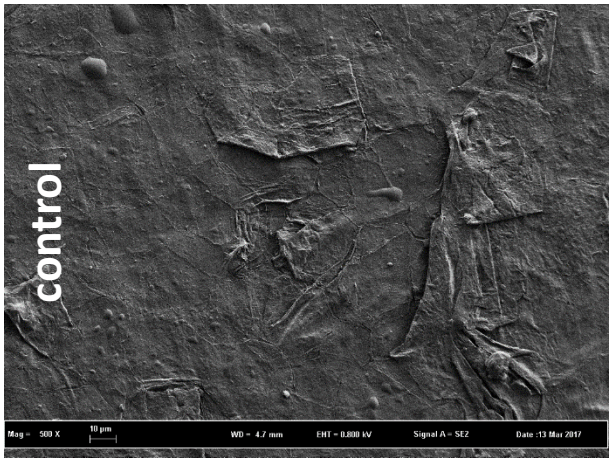
The sample of stratum corneum used was provided by Novitom. No particular surface treatment was required.

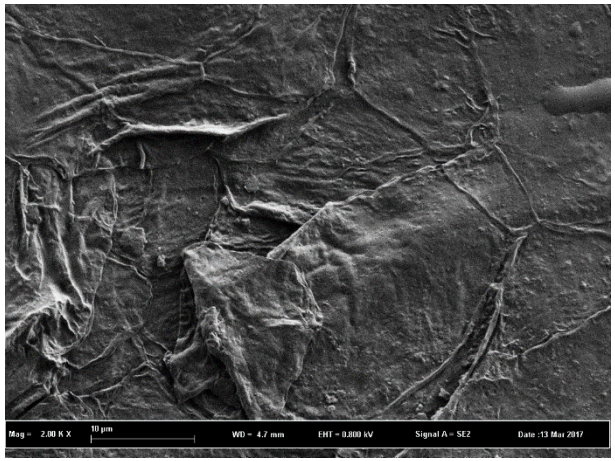
The product (S-100 diluted to 30%) was applied to the stratum corneum in 3 successive sprays with drying between each spraying; after the last spraying the SC was left in the open without being dried.
The samples were observed 10 min after the last spraying.

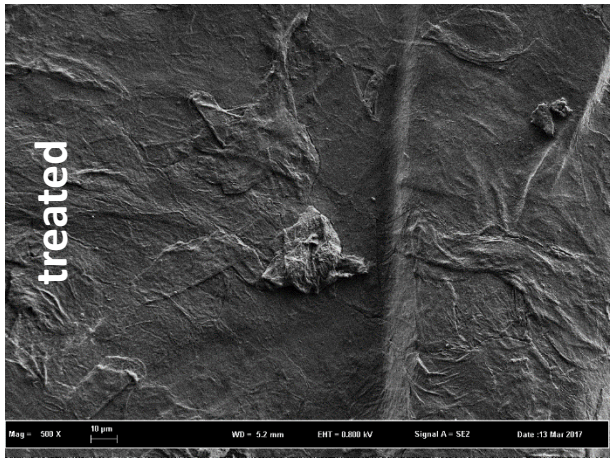
The images were acquired at x500, x1000, x2000 and x5000 magnifications.

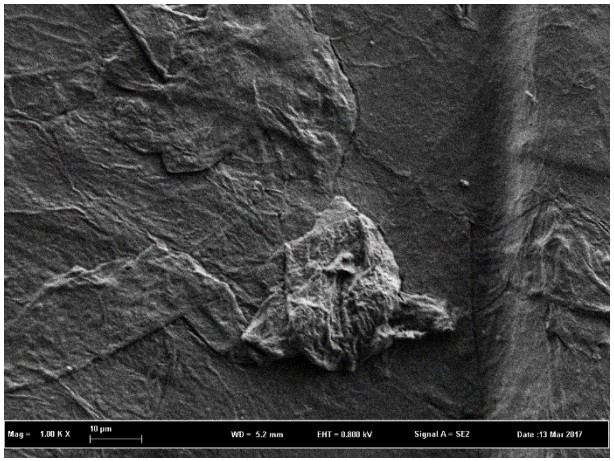
The scale bars are 10µm for x500, x1000 and x2000 images.

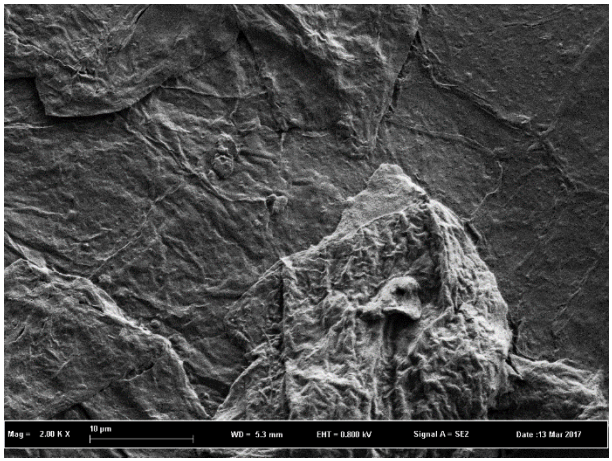
Comparison control - treated











No difference is detected prior and after treatment; the surface of the stratum corneum displays a similar aspect.

Conclusion: Adwatis water does not alter the surface state of the stratum corneum. It has no particular effect on micro-roughness, cohesion and surface corneocyte. No phenomenon of desquamation was observed either. Adwatis water can therefore also be considered as a neutral product that does not alter the surface state of the stratum corneum.