STUDY ON THE PERMEATION OF SUBSTANCES THROUGH THE SKIN LAYERS BY RAMAN SPECTROSCOPY IN THE *IN VITRO* MODEL

STUDY'S DESCRIPTION:

Study on the permeation of substances through the skin layers by Raman spectroscopy in the *in vitro* model
The Raman spectroscopy technique can be used to determine the depth of penetration of substances (permeation) through skin layers in an *in vitro* model.

Name of the device used for study
Raman spectrometer (Witecalpha 300 RSA+) is equipped with confocal microscope, AFM atomic force microscope, polyferometer.

Target client's group
Clients from cosmetic industry, development departments, marketing departments.

Kinds of samples
- samples that will be qualified for skin testing in a pilot study
- samples in cream, paste, ointment, liquid and semi-liquid form
- samples having the status of cosmetic, medical device, medicine in the form described above

Pilot study
The goal of pilot study is to determine whether the spectrum of the main ingredient of a given formulation will be distinguishable from the spectra that the skin components generate. The client will provide the main ingredient of the test formulation in a quantity of **5 ml**. The results of the pilot study will be presented to the client in the form of a short report explaining the validity (or lack thereof) of the study using human skin.

Skin permeation test procedure by means of Raman spectroscopy in an *in vitro* model
The test is conducted on human skin scrapings. The test formulation is applied to the thawed skin scrap (enough to cover the entire skin surface). The skin with the applied sample is then incubated in a hothouse at 37°C with access to CO_2. The skin is kept moist at all times (cell medium with appropriate antibiotics or PBS solution with appropriate antibiotics).

After a defined incubation time (depending on the arrangements with the client, up to 4 h as standard), a cross section is taken through the skin layers. The material thus prepared is placed on slides for Raman measurements. The samples are moistened with cell medium to prevent the skin from drying out during the measurements.
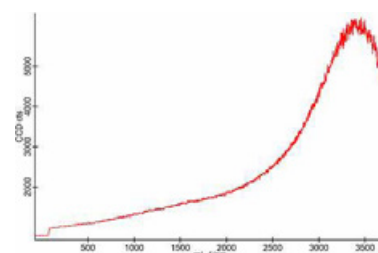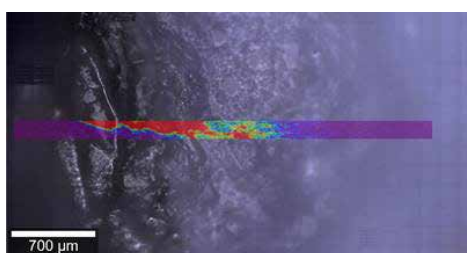
Samples prepared as described above are mapped by Raman spectroscopy. Based on previously measured patterns of the test substance, its marker bands are indicated. On the recorded maps, the penetration depth of the substance through the skin layers is determined (based on the previously indicated marker bands of the substance). The measurement is performed in 3 biological repetitions and 3 technical repetitions for statistical confirmation of the penetration depth (a total of 9 maps are obtained for one sample).

The advantages of *in vitro* permeation study:

- the test demonstrates whether the formulation overcomes the epidermal barrier and provides a numerical value representing the depth of penetration into the skin
- the test makes it possible to compare formulations with the same main ingredient and to determine which formulation allows the best penetration through the epidermis
- the test is a good complement to the results obtained from the Franz cell test
- the maps obtained during the test can be used as input for marketing or sales materials, showing how a particular formulation compares with the competition, for example

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They trusted us:

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