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Validation of the combined ATR-FTIR/tape stripping technique for monitoring the distribution of surfactants in the stratum corneum

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ABSTRACT

The physical presence of surfactants in the skin is linked to their skin irritation potential. Combined ATR-FTIR spectroscopy and tape stripping experiments in vitro on porcine ear skin were used to investigate the spatial distribution of sodium lauryl ether sulfate (SLES) in the stratum corneum and to assess its effects on conformational order of stratum corneum intercellular lipids, secondary structure of keratin and skin hydration. It was possible to monitor the spatial distribution of SLES in the stratum corneum for the first time by subtracting spectra of untreated from treated skin samples and without the need of a perdeuterated form. This method of analysis was evaluated by addressing potential error sources such as differences in removed amounts of corneocytes and intra-individual changes in stratum corneum composition as a function of depth. The obtained results indicate a penetration of SLES into deep layers of the stratum corneum. Furthermore, SLES treatment led to significantly decreased skin hydration levels, whereas the secondary structure of keratin remained nearly unaffected. The reliability of this semi-quantitative method of analysis was confirmed by receiving a coefficient of determination of 0.9963 after making a correlation of deep depended absorbances of two different characteristic bands with different absorption coefficients.

1. Introduction

In the field of skin research, ATR-FTIR spectroscopy is mainly used to investigate the molecular effects of different formulations or excipients on the conformational order of stratum corneum lipids and proteins. Due to a probing depth of a shallow micrometer, a majority of these studies only provide molecular level information about the skin surface (Hasanovic et al., 2011; Rubio et al., 2013; Saad et al., 2012; Schwarz et al., 2013).

The stratum corneum, the uppermost layer of the skin, is a unique barrier membrane. It provides a vital barrier function and protects the skin from the excessive loss of water and the ingress of foreign materials. It consists of corneocytes embedded in a lamellar lipid matrix, which primarily provides the excellent barrier function (Bommannan et al., 1990; Hadgraft and Lane, 2011).

Surfactants, widely used in components of personal care products and drug delivery systems, are recognized to diminish the properties of the stratum corneum. The spatial distribution of surfactants seems to play an important role when it comes to skin irritation and damage (Mao et al., 2012). Thus, the uptake and distribution of surfactants in the stratum corneum is of great interest. Anionic surfactants, where the hydrophilic part carries a negative charge, are well-known skin irritants. Especially the effects of sodium dodecyl sulfate (SDS) on the skin have been studied extensively. Molecular influence of SDS on the stratum corneum such as binding and denaturation of skin surface proteins and solubilization or disorganization of intercellular lipids were reported over the last years (Mao et al., 2012; Saad et al., 2012; Som et al., 2012; Tsang and Guy, 2010).

Therefore, the use of SDS decreased in daily practice. SDS is now mostly replaced by its ethoxylated milder analogon sodium lauryl ether sulfate (SLES) (Bárány et al., 2000; Charbonnier et al., 2001; Tsang and Guy, 2010). It appears in a variety of cosmetic products

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such as cleansing agents, emulsifiers, stabilizers and solubilizers in a concentration range from 0.1 to 50% (Robinson et al., 2010). However, although the spatial distribution of perdeuterated SDS was investigated in detail by confocal Raman and infrared microscopy in a recent study by Mao et al. (2012); detailed information concerning the spatial distribution of its ethoxylated, more often used analogon SLES in the stratum corneum is still missing.

Combined with a tape stripping procedure, the ATR-FTIR technique can be employed to detect exogenous substances in different layers of the stratum corneum. However, this approach is rarely used since characteristic bands of exogenous substances appear in the less attention paid fingerprint region and mostly overlap with typical skin bands. Unless substances are available in perdeuterated form, it has proved difficult to quantify their uptake into the stratum corneum (Hathout et al., 2010; Schwarz et al., 2013).

Therefore, the aim of the present study was to track the penetration of SLES from the spatial distribution of its characteristic vibrational bands in different layers of the stratum corneum. SLES has intense bands arising from the sulfate headgroup (Viana et al., 2012). These bands are overlapping with modes from the skin and are therefore mostly not further analyzed. However, spectral subtraction, as one of the main tools used to attack the problem of mixture spectra by simplifying the spectrum itself, is mainly overlooked (Smith, 1998). In this study, spectral subtraction of untreated from SLES treated skin was used to monitor the distribution of SLES in different layers of the stratum corneum. Possible error sources that arise during this experimental setup such as differences in removal of corneocytes through tape stripping or intra-individual changes in stratum corneum composition as a function of depth were analyzed in detail and taken in consideration.

In addition to tracking the penetration of exogenous material through skin, ATR-FTIR spectroscopy can monitor molecular structure changes (Mao et al., 2012). Due to a reported impact of SDS on water permeability and skin proteins, effects of its ethoxylated analogon SLES on skin hydration and secondary structure of keratin were investigated in detail (Lee et al., 2013; Som et al., 2012).

Although the combination of ATR-FTIR spectroscopy and tape stripping experiments is a known technique, its application for tracking exogenous substances in the stratum corneum is so far limited to deuterated compounds. Therefore, the practical value of the described approach is significant, providing a tool for investigating and comparing the penetration of a variety of surfactants as well as obtaining information about the molecular effects on stratum corneum structure.

2. Materials and methods

2.1. Materials

Sodium laureth sulfate (SLES) was purchased from Dr. Temt Laboratories (Vienna, Austria) as aqueous solution (28%, w/w). Tesa film crystal clear tape (Product number 57,859–00000, Vienna, Austria) was used to remove the layers of the stratum corneum during tape stripping experiments.

2.2. Methods

2.2.1. Skin tissue

Pig ears were obtained from a local abattoir (Totzenbach, Austria) and stored at −24 °C up to a maximum of 6 months. At the beginning of the procedure the pig ears were defrosted, cleaned with cold water and blotted dry with a soft tissue. Afterwards full-thickness skin was removed from the cartilage with a scalpel and the hair was carefully clipped with a scissor. Samples were cut into pieces of 2 cm × 7.5 cm and pinned onto pieces of Styrofoam with needles on each side.

2.2.2. Formulations

The used surfactant solution was prepared by diluting the purchased 28% (w/w) aqueous solution to a 15% (w/w) aqueous preparation of SLES in distilled water. This percentage was chosen due to commonly used 15–20% aqueous solutions of surfactants for skin irritation testing (Bárány et al., 2000; Hall-Manning et al., 1998).

2.2.3. Combined ATR-FTIR and tape stripping experiments

80 μl of 15% aqueous solution of SLES were applied on the porcine ear skin samples and incubated for 1 h at the skin surface temperature of 32 °C. After this time period, the samples were blotted dry with a soft tissue to ensure complete removal of the formulation from the skin surface. To the end, a strip of adhesive tape was placed on the skin sample and removed in one continuous movement. 20 sequential tape strips were removed in this manner and ATR-FTIR spectra were recorded from the skin surface prior to the tape stripping procedure as well as after each removed tape strip. Experiments were performed in triplicate ($n = 3$). In addition to the samples treated with SLES, other porcine ear skin samples were incubated with 80 μl distilled water for 1 h at 32 °C. In order to eliminate error sources caused by intra-individual variations in stratum corneum composition, these samples were tape stripped in the same manner and analyzed regarding their lipid content and skin hydration as described later.

Porcine ear skin samples were placed stratum corneum facing down on the ZnSe ATR crystal of an ATR-FTIR spectrometer (Tensor 27, Bio-ATR I tool, Bruker Optics, Germany) equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector. Spectra were recorded in double sided mode at the skin surface temperature of 32 °C as the average of 60 scans in the frequency range 4000–870 cm^{-1} at a resolution of 4 cm^{-1} using Blackman–Harris 3-term apodization and a zero filling factor of 8.

In addition, ATR-FTIR spectra of the 15% aqueous solution of SLES were recorded on the same tool and under same conditions.

2.2.4. Infrared data analysis

Data analyses such as determination of peak position and calculation of peak areas were performed using OPUS 5.5 software (Bruker Optics, Germany).

Consideration of dependency of the ATR-absorbance on the degree of contact between the crystal and the sample is of great importance for quantitative analysis by ATR-FTIR. Therefore, the amide II absorbance was used as an internal standard to account for this variability (Klimisch and Chandra, 1986; Saad et al., 2012).

The intensity ratio of the symmetric CH_2 stretching band at ~2850 cm^{-1} to the amide II absorbance at ~1540 cm^{-1} can be used to receive information about the lipid content of the stratum corneum. Furthermore, calculating of the amide I to amide II absorbance ratio reflects stratum corneum hydration (Hathout et al., 2010). In this method a straight line is drawn between the points intersection of the measured absorbance and the two frequency limits defined. The area above this line will be integrated. The frequency limits for integration were (1) 2861–2844 cm^{-1} for the symmetric CH_2 stretching mode and (2) 1568–1508 cm^{-1} for the amide II absorbance, respectively.

2.2.5. Stratum corneum distribution of SLES

Most characteristic bands of SLES were overlapping with typical bands origin from the skin. To overcome this problem, spectral subtraction was used as a tool to simplify the obtained spectra. Therefore, spectra of the control samples, only incubated with

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