In vivo analysis of physiological skin parameters: Confocal Raman spectroscopy and classical biophysical techniques

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Introduction

New drug delivery systems have to overcome the skin barrier without causing irritation. Thus, knowledge of the skin composition is essential to obtain reliable data about the impact of dermal products. Besides the formulations' physicochemical properties and stability, its influence on skin physiology is an important aspect in the development of new dermal drug delivery systems.

We have recently developed novel concentrated water-in-oil (W/O) emulsions based on a non-ionic silicone surfactant. The aim of this study was to assess the effect of these formulations on physiological skin parameters of healthy volunteers after repeated application. To this end, confocal Raman spectroscopy (CRS) and classical biophysical techniques were used.

Experimental Methods

Study design

The study was conducted with approval from the Ethics Committee of the Medical University of Vienna. Two selected formulations (Tab. 1) were applied daily on the volar forearm of ten randomly chosen participants (27 ± 3 years of age). The other forearm was left as the untreated control. Over a period of four weeks, the skin composition was characterised in regular intervals.

Tab. 1: Composition of the investigated W/O emulsions in % (w/w).

	IPM_2/18	PAR_2/18
Emulsifier 10	2	2
Liquid paraffin	-	18
Isopropyl myristate	18	-
Water phase[a]	80	80

[a] Composed of distilled water containing 1% (w/w) sodium chloride.

Measurement of physiological skin parameters

The transepidermal water loss (TEWL) was measured with the closed-chamber device AquaFlux® (Biox Ltd., UK). The condensed-chamber probe measures the water evaporating from the skin in g/m²/h; in this way, increased skin water loss, indicating barrier damage, can be detected [1,2]. The capacitance devices Corneometer® CM 825 (C+K electronic GmbH, DE) and Epsilon® (Biox Ltd., UK) were used for the evaluation of skin hydration. The sebum amount at the skin surface was measured with a Sebumeter® (C+K electronic GmbH, DE) and the skin surface pH value was determined with a Skin-pH-Meter PH 905 (C+K electronic GmbH, DE).

In vivo CRS experiments were carried out using a confocal Raman microspectrometer (gen2 Skin Composition Analyzer, River Diagnostics, NL) with two incorporated lasers (671 nm and 785 nm). All spectra collected were analysed using SkinTools® software version 2.0. For the calculation of the depth concentration profiles of the natural moisturizing factor (NMF) and urea, a least-squares fitting algorithm based on the endogenous skin components was used. Water profiles were generated by calculating the water content from the water to protein ratio [3]. The thickness of the stratum corneum (SC) was determined through water concentration profiles [4].

Calculation of parameter changes

The influence of the emulsions on the skin parameters was calculated after Eq. (1), including the control values of the untreated forearm.

$$\text{Parameter changes (\%)} = \left(\frac{T_4}{C_4} \Big/ \frac{T_0}{C_0} - 1\right) \cdot 100 \qquad (1)$$

T_4 and C_4 are the mean values of the treated and control forearm after the formulation application period of four weeks, and T_0 and C_0 are the respective mean basic values at the study start.

References
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Results

Fig. 1 shows the effect of regular treatment on the TEWL, skin hydration and skin permittivity. A trend towards slightly decreased mean TEWL values was observed for both formulations. Continuous treatment led to a change of skin hydration towards higher Corneometer values. Skin permittivity mapping with the fingerprint sensor Epsilon® revealed similar trends to the Corneometer capacitance measurements. In Fig. 2, representative skin permittivity maps are presented.

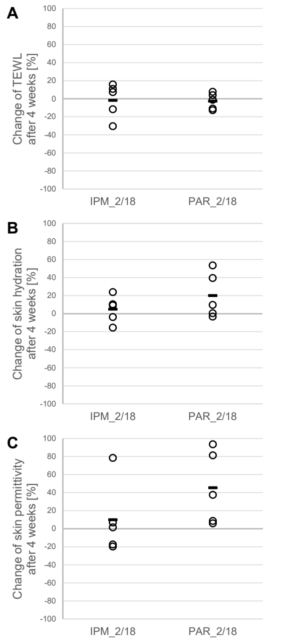

Fig. 1: Effect of the emulsions after 4 weeks of regular application on (**A**) TEWL, (**B**) skin hydration and (**C**) skin permittivity. Parameter changes are expressed as single values (n = 5) and corresponding mean.

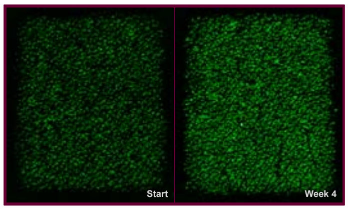

Fig. 2: Side-by-side comparison of representative capacitive images of human forearm skin acquired with the fingerprint sensor Epsilon® at the beginning of the study and after 4 weeks of treatment with PAR_2/18.

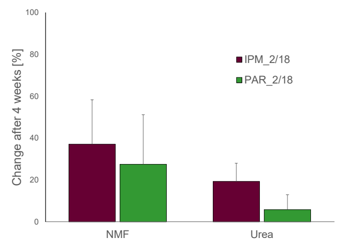

Fig. 3: Changes of relative NMF and urea concentration in the human volar forearm after 4 weeks of treatment. Parameter changes were averaged over the outermost 10 µm of the SC (n = 5 ± SE).

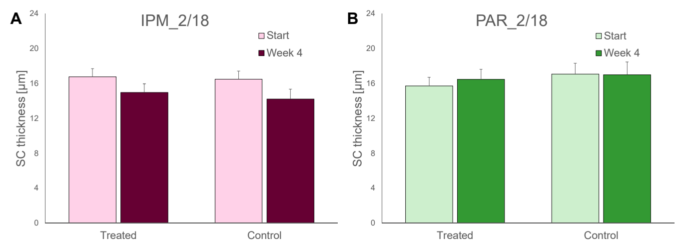

Fig. 4: Comparison of the SC thickness of the treated and control forearm at the beginning of the study and after a four-week treatment period (**A**) in the IPM_2/18 group and (**B**) in the PAR_2/18 group (means ± SE, n = 5).

The NMF content showed a considerable increase after four weeks in both groups (Fig. 3). Mean urea levels did also increase after four weeks of treatment, albeit not to the same extent as NMF levels. CRS-derived SC thicknesses of the participants varied between 12 and 21 µm. A comparison of the mean SC thickness for both study groups is given in Fig. 4. No significant differences over time were found.

In summary, both tested W/O emulsions exhibited a positive influence on physiological skin parameters. A combination of complementary techniques proved beneficial for the evaluation of the influence of the dermal formulations on barrier integrity and irritation potential.

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