EFFICACY EVALUATION OF DIFFERENT CREAM FORMULATIONS ON HEALTHY SKIN PROPERTIES

MIRELA MOLDOVAN1*, LORELAI CIORTEA2

*University of Medicine and Pharmacy “Iuliu Hatieganu”, Faculty of Pharmacy, Department of Dermopharmacy and Cosmetics, 41, V.Babeş Street, 400012, Cluj-Napoca, Romania, Tel/Fax: +40264450531,
2 Biox System Ltd, 103 Borough Road, SE1 0AA, London, UK.
*corresponding author: mmoldovan@umfcluj.ro

Abstract
In this study three cream formulations were compared in order to assess the influence of different kind of excipients on the skin barrier and stratum corneum hydration. The first cream was formulated based on lipophilic emollients: petrolatum, paraffin oil, cetylstearyl alcohol, cetyl palmitate and paraffin, the second cream was formulated based on emollients (cetylstearyl alcohol, cetyl palmitate) and humectants (urea and glycerin), both as oil-in-water emulsions, and the third cream contained emollients (paraffin oil, cetyl palmitate, cetylstearyl alcohol) and humectants (urea and glycerin) in a multiple emulsion system, water1-oil-water2 type (W1/O/W2), of second order and three components.

The formulations were tested on the forearm of 10 healthy volunteers. The measurements were performed at room temperature (22 ± 2°C) and 45% ± 2% relative humidity. Skin conditions in terms of water content of the stratum corneum and transepidermal water loss (TEWL) were analyzed before creams application and at several time-intervals after cream application, during 4 hours. The best hydration was obtained when the multiple emulsions were used and also the duration of hydration was the greatest. The TEWL values are not significantly influenced by the studied formulations.

Keywords: moisturizers, skin hydration, transepidermal water loss (TEWL)
Introduction

Cosmetic creams are generally used to improve skin appearance. The way the skin looks is influenced mainly by the water content of stratum corneum and the quantity and quality of skin surface lipids [3, 9, 10]. The preservation of water content in the stratum corneum depends on its integrity, which is assured by the lamellar lipids and by the corneocyte adhesion. The water content of stratum corneum is a result of a balance between the water supply at this level from internal skin layers and the loose of water by evaporation from the skin surface.

Transepidermal water loss (TEWL) is the normal, constitutive loss of water vapor from the skin in the absence of sweat gland activity and is commonly ascribed to be a measurement of skin barrier function, more precisely a measurement of the skin water barrier function [2, 5].

Both water content of the stratum corneum and TEWL might be influenced by agents as emollients. These agents may be hydrophilic or lipophilic. There are two mechanisms by which these agents modify the stratum corneum hydration. Lipophilic emollients provide a surface film of lipids which soften the stratum corneum by increasing its hydration and reduce the water lose from the epidermis, helping to keep the skin moist and flexible and facilitating the enzymatic reactions that drive to the stratum corneum maturation [1, 4, 9]. Hydrophilic emollients, also called humectants, may act by absorbing water from the environment or from a cosmetic product or may help another substance to retain water in stratum corneum.

Emollients and humectants may be formulated in different type of formulations; creams are preferable because they tend to be thicker, more occlusive, and therefore more effective [4].

The aim of this study was to evaluate the moisturizing effect of three different types of cosmetic creams on healthy skin hydration and skin barrier properties after a single period of application. The first cream was formulated with more or less occlusive lipophilic emollients: paraffin, paraffin oil and petrolatum. As mentioned, they are meant to provide an oily layer which helps to retain water in the stratum corneum by reducing the epidermal water loss. The second cream was formulated with humectants: urea, glycerin and allantoin, the last being also a soothing agent. Glycerin is meant to provide water and urea to retain water in the skin superficial layers. Both cream 1 and cream 2 were oil /water (O/W) emulsions, where sodium laurylsulphate 1% was used as surfactant. Cream 3, formulated as a multiple emulsion, contains a lipophilic emollient: paraffin oil and two humectants: urea and glycerin. Two non-ionic surfactants were used – polyoxiethylene (20) sorbitan monooleate as hydrophilic surfactant and sorbitan monooleate
as lipophilic surfactant. Urea was included in the internal phase of the multiple emulsions of water1/oil/water2 (W1/O/W2) type.

Cetylstearilic alcohol and cetyl palmitate were used in all formulations, at the same concentration, as the oily phase of the each emulsion, so for the interpretation we focused on the influence of the mentioned lipophilic emollients and humectants.

The skin parameters observed were the stratum corneum hydration level and TEWL.

**Materials and methods**

**Materials**
All substances used were of pharmaceutical purity.

**Methods**

**Formulations**
Three different formulations of cosmetic creams were tested (Table 1), containing different types of moisturizers.

<table>
<thead>
<tr>
<th>Moisturizers in cream formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cream 1</strong> (O/W emulsion)</td>
</tr>
<tr>
<td>Lipophilic emollients (%)</td>
</tr>
<tr>
<td>Petrolatum - 10</td>
</tr>
<tr>
<td>Paraffin oil - 10</td>
</tr>
<tr>
<td>Paraffin - 0.5</td>
</tr>
<tr>
<td>Cetylstearilic alcohol - 8</td>
</tr>
<tr>
<td>Cetyl palmitate - 1</td>
</tr>
</tbody>
</table>

**Preparation**
Both O/W emulsions were prepared by heating separately the water and oily phases, followed by mixing and homogenization for 5 minutes at 1500 rpm (Heidolph, Germany).

The multiple emulsions were prepared by a two-step method [6, 11]. The primary emulsion was obtained at 70°C by incorporating the aqueous phase containing the humectants into the oily phase which contained the W/O surfactant, after heating, with stirring at 7000 rpm for 10 minutes. The resulting W/O primary emulsion was dispersed into the aqueous phase containing the hydrophilic surfactant and a gelling polymer, under stirring: 20 minutes, at 700 rpm, when the W1/O/W2 multiple emulsion was obtained.

All samples were freshly prepared and had the pH values of 5.2–5.8.
Subjects

Ten female healthy volunteers (N = 10), between 20-25 years old, participated in the study. All participants had normal to slightly dry skin. Written informed consent was obtained for all participants prior to the study, they were informed about the nature of the test and about the possible adverse reactions. Neither of the participants used any cosmetic cream in the tested area two weeks before the study was carried out.

All measurements were performed in controlled conditions of relative humidity (45%±2%) and temperature (22°C±2°C). Prior to the measurements, the volunteers were asked to stay in the test room for at least 1 h before the measurements, so the skin could acclimatize at room conditions and also they were not allowed to perform any physical effort.

The test area was the forearm of each volunteer. Creams were applied in an uniform layer resulted from applying 2 mg/cm² of the test product. The remaining product was carefully removed from every tested site before the measurements with paper towel. All measurements were carried out according to the relevant guidelines [5].

Several measurements were performed: before applying the cream, immediately after cream application and then at several time intervals after creams application: 1, 2, 3 and 4 hours respectively for skin hydration measurements and 1, 2 and 3 hours after creams application for TEWL measurements.

Determination of skin hydration in the superficial epidermal layers

The water content of stratum corneum was measured using Moisture Checker for skin (Taberna pro Medicum, Germany), a device which measures the skin capacitance in the contact area, which is about 1 cm², the depth being tens of microns from where the sensor was applied [8]. This is the most common method to evaluate the skin water content, as it has great reproducibility and can detect even the slightest changes in the hydration level. The measurement criterium is based on the different dielectric constants between water (81) and other of the stratum corneum (<7). The capacitance differences are recorded according to the water content, by a very precise capacitor. The results are expressed as percents [12].

Determination of TEWL

The term TEWL generally refers to the total amount of water vapor lost through the skin (water flow from the skin) which is registered in g/m²h. In order to measure the TEWL, the AquaFlux AF103 (Biox System Ltd, UK) was used, which has a cylindrical measurement chamber, closed at
one end, maintained below the freezing temperature of water. The open end is placed in contact with the skin and acts as a measurement orifice.

**Statistical analysis**

Statistical analysis was performed with ANOVA test with a single factor, Analyse-it + General Software 1.73. A p-value below 0.05 was considered significant.

**Results and discussion**

*Determination of the skin hydration*

All participants had normal values of hydration. The baseline stratum corneum hydration before applying the cream was 31.13±1.27%.

Hydration values, represented as the difference between the hydration value after cream application and the hydration value before cream application at each tested site at different time intervals, are shown in Figure 1. A significant influence of all tested formulations on stratum corneum hydration was observed (p<0.0001).

![Figure 1](image)

*Figure 1*

Variation in time of the hydration effect after cream application (N=10, mean ±SD); 1 represents the values determined immediately after application, 2, 3, 4 and 5 represent the values determined after 1, 2, 3 and respectively 4 hours after creams application.

Cream 1, the O/W emulsion formulated only with lipophilic emollients, provided the lowest hydration effect on skin, probably due to the
fact that even if they may have a hydrating effect by reducing water evaporation at the skin surface, their main role being to improve skin flexibility and smoothness and thus improving its appearance.

Cream 2, the O/W emulsion containing hydrophilic emollients, which have water binding capacity, provided a higher hydration effect on skin compared to cream 1. Glycerol, as one of the most effective humectants, is able to actually enhance water absorption from the inner layer of the skin, from dermis into epidermis, or to absorb water from the external environment when its humidity is higher than the skin hydration [7]. Urea binds the water supplied by the atmospheric condition or by the aqueous phase of the product applied on the skin. It acts on skin cells exposing their water binding sites, thus enabling the cell to absorb and retain additional moisture. This two humectants provide water into the skin after the cream application, the hydration value being the highest immediately after application. This high hydration value may be maintained due to the lipophilic emollients which provide a more or less occlusive layer and thus reducing the evaporation of water which already exists in the skin, supplied by the hydrophilic emollients and also by the external aqueous phase of the emulsion.

The results had shown that, in the condition of this study, after a single application and during four hours and for the same emulsion type, humectants provide more water to the skin than the lipophilic emollients, as we can see from the results presented in figure 1.

Cream 3, the W1/O/W2 multiple emulsion provided the most important hydrating effect, at each of the tested time intervals. This high level of hydration was maintained almost constant during four hours after cream application. This fact may be due to the delayed release of urea from the internal phase of the multiple emulsion, as in the literature there is mentioned the release more or less delayed of the component included in the internal phase of a multiple emulsion. Thus, a high level of hydration of the stratum corneum may be ensured for longer periods of time by the multiple emulsions than the simple emulsions.

Determination of transepidermal water loss (TEWL)

All participants had values of TEWL situated in the normal range, the baseline TEWL values before applying the cream was 15.4±2.4 g/m²h.

In figure 2 are represented the TEWL values as difference between the TEWL value before cream application and TEWL after cream application for every site tested, at each hour, from 1 to 3 hours.
As we can see from the figure 2, the first and second cream lead to an increase of the TEWL values immediately after cream application, probably due to the water content of the product. The TEWL values were lower than the initial values for the third cream immediately after application and then at all three studied intervals: 1, 2 and 3 hours, suggesting that it may have a certain occlusive effect. The inter-individual variations obtained were quite important.

Significant difference between TEWL values measured was obtained only for the values registered immediately after the creams application (p < 0.01). For all other investigated times, the TEWL are not influenced by the type of formulation, as no significant difference between formulations was obtained (p > 0.05).

Conclusions

Three creams were formulated, based on different types of moisturizing excipients: emollients and humectants, as two different carrier systems: O/W emulsion and W₁/O/W₂ multiple emulsion.

In the conditions of this study, the best hydration effect and the longest duration effect was provided by the W₁/O/W₂ multiple emulsion
with both lipophilic emollients and humectants. Comparing the two types of emollients in simple O/W emulsion, humectants were able to increase *stratum corneum* hydration more than the lipophilic emollients. The barrier function, as evaluated by the TEWL values, was significantly influenced by the three types of creams, only immediately after application, and was not significantly influenced at 1, 2 and 3 hours after application.

**References**

3. Dal’Belo, S.E., Gaspar, L.E., Gonçalves, P.M.B, Campos, M. Moisturising effect of cosmetic formulations containing *Aloe vera* extract in different concentrations assessed by skin bioengineering techniques. *Skin Res. Technol.*, 2006, 12, 241-246

*Manuscript received: January 20th 2010*