Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 271 (2004) 269–280
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Vehicle-controlled effect of urea on normal and SLS-irritated skin

S. Savic [a], S. Tamburic [b,*], M. Savic [c], N. Cekic [d], J. Milic [a], G. Vuleta [a]

[a] *Institute of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia and Montenegro*
[b] *London College of Fashion, The London Institute, 20 John Prince's Street, London W1M 0BJ, UK*
[c] *Institute of Pharmacology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia and Montenegro*
[d] *DCP Hemigal, Leskovac, Serbia and Montenegro*

Received 16 June 2003; received in revised form 6 November 2003; accepted 20 November 2003

Abstract

It is known that, depending on the concentration, treatment with urea could improve skin barrier function, despite its penetration-enhancing properties. This controversial skin effect of urea has been explored systematically in this study in terms of the effect of vehicle on the performance of urea. In the first part, a series of four semi-solid emulsions with 5% (w/w) urea, varying in the type of emulsion, nature of emulsifier and polarity of oil ingredients, have been evaluated with regard to their skin hydrating and transepidermal water loss (TEWL)-modifying properties. Placebo samples were tested alongside the urea-containing ones. Two best performing moisturisers from the above were chosen for the second part of the study, in which sodium lauryl sulphate (SLS)-irritated skin was treated with both placebo and urea-containing samples. In addition to TEWL and skin hydration level, the erythema index (EI) was measured before, during and after the treatment. The results have shown that barrier-improving and hydrating abilities of urea are bi-directional and dependent on both the type of vehicle used for its delivery and the state of skin.

Keywords: Urea; Moisturiser; Skin hydration; TEWL; Erythema index; Emulsion

1. Introduction

In the treatment of dermatoses accompanied with dry and scaly skin, it is essential to use highly moisturising products, mostly in the form of emulsions. In the process of developing an effective moisturising emulsion, two aspects are considered crucial. Firstly, its physico-chemical characterisation (e.g. by rheology, thermal analysis, microscopic studies and X-ray diffraction) in order to obtain the information on its physical stability and microstructure, which affects the evaporation of water and the release of active, i.e. bioavailability (Peramal et al., 1997; Erös et al., 2003). Secondly, it is important to perform clinical assessment by means of appropriate bioengineering methods and using well-defined skin parameters (Fischer et al., 2001).

It is well established that applying of urea-containing preparations to the skin improves its barrier function and increases water content of the stratum corneum (SC) in normal and dry skin conditions (Serup, 1992; Swanbeck, 1992; Lodén, 1996, 2000; Lodén et al., 1999, 2001; Lodén and Andersson, 1996; Bettinger and Maibach, 1997). Although urea has been found to enhance penetration of some drugs (Beastall et al., 1986; Kim et al., 1993), it also decreases skin sensitivity to sodium lauryl sulphate (SLS)-induced irritation (Lodén, 1996; Lodén et al., 1999). This controversial skin effect has not been investigated systematically in terms of the influence of vehicle on the performance of urea.

* Corresponding author. Tel.: +44-207-514-7722; fax: +44-207-514-7626.
E-mail address: d.tamburic@lcf.linst.ac.uk (S. Tamburic).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2003.11.033

It is known that a large number of emulsifiers, including commonly used non-ionic surfactants (ethoxylated and propoxylated derivatives and polyol esters) could affect the properties of lamellar lipids of intercellular matrix of the SC, resulting in significant increase in transepidermal water loss (TEWL) (Idson, 1991; Bárány et al., 2000). Recently, emulsifiers of sugar ether type were proposed as an alternative to traditionally used polyoxyethylene derivatives in the stabilisation of oil in water (o/w) emulsions (Desai, 1990; Simon et al., 1998; Aikens and Friberg, 2000). Therefore, it was of interest to study the effect of sugar ether-based emulsifier vehicle in comparison to ethoxylated one on the action of urea. Furthermore, emulsions of water in oil (w/o) type with high percent of water (60–70%), based on silicon emulsifiers are known to be useful as skin moisturisers, and comparable in their effect to the conventional choice in dermatological practice, the Cold cream (Deitz, 2002). The above-mentioned types of emulsion systems were chosen for this study as examples of different vehicles, which may or may not cause alterations in the effect of urea on the skin.

The aim of the study was to evaluate long-term effects of daily use of four different types of emulsions, with and without urea as a moisturising agent, on both normal and SLS-irritated human skin. Skin parameters observed were TEWL, SC hydration level and erythema index (EI). The investigation was composed of two parts, both using double-blind randomised study design. In part I, the TEWL, electrical capacitance (EC) and sebum content (as a measure of any cream residue) on a flexor side of both forearms were evaluated. This was done on two groups of 15 volunteers during a 3-week treatment with the four test emulsions. In part II, the effects on barrier integrity, SC hydration level and erythema of SLS-irritated hand skin were evaluated in additional two groups of 10 volunteers, each treated for 5 days with two creams, chosen from part I.

2. Material and methods

2.1. Subjects

In total 50 healthy individuals (all women) without any history or clinical signs of dermatological diseases, with normal to moderately dry skin, completed the study. During the test period the subjects were allowed to wash normally, but not to use any other skin care products on their arms. Informed consent was obtained from all volunteers and the study was approved by the local ethics committee. The study was performed from the beginning of November to the end of December.

2.2. Test samples

Four different emulsion vehicles were tested. Sample F1 was Cold cream (Unguentum emolliens, Ph.Jug. IV), with F1p being a placebo sample and F1a urea-containing (active) cream. The Cold cream was made of (in w/w): cetaceum (5%), bees wax (12.0%), liquid paraffin (56%), sodium tetraborate (0.5%) and purified water (19%). The remaining three samples were formulated with an equal amount (21%) and equal composition of oil phase, with the exception of some co-emulsifiers and consistency promoters (Table 1). Sample F2 was an o/w emulsion based on a non-ionic emulsifier of sugar ether type (cetearyl glucoside and cetearyl alcohol as Montanov 68®, kindly provided by Seppic, France); with an appropriate co-emulsifier. Sample F3 was a w/o emulsion based on silicon emulsifier (cetyl dimethicone copolyol), while sample F4 was the conventional o/w emulsion with the combination of two ethoxylated non-ionic emulsifiers (ceteareth-12/ceteareth-20), as emulsion stabilisers. In urea-containing samples (F1a–F4a), purified water was replaced with 5% of urea. All samples were freshly prepared, having the pH vales of 5.4–5.7.

2.3. Experimental design

In part I, 30 volunteers were divided in two groups (placebo and urea cream users), as reported in similar studies (Derde et al., 2000; Held et al., 2001):

Group 1 (15 volunteers, mean age: 24.2 ± 0.9): the flexor sides of both forearms were treated with the four placebo samples, using precisely delineated and marked cardboard ruler (with three empty spaces in the form of rectangles, each being 9 cm^2). Samples F1p and F2p were applied at the left, and F3p and F4p at the right forearm. At each forearm one rectangle was left as an untreated control.

Table 1
Composition of placebo emulsions (F2p–F4p); in active samples (F2a–F4a) 5% of purified water was replaced with urea

Ingredients/INCI	Sample % (w/w)		
	F2p	F3p	F4p
Oil phase			
Petrolatum	3.00	3.00	3.00
Mineral oil	3.00	3.00	3.00
Prunus dulcis	3.00	3.00	3.00
Isopropyl miristate	3.50	3.50	3.50
Caprylic/capric triglyceride	3.00	3.00	3.00
Coco-caprylate/caprate	4.00	4.00	4.00
Cetearyl alcohol	1.00		1.00
Polyglyceryl-4-isostearate		1.00	
Bees wax		1.20	
Hydrogenated castor oil		0.80	
Dimethicone	0.50	0.50	0.50
BHT, ascorbyl palmitate	0.05	0.05	0.05
Emulsifier			
Cetearyl glucoside (and) cetearyl alcohol	5.00		
Ceteareth-12			2.00
Ceteareth-20			2.00
Cetyl dimethicone copolyol		2.00	
Water phase			
Propylene glycol	3.00	3.00	3.00
Preservatives blend	0.50	0.50	0.50
Sodium chloride		0.5	
Aqua	70.45	70.95	71.45

Group 2 (15 volunteers, mean age: 22.6 ± 0.5): active creams with 5% urea in different vehicles (samples F1a–F4a) were applied in the same manner as placebo samples.

During part I of the study, subjects were treated for 21 days twice daily, in the morning and in the evening. TEWL, EC and sebum content were measured before their entry into the study (baselines), 30 min after the first application of the samples and then on days 7, 14 and 21, as well as 3 days upon the last application of the tested samples. Each measurement was carried out 10–15 h after previous treatment, i.e. the products were applied in the evening and the measurements were taken on the following day. The subjects were instructed to wash their arms normally and not to administer any product in the morning before the measurement. Based on the results of the first phase of the experiment, two emulsions were chosen for the part II of the study.

In part II, the skin on the back of the hand, previously exposed to 2% (w/w) SLS solution was treated (method outlined below). TEWL, EC, EI and sebum content were measured before the SLS exposure (baseline), 12–15 h upon the last immersion of the hand, and finally 12–15 h after the last application of the emulsions. The exposure of the skin of both hands to SLS solution was carried out 1 day after the baselines were taken, and was performed on 2 consecutive days. Following irritation, a 5-day long treatment with the two chosen creams was carried out. Participants were advised to wash their hands as usually.

Twenty volunteers that took part in this phase of the study were divided in two groups. Group 3 (10 individuals, mean age: 22.7 ± 0.8): dorsal side of the left hand was treated with the sample F2, using precisely delineated and marked cardboard ruler, so that the left half of the hand was treated with placebo and the right with urea-containing cream (each area 16 cm^2). The right hand served as an untreated control. Group 4 (10 individuals, mean age: 25.6 ± 3.6): treated with F3p and F3a in the same manner as group 3.

2.4. Sodium lauryl sulphate exposure

Experimental irritation was elicited by immersion of both hands into 2% (w/w) SLS solution (Ph.Eur.), warmed at 40 °C. Immersion took place for 10 min twice daily for 2 consecutive days, with an interval of at least 4 h between immersions (Ramsing and Agner, 1997; Fluhr et al., 2001).

2.5. Instrumental evaluation

All skin tests were performed after adaptation of participants to room conditions (30 min at 21 ± 1 °C and 45 ± 5% RH). The following order of measurements was performed: TEWL, EI (during part II of the study), skin capacitance and sebum content.

All measurements were carried out according to the relevant guidelines (Pinagoda et al., 1990; Fullerton et al., 1996; Berardesca, 1997; Rogiers, 2001).

TEWL was evaluated as an indicator of the skin barrier integrity using Tewameter TM 210. Electrical capacitance, indicating the hydration level of the outer epidermis, was measured by Corneometer CM 825 and the results presented in relative corneometer units (rcu). EI, as an indicator of the skin irritation,

was detected by Mexameter MX 18. Possible presence of the cream residue at the skin surface was checked by Sebumeter SM 810 (all instruments by Courage + Khazaka, Germany). An average of three measurements was used in all the cases, except for the EI (five readings).

2.6. Statistical analysis

The values of all observed parameters are given as means ± S.D.

In part I, following a positive testing for normality, parametric tests were used. Data involving values of parameters measured on forearm areas treated by different samples (F1 through F4, active and placebo, as well as the control site), at distinct time points, were analysed by the one-way within-subjects (repeated measures) ANOVA, followed by Tukey's *t*-test, where appropriate (Kuss and Diepgen, 1998; Fluhr et al., 2001). Differences between placebo and corresponding active cream-treated groups at distinct time points, were checked by Student's unpaired *t*-test.

In the second part of the study, due to inhomogeneity of the data, Friedman rank variance analysis with consecutive Dunn's comparisons was used to evaluate the statistical difference between the values of measured parameters (TEWL, EC, EI) at different time points (baseline, upon SLS irritation, and upon 5-day treatment), for each site (placebo, active treatment and control) separately. Differences between values obtained at the same time point, but for different regions of the tested hand skin (placebo versus active treatment versus control) were analysed by the one-way within-subjects (repeated measures) ANOVA followed by Tukey's *t*-test (Kuss and Diepgen, 1998; Fluhr et al., 2001).

$P < 0.05$ was considered significant for all test methods. Statistical analyses were performed with commercial statistical software Stat for Windows R. 5.0.

3. Results

All participants reported strict compliance with the instructions, including washing their arms in the morning prior to measurements. This was confirmed by the lack of the cream residue on the skin surface, measured by Sebumeter (absolute values being within the range 0–2 μg/cm^2, both at treated and control sites, as well as at each time point during the study).

3.1. Part I

The results of the first part of the study are presented in Tables 2 and 3and Figs. 1–4. Table 2

Table 2
TEWL (g/m^2/h) of placebo samples (F1p through Fp4) and active samples (F1a through F4a), with corresponding control values, during the test period; results are given as means ± S.D.[a]

Sample	Time (min/day)					
	Baseline	30 min	7 days	14 days	21 days	3 days upon treatment
F1p left	8.6 ± 2.1	8.1 ± 2.9	7.5 ± 1.2^{2,4}	6.6 ± 1.2^{4}	5.9 ± 1.6^{4}	5.5 ± 1.5^{3,4}
F2p left	7.3 ± 1.3	7.8 ± 2.5	10.3 ± 3.9^{*1,3}	7.9 ± 1.4^{4}	6.4 ± 1.6^{4}	6.6 ± 1.^{3,4}
F3p right	7.1 ± 2.0	6.1 ± 1.3	7.2 ± 1.4^{2,4}	6.6 ± 1.2^{4}	7.2 ± 1.7^{4}	6.8 ± 1.7^{1,4}
F4p right	8.7 ± 2.5	8.2 ± 1.6	10.9 ± 2.5^{*1,3}	9.8 ± 2.9^{*1,2,3}	10.2 ± 3.2^{*1,2,3}	9.5 ± 1.3^{*1,2,3}
Control left	7.4 ± 2.1	7.7 ± 2.3	7.8 ± 1.3	7.1 ± 1.9	6.6 ± 1.7	6.7 ± 1.3
Control right	7.7 ± 1.9	7.8 ± 1.8	8.1 ± 2.4	6.8 ± 1.7	6.3 ± 1.8	5.9 ± 1.5
F1a left	7.3 ± 2.4	8.3 ± 3.0	9.9 ± 2.6^{*3,4}	6.7 ± 1.8^{4}	6.5 ± 2.4^{2,3,4}	7.8 ± 1.9^{*2,3,4}
F2a left	6.5 ± 1.3	7.6 ± 1.7	9.6 ± 2.1*	6.6 ± 1.2^{4}	5.2 ± 2.0^{*1}	5.7 ± 2.0^{1}
F3a right	7.7 ± 2.3	6.7 ± 1.9	8.6 ± 2.9^{1}	4.7 ± 2.3 ^{*1,2,4}	5.2 ± 1.8^{*1}	5.7 ± 1.3^{1}
F4a right	7.3 ± 2.1	6.2 ± 2.0	8.3 ± 2.1^{1}	5.8 ± 1.9^{1,2,3}	5.3 ± 1.8^{*1}	6.5 ± 1.9^{1}
Control left	7.5 ± 1.8	7.1 ± 2.3	7.5 ± 1.7	6.1 ± 1.9	6.5 ± 2.4	6.1 ± 2.1
Control right	7.8 ± 2.2	7.5 ± 1.9	7.9 ± 3.0	5.9 ± 2.0	6.4 ± 2.7	6.3 ± 1.9

[a] On the given time point (baseline, 30 min, 7, 14, 21, 3 days upon treatment withdrawal), significant group differences ($P < 0.05$) according to Tukey's post hoc analysis were given related to corresponding control values (∗), or to values obtained under treatments with samples F1p through F4p, i.e. F1a through F4a (numbers 1 through 4 in superscript, respectively).

Table 3
Electrical capacitance (EC, rcu) of placebo samples (F1p through F4p) and active samples (F1a through F4a), with corresponding control values, during the test period; results are given as means ± S.D.[a]

Sample	Time (min/day)					
	Baseline	30 min	7 days	14 days	21 days	3 days upon treatment
F1p left	29.4 ± 3.7	35.9 ± 5.2*	37.8 ± 4.1*2,3,4	36.5 ± 4.5*2	33.7 ± 3.7*2	30.7 ± 3.4*2
F2p left	27.4 ± 2.8	35.5 ± 3.9*	45.5 ± 3.1*1,3,4	46.1 ± 3.2*,1,3,4	44.4 ± 3.9*1,3,4	36.6 ± 3.8*1,3,4
F3p right	27.1 ± 2.9	32.3 ± 3.2*	33.9 ± 3.0*1,2	33.7 ± 4.0*2	30.9 ± 2.6*2	29.4 ± 2.7*2
F4p right	26.4 ± 2.6	33.5 ± 2.5*	33.9 ± 3.5*1,2	32.5 ± 3.2*2	29.6 ± 3.2*2	29.3 ± 3.3*2
Control left	25.8 ± 2.4	27.3 ± 2.1	24.7 ± 3.1	23.3 ± 2.7	23.2 ± 2.5	22.6 ± 2.2
Control right	27.0 ± 2.6	26.9 ± 4.1	25.9 ± 2.7	22.7 ± 3.5	22.5 ± 3.8	22.7 ± 2.7
F1a left	26.1 ± 5.0	30.3 ± 2.9*	36.0 ± 3.1*	33.0 ± 3.0*4	35.5 ± 3.1*3,4	30.1 ± 3.2*
F2a left	27.4 ± 2.8	33.1 ± 3.2*	38.8 ± 2.8*	33.9 ± 2.8*4	35.7 ± 2.9*3,4	31.7 ± 2.2*
F3a right	24.7 ± 2.8	24.4 ± 2.3	38.8 ± 3.0*	33.2 ± 2.8*4	38.5 ± 3.2*1,2,4	31.9 ± 2.8*
F4a right	25.0 ± 2.4	29.8 ± 2.7*	37.5 ± 3.2*	26.7 ± 1.81,2,3	32.2 ± 2.1*1,2,3	29.6 ± 2.4*
Control left	24.7 ± 2.1	24.9 ± 2.4	24.2 ± 3.1	23.5 ± 3.2	23.1 ± 2.7	22.5 ± 2.7
Control right	25.3 ± 2.8	26.9 ± 3.1	26.9 ± 2.7	23.3 ± 3.0	22.3 ± 2.7	22.0 ± 2.7

[a] The same as Table 2.

summarises the means for TEWL after treatments with placebo (group 1, samples F1p–F4p) and active (group 2, samples F1a–F4a) samples and presents the significance levels between samples, mutually and related to untreated control. In the same manner, Table 3 sums up the results for EC of placebo and urea-containing creams. In addition, graphical representation of percentage change related to corresponding control value for each emulsion type (F1–F4) are shown in Figs. 1–4 (*t*-test, $P < 0.05$).

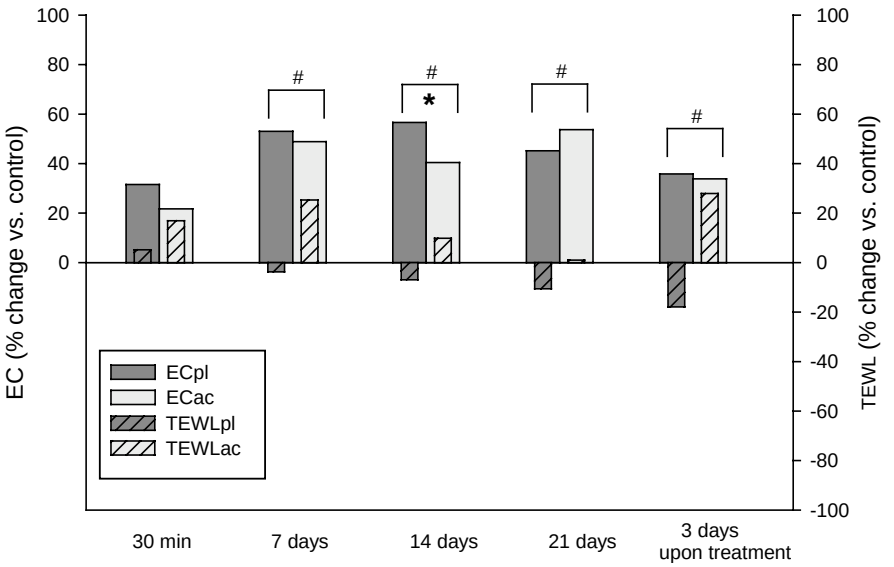

Fig. 1. The effect of topical application of sample F1 (Cold cream) ($n = 15$) without urea (F1p, dark grey bars) and with 5% urea (F1a, light grey bars) on EC (open bars—wider) and TEWL (hatched bars—narrower), related to control (percent change). Differences between placebo and active cream-treated groups at distinct time points were checked by Student's unpaired *t*-test, significant differences ($P < 0.05$) being marked with (#) for TEWL, and with (∗) for EC.

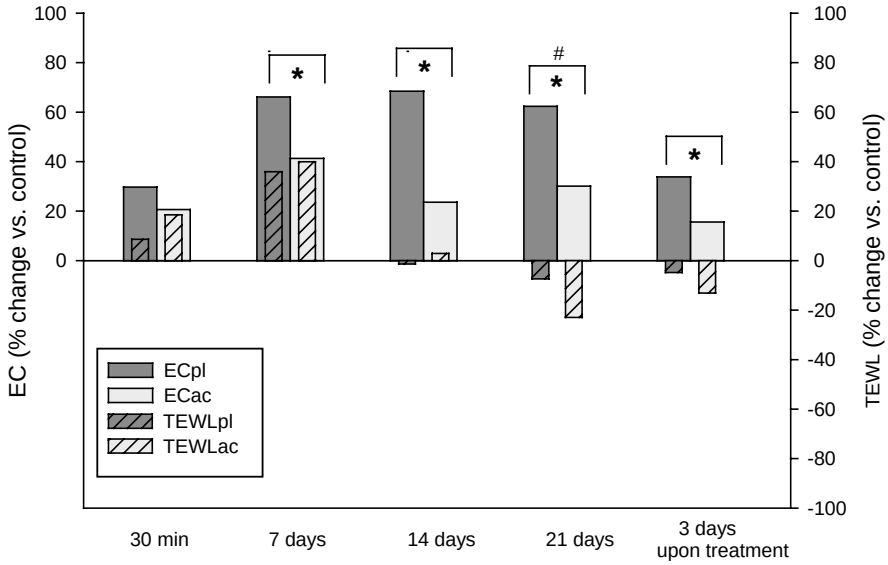

Fig. 2. The effect of topical application of sample F2 (sugar ether-based cream) ($n = 15$) without urea (F2p, dark grey bars) and with 5% urea (F2a, light grey bars) on EC (open bars—wider) and TEWL (hatched bars—narrower), related to control (percent change). Statistics as in Fig. 1.

Control TEWL and EC values did not significantly fluctuate within the two groups of participants, nor between these groups, during the entire test period.

3.1.1. Transepidermal water loss

Thirty minutes after cream application there was no significant change of TEWL related to corresponding control, neither with placebo nor with active samples

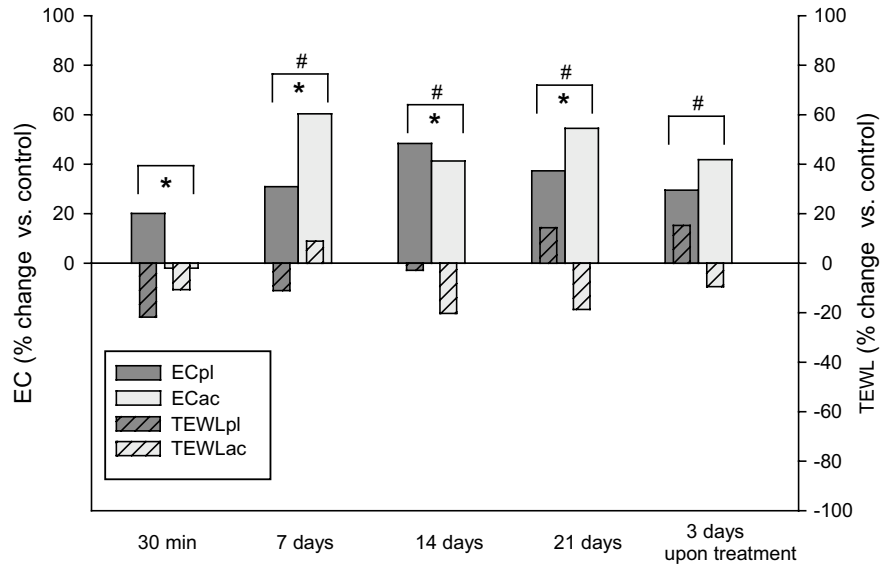

Fig. 3. The effect of topical application of sample F3 (silicone copolyol-based cream) ($n = 15$) without urea (F3p, dark grey bars) and with 5% urea (F3a, light grey bars) on EC (open bars—wider) and TEWL (hatched bars—narrower), related to control (percent change). Statistics as in Fig. 1.

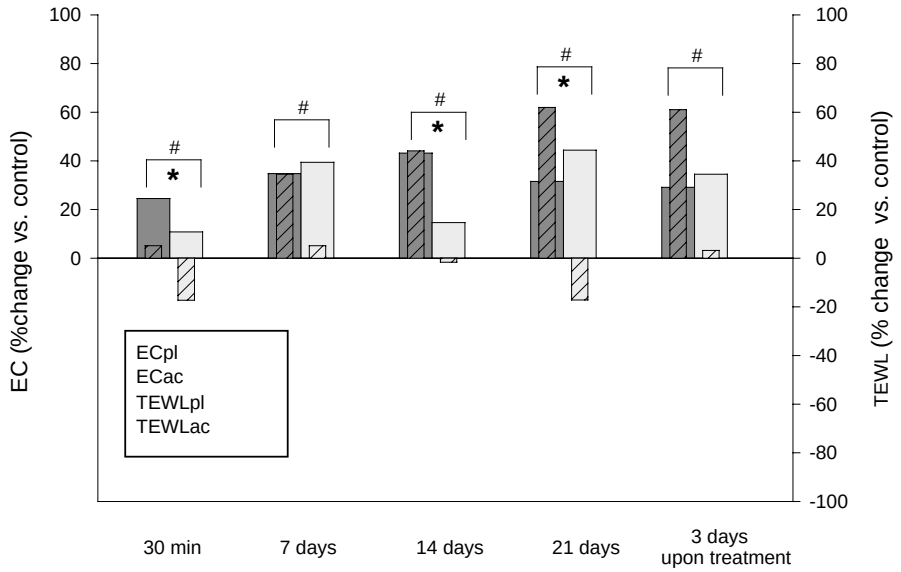

Fig. 4. The effect of topical application of sample F4 (etoxylated non-ionics-based cream) ($n = 15$) without urea (F4p, dark grey bars) and with 5% urea (F4a, light grey bars) on EC (open bars—wider) and TEWL (hatched bars—narrower), related to control (percent change). Statistics as in Fig. 1.

(Table 2). During the treatment period, different trends of TEWL alterations in different emulsion samples were observed (Table 2). In sample F1 (Cold cream), placebo and urea-containing emulsions performed in two opposite ways. Placebo sample (F1p) tended to reduce TEWL during the entire test period, whereas TEWL measured at the final time point in urea-containing sample (F1a) was significantly higher compared to the corresponding control and other samples (Table 2). Comparison of means of TEWL percentage changes (related to control) showed that placebo sample improved the skin barrier significantly better than active sample after 7, 14, 21 and 24 days of test duration (Fig. 1).

In sample F2, placebo and active cream similarly affected TEWL, with pronounced increase after 7 days of treatment, and almost linear decrease until the end of the experiment (Table 2, Fig. 2). Sample F3p did not affect TEWL significantly compared to control, whereas its active sample (F3a) did reduce it significantly, which was especially pronounced after 14 days (Table 2, Fig. 3).

In sample F4, placebo emulsion F4p significantly increased TEWL compared to control and other placebo samples after 14, 21 and 24 days of treatment (Table 2). On the contrary, its urea-containing counterpart (F4a) has produced a significant decrease in TEWL throughout the study (Table 2, Fig. 4).

3.1.2. Electrical capacitance

For all samples, either placebo or active, SC hydration level was significantly improved compared to corresponding control at all time points, except for sample F3a at 30 min after application (Table 3). The effect of emulsion formulation was clearly detectable, the emulsion F2 showing the best moisturising potential (Table 3). Furthermore, sample F2p hydrated skin significantly better than other placebo samples from day 7 until the end of the test (Table 3). Interestingly, F2p has performed better than its corresponding urea-containing sample F2a (Fig. 2). In other emulsion formulations, no consistent trend of moisturisation effects could be discerned (Figs. 1, 3 and 4).

3.2. Part II

Based on the results of part I, the sugar-based o/w emulsion (F2) and the light silicone-based w/o emulsion (F3) were chosen for further experiments.

Table 4
TEWL (g/m^2/h), EC (rcu), and EI (arbitrary units) for group 3, treated with F2 (placebo and active cream); results are given as means ± S.D.[a]

Sample	Baseline (1)	Upon SLS irritation (2)	Upon 5 days treatment (3)	*P*-values (Friedman's test)
TEWL				
Placebo (p)	13.1 ± 5.9	13.3 ± 4.5^{c,a}	11.3 ± 3.5^{c}	0.497
Active cream (a)	13.6 ± 5.7	15.3 ± 4.9^{p}	12.1 ± 5.4^{c}	0.007$^{\#,2:3}$
Control (c)	13.2 ± 3.1	15.4 ± 5.3^{p}	14.5 ± 4.6^{p,a}	0.150
P-values (ANOVA)	0.244	0.038*	0.034*	–
EC				
Placebo (p)	26.8 ± 5.7	19.7 ± 4.7	25.4 ± 6.8^{a,c}	<0.001$^{\#,1:2,2:3}$
Active cream (a)	25.8 ± 5.2	19.2 ± 3.9	31.2 ± 4.9 p,c	<0.001$^{\#,1:2:3}$
Control (c)	24.1 ± 5.7	19.5 ± 3.4	20.2 ± 5.3^{p,a}	<0.001$^{\#,1:2:3}$
P-values (ANOVA)	0.078	0.899	<0.001*	–
EI				
Placebo (p)	236.8 ± 57.0	261.0 ± 50.3	255.3 ± 48.8^{c}	0.497
Active cream (a)	250.4 ± 50.6	260.9 ± 29.0	238.2 ± 84.0c	0.905
Control (c)	259.9 ± 52.0	285.5 ± 51.5	340.8 ± 84.6^{p,a}	0.014$^{\#,1:3}$
P-values (ANOVA)	0.067	0.069	0.003*	–

[a] Significant differences ($P < 0.05$) are marked with (∗) (repeated measures ANOVA, data in columns) and (#) (Friedman's test, data in rows). Although the second test is a non-parametric one, the values of parameters are for the sake of simplicity given as means with standard deviations. Values participating in significant post hoc comparisons are given in letters after the corresponding values for Tukey's test, and in numbers after the *P*-values for Dunn's test.

Sample F2, particularly placebo F2p, exhibited the best moisturising potential, with a reasonable effect on the skin barrier integrity. On the other hand, active sample F3a has shown a more favourable profile of both SC hydration and TEWL than its placebo sample. The SLS-irritated hand skin was treated for 5 days and the effects obtained for placebo and urea-containing samples of F2 and F3 were compared with a non-treated control. Results of the part II of the study are presented in Tables 4 and 5.

Table 5
TEWL (g/m^2/h), EC (rcu), and EI (arbitrary units) for group 4, treated with F3 (placebo and active cream); results are given as means ± S.D.[a]

Sample	Baseline (1)	Upon SLS irritation (2)	Upon 5 days treatment (3)	*P*-values (Friedman' test)
TEWL				
Placebo (p)	12.3 ± 3.2	17.7 ± 6.3	13.9 ± 4.5^{c}	0.003$^{\#,1:2,2:3}$
Active cream (a)	13.7 ± 3.6	17.9 ± 6.3	12.7 ± 3.4^{c}	0.002$^{\#,1:2,2:3}$
Control (c)	15.2 ± 8.8	17.4 ± 8.02	18.4 ± 6.6^{p,a}	0.150
P-values (ANOVA)	0.175	0.380	<0.001*	–
EC				
Placebo (p)	25.9 ± 6.3	21.7 ± 5.5	25.9 ± 7.7^{a,c}	<0.001$^{\#,1:2,2:3}$
Active cream (a)	25.7 ± 4.6	21.5 ± 4.9	31.6 ± 6.6p,c	<0.001$^{\#,1:2:3}$
Control (c)	24.9 ± 5.4	19.9 ± 5.1	16.7 ± 4.7^{p,a}	<0.001$^{\#,1:2:3}$
P-values (ANOVA)	0.158	0.213	<0.001*	–
EI				
Placebo (p)	257.7 ± 41.4	244.3 ± 57.6	241.8 ± 33.9c	0.150
Active cream (a)	259.3 ± 44.8	243.0 ± 44.2	227.3 ± 41.4c	0.670
Control (c)	278.6 ± 42.3	276.8 ± 42.8	305.1 ± 71.6^{p,a}	0.097
P-values (ANOVA)	0.074	0.054	0.002*	–

[a] The same as Table 4.

3.2.1. Transepidermal water loss

After SLS irritation, TEWL increase was recorded in both groups of participants (Tables 4 and 5). However, Friedman analysis followed by Dunn's test revealed significant difference between irritated skin and baselines only in the case of group 4 (Table 5). In group 3, a 5-day treatment with placebo (F2p) and active (F2a) sample produced significant TEWL decrease compared to untreated control (Table 4). In group 4, application of both placebo and active F3 sample resulted in significant barrier repair of the irritated skin (Friedman/Dunn's test, Table 5).

3.2.2. Electrical capacitance

SC hydration levels were significantly lowered after SLS-induced irritation in both groups of participants at all tested sites. Treatment with both placebo samples (F2p and F3p) significantly enhanced hydration level, compared to the values of irritated skin, whereas active samples F2a and F3a increased the skin moisture level significantly in relation to both irritated sites and baselines (Friedman/Dunn's test, Tables 4 and 5). Using repeated measures ANOVA, significant differences between active and placebo samples (i.e. F2p versus F2a and F3p versus F3a), as well as related to untreated control, were found in both groups (Tables 4 and 5).

3.2.3. Erythema index

EI has significantly increased in control sites compared to those treated with placebo and active samples in both groups (repeated measures ANOVA, Tables 4 and 5), indicating that the treatment with either placebo or urea-containing samples favourably affected SLS-irritated skin.

4. Discussion

In part I of this study, the effect of four different types of emulsion vehicles, with and without urea, on skin barrier function and SC hydration level were studied. It was of interest to establish relationship between emulsion composition (type of emulsion, nature of emulsifier, polarity of oil ingredients) and the effect of urea on the skin.

The most important phenomenon taking place after application of skin care emulsions is the evaporation of 90% of its water within 15–20 min, leading to pronounced structural changes of emulsion placed on the skin surface (Bruno et al., 1993; Held et al., 1999; Aikens and Friberg, 2000). It was intriguing to find no significant changes in TEWL 30 min after application of emulsions (Table 2). At the same time, EC was found to be significantly higher than corresponding control values in all placebo, but not in all urea-containing samples (Table 3). It is known that the evaporation phase is followed by the lipidisation phase, in which emulsion lipids penetrate into the epidermis and cause the increase of skin hydration level (Aikens and Friberg, 2000). Therefore, it could be speculated that a pronounced lipidisation phase in these samples has led to an insignificant TEWL changes, but a considerable increase in skin moisturisation. This is in line with the findings of Bruno et al. (1993) and Held et al. (1999).

During the first 30 min after the sample application, Cold cream (F1) with only 19% of water has shown a similar trend of EC and TEWL changes as other samples, with more than 70% of water. This proves that, at least in the case of the samples used in this study, the differences in inherent capacitance of moisturising creams were not the main source of an increased skin hydration (Jemec et al., 2000) and point out the significance of the lipidisation phase. However, the mechanism of moisturisation of Cold cream rich in non-polar lipid ingredients (petrolatum and mineral oil) should be distinguished from the effect of other samples with 21% of predominantly polar oil phase. It was shown that petrolatum could enter the human skin and even get incorporated within the intercellular lamellar bilayers of the SC, thus assisting barrier integrity and skin hydration (Ghadially et al., 1992). Subsequent studies revealed that this rapid improvement in barrier function by petrolatum is mainly due to the interaction restricted to the SC (Mao-Qiang et al., 1995). Hence, the influence of non-physiologic lipids exemplified by petrolatum can be clearly delineated from the effects of physiologic lipids (Mao-Qiang et al., 1995; Man et al., 1996). An improvement in skin barrier function during long-term treatment was shown in other studies using urea in occlusive creams similar to Cold cream (Lodén, 1996; Ramsing and Agner, 1997). However, this kind of emulsion is characterised by unfavourable aesthetics (low spreadability and sticky feel), which is likely to affect users' compliance.

The sample of light w/o emulsion based on silicone emulsifier has been found to enhance EC (Table 3), whereas TEWL remains constant (placebo, F3p) or significantly decreases (urea-containing sample, F3a) (Table 2). Overall, it has performed significantly better than the traditional w/o emulsion (Cold cream), both as placebo and urea-containing cream. This is not in line with the finding that moisturisers with higher lipid content have better barrier repairing and moisturising effects, shown in the case of SLS-irritated skin (Held et al., 2001). The high performance of sample F3 can be related to the mobility of the polar oil phase and its ability to penetrate into the intercellular lipid lamellae much easier than non-polar oil ingredients (Lodén and Andersson, 1996), thus incorporating in the SC and supporting or replacing the endogenous lipids of intercellular bilayers (Denda et al., 1994). Moreover, the addition of urea (F3a) enhanced the beneficial effect of placebo sample F3p (Fig. 3), which is in line with the findings of Wohlrab (1986) and Bettinger et al. (1994) concerning different w/o emulsion formulations.

The comparison of the effects of o/w emulsion vehicles on the performance of urea reveals the importance of the emulsifier type. In the case of sample F2, a non-ethoxylated non-ionic emulsifier of sugar ether type was used, whereas in cream F4 a combination of two non-ionic ethoxylated emulsifiers with 12 and 20 ethylene oxide (EO) units was employed. Placebo sample F2p has improved skin hydration significantly better than urea-containing F2a cream, whereas the effect on skin barrier was slightly more favourable with the active sample (Fig. 2). Both F2 samples exhibited significant increase in TEWL after 7 days of treatment, with the tendency of beneficial skin barrier effects being observed at the end of the study. On the other hand, placebo cream F4p was found to increase TEWL, indicating skin barrier impairment, while the active cream F4a had an opposite effect (Fig. 4).

It has been shown that emulsifiers of sugar ether type from vegetable origin cause lower lipid depleting effect and smaller alteration of superficial cutaneous proteins (Simon et al., 1998). The head groups of the sugar-based emulsifiers contain many free hydroxyl groups, which bind water and provide additional moisturisation (Aikens and Friberg, 2000). Despite that, it was surprising to find out that placebo sample F2p hydrated skin better than all others, including its urea-containing counterpart. The rationale for this behaviour may be found in the emulsion structure. As these creams are stabilised by lamellar gel phase (unpublished data), where a portion of water phase is fixed between lamellar lipid bilayers as “depo” or interlamellar water, that portion of water is probably responsible for sustained skin moisturisation (Junginger, 1997). On the other hand, urea is a hygroscopic substance with a pronounced water-binding capacity (Swanbeck, 1992; Bettinger and Maibach, 1997). It could be speculated that urea binds and withholds the “depo” water within the cream, thus limiting the moisturising potential of active sample F2a.

The skin performance of the sample F4 appears to be linked to the ethoxylated nature of emulsifiers. It is known that emulsifiers incorporated in moisturisers could damage skin barrier, which is reflected in the TEWL increase (Bárány et al., 2000). The effect is possibly due to alterations in intercellular lipid matrix and its possible fluidisation, affecting the permeability of SC and bioavailability of topical drugs or dermo-cosmetics (Bárány et al., 2000). It is also known that emulsifier irritancy increases with the EO units number (Bárány et al., 2000), which was 12 and 20, respectively. The restorative effect of urea-containing cream F4a most probably relies on the favourable effect of urea itself (Lodén, 1996).

Claims for dermo-cosmetic moisturisers should ideally be substantiated using a combined strategy, i.e. testing on normal as well as on SLS-irritated human skin (Derde et al., 2000; Held et al., 2001). In the latter case, the measurement of skin colour has been suggested as a useful method for an objective evaluation of the moisturising effect on the SLS-induced skin damage (Clarys et al., 2000; Derde et al., 2000; Held et al., 2001; Paye et al., 2001). While Mexameter has been found inappropriate for detecting irritation response after 6-h period of application of 2% SLS (Fluhr et al., 2001), in the case of prolonged irritation (12 h overnight closed patch of 0.5% SLS), EI measured by Mexameter was shown to be an adequate parameter for objective evaluation of skin irritation (Clarys et al., 2000). It has also been found that the hand and forearm skin respond similarly to surfactants (Paye et al., 2001).

In part II of this study, the soaking of hands in the warm 2% SLS solution for 2 consecutive days, 10 min twice daily, has produced a significant decrease of skin hydration in both groups of participants,

but mainly insignificant changes of TEWL and EI (Tables 4 and 5). A 5-day treatment with placebo (F2p, F3p) and urea-containing samples (F2a, F3a) has significantly accelerated skin barrier repair, judged by TEWL and EC measurements, and decreased the EI, compared to the control hand (Tables 4 and 5). The unfavourable values of these parameters have been detected in control hands of both groups during the study (Tables 4 and 5), indicating that the process of physiological repair of the skin has not started (winter season). This was in agreement with an earlier report (Ramsing and Agner, 1997).

In contrast to results on normal skin, urea-containing emulsion stabilised with sugar ether-based emulsifier (F2a) hydrated skin significantly better than its placebo sample (Table 4). It should be noted that the hydration level of the skin after SLS irritation was significantly lower than in normal skin. It has been suggested that the main impact of urea is on the free water in SC, and not on the primary or secondary water (Bettinger and Maibach, 1997). We speculate that this effect of urea-containing cream could be attributed to the ability of the hygroscopic molecule of urea to help compensate the free water depleted from the damaged skin. It is possible that urea (sample F2a) promotes the shift of "depo" water, interlamellary fixed within the F2 emulsion vehicle, into the dehydrated SC. This effect has not been seen in normal skin, which shows a clear difference in response of normal and damaged skin to urea, most probably due to an altered water concentration gradient. Otherwise, both F2 and F3 emulsions have shown quite similar effects on SLS-irritated skin, enhancing its barrier function and hydration level.

In conclusion, the present study has confirmed that the higher quantity of lipids within emulsion formulation does not necessarily bring better moisturisation of normal skin and that the type of the oil phase and emulsion formulation play important roles in this process. It was also confirmed that the use of ethoxylated non-ionic emulsifiers could induce barrier impairment, followed by TEWL increase. It was shown that the new generation of sugar-based emulsifiers do significantly contribute to the moisturisation potential of creams, but could also decrease the bioavailability of urea and, possibly, other hygroscopic substances of comparable water-binding capacity.

It is feasible to formulate effective moisturising emulsion stabilised with sugar ether-based surfactant with a relatively low content of non-physiological lipids and without traditional humectants, intended for normal skin care. Different effectiveness of test samples in the treatment of normal and SLS-irritated skin underlines the rule that "each skin has an appropriate moisturiser", therefore, the claims for each product have to be made with respect to its intended use.

Acknowledgements

This work was supported by the FIP Fellowship for year 2003, awarded to Snezana Savic by the FIP Foundation for Education and Research.

References

Aikens, P.A., Friberg S.E., 2000. Emulsifiers. In: Lodén, M., Maibach, H.I. (Eds.), Dry Skin and Moisturizers. CRC Press LLC, Boca Raton, pp. 183–201.

Bárány, E., Lindberg, M., Lodén, M., 2000. Unexpected skin barrier influence from nonionic emulsifiers. Int. J. Pharm. 195, 189–195.

Beastall, I., Guy, R.H., Hadgraft, J., Wilding, I., 1986. The influence of urea on percutaneous absorption. Pharm. Res. 3, 294–297.

Berardesca, E., 1997. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. Skin Res. Technol. 3, 126–132.

Bettinger, J., Maibach, H.I., 1997. SC water-binding capacity. Cosmet. Toiletries 112, 49–53.

Bettinger, J., Gloor, M., Gehring, W., 1994. Influence of a pretreatment with emulsions on the dehydration of the skin by surfactants. Int. J. Cosmet. Sci. 16, 53–60.

Bruno, R., Langlois, C., Friberg, S.E., 1993. Evaporation from a complex emulsion system. J. Soc. Cosmet. Chem. 44, 23–34.

Clarys, P., Alewaeters, K., Lambrecht, R., Barel, A.O., 2000. Skin color measurements: comparison between three instruments: the Chromameter®, the DermaSpectrometer®, and the Mexameter®. Skin Res. Technol. 6, 230–238.

Deitz, T., 2002. Skin smoothing sensation—a novel silicone-based o/w emulsifier. SOFW 128, 22–32.

Denda, M., Koyama, J., Namba, R., Horii, I., 1994. Stratum corneum lipid morphology and transepidermal water loss in normal skin and surfactant-induced scaly skin. Arch. Dermatol. Res. 286, 41–46.

Derde, M.P., Roseeuw, D., Rogiers, V., 2000. Claim substantiation and efficiency of hydrating body lotions and protective creams. Contact Dermat. 42, 227–234.

Desai, N.B., 1990. Esters of sucrose and glucose as cosmetic materials. Cosmet. Toiletries 105, 99–107.

Erös, I., Kónya, M., Csóka, I., 2003. Study of the structure of coherent emulsions. Int. J. Pharm. 256, 75–84.

Fischer, T.W., Wigger-Alberti, W., Elsner, P., 2001. Assessment of dry skin: current bioengineering methods and test designs. Skin Pharmacol. Appl. Skin Physiol. 14, 183–195.

Fluhr, J.W., Kuss, O., Diepgen, T., Lazzerini, S., Pelosi, A., Gloor, M., Berardesca, E., 2001. Testing for irritation with a multifactorial approach: comparison of eight non-invasive measuring techniques on five different irritation types. Br. J. Dermatol. 145, 696–703.

Fullerton, A., Fischer, T., Lahti, A., Wilhelm, K.P., Takwaki, H., Serup, J., 1996. Guidelines for measurement of skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermat. 35, 1–10.

Ghadially, R., Halkier-Sorensen, L., Elias, P.M., 1992. Effects of petrolatum on stratum corneum structure and function. J. Am. Acad. Dermatol. 26, 387–396.

Held, E., Sveinsdóttir, S., Agner, T., 1999. Effect of long-term use of moisturizer on skin hydration, barrier function and susceptibility to irritants. Acta Derm. Venereol. 79, 49–51.

Held, E., Lund, H., Agner, T., 2001. Effect of different moisturizers on SLS-irritated human skin. Contact Dermat. 44, 229–234.

Idson, B., 1991. Effects of emulsifiers on skin. Cosmet. Toiletries 106, 43–51.

Jemec, G.B.E., Na, R., Wulf, H.C., 2000. The inherent capacitance of moisturising creams: a source of false positive results? Skin Pharmacol. Appl. Skin Physiol. 13, 182–187.

Junginger, H.E., 1997. Multiphase emulsions. In: Rieger, M.M., Rhein, L.D. (Eds.), Surfactants in Cosmetics. Marcel Dekker, New York, pp. 155–182.

Kim, C.K., Kim, J.J., Chi, S.C., Shin, C.K., 1993. Effect of fatty acid and urea on the penetration of ketoprofen through rat skin. Int. J. Pharm. 99, 109–118.

Kuss, O., Diepgen, T., 1998. Proper statistical analysis of transepidermal water (TEWL) measurements in bioengineering studies. Contact Dermat. 39, 64–67.

Lodén, M., 1996. Urea-containing moisturizers influence barrier properties of normal skin. Arch. Dermatol. Res. 288, 103–107.

Lodén, M., 2000. Urea. In: Loden, M., Maibach, H.I. (Eds.), Dry Skin and Moisturizers. CRC Press LLC, Boca Raton, pp. 243–250.

Lodén, M., Andersson, A.C., 1996. Effect of topically applied lipids on surfactant-irritated skin. Br. J. Dermatol. 134, 215–220.

Lodén, M., Andersson, A.C., Lindberg, M., 1999. Improvement in skin barrier function in patients with atopic dermatitis after treatment with a moisturizing cream (Canoderm®). Br. J. Dermatol. 140, 264–268.

Lodén, M., Andersson, A.C., Frödin, T., Öman, H., Lindberg, M., 2001. Instrumental and dermatologist evaluation of the effect of glycerine and urea on dry skin in atopic dermatitis. Skin Res. Technol. 7, 209–213.

Man, Q.M., Feingold, K.R., Thornfeldt, C.R., Elias, P.M., 1996. Optimization of physiological lipid mixture for barrier repair. J. Invest. Dermatol. 106, 1096–1101.

Mao-Qiang, M., Brown, B.E., Wu-Pong, S., Feingold, K.R., Elias, P.M., 1995. Exogenous nonphysiologic vs. physiologic lipids. Divergent mechanisms for correction of permeability barrier dysfunction. Arch. Dermatol. 131, 809–816.

Paye, M., Cartiaux, Y., Goffin, V., Pierard, G.E., 2001. Hand and forearm skin: comparison of their respective responsiveness to surfactants. Skin Res. Technol. 7, 78–83.

Peramal, V.L., Tamburić, S., Craig, D.Q.M., 1997. Characterisation of the variation in the physical properties of commercial creams using thermogravimetric analysis and rheology. Int. J. Pharm. 155, 191–198.

Pinagoda, J., Tupker, R.A., Agner, T., Serup, J., 1990. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermat. 22, 164–178.

Ramsing, D.W., Agner, T., 1997. Preventive and therapeutic effects of a moisturizer. Acta Derm. Venereol. 77, 335–337.

Rogiers, V., EEMCO Group, 2001. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. Skin Pharmacol. Appl. Skin Physiol. 14, 117–128.

Serup, J., 1992. A double-blind comparison of two creams containing urea as the active ingredient. Acta Derm. Venerol. Suppl. 177, 34–38.

Simon, P., Veyrat, S., Piquemal, P., Montastier, C., Gofin, V., Pierard, G., 1998. Surfactants and the skin. Cosmet. Toiletries 113, 69–72.

Swanbeck, G., 1992. Urea in the treatment of dry skin. Acta Derm. Venereol. Suppl. 177, 7–8.

Wohlrab, W., 1986. (Urea and the skin). Schweiz Rundsch. Med. Prax. 75, 201–204.