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In vivo evaluation of the effects of moisturisers on transepidermal water loss using factorial designs

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Abstract

The effect of topical applications of pyrrolidone carboxylic acid (PCA), sodium lactate (NaL) and urea on in vivo transepidermal water loss (TEWL) in healthy volunteers was studied. The moisturizing compounds were applied both singly and as mixtures using a 2^2 factorial design. It is shown that all three compounds increased TEWL and that moreover, urea and PCA exerted synergism. No such interaction was observed between urea and sodium lactate. The study provides a rational basis for the co-formulation of urea and PCA in moisturising products for topical use.

Keywords: Emollient; Skin hydration; Xerosis; Moisturisation; Humectant; Urea; Pyrrolidone carboxylic acid; Sodium lactate

1. Introduction

Dry skin is a feature of a number of clinical conditions and often palliative treatment with a moisturizer or emollient is an important aspect of the management of such conditions. More generally, moisturising products are widely used to soften the skin and enhance its physical attractiveness. For this reason there has been much work on skin hydration and the formulation of moisturising products. Blank, for example, has made important contributions towards our present understanding of skin physiology by identifying the importance of water in maintaining skin flexibility and softness (Blank, 1952, 1953, 1985; Blank et al., 1984). He further paved the way for others to show that various compounds, now commonly and collectively referred to as the natural moisturising factor act together to hold water in the stratum corneum (Jacobi, 1967; Idson, 1978; Clar and Fourtanier, 1984; Sakamoto, 1984; Imokawa and Hattori, 1985; Deniker, 1986; Immokawi et al., 1986, 1989). Despite the fact that most moisturising factors are formulated as compound products to exert their effects, most reported studies focus on the effects of single agents rather than on their interactions. For studying such interactions, experimental designs capable of identifying such effects should be used.

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Those designs collectively known as factorial designs are now increasingly used for studying interactive effects of drugs, preservatives and insecticides among many others. In this report we describe the use of factorial design for studying the effect of various moisturisers.

2. Materials and methods

The following were obtained from the indicated sources: Evaporimeter (Servo-Med, Sweden) dual probe model (EP1D); universal stands (no.2016); disposable glass micropipettes (Analtech Inc.); temperature plate (ETI Instruments Ltd); plotter (Gallenkamp Omniscribe); urea (BDH Chemicals Ltd, Poole, U.K.); pyrrolidone carboxylic acid or PCA and sodium lactate (Sigma).

2.1. Variations in anatomical site

Four Caucasian female volunteers aged between 19 and 38 years (average 25.25 years) with no previous history of dermatological disorders were asked to refrain from using any form of lotion, moisturiser or soap for a 24 h period prior to experimentation. The volunteers were placed in a supine position in a cool room (19–20°C) with an ambient relative humidity of 51–62% (mean $56.25 \pm 4.78\%$) for a 15 min rest period before measurement commenced. After this time the TEWL through a number of anatomical sites was recorded under carefully controlled conditions. The sites tested are listed in Table 1 and variations due to the use of the protective covers no. 2017 and 2018 were also investigated. All the transepidemal water loss (TEWL) readings were recorded using a standardised procedure:

- at time $t = 0$ s, the probes are placed lightly on the skin sites;
- at time $t = 40$ s, the 10 s damping filter is depressed;
- at time $t = 55$ s, the 20 s damping filter is depressed;
- at time $t = 75$ s, the final reading is recorded.

This procedure was repeated three times at each site, allowing a 5 min period between readings. The probe was placed in the universal stand provided and was manoeuvred by holding the base of the stand so that there was no direct contact with the probe itself thereby eliminating any fluctuations in readings caused by heat transfer from hand to probe (Pinnagoda et al., 1990). In addition, the operator wore a face mask to decrease local air currents caused by breathing. For uniformity, the same probe was used on all the volunteers.

Table 1
Anatomical sites through which water loss was monitored

Anatomical site	Description
Forehead	between the eyebrows in the region of the glabella bone
Anterior aspect of forearms	12 cm from the wrist
Posterior aspect of forearms	12 cm from the wrist
Dorsal aspect of hands	halfway between second and third metacarpals
Anterior aspect of lower legs	15 cm from the ankle

Table 2
Preparation of solutions of components of the natural moisturising factor

	Low (L) w/w	High (H) w/w
Urea	10%	20%
PCA	2%	5%
Sodium lactate	5%	10%

2.2. Factorial design experiments

Urea, PCA and sodium lactate were made up as w/w solutions in propylene glycol (PG) as summarised in Table 2. Sodium lactate was purchased as a solution of between 70 and 72% w/w in water. Throughout the investigations the strength was taken as 70% w/w and the propylene glycolic solutions made up accordingly. In an initial investigation the factorial design study was carried out on one female Caucasian volunteer (age 25 years).

The volunteer refrained from using any lotion, moisturiser or soap on the left arm for 24 h prior to experimentation. At least 24 h elapsed before the site for moisturiser application was re-used,

Table 3
Transepidermal water loss [a] (g/m^2 per h) in normal subjects (female) at different exposed sites of the body surface at 55±5% relative humidity and 20±1°C

Subject:	1			2			3			4		
Method [b]:	1	2	3	1	2	3	1	2	3	1	2	3
Forehead	14.7±0.1	13.5±0.2	9.6±0.1	17.6±0.2	13.7±0.2	11.8±0.2	15.0±0.2	11.8±0.2	10.8±0.2	14.7±0.2	12.6±0.4	11.4±0.4
Left anterior forearm	4.1±0.2	3.6±0.1	2.0±0.1	4.4±0.2	3.3±0.2	2.4±0.2	5.8±0.3	4.3±0.2	3.1±0.3	4.0±0.2	3.4±0.3	2.7±0.2
Left posterior forearm	4.0±0.2	3.6±0.3	1.9±0.2	3.3±0.1	2.4±0.2	1.7±0.2	5.4±0.2	4.7±0.1	3.9±0.2	4.2±0.1	3.5±0.1	2.7±0.2
Right anterior forearm	4.0±0.2	3.0±0.2	2.2±0.2	4.2±0.1	3.0±0.2	2.6±0.1	5.5±0.3	4.2±0.1	3.3±0.2	4.6±0.4	3.2±0.1	2.8±0.1
Right posterior forearm	4.2±0.3	3.1±0.2	2.2±0.1	5.3±0.3	3.3±0.2	1.7±0.2	5.5±0.1	4.8±0.1	3.1±0.1	4.5±0.1	3.7±0.1	3.3±0.1
Left dorsum of hand	4.3±0.2	3.8±0.1	2.6±0.5	7.3±0.2	6.1±0.1	3.5±0.3	8.2±0.2	6.6±0.2	4.5±0.1	8.0±0.2	6.3±0.2	4.9±0.2
Right dorsum of hand	4.4±0.1	3.4±0.3	2.3±0.1	7.7±0.1	6.5±0.2	3.8±0.1	8.3±0.1	6.3±0.2	5.0±0.1	7.9±0.3	6.8±0.2	5.2±0.2
Left anterior lower leg	4.1±0.3	2.8±0.3	2.0±0.1	4.9±0.2	3.0±0.2	1.9±0.2	6.1±0.1	4.6±0.3	3.1±0.1	3.5±0.1	2.9±0.2	3.0±0.1
Right anterior lower leg	3.7±0.2	2.9±0.2	1.9±0.2	4.6±0.3	3.4±0.3	2.7	5.4±0.2	4.6±0.1	3.6±0.1	3.4±0.1	2.9±0.3	2.8±0.1

[a] Each water loss value is recorded as the mean±SD ($n = 3$).

[b] Method 1 represents water loss measurement with none of the covers in place. Method 2 represents water loss measurements with gold cover no. 2018 (without grid) positioned over the teflon capsule of the Evaporimeter probe. Method 3 represents water loss measurements with gold cover no. 2017 (with grid) positioned over the teflon capsule of the Evaporimeter probe.

Table 4
Some of the reported TEWL values (±SD) in g/m^2 per h obtained with the Evaporimeter and the conditions under which they were obtained

Site	Ambient temperature; RH; Premeasurement rest time	Mean TEWL ± SD (g/m^2 per h)	Season; Year; Country	Reference
Forehead	22.1 ± 0.9°C; 31 ± 4%; 40 min	16.5 (n = 10)	1977; Sweden	Nilsson (1977)
Palm	20–23°C; 18–28%	37.4 ± 13.53 (n = 10)	Winter; 1985; Denmark	Blichman and Serup (1987)
Anterior forearm	20°C; 15 min	6.46 ± 2.08 (n = 44)	Spring; 1988; The Netherlands	Pinnagoda et al. (1990)
Anterior forearm	22–23°C; 55%; 10 min	4.7 ± 0.1 (n = 50)	Summer; 1985; Singapore	Coenraads et al. (1986)
Anterior forearm	22–23°C; 18–28%	5.7 ± 2.0 (n = 10)	Winter; 1985; Denmark	Blichman and Serup (1987)
Anterior forearm (mid-region)	20–22°C; 59–67%; 15 min	2.8 ± 1 (n = 10)	1988; U.S.A.	Van der Valk and Maibach (1989)
Anterior forearm (wrist region)	20–22°C; 59–67%; 15 min	5.4 ± 3.3 (n = 10)	1988; U.S.A.	Van der Valk and Maibach (1989)
Posterior forearm	22–23°C; 55%; 10 min	4.2–4.6 (n = 50)	Summer; 1985; Singapore	Coenraads et al. (1986)
Upper back	22.1 ± 0.9°C; 31 ± 4%; 40 min	6.0 (n = 10)	1977; Sweden	Nilsson (1977)
Upper back	22°C; 42%; 10 min	5.5 ± 2.61 (male; n = 23) 2.9 ± 1.62 (female; n = 15)	Summer; 1987; Singapore	Goh and Chia (1988)

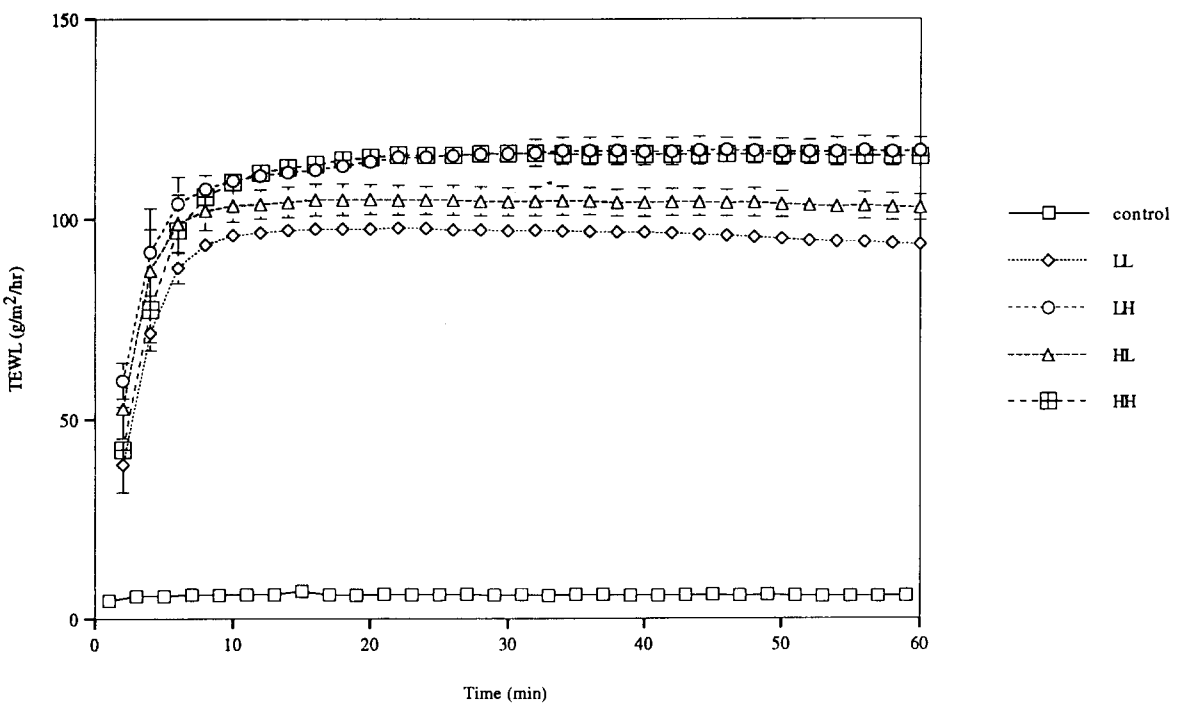

Fig. 1. Effect of application of various propylene glycolic combinations of urea and PCA on the rate of transepidermal water loss (g/m^2 per h). LL = 10% urea and 2% PCA; LH = 10% urea and 5% PCA; HL = 20% urea and 2% PCA and HH = 20% urea and 5% PCA. The results were recorded manually from the digital display.

Table 5
Analysis of variance for the effect of urea/PCA combinations applied to posterior forearm skin on transepidermal water loss using a 2×2 factorial design

Source	DF	SS	MS	*F* value	*P*
Urea	1	41.89	41.89	5.88	0.041
PCA	1	874.15	874.15	122.77	< 0.0001
Interaction	1	66.84	66.84	9.39	0.015
Error	8	56.96	7.12		
Total	11	1039.84			

PCA, pyrrolidone carboxylic acid; DF, degrees of freedom; SS, sum of squares; MS, mean square error; *P*, significance probability.

and the same probe was used to monitor TEWL at this site throughout all the experiments.

After a 15 min rest period, while the volunteer remained seated in a cool room (19–21°C), 5 μl of the relevant moisturiser solution was applied to the left posterior forearm at a site delineated 12 cm from the wrist in a 2 × 2 design. A second site at 8 cm from the wrist was used as a control site and remained untreated. The moisturiser combinations were applied to the skin 90 min before TEWL measurement began. The pretreatment time ensured that the moisturiser solutions had penetrated the skin (the site appeared 'dry' after 30–40 min). After this time, the volunteer put on a face mask and the probes, held in the universal stands, were lowered onto the treated and control skin sites. The protective gold covers (no. 2018 without grid) were used in all the factorial design studies carried out. A plotter recorded the analogue readings and, in addition, the digital readings were recorded manually for a period of 60 min during which time the volunteer remained as still as possible.

2.3. Control study on individual effect of moisturisers

A control study investigated the effects of PG on the TEWL values through a test skin site on the left posterior forearm and compared the result with the effect of either urea, PCA or sodium lactate made up in PG as described previously.

3. Results and discussion

In order to establish baseline values under our experimental conditions, transepidermal water loss (TEWL) was measured with the evaporimeter at nine exposed sites of the body surface in four female subjects with the results shown in Table 3. Those values compare well with those reported in the literature (Table 4).

3.1. Factorial study

3.1.1. Joint effects of urea and PCA

Fig. 1 shows the effect of application of the different combinations of urea and pyrrolidone carboxylic acid on the rate of transepidermal water loss (TEWL). There is a rapid rise in TEWL following each application leading to an apparent equilibrium TEWL value. This steady-state TEWL was therefore chosen as the statistic to use in the hypothesis tests. The results of analysis of variance appropriate for a 2 × 2 factorial design are shown in Table 5.

The statistical analyses show that at the usual 5% significance level the concentrations of both urea and PCA alter the TEWL. While increasing the concentration of urea from 10 to 20% w/w increases TEWL values in the presence of 2%

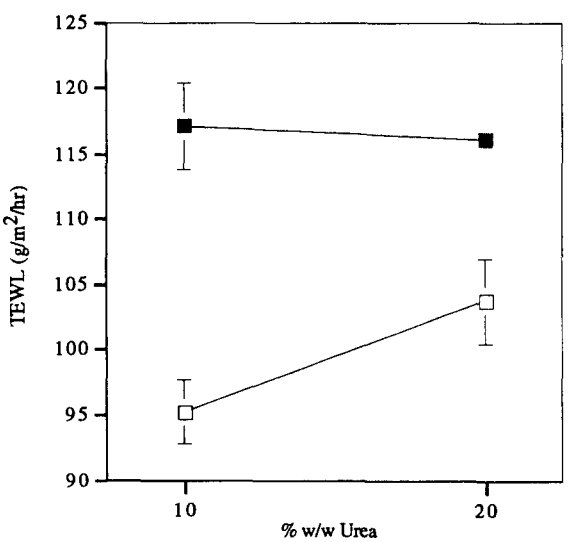

Fig. 2. Effect of urea on TEWL in the presence of 2% PCA (□) and 5% PCA (■).

w/w PCA (Fig. 2), it appears to have no additional effect on the effect of 5% PCA (Table 5). However, increasing PCA concentration from 2 to 5% w/w increases the TEWL significantly ($p < 0.001$, Table 5) in the presence of both 10 and 20% w/w urea (Fig. 3).

More interestingly, the results also reveal that the magnitude of effects of altering the concentration of urea depends on the concentration of the PCA present and vice versa. In other words, urea and PCA interact with each other ($p = 0.015$, Table 5).

3.1.2. Joint effect of urea and sodium lactate

Application of combinations of urea and sodium lactate showed effects similar to those seen with urea and PCA mixtures (Fig. 4). Analysis of variance shows once again that altering the concentrations of both urea and sodium lactate altered the effects of the two compounds on the TEWL (Table 4). From Fig. 5, it is apparent that increasing the sodium lactate concentration from 5 to 10% w/w in the presence of both 10 and 20% w/w urea resulted in a decrease in the TEWL values which was shown to be statistically significant ($p < 0.001$, Table 6). Increasing urea

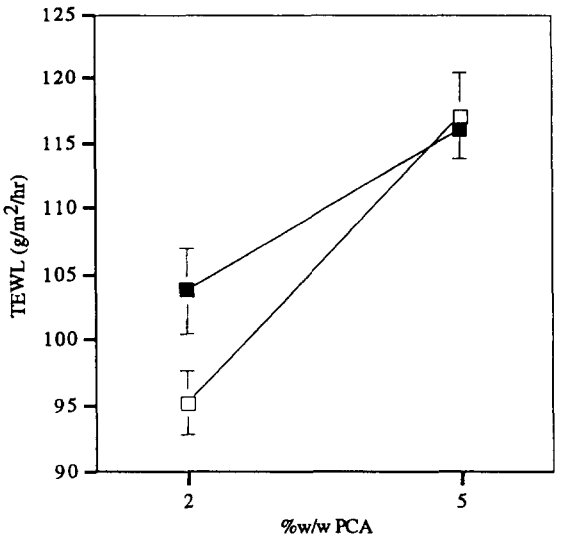

Fig. 3. Effect of PCA on TEWL in the presence of 10% urea (□) and 20% urea (■).

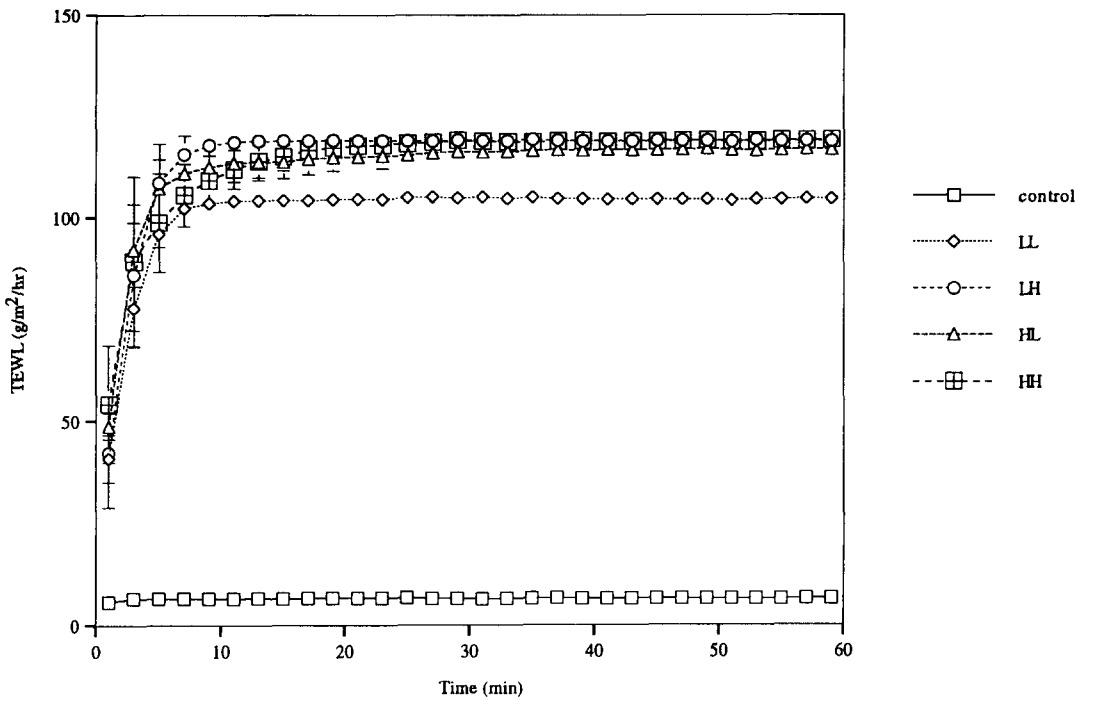

Fig. 4. Effect of application of various propylene glycolic combinations of urea and sodium lactate on the rate of transepidermal water loss (g/m^2 per h). LL = 10% urea and 5% sodium lactate; LH = 10% urea and 10% sodium lactate; HL = 20% urea and 5% sodium lactate and HH = 20% urea and 10% sodium lactate.

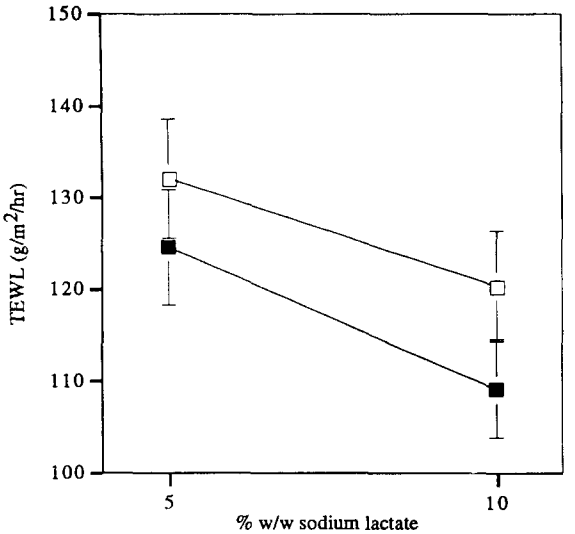

Fig. 5. Effect of sodium lactate on TEWL in the presence of 10% urea (□) and 20% urea (■).

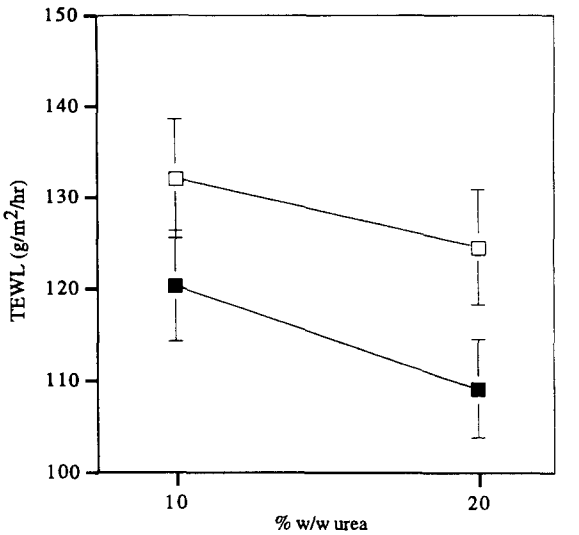

Fig. 6. Effect of urea on TEWL in the presence of 5% sodium lactate (□) and 10% sodium lactate (■).

concentration from 10 to 20% w/w in the presence of both 5 and 10% w/w sodium lactate significantly decreased TEWL ($p = 0.006$, Table 6). In this case, however, urea and sodium lactate did not exhibit any interactions ($p = 0.502$, Table 6).

3.1.3. *Effect of individual components of the NMF*

Propylene glycol, the vehicle used for dissolving the moisturisers, also increased TEWL. Indeed, its effect was more pronounced than that produced when urea or PCA was added to it. Table 7 shows the TEWL values observed while Table 8 gives the ANOVA results for the individual applications of moisturisers (in propylene glycol) compared to propylene glycol itself. Urea was seen to exert a significant decreasing effect on TEWL compared to propylene glycol alone, PCA or sodium lactate. This effect has been shown previously by others both in vivo (Grice et al., 1973; Serup, 1992) and in vitro (McCallion and Li Wan Po, 1994). PCA and sodium lactate were not significantly different from each other (at the 95% confidence level) but yielded significantly higher TEWL values than urea.

A recent study has suggested that small volatile molecules such as propylene glycol, methanol, ethanol and acetone, adsorb onto the probe of the Evaporimeter resulting in artificially elevated

Table 6
Analysis of variance for the effect of urea/sodium lactate combinations, applied to the skin of the posterior forearm, on transepidermal water loss using a factorial design

Source	DF	SS	MS	*F* value	*P*
Urea	1	262.08	262.08	14.17	0.006
Sodium lactate	1	551.08	551.08	29.80	< 0.001
Interaction	1	9.12	9.12	0.49	0.502
Error	8	147.95	18.49		
Total	11	970.22			

DF, degrees of freedom; SS, sum of squares; MS, mean square error; *P*, significance probability

Table 7
Effect of propylene glycol and propylene glycolic solutions of PCA, sodium lactate and urea on transepidermal water loss (g/m^2 per h)

Replicate	PCA	Sodium lactate	Urea	PG
1	134.46	132.49	93.85	150.45
2	128.1	132.64	95.69	151.9
3	129.00	125.43	97.32	149.09

PCA = 5 μl of 5% w/w PCA in PG and 5 μl PG; sodium lactate = 5 μl of 10% w/w sodium lactate in PG and 5 μl PG; urea = 5 μl of 20% w/w urea in PG and 5 μl PG; PG = 10 μl PG.

Table 8
Statistical analysis of the steady-state TEWL values obtained after four different treatments (application of PCA, urea, sodium lactate or PG to posterior forearm)

Source	DF	SS	MS	F	P
Treatment	3	4674.63	1558.21	184.38	< 0.001
Error	8	67.61	8.45		
Total	11	4742.23			

readings (Morrison, 1992). The author suggests that ideally a period of 2–3 h elapses after product application before readings are taken to reduce this effect. In the present study, 1.5 h elapsed between application and the first measurement at which time the skin appeared and felt dry. Despite this, differences in TEWL with different applications were apparent and stable and serve to show the usefulness of factorial design used in our experiments.

3.2. Conclusions

Components of the NMF were chosen for investigation of potential additive effects using factorial design experiments since it has been suggested that such components combine to yield their moisturising effects (Jacobi, 1967; Clar and Fourtanier, 1981). Urea, sodium lactate and pyrrolidone carboxylic acid were selected for investigation because although their precise modes of action are not completely understood, they are known to act in different ways as discussed already. Propylene glycol and PCA have humectant and hydroscopic properties. Application of these substances will cause water to diffuse across the stratum corneum, not in response to a concentration gradient, but in response to the attractive forces of the applied substance. Urea and sodium lactate can break hydrogen and ionic bonds, respectively, and will therefore alter the characteristics of the stratum corneum. In fact, hydration of the stratum corneum causes it to swell which again alters the characteristics of the membrane through which water is diffusing. All these factors influence the very assumptions on which the theory of transport of water through the stratum corneum is based (Scheuplein and Blank, 1971).

The results serve to demonstrate the potential for factorial design experiments and provide a framework for future procedures involved with investigating interactions between moisturising substances.

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