Responsive corneosurfametry following \textit{in vivo} skin preconditioning

E. Uhoda, V. Goffin and G. E. Pierard
Department of Dermatopathology, University Hospital of Liège, Liège, Belgium

Skin is subjected to many environmental threats, some of which alter the structure and function of the stratum corneum. Among them, surfactants are recognized factors that may influence irritant contact dermatitis. The present study was conducted to compare the variations in skin capacitance and corneosurfametry (CSM) reactivity before and after skin exposure to repeated subclinical injuries by 2 hand dishwashing liquids. A forearm immersion test was performed on 30 healthy volunteers. 2 daily soak sessions were performed for 5 days. At inclusion and the day following the last soak session, skin capacitance was measured and cyanoacrylate skin-surface strippings were harvested. The latter specimens were used for the \textit{ex vivo} microwave CSM. Both types of assessments clearly differentiated the 2 hand dishwashing liquids. The forearm immersion test allowed the discriminant sensitivity of CSM to increase. Intact skin capacitance did not predict CSM data. By contrast, a significant correlation was found between the post-test conductance and the corresponding CSM data. In conclusion, a forearm immersion test under realistic conditions can discriminate the irritation potential between surfactant-based products by measuring skin conductance and performing CSM. \textit{In vivo} skin preconditioning by surfactants increases CSM sensitivity to the same surfactants.

\textbf{Key words:} corneosurfametry; forearm immersion test; irritant contact dermatitis; surfactant.

A number of studies on repeated irritant exposures used occlusive patch tests (7, 8), but this procedure does not exactly reproduce the consequences of usual occupational or domestic duties. Exaggerated hand and forearm immersion tests have been used for testing dishwashing liquids (9). However, a non-exaggerated method might be better suited and likely represents a more relevant procedure (10).

Irritant contact dermatitis is a clinical response of the skin to a variety of external chemical and physical threats. Subjects can respond differently when exposed to the same irritant under different conditions. This is particularly true for surfactant-containing products (1). The cumulative effect of subliminal alterations of the skin has already been stressed among the many factors responsible for such variable clinical expression of irritancy (2–6). In daily life, cumulative irritant contact dermatitis occurs when exposures to irritant are too frequent in relation to the skin recovery time.

A series of \textit{in vitro} tests have been designed to predict the irritation potential of surfactants (11, 12). The search for simple and reproducible predictive tests led to the development of the corneosurfametry (CSM) bioassay performed on human stratum corneum (SC) (11, 13–17). Microwave CSM (18, 19) is a variant of the ancillary CSM. CSM data yielded on normal SC were shown to be different from those gained from subjects sensitive to surfactants or suffering from atopic xerosis (15–17).

The aim of the present study was to correlate the individual proclivity to develop surfactant-induced decrease in skin capacitance with data yielded by microwave CSM.

\textbf{Materials and Methods}

The study was conducted during winter on healthy, fully informed and consented adult volunteers according to the principles of good clinical practice.
At inclusion, series of 3 cyanoacrylate skin-surface strippings (CSSSs) were harvested from the volar aspect of the forearms in each of 30 phototype II and III adult healthy panellists. None of the volunteers suffered from atopy or dry skin. These samples were stored for further laboratory assessments testing water and products A and B. 10 days later, the same panellists entered a forearm immersion test comparing 2 surfactant-based test products. These products were hand dishwashing liquids at a surfactant concentration of 42–45%. Products A and B were mainly composed of anionic and non-ionic surfactants, respectively.

For 5 days prior to the test and for the test period, panellists were asked not to apply any topical product to their forearms. The dishwashing liquids A and B were diluted at 0.2% (v/v) in 2 l of tap water at 40˚C. Temperature at the end of a soak session ranged from 35 to 37˚C. The randomized assignment of a given product to a particular forearm was kept constant for the duration of the test. Panellists soaked their forearms in solutions of products A and B ×2 daily for 5 days. There was a period of 5 h between the 2 daily soak sessions. When soaking, subjects followed successive cycles with the forearms in the solutions for 20 s and out of the solutions for 30 s (12 full cycles over each 10-min soak session). At the end of each session, the forearms were rinsed under running tap water and patted dry without rubbing.

Capacitance measurements of the skin-surface hydration/dehydration were performed using the Corneometer® CM 820 (C+K Electronics, Cologne, Germany) following the recommendations made by the EEMCO group (20). Measurements were taken before the 1st soak session (D1) and on the morning following the last soak session (D6), after the panellists had waited for 30 min, at rest, in a room at 21 ± 1°C and 47 ± 2% relative humidity. At each evaluation time, 3 replicate measurements of skin capacitance were performed and averaged on each forearm.

In each panellist, a second series of 2 CSSSs was harvested from each forearm after measuring skin capacitance at D6. Samples from each forearm served to test water and the dishwashing liquid corresponding to the one used in the preconditioning procedure for that forearm. CSSSs collected on non-exposed skin at inclusion and on surfactant-preconditioned skin at D6 were used for the microwave CSM bioassay, as previously described (18, 19). In short, 1 CSSS from each panellist at both collection times was immersed in a plastic flask containing one of the tested hand dishwashing liquids, or water as negative control. After placing in a microwave oven (Philips M642 sensor) with a 500-ml water load, microwave CSM was run at 750 W for 30 s. Samples were then thoroughly rinsed in tap water, air dried and stained for 3 min using a toluidine blue-basic fuschin solution in 30% ethanol. Reflectance colourimetry (Chroma Meter CR200 Minolta, Osaka, Japan) was performed after placing the samples on a white reference plate. The L* and Chroma C* values were recorded to derive the colourimetric index of mildness (CIM), which - corresponds to the difference between the L* and Chroma C* values. A previous study indicated that 750 W microwaving for 30 s yielded CIM values ranging from below 0 for harsh irritant products to about 70 for water (18).

The mean (M) and SD of CIM and skin capacitance values were calculated for each test product at both evaluation times. Coefficients of variation (CV = 10^2 SD/M) were also calculated. The statistical analysis was performed on a Hewlett Packard 48 G microprocessor. Comparisons were made using the two-tailed paired Student’s t-test. A correlation between data yielded at inclusion and at completion of the in vivo tests was searched for by the Spearman’s regression model analysis. The best relationship, i.e. linear, logarithmic, exponential or power, was chosen on the basis of the highest coefficient of correlation r. A P-value of 0.05 was taken as the level of significance.

**Results**

At entry to the study, skin capacitances were similar on the forearms randomized for testing the 2 hand dishwashing liquids (Fig. 1). Theirdishwashing liquids, or water as negative control. After placing in a microwave oven (Philips M642 sensor) with a 500-ml water load, microwave CSM was run at 750 W for 30 s. Samples were then thoroughly rinsed in tap water, air dried and stained for 3 min using a toluidine blue-basic fuschin solution in 30% ethanol. Reflectance colourimetry (Chroma Meter CR200 Minolta, Osaka, Japan) was performed after placing the samples on a white reference plate. The L* and Chroma C* values were recorded to derive the colourimetric index of mildness (CIM), which - corresponds to the difference between the L* and Chroma C* values. A previous study indicated that 750 W microwaving for 30 s yielded CIM values ranging from below 0 for harsh irritant products to about 70 for water (18).

The mean (M) and SD of CIM and skin capacitance values were calculated for each test product at both evaluation times. Coefficients of variation (CV = 10^2 SD/M) were also calculated. The statistical analysis was performed on a Hewlett Packard 48 G microprocessor. Comparisons were made using the two-tailed paired Student’s t-test. A correlation between data yielded at inclusion and at completion of the in vivo tests was searched for by the Spearman’s regression model analysis. The best relationship, i.e. linear, logarithmic, exponential or power, was chosen on the basis of the highest coefficient of correlation r. A P-value of 0.05 was taken as the level of significance.

![Fig. 1. Mean and SD of skin capacitance expressed in arbitrary units (AU) before and after 10 soak sessions using anionic (A) and non-ionic (B) hand dishwashing liquids. Skin capacitance decreased significantly (P < 0.01) after 10 soak sessions with each of the products.](image-url)
CVs were lower than 3%. At completion of the 5-day forearm immersion test, the skin showed a faint erythema in 43.3% (13/30) of the forearms soaked with product A, and in 23.3% (7/30) of the sites soaked with product B. A discrete skin dryness was focally present on 6.6% (2/30) of the forearms in contact with product A. Similar changes were not seen on the other forearm. Skin capacitance was significantly decreased ($P < 0.001$) at the sites that had been in contact with products A and B (Fig. 1). CV was higher for product A (6.9%) than that for product B (3.5%). In addition, skin capacitance was significantly more reduced ($P < 0.001$) for product A than that for product B.

The modest positive linear correlation between skin capacitances at both test sites at inclusion ($r = 0.67$) was lost at completion of the forearm immersion test ($r = 0.49$). No correlation was found between the capacitance values yielded at entry and at completion of the soaking sessions for both products A ($r = 0.16$) and B ($r = 0.10$).

At inclusion, control CSM performed with water showed that CIM values (68.4 ± 1.3) were in the normal range for intact SC. Water CIM dropped significantly after in vivo SC preconditioning with products A (33.8 ± 9.6, $P < 0.001$) and B (46.2 ± 7.5, $P < 0.001$). CSM performed on intact SC at entry to the study showed that CIM yielded for the dishwashing liquid A was significantly lower ($P < 0.001$) than that for product B (Fig. 2). Both of these dishwashing CIM values were significantly lower ($P < 0.001$) than that of the water. The differences between the water CIM and both dishwashing CIM were increased when CSM was performed on the compromised SC at completion of the 5-day forearm immersion test (Fig. 2). The CIM reductions after in vivo skin challenge were statistically significant ($P < 0.001$) for each of the products. CV of CIM data at inclusion reached 10% for product A and 7.4% for product B. They increased considerably after the soaking sessions, reaching 90.7 and 76.6% for products A and B, respectively.

No correlations were found for interproduct CIM comparisons at both evaluation times. The $r$-values for evaluation times D0 and D6 reached 0.23 and 0.26, respectively. No correlations were found either between CIM values gained before and after the 5-day forearm immersion test using products A ($r = 0.23$) and B ($r = 0.25$).

At entry to the study, no relationships were found between skin capacitances and CIM for both products A ($r = 0.30$) and B ($r = 0.42$) (Fig. 3a,b). By contrast, significant linear correlations were found between skin capacitances and CIM for both products A ($r = 0.88$) and B ($r = 0.86$) after completing the 5-day forearm immersion test (Fig. 4a,b).

![Fig. 2. Corneosurfametry bioassay. Mean and SD of the colourimetric index of mildness (CIM) before and after 10 soak sessions using anionic (A) and non-ionic (B) hand dishwashing liquids. CIM values decreased significantly ($P < 0.01$) after skin preconditioning by 10 soak sessions with each product.](image)

![Fig. 3. Scatterplot between skin capacitance and values of colourimetric index of mildness (CIM) before in vivo soak sessions with hand dishwashing liquids. (a) Anionic product A, $r = 0.23$; (b) non-ionic product B, $r = 0.25$.](image)
One of the primary concerns in designing new hand dishwashing liquids should be the selection of those agents which will perform effectively and have no or minimal undesirable effects on the skin. Many methods and test designs for the estimation of potential irritancy by surfactants have been described over the past decades. Despite much progress, no single-test method correlates precisely with the variety of events which occur during interactions between skin and surfactants. Some hand dishwashing liquids may be responsible for skin alterations ranging from discrete dryness to more severe irritant contact dermatitis (10). The effect of temperature on the skin and surfactant interaction is important to consider, particularly for these products (9, 21). The present laboratory-controlled test was performed in a non-exaggerated design representing realistic conditions with regard to daily life. The SC alterations were assessed on a body site relatively protected from environmental threats compared to the SC of the hands. As a result, the forearm SC is better representative of an intact structure than the hand SC (22).

The hydration state of the SC largely determines its physical properties, including softness, smoothness and flexibility. After surfactant damage to the SC, the skin surface becomes harsh, rough and scaly. This is invariably accompanied by a decrease in water content of the SC. Measuring electrical capacitance is an indirect method for assessing variation in SC moisture. Such a sensitive method allows the disclosure of SC alterations well before skin chapping becomes readily visible (10, 20, 23–25). For both test products, the capacitance level found in this study after 10 soak sessions was decreased from baseline but was still higher than that reported after 8 soak sessions performed on the hands (10). This stresses the intrinsic regional variability in SC reactivity (22) and perhaps the influence of the inevitably compromised status of the hand SC by daily life.

The ancillary CSM and its variant microwave CSM are predictive bioassays that have shown their relevance in various experiment studies (11, 13–19, 21,22). These are inexpensive and rapid procedures allowing simultaneous comparisons of many products or concentrations, up to 2 dozen, in a couple of hours. Neat products can be tested without any toxic or irritant risk for the panellists. These characteristics are helpful in comparative screening programs using products of unknown or proven irritancy potential. In such instances, it is ethically wise to use CSM or any other *in vitro* test before planning a hazardous *in vivo* test.

The more aggressive potential of the anionic based product A compared to the non-ionic-based product B was previously shown in an earlier hand immersion test (10). Basically, the reduction in skin surface capacitance was paralleled by a drop in CIM values. A significant correlation was found between these *in vivo* and *ex vivo* assessments at the end of the 10 soak sessions. This finding indicates that skin capacitance may be checked to select CSSS donors for a CSM study. Indeed, in the present study, infraclinical or barely visible irritation considerably influenced the CSM data (not shown). The CSM information gained from intact SC indicates only the irritation potential of the tested products. By contrast, the same bioassay performed on compromised SC is a function of the interindividual variability in the skin susceptibility to a given surfactant and the irritation potential of the tested product. It allows the screening of individuals who might suffer from irritant contact dermatitis after repeated subclinical threats. It is noteworthy that interindividual susceptibility to surfactants was shown by increasing values in CV
which mirrored the drop in CIM values. This situation probably reflects the lay concept of sensitive skin to surfactants expressed by consumers (15, 26).

In short, the present findings indicate that compromising SC in vivo increases the CSM ability to discriminate the irritation risk among different surfactant-based products. Thus, it should be stressed that CSM data can only be interpreted when the SC status is controlled before CSSS sampling. This study using microwave CSM clearly confirms the deleterious effect of repetitive discrete surfactant threats to the SC at a rhythm exceeding the natural repair mechanisms. The presently described responsive CSM design could also be used to document any benefit from specific surfactant combinations (8) and any protective effect of so-called barrier creams.

References

Address:
Prof. G. E. Piérard, MD, PhD
Department of Dermatopathology
CHU Sart Tilman
B-4000 Liège
Belgium
Tel: +32 4 3662408
Fax: +32 4 3662976
e-mail: gerald.pierard@ulg.ac.be