

An open assay model to induce subclinical non-erythematous irritation

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To avoid the acute irritation and dryness that a single occlusive surfactant application (24-h patch test) may cause, and to approximate clinical use, an open application model was chosen to define subclinical non-erythematous irritation in the stratum corneum. This human test combined a supervised washing at the laboratory with at-home use of the test products by the subjects. Effects of washing with the surfactants on the dorsal hand and volar forearm were compared. The results demonstrated that this situational use model permits the exploration of subclinical surfactant-induced irritation. The forearm appeared to be more discriminative as compared to the dorsal hand. Squamometry proved to be a sensitive, complementary assessment method for detecting surfactant-induced subclinical skin surface alterations and for differentiating surfactant effects in this open assay.

Key words: sodium lauryl sulfate (SLS); sodium laureth sulfate (SLES); squamometry; irritancy; irritant contact dermatitis; non-erythematous irritation; repeated open application; use test; surfactants; visual assessment; bioengineering methods. © Munksgaard, 2000.

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Consumers are not typically exposed to surfactants under the occlusive application that is found in patch tests and that is frequently used to determine the irritation potential of surfactants (1, 2). Consumer contact with surfactants is repetitive, for brief periods, and via open application, such as through hand washing or personal cleansing. An open application test, using the exaggerated hand wash method, has permitted exploration of subclinical (non-erythematous) surfactant effects (3). To refine this model and to compare the effects of surfactants in the absence of any inflammatory reaction, 3 daily controlled washes at the laboratory were combined with typical volunteer use at home as the test model.

2 surfactants, sodium lauryl sulfate (SLS) and sodium laureth sulfate (SLES), were compared. Stratum corneum alterations were documented through clinical evaluations (visible scores of erythema and dryness), instrumental analysis (skin conductance and evaporimetry), and squamometry. These measurements were made on the dorsal hand and volar forearm. Volunteer comportment and consumption analysis was also conducted. Squamometry proved to be a sensitive assessment method for detecting

surfactant-induced, subclinical skin alterations and for differentiating between the surfactants as to their effect on the stratum corneum.

Materials and Methods

20 volunteers, aged 43 ± 10 years old (mean $\pm$ SD), provided informed consent. The University of California Committee on Human Research approved the study. None had a history of atopic dermatitis or overt skin disease. The study was conducted in April 1998. Each subject used each of the 2 solutions for 1 week with a 1-week rest between. A comparison of the baseline values for each of the measured parameters indicated no differences and that a sufficient recovery time for the skin had occurred after the 1st week's exposure. Product assignment was balanced within the 2 groups (10 volunteers each) during the 1st and the 3rd weeks using a crossover design. The 2nd week was a rest period, in which the panelists used only a standard, mild cleansing bar at home, which was provided to them.

The 2 surfactants were compared at the same concentration (5% w/v) in distilled water. The 2

surfactants were sodium lauryl sulfate (Stepan, Northfield, IL) and sodium laureth sulfate (Henkel, Hoboken, NJ).

Controlled wash procedure at the laboratory (weeks 1 and 3)

A technician supervised 3 successive washes by the panelists as follows. For each wash, the technician poured 4 ml (2 phases of 2 ml) of 1 surfactant solution into both palms of the panelist. The panelist rubbed his hands in a circular motion 10× and washed both his dorsal hands and volar forearms for 1 min with the lather. Hands and forearms were rinsed with tepid tap water for 15 s (water hardness: 58 µg/l CaCO₃), followed by a 2-min rest. This washing procedure was repeated 2×. After the last wash, the hands and forearms were gently dried with a paper towel without rubbing. The washing procedure was repeated daily during week 1 from day 0 to day 4 as well as on day 7 and during week 3 from day 14 to day 18 as well as on day 21.

Panelists used the same surfactant solution at home (weekend included). A diary was kept by the panelists that included the date, time, and the purpose of the surfactant use (hand washing, showering, or bathing).

Evaluation and measurements

Clinical evaluations of the non-dominant dorsal hand and volar forearm was performed for erythema and skin dryness on days 0 and 14 before the 1st controlled washings (baseline) of each week and on days 7 and 21, 1 h after the last controlled wash. The scoring scales were defined as follows:

Erythema

- 0 no redness
- 1 very slight redness
- 2 slight redness
- 3 moderate redness
- 4 strong redness
- 5 very strong redness
- 6 extreme redness.

Dryness

- 0 no dryness; well hydrated and completely smooth
- 1 very slight; dry without scaling, no whitening in the ridges
- 2 slight dryness; dry with possibly very slight powdering
- 3 moderate dryness; scaling is obvious, mainly on limited areas; scales are small

- 4 strong dryness; same as score of 3, but on a larger surface
- 5 very strong; scales are larger in size in some areas; small cracking can be seen; slightly rough surface
- 6 extreme dryness; large scales on large surface+cracking+roughness.

Before the 1st controlled washings on day 0 and day 14 and 1 h after the last controlled wash on days 7 and 21, instrumental measurements were successively conducted on the dorsal hand and volar forearm, on the non-dominant hand side. All measurements were performed after a rest period of 1 h at a room temperature of 20±2°C and relative humidity 40–55%.

Capacitance as a parameter for stratum corneum hydration was measured in triplicate with a capacitance meter (Corneometer CM 820 PC, Courage & Khazaka, Koln, Germany). The probe head, consisting of a condenser, was applied to the skin surface at constant pressure. The measuring principle is based on the distinctly different dielectric constant of water as compared to the dielectric constant of most other materials found in the stratum corneum (4).

Transepidermal water loss (TEWL) documents the integrity of stratum corneum water barrier function (5) and is a sensitive measure of surfactant-induced skin barrier alteration (6–9). It was measured with an evaporimeter (Tewameter TM 210, Courage & Khazaka, Koln, Germany). The measuring principle and guidelines have been described in detail elsewhere (5, 10).

Erythema was quantified by skin color reflectance measurements using a tristimulus colorimeter (Chroma Meter CR 300, Minolta, Osaka, Japan) using the published guidelines (11). In the $L^*a^*b^*$ colorspace, a^* represents the color spectrum from total green to pure red and is known to correlate closely with erythema (12, 13).

Finally, 1 stratum corneum stripping using tape discs (D-squame, Cuderm Corporation, Dallas, TX, USA) was collected from the dorsal hand and volar forearm. 1 additional stripping was collected from these same sites on days 7 and 21 before the last washings at the laboratory.

6 randomized sites were sampled and evaluated on the dorsal hand and volar forearm: 1 for each of the measurement times.

Squamometric analysis

The tape discs were stained for 30 s with 1 drop of a solution of toluidine blue and basic fushsin, polychrome multiple stain (PMS, Delasco, Council Bluffs, IA, USA). The tape discs are gently rinsed with tap water and allowed to air dry. The tape

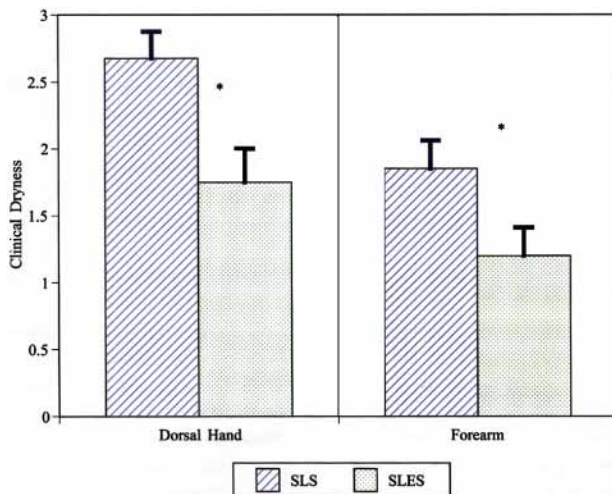


Fig. 1. Increase in clinical dryness after 1 week of surfactant use. The use of SLS generated more skin dryness than the use of SLES on both the hands and the forearms. * The 2 surfactants are significantly different at $p \leq 0.05$.

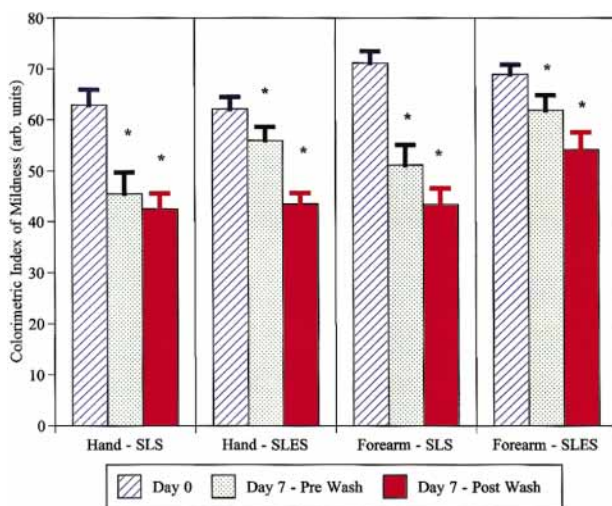


Fig. 2. Colorimetric index of mildness (CIM) results are presented as a function of evaluation. Both SLS and SLES caused CIM to decrease with usage on both the hands and the forearms. * The 2 surfactants are significantly different at $p \leq 0.05$.

discs are attached onto a glass slide. The glass slide is placed onto a white reference plate, and the color of each tape disc is determined by colorimetry using the Minolta Chroma Meter. Calculation of the Chroma C^* values were done after $(a^2 + b^2)^{1/2}$ using the measured values. This parameter combines the values of the red and blue chromaticities, which are the predominant colors of PMS (14–16). The calculation of the Colorimetric Index of Mildness (CIM) was performed by subtracting the Chroma C^* value from the luminance value, L^* (17). Finally, a trained person

scored the discs with a microscope at $20\times$ magnification according to the following scales (18):

Interorneocyte cohesion

- 0 large sheet
- 1 large clusters+few isolated cells
- 2 small clusters+many isolated cells
- 3 clusters in disruption, most cells isolated
- 4 all cells isolated, many cases of lysis.

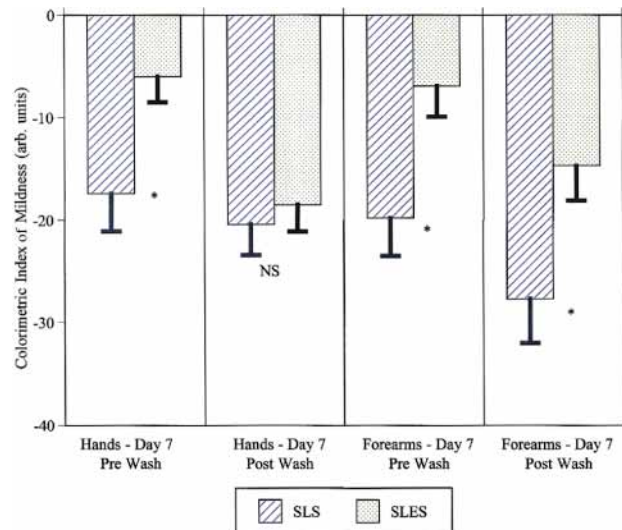


Fig. 3. Change in the colorimetric index of mildness (CIM) values. SLS causes more of an effect on the CIM values before the day-7 washings for the dorsal hands and both before and after the day-7 washings for the volar forearms. * The 2 surfactants are significantly different at $p \leq 0.05$.

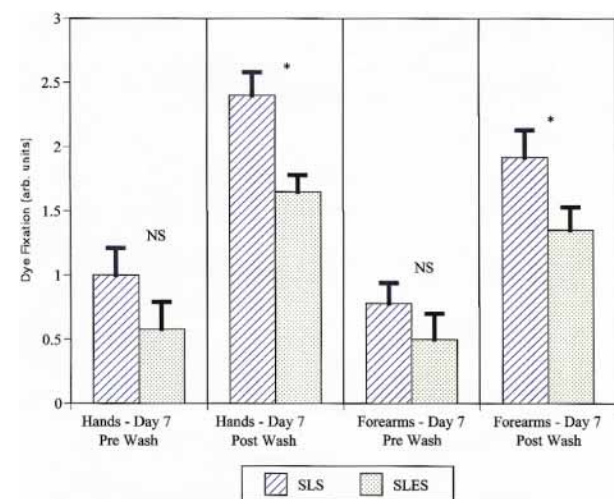


Fig. 4. The damaging effect of surfactants is shown by the amount of dye that is fixed to the cells. The more damage that occurs, the more dye that is fixed to the cells. Note that the only significant difference between the 2 surfactants occurs after the day-7 washings on both hand and forearm. * The 2 surfactants are significantly different at $p \leq 0.05$.

Amount and distribution of dye found in cells

- 0 no staining
- 1 staining between cells or slight staining in cells
- 2 moderate staining in cells
- 3 large amount of dye in cells, but uniform
- 4 important staining in all cells, often with grains.

Statistical analysis

Paired *t*-tests (confidence level=95%) were conducted on the differences calculated between the before and after treatment data to determine if there were differences between the effects of the 2 surfactants. The analyses were conducted separately for the dorsal hand and the volar forearm.

Results

Instrumental and clinical

Instrumental evaluations of skin capacitance and transepidermal water loss did not reveal a significant difference between the skin sites exposed to the 2 test products. Clinically, 5% SLS generated statistically ($p \leq 0.05$) more skin dryness (Fig. 1) than did 5% SLES after 7 days of testing on both the dorsal hand and the forearm. Erythema was not generated by either product at either site.

Squamometry

There was a significant difference ($p \leq 0.05$) in the effect of the 2 surfactants on the skin as measured by squamometry on the tape strippings, with the SLS solution causing more damage than the SLES solution. Both treatments caused chroma C^* values to increase with usage (data not shown). SLS caused a significantly larger increase in the C^* values than SLES on the forearm before the 3 washings on day 7. 1 h after these washings, the difference disappeared, however, SLS still caused a trend ($p \leq 0.10$) toward a larger increase in C^* values than did SLES. No significant differences were detected between the effects of SLS and SLES on C^* values on the tape strippings taken from the dorsal hands.

Both treatments caused the colorimetric index of mildness (CIM) values to significantly decrease with usage (Fig. 2). 1 h after the last day-7 wash, SLS caused a significantly larger decrease ($p \leq 0.05$) in the CIM values than did SLES on the volar forearms. There were no differences observed on the dorsal hands (Fig. 3). There were also significant differences ($p \leq 0.05$) between the effects of the 2 surfactants on the CIM data before the washings on day 7 for both the dorsal hand and the volar forearm (Fig. 3).

There were no significant differences in the effects of the surfactants on cell cohesion or dye fixation before the washings on day 7 for both the dorsal hand and the volar forearm. Cohesion assessment analysis did show a significant difference on the dorsal hand 1 h after the last day-7 wash (data not shown). Dye fixation data revealed significant differences ($p \leq 0.05$) between the 2 surfactants on both the dorsal hand and the volar forearms 1 h after the last washings on day 7 (Fig. 4).

Panelists used the same amount of SLS and SLES during the course of the study.

Discussion

Bioengineering measurements are not always sufficiently sensitive to reveal significant differences between these 2 surfactants (Charbonnier et al., unpublished results). In the current study the clinical dryness assessments distinguished the skin sites washed with SLS from those washed with SLES. Once again (3), squamometry documented subclinical effects on the stratum corneum, e.g., non-erythematous irritation, that were induced upon washing with these 2 surfactants. Chroma C^* , CIM, and microscopic examination of cell cohesion and dye fixation were sufficiently sensitive to reveal differences between the effects of 5% SLS from those of 5% SLES.

Most results were observed 1 h after the day-7 washings, but in addition, statistical analysis of the CIM and the chroma C^* values revealed a significant difference between the effects of SLS and SLES before these day-7 washings, which was after the panelists used the products ad-lib over a weekend. This shows that squamometry is more sensitive than conventional methods, either visual or instrumental, in observing subclinical skin changes in an open application assay. Protected sites (like forearm) seemed to be more discriminating in this open assay. Even when the non-dominant side was chosen to control the left-hand/right-hand variability, the hands, especially the dorsum, are more exposed to external stresses, which probably enhances the effect of the surfactants.

Conclusion

Our goal was to observe skin surface modifications induced by an open test with regard to discriminating between 2 surfactant solutions on the volar forearm and the dorsal hand. Squamometry is a sensitive method for the detection of the interactions of surfactants with the stratum corneum and a complementary method to conventional clinical evaluation, skin color, TEWL, and hydration measurements, particularly when subclinical alter-

ations are to be detected. It was capable of distinguishing between the effects of the 2 surfactants on the stratum corneum. In fact, the volar forearm was a more discriminating site than the dorsal hand. Compared to an exaggerated hand washing procedure (3), this open assay, especially on the forearm, may better approximate consumer surfactant use. Thus, squamometry differentiates the irritancy of surfactants at low concentrations (3) during very short exposures (19), in controlled-laboratory conditions (20), and now in an open use assay.

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