

# Influence of temperature on irritation in the hand/forearm immersion test

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As indicated by *in vitro* experiments the penetration of irritants through the skin is significantly influenced by the temperature of the solution. *In vivo* experiments, demonstrated equally a significant influence of temperature in surfactant-induced skin irritation. In order to evaluate the irritant potential of detergent solutions under normal user conditions, we used the hand/forearm immersion test. We compared 2 detergents with different anionic character in a repetitive immersion protocol (30 min immersion on 4 consecutive days). The solutions were tested at 2 temperatures (37°C and 40°C). The irritation was quantified by assessment of the stratum corneum barrier function (transepidermal water loss), skin redness ( $a^*$  colour parameter) and skin dryness (capacitance method). Both detergents affected the integrity of the skin in a significant way. The anionic content as well as the temperature of the solutions were found to be determinative for the irritant potential, with a stronger response for higher anionic content and temperature, respectively.

**Key words:** detergents; temperature; irritation; immersion test; bioengineering methods; transepidermal water loss; colorimetry; capacitance. © Munksgaard, 1997.

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The hand/forearm immersion test is a laboratory technique very similar to normal user conditions of dishwashing detergents. The experimental conditions must be well-standardized, since the development of irritation is influenced by several factors, such as duration of immersion, concentration of the products, number of repetitions, etc.

As indicated by the *in vitro* results of Emilson et al. (1), the penetration of irritants through the skin is significantly influenced by the temperature of the solution. *In vivo* experiments indicate equally increased penetration as a function of increased temperature (2), resulting in increased irritation of the applied irritant (3-5). The latter was demonstrated by the results of Berardesca et al. (4) using repeated open applications of sodium lauryl sulfate (SLS) solutions at different temperatures (4°, 20° and 40°C), and by the results of Øhlenschläger et al. (5) using repeated immersions in an SLS solution at 20° and 40°C.

More recently developed detergents (non-ionic) seem to be milder compared to the classical ionic detergents containing lauryl sulfate (6). In our experiment, we compared a moderate anionic detergent (a sodium laureth sulfate-1,2 ethoxylate) with

a milder detergent where half of the anionic molecules were replaced by non-ionic molecules (lauryl glucoside). The solutions were tested at temperatures very close to the temperatures used under domestic conditions (37° and 40°C). Irritation was assessed by means of noninvasive biophysical measurements (7).

## Materials and Methods

### Subjects

2 groups of 6 healthy volunteers (male and female aged between 18 and 26 years) were selected for the study. The subjects were informed concerning the study procedure and gave their written consent. One group participated in the evaluation of the detergents at 37°C, and the other at 40°C.

### Products

2 dishwashing detergent solutions were compared. 1 product (product 1) contained solely an anionic surfactant (sodium laureth sulfate with 1-2 ethoxylations). The 2nd product (product 2) contained a mixture of sodium laureth sulfate (SLES) with 1-2 ethoxylations and a nonionic surfactant

(lauryl glucoside). Both detergents were of the highest technical purity. 1% solutions of the products were made with tap water and heated to 37°C or 40°C by a heater/stirrer in plastic tanks.

### Immersion method

The subjects placed one hand and forearm in the tank containing the anionic detergent while the other hand and forearm were placed in the tank containing the combined surfactants according to a randomized model. The study was conducted under double-blind conditions, since products were coded for the volunteers and the experimenter. The hand/forearm was placed in the tank for 30 min. When immersion time had expired, the arm was adequately rinsed with tap water and dried by patting with soft towels. The same immersion procedure was repeated 4× over 4 consecutive days (24 h interval).

### Measurements

Measurements were performed before the 1st exposure (immersion 0), 24 h after the 2nd exposure and 24 h after the 4th exposure (immersions 2 and 4, respectively). Skin irritation was assessed as follows.

(i) Transepidermal water loss (TEWL). The measurements were performed using the Tewameter TM210 (Courage & Khazaka, Koln, Germany) according to the guidelines of Pinnagoda et al. (8).

(ii) Skin colour. Skin colour was evaluated by means of the Chromameter CR200 in the  $L^* a^* b^*$  measuring mode (Minolta Camera Co., Ltd., Osaka, Japan). The  $a^*$  colour parameter was selected as a sensible criterion for detection of skin redness (9).

(iii) Water content. The water content of the stratum corneum was assessed using the capacitance technique (Corneometer CM820, Courage & Khazaka, Koln, Germany), as proposed by Barel and Clarys (10).

Measurements were carried out under controlled

environmental conditions (temperature  $20^\circ\text{C} \pm 2^\circ\text{C}$ , relative humidity  $45\% \pm 5\%$ ). All volunteers underwent an acclimatization period of 30 min before every measurement. All measurements were performed on the dorsal side of the hand and on the volar aspect of the forearm (at the midpoint between wrist and elbow).

Effects on the different parameters after the immersions were statistically evaluated using the Friedman test combined with a Wilcoxon test. The Mann-Whitney  $U$ -test was used for comparisons between the 2 populations. The differences in baseline values were taken into account by using baseline values adjusted to that particular test. A paired  $t$ -test was used for comparison of products within the same test group. The significance level was set at 5%.

### Results

Tables 1–3 detail the results of the immersion procedures carried out at 37 and 40°C, respectively. The differences in baseline TEWL and stratum corneum capacitance values found between the test groups remained within the reported range of inter-individual variability of these parameters (8, 9). Repetitive exposure of the skin to both detergents at 37°C provoked a significant increase in TEWL and only moderate or no alterations of skin colour ( $a^*$  parameter) and stratum corneum capacitance values (Friedman test). The same immersion protocol carried out at higher temperature (40°C) indicated a more pronounced skin response after exposure to the solution with the highest anionic content (Tables 1–3).

At both temperatures, no significant differences were detected between the 2 products at any measurement interval ( $t$ -test). However, due to the small sample size ( $n=6$ ), statistical type II errors may easily occur. TEWL seemed to be more sensitive compared to the  $a^*$  colour parameter and the stratum corneum capacitance measurements, in order to demonstrate the temperature effect (Mann-Whitney  $U$  test).

Table 1. TEWL values ( $\text{g/m}^2\text{h}$ ) measured before (im=0) and 24 h after the 2nd (im=2) and 4th (im=4) immersions

| im | Hand      |           |           |            | Forearm   |           |           |          |
|----|-----------|-----------|-----------|------------|-----------|-----------|-----------|----------|
|    | prod 1    |           | prod 2    |            | prod 1    |           | prod 2    |          |
|    | 37°C      | 40°C      | 37°C      | 40°C       | 37°C      | 40°C      | 37°C      | 40°C     |
| 0  | 14.8±5.5  | 18.4±4.5  | 14.1±3.5  | 18.6±3.4   | 8.9±3.7   | 13.6±4.6  | 8.3±4.0   | 12.8±5.6 |
| 2  | 17.6±5.2  | 24.2±5.1* | 14.5±4.2  | 26.2±11.3* | 11.8±4.6  | 18.3±6.6* | 11.4±4.5* | 21.7±11* |
| 4  | 20.9±4.9* | 28.0±7.0* | 17.8±4.6* | 34.1±11.9* | 13.9±4.8* | 23.2±7.8* | 14.0±5.7* | 28.8±11* |

\*Values differ significantly from values before immersion (Friedman Anova). A continuous line indicates a significant temperature effect for the evaluated product (Mann Whitney  $U$ -test, performed on baseline adjusted values), ( $n=6$ ).

Table 2.  $a^*$  colour values (arbitrary  $a^*$  units) measured before (im=0) and 24 h after the 2nd (im=2) and 4th (im=4) immersions

| im | Hand    |          |         |          | Forearm |          |         |          |
|----|---------|----------|---------|----------|---------|----------|---------|----------|
|    | prod 1  |          | prod 2  |          | prod 1  |          | prod 2  |          |
|    | 37°C    | 40°C     | 37°C    | 40°C     | 37°C    | 40°C     | 37°C    | 40°C     |
| 0  | 8.4±1.1 | 7.1±1.5  | 7.9±1.2 | 7.2±1.4  | 5.4±0.8 | 4.6±0.8  | 5.4±0.8 | 4.5±0.9  |
| 2  | 7.7±1.6 | 8.1±1.7  | 7.2±1.0 | 8.4±1.7* | 5.5±0.9 | 5.2±0.7  | 5.2±0.6 | 5.4±0.8* |
| 4  | 7.6±1.2 | 8.7±1.7* | 7.6±1.0 | 9.5±2.3* | 5.6±0.7 | 5.9±0.7* | 5.4±0.7 | 6.5±0.7* |

\*Values differ significantly from values before immersion (Friedman Anova). A continuous line indicates a significant temperature effect for the evaluated product (Mann Whitney *U*-test, performed on baseline adjusted values), ( $n=6$ ).

Table 3. Stratum corneum capacitance values (arbitrary capacitance units) measured before (im=0) and 24 h after the 2nd (im=2) and 4th (im=4) immersions

| im | Hand   |       |        |       | Forearm |       |        |        |
|----|--------|-------|--------|-------|---------|-------|--------|--------|
|    | prod 1 |       | prod 2 |       | prod 1  |       | prod 2 |        |
|    | 37°C   | 40°C  | 40°C   | 40°C  | 37°C    | 40°C  | 37°C   | 40°C   |
| 0  | 65±9   | 57±11 | 64±9   | 57±9  | 65±6    | 57±9  | 65±8   | 60±8   |
| 2  | 63±10  | 58±10 | 60±8   | 53±5  | 66±6    | 57±5  | 65±7   | 50±8*  |
| 4  | 63±7   | 50±6  | 60±6   | 42±9* | 67±7    | 56±10 | 60±6   | 52±12* |

\*Values differ significantly from values before immersion (Friedman Anova). A continuous line indicates a significant temperature effect for the evaluated product (Mann Whitney *U*-test, performed on baseline adjusted values), ( $n=6$ ).

### Discussion

The hand/forearm immersion protocol, by reproducing the in-use conditions of detergents in a more realistic way, seems to be a good method for the investigation of the irritation/mildness properties of detergents used daily. Our results indicate a temperature-dependent irritation effect which was equally product dependent. Moreover, the skin parameters used to quantify the irritation seem to be sensitive enough to discriminate between the irritation/mildness properties of 2 dishwashing products within 4 days. Our results are in complete agreement with the literature on bioengineering techniques for the objective quantification of irritation, as we found an increase in TEWL (11) and  $a^*$  parameter (9, 12) and a decrease in stratum corneum capacitance (10) characterizing the irritation.

The results obtained in our experiments point to the importance of temperature in irritation protocols. The influence of temperature has already been demonstrated by the *in vitro* experiments of Emilson et al. (1). These authors measured SLS penetration through excised skin at different temperatures. SLS penetration was found to increase as a function of temperature, which was explained by temperature-dependent disorganization of stratum corneum barrier function (1). These *in vitro* findings were confirmed by *in vivo* studies evaluating the irritancy of SLS solutions at different temperatures, in a repeated open application protocol at 4°, 20° and 40°C (4), and a repeated im-

mersion protocol at 20° and 40°C (5), respectively.

Our results were obtained with more complex domestic dishwashing products, instead of more standard SLS solutions. However, our present results corroborate the previous studies, since they also indicate stronger irritant responses at increased temperature (4–5). Moreover, our findings add some further insight into the shape of the irritant capacity curve as a function of temperature, as mentioned by Øhlenslæger et al. (5). They indicate the existence of a significant skewness to the left of this curve, with much stronger irritant potential at temperatures near 40°C. These findings may point to the importance of fluidity of stratum corneum lipids in the percutaneous penetration process, a hypothesis equally proposed by Berardesca et al. (4) as an explanation of increased irritancy at higher temperatures. According to Forslind (13), fluidization of stratum corneum lipids occurs from 37°C upwards. This is above the normal temperature of the skin, but still at a physiologically acceptable level.

The irritant properties of SLS are well-documented, since SLS is used as a standard model irritant in many experimental protocols (14). In order to obtain milder detergents, SLS is often replaced by less irritant anionic or non-ionic surfactants. The more recently developed non-ionic and amphoteric detergents are known to be milder compared to the SLS solutions used earlier (15, 16).

Equally, as demonstrated by our results, the replacement of SLES by non-ionic surfactants seems to result in milder products. We found weaker irri-

tancy for the dishwashing product containing a mixture of anionic and non-ionic surfactants, compared to the product containing solely the anionic surfactant. This is in complete agreement with the paper of Effendy & Maibach (15), reporting on the decreased irritancy of mixtures of non-ionic and anionic surfactants compared to anionic surfactants alone.

However, the findings of the present study demonstrate that even mild products may become irritant when repeatedly brought into contact with the skin at 40°C.

In conclusion, our results indicate that temperature is an important variable in the evaluation of the mildness/irritant potential of detergents. The hand/forearm immersion test seems to be a reliable method very close to the real situation of product use. Instrumental determination of skin parameters in irritation assessment permits objective discrimination between products tested in daily use. Both detergents, even at low concentrations (1%), affected the integrity of the skin after 4 consecutive immersions at 40°C. This was clearly demonstrated by the increase in TEWL, modification of skin colour indicating redness, and dehydration of the upper layers of the epidermis.

The irritation provoked in our experiments was product-dependent, with a stronger response as a function of concentration of anionic surfactants. The differences in mildness/irritation properties of the 2 test substances became clearer when using a repetitive immersion protocol with a solution temperature at 40°C, compared to a solution temperature of 37°C.

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