Milan, 28th April 2005

# ALLEVIATING AND REGENERATING EFFECT ON THE BARRIER AFTER IRRITATION CAUSED BY SODIUM LAURYL SULFATE AND UV-RAYS

Method: Ref. E12C + E14C + E13C

Orderer: ATLANTIC ITALIA srl
Piazza Piola, 4
20133 Milan

Product: MELEM
Ref. ISPE 77/05/01-87/05

Date of the beginning of trial: 15.4.2005.

Date of completion: 26.4.2005.

## ETHIC AND QUALITY CRITERIA

This study was conducted in accordance with the methods of quality management system according to general principles of correct laboratory practice (GLP) and correct clinical practice (GCP) and according to the principles determined based on the Declaration of the World healthcare organisation from Helsinki.

## QUOTATIONS

Information stated in this report refer exclusively to the tested product.
This report can only be reproduced entirely.

ISPE S.r.l.
Direttore del Laboratorio
Dott. Luigi Rigano

ISPE S.r.l.
Laboratory director
Dr. Luigi Rigano
(own signature)

CONTENT

## 1. SAMPLE SHEET

REF. SAMPLES MELEM
Ref. ISPE 77/05/01-87/05

Date of sample reception: 7th March 2005

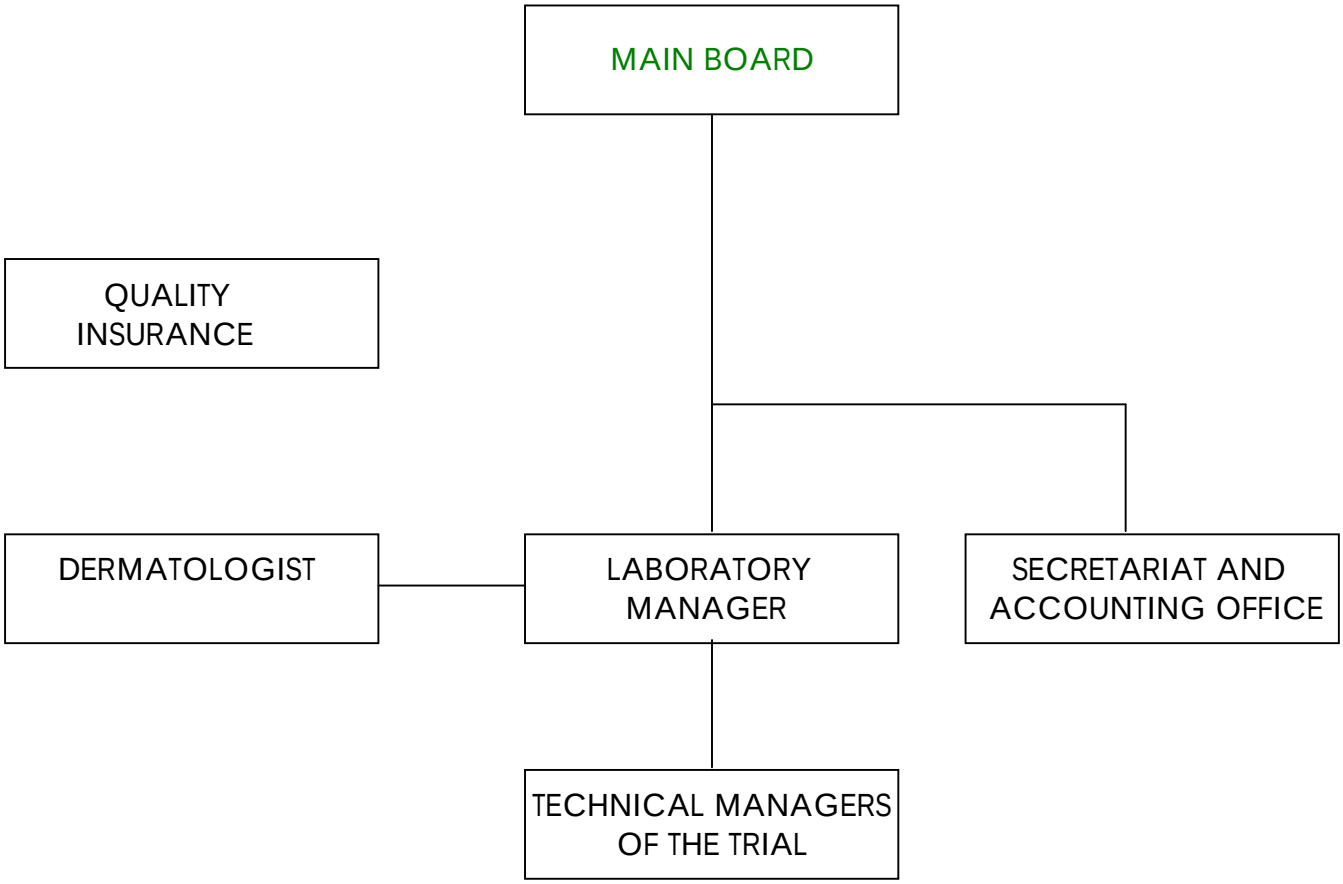
PRODUCT: - PHYSICAL FROM: - rigid substance
- COLOUR: - pale yellow

FORMULA: - KNOWN /X/
- OTHER INFORMATION / /

OTHER INFORMATION REGARDING THE SAFETY/EFFICACY OF THE PRODUCT:
none

ARCHIVE: 1 counter-sample with the quotation ref. ISPE 77/05/01-87/05 and documentation regarding this study are stored for 3, i.e. 10 years in our archives. After this period they are removed, unless the party requests otherwise.

## ORGANOGRAM


MAIN BOARD
QUALITY INSURANCE
DERMATOLOGIST
LABORATORY MANAGER
SECRETARIAT AND ACCOUNTING OFFICE
TECHNICAL MANAGERS OF THE TRIAL

ALLEVIATING AND REGENERATING EFFECT ON THE BARRIER AFTER IRRITATION CAUSED BY SODIUM LAURYL SULFATE AND UV RAYS (Ref. E12C + E14C + E13C)

## 3. METHOD PRINCIPLE

The scope of trial is to assess the alleviating and regenerating effect on the protective skin layer of a cosmetic product on skin irritated by sodium lauryl sulphate (SLS) and ultraviolet rays for the experiment in comparison with untreated segment.

The trial is conducted on volar forearm of 12 subjects on 4 randomised segments:

- on the first area SLS, and then the concerned product are applied;
- on the second area SLS is applied without the concerned product (control area);
- the third area is exposed to UV rays, and then the concerned product is applied;
- the fourth area is exposed to UV rays without the application of concerned products (control area);

Measurements with instruments for skin colorimetry and transepidermal loss of water are conducted on two segments treated with SLS according to the following drawing from the study:

- basic measurement ($T_0$);
- 24 hours after the application of SLS ($T_{1sls}$) for measuring damage due to caused irritation;
- treating with concerned product of one segment during 3 consecutive days;
- measuring after 48, 72 and 96 hours from the application of SLS ($T_{2sls}$, $T_{3sls}$, $T_{4sls}$) for assessing of alleviating and regenerating effect of the concerned product in comparison with irritating damage caused by SLS.

On two segments exposed to UV rays instrumental measurements of skin colorimetry are conducted:

- basic measurement ($T_0$);
- 24 hours after exposure to UV rays ($T_{1UV}$) for measuring damage due to caused irritation;
- treating with concerned product of one segment during 3 consecutive days;
- measuring after 48, 72 and 96 hours since exposure to UV rays ($T_{2UV}$, $T_{3UV}$, $T_{4UV}$) for assessing alleviating and regenerating effect of the concerned product in comparison with irritating damage caused by exposure to rays.

## 4. SUBJECT SELECTION

### 4.a. Selection and inclusion criteria

On the beginning of trial each subject read and signed the consent form composed by the investigators. 12 volunteers of both genders were involved (average maternal age). Selection of subjects was conducted according to the criteria for inclusion and exclusion which are stated further in the text.

### 4.b. Selection criteria

Race: Caucasian
Age: adults between the ages of 18 and 55
Gender: men and women
Health condition: no pathologies in the period immediately before or during the trial
Understanding the Italian language
Possibility of contact on the residing address
Phototypes I, II and III (according to Fitzpatrick classification)

### 4.c. Non-acceptance criteria

- Subjects who do not fulfil the criteria stated in 4.b.
- Subjects who are treated with topic or systematic therapy with any medication which could affect the outcome of trial.
- Pregnant and nursing women.
- Subjects who have some kind of skin disease.
- Subjects who have already shown intolerance to medications and/or cosmetic products.
- Subjects who have recently been exposed to intensive doses of UVA + UVB radiation.

### 4.d. Drop-out (termination)

Reasons for termination of trial can be:
- such independent decision of the subject
- reasons not connected with analysis (e.g. appearance of disease, chirurgic treatments, etc.)
- reasons connected with the analysis (e.g. the appearance of irritating reactions, allergies, etc.)

Instances of termination which happen during trial have to be stated.

### 4.e. Limitations

During the entire trial period subjects are forbidden to use cosmetic products on which are not subject of this trial on tested area (crèmes, lotions, etc.) and longer exposure to UVA and UVB rays.

# 5. EQUIPMENT

## 5.a. CROMAMETER CR300

It is a portable colorimeter on the basis of reflection with a double canal, an inbuilt micro calculator, an indicator based on liquid crystals and source of light based on xenon in the measuring head. Measuring area is of 8 mm diameter.
As a sample for calibration one white board is used (supplier Minolta). The system of numbering colours which is used to read the L*a*b* system where:
L* equals colour brightness
a* and b* show bicoloured axes, a* shows red – green axis, and b* yellow – blue.

In this trial, parameter a* was taken into consideration as the index of skin redness.

## 5.b. TEWAMETER TM210

Evaporimeter (TEWAMETER TM 210 Courage & Khazaka) measures water steam which is released by a certain surface, based on the Fick's law of diffusion:

$$dm / dt = + D * A * do / dl$$

where: dm /dt = diffusion stream
A = coefficient of distribution water/skin
do = change of concentration
dl = covered area
D = coefficient of water diffusion in the air

Diffusion stream dm/dt brings the amount of water steam (g/m2) which is transferred in the unit of time (h), diffusion stream do/dl determines the amount of water steam which passes vertically through the skin surface, and refers to the changes on the covered area.
The evaporimeter has a cylindrical probe which contains five sensors. The moist which evaporates from skin surface passes through the cylindrical part of the probe.
Saturation gradient which forms is indirectly measured by a few sensors (temperature and relative moisture), which is analysed by one microprocessor. TEWL values are expressed in g/h m2.

### 5.c. UV solar multiport simulator model 601

The instrument which is used as a simulator of sun rays (solar multiport simulator model 601) consists of a base with the possibility of regulation to which a system of 6 independent lights is attached, which guarantee a monotonous and constant distribution of radiation energy. The xenon light emits a continued spectrum from 290 to 400 nm.
The source of light is set in direct contact with the skin of the subject.

## 6. METHODOLOGY

### 6.a. The way of conducting the trial

The trial was conducted in an air-conditioned chamber (24ºC; 50% rh). Volunteers were asked not to rub or wipe forearms 3 hours before the trial beginning.

On the volar forearm of chosen subjects 4 skin areas are marked off:

- the first area will be treated with SLS, and after that with the concerned product
- the second area will be treated with SLS, but not with the concerned product (control area)
- the third area will be exposed to UV rays, and then treated with the concerned product
- the fourth area will be exposed to UV rays (but not treated with the concerned product, control area).

Chosen areas were randomly selected between different subjects to avoid regional differences in skin reactions.

After basic measurement of transepidermal values water loss skin colorimetry ($T_0$), on two of four selected segments (tested area and control area) the water solution of 2% sodium lauryl sulphate was oclusively placed. For this purpose Finn Chambers (Bracco) were used, aluminium cells of 8 mm diameter.

After 24 hours, patches which contain the SLS solution were removed and measurements with instruments were repeated ($T_{1sls}$) to measure the caused irritation damage.

After that, volunteers received the concerned product which they took home, on one of two described segments, they applied it twice a day for 3 consecutive days.

After 48, 72 and 96 hours from application of SLS ($T_{2sls}$, $T_{3sls}$, $T_{4sls}$) colorimetry measurements and control TEWL measurement were conducted for the assessment of alleviating and regenerating effect of concerned product according to damage caused by SLS irritation.

The remaining two segments (treated and control area), after basic measurements of skin colorimetry ($T_0$) ere exposed to a dose of UVA+UVB rays of 2MEDu (MEDu = minimal eritematogen dose on unprotected skin),
MED is determined based on the phototype to which the subject belongs.

After 24 hours from the exposure to UV rays on two areas, the measurements with instruments were repeated ($T_{1UV}$) to measure the damage caused by irritation.

In the case the product was also applied by subjects themselves at home, two times a day on two segments, during 3 consecutive days.

After 48, 72 and 96 hours from the exposure to radiation /$T_{2UV}$, $T_{3UV}$, $T_{4UV}$) control colorimetry measurements were conducted to assess the alleviating effect of the concerned product according to the damage caused by irritation from exposure to light.

## 6.b. Mathematic analysis

For both parameters which were followed on four skin zones (2 treated segments + two control segments) in different time readings, the mean value and standard deviation were calculated.

Information obtained by instruments in different time were statistically compared by Variance analysis and Tukey test.
The comparison between the groups of information was statistically significant for the value $p \leq 0,05$.

## 7. REZULTATS: TABLES AND DIAGRAMS

### 7.a. Model of irritation damage caused by sodium lauryl sulphate (SLS)

The tables show mean value, standard deviation and statistical comparison of values of transepidermal water loss and values of registered colorimetry according to two segments damaged by SLS in different time during the control.

Table 1: mean value and standard deviation, value of transepidermal water loss

| TEWL | $T_0$ | $T_{1sls}$ | $T_{2sls}$ | $T_{3sls}$ | $T_{4sls}$ |
|---|---|---|---|---|---|
| Melem | 8,41 ±1,90 | 43,67 ±10,36 | 27,61 ±12,57 | 22,03 ±8,36 | 14,82 ± 5,27 |
| Control | 8,76 ±1,77 | 40,09 ±13,08 | 35,10 ±14,45 | 27,73 ±12,58 | 23,92 ± 12,00 |

Table 2: statistical comparison between times (Variance analysis and Tukey test)

| TEWL | $T_0$ vs. $T_{1sls}$ | $T_0$ vs. $T_{2sls}$ | $T_0$ vs. $T_{3sls}$ | $T_0$ vs. $T_{4sls}$ |
|---|---|---|---|---|
| Melem | p< 0,001 | p< 0,001 | p< 0,001 | p> 0,05 (n.s.) |
| Control | p< 0,001 | p< 0,001 | p< 0,001 | p< 0,05 |

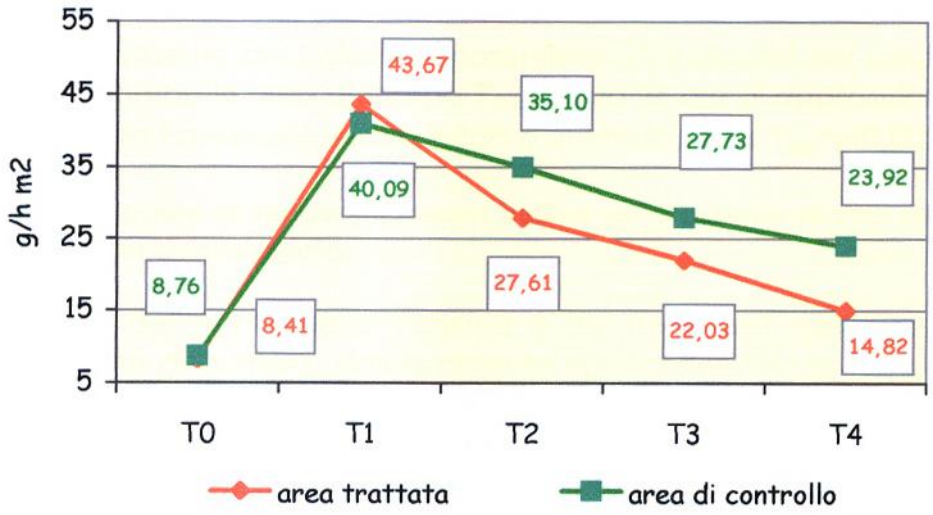
A significant increase of evaporimetric values registered in time of $T_{1sls}$ on both areas (after 24 hours from occlusive application of SLS) shows that skin barrier was damaged ($T_0$ vs. $T_{1sls}$ = p< 0,001).
The decrease of value in time after that shows a gradual regeneration of barrier functionality.
Based on the outcome of Variance analysis and Tukey test, on the area treated with the concerned product barrier damage, statistically significant until the time $T_{3sls}$ ($T_0$ vs. $T_{2sls}$/ $T_{3sls}$ = p< 0,001), was alleviated 72 hours after its appearance. Actually, the comparison between the basic value $T_0$ and final $T_{4sls}$ shows that they are not significant ($T_0$ vs. $T_{4sls}$ = p> 0,05) thus showing that the difference between these two values is negligible.

However, the damage of barrier caused by SLS on the control area is the result which is statistically high until the final control: $T_0$ vs. $T_{4sls}$ = p< 0,05.

Table 3: statistical comparison of two segments (Variance analysis and Tukey test)

| TEWL | $T_{1sls}$ vs. $T_{1sls}$ | $T_{2sls}$ vs. $T_{2sls}$ | $T_{3sls}$ vs. $T_{3sls}$ | $T_{4sls}$ vs. $T_{4sls}$ |
|---|---|---|---|---|
| Melem vs. control | p>0,05 (n.s.) | p>0,05 (n.s.) | p>0,05 (n.s.) | p<0,05 |

The statistical comparison of two concerned areas has shown that 72 hours since the appearance of damage to the barrier $T_{4sls}$ values registered on the control area significantly higher than those measured on treated area (treated $T_{4sls}$ vs. control $T_{4sls}$ p <0,05). The conclusion is the following: by applying the product faster skin barrier regeneration occurred in comparison to the untreated segment.




• treated area ■ control area

Diagram 1: in diagram TWEL values are shown on two concerned areas in different control times.

Table 4: mean value and standard deviation, colorimetry values (parameter a*)

| TEWL | $T_0$ | $T_{1sls}$ | $T_{2sls}$ | $T_{3sls}$ | $T_{4sls}$ |
|---|---|---|---|---|---|
| Melem | 7,00 ±1,15 | 11,27 ±3,19 | 10,34 ±2,69 | 9,12 ±2,23 | 8,93 ± 1,95 |
| Control | 7,16 ±0,95 | 10,29 ±2,04 | 11,31 ±3,29 | 10,96 ±2,80 | 11,12 ± 2,84 |

Table 5: statistical comparison between time (Variance analysis and Tukey test)

| TEWL | $T_0$ vs. $T_{1sls}$ | $T_0$ vs. $T_{2sls}$ | $T_0$ vs. $T_{3sls}$ | $T_0$ vs. $T_{4sls}$ |
|---|---|---|---|---|
| Melem | p< 0,001 | p< 0,001 | p>0,05(n.s.) | p> 0,05 (n.s.) |
| Control | p< 0,001 | p< 0,001 | p< 0,001 | p< 0,001 |

A significant increase of parameter value a*, which are indicated by skin redness marked on both areas in time $T_{2sls}$ (24 hours after the occlusive application of SLS) testifies that skin irritation occurred ($T_0$ vs. $T_{1sls}$ = p< 0,001).

The decrease in value after that time shows a gradual return to the initial situation when the skin wasn't damaged.

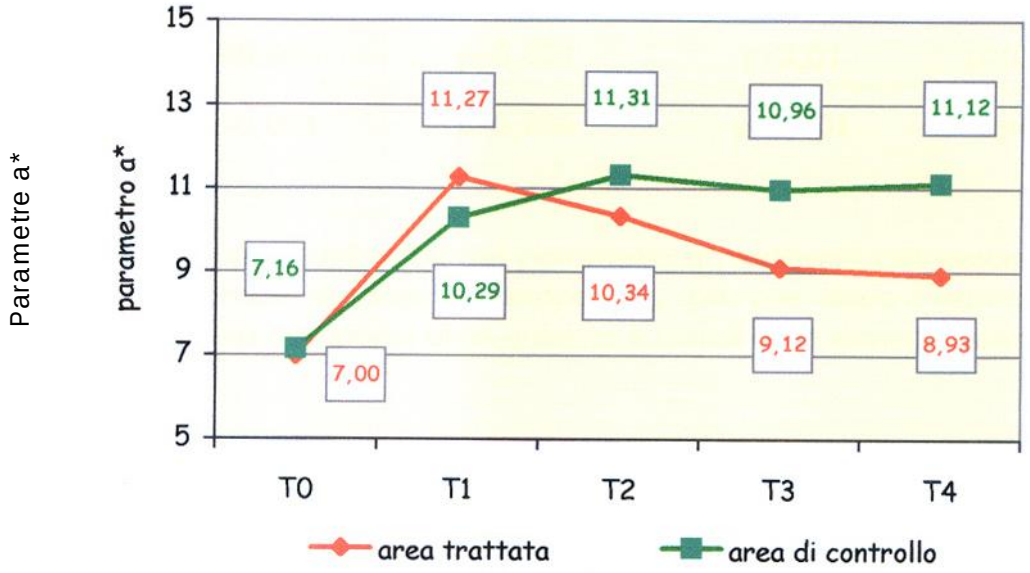
Based on the outcome of Variance analysis and Tukey test, on the area treated with concerned product, barrier damage, statistically significant until the time $T_{2sls}$ ($T_0$ vs. $T_{2sls}$ = p< 0,001), was alleviated already 48 hours after its appearance. Actually, the comparison between the basic value and control times $T_{3sls}$ and $T_{4sls}$ leads to the outcome which is not significant ($T_0$ vs. $T_{3sls}$ /$T_{4sls}$ = p> 0,05) showing in this way that the difference between these values is negligible.

However, on control area the damage caused by SLS give a statistically significant result until the final control: $T_0$ vs. $T_{4sls}$ = p< 0,001.

Table 6: statistical comparison of two segments (Variance analysis and Tukey test)

| Colorimetry | $T_{1sls}$ vs. $T_{1sls}$ | $T_{2sls}$ vs. $T_{2sls}$ | $T_{3sls}$ vs. $T_{3sls}$ | $T_{4sls}$ vs. $T_{4sls}$ |
|---|---|---|---|---|
| Melem vs. control | p>0,05 (n.s.) | p>0,05 (n.s.) | p>0,05 (n.s.) | p<0,05 |

Statistical comparison of two areas has shown that 72 hours from the appearance of damaged barrier $T_{4sls}$ values registered on control area were significantly higher than those measured on treated area (treated $T_{4sls}$ vs. control $T_{4sls}$ p <0,05). The conclusion is the following: by the application of the concerned product a faster regeneration of skin barrier caused by SLS irritation occurred in regard to the untreated segment.




• treated area ■ control area

Diagram 2: in diagram the values of parameter a* (skin redness) are stated, noted on two concerned areas in different control times.

## 7.b. Model of photo-inducted skin damage (UV)

Tables show mean value, standard deviation and statistical comparison of values of skin colorimetry in regard to two segments damaged by UV rays in different control times.

### Table 7: mean value and standard deviation, colorimetry values (parameter a*)

| Colorimetry | $T_0$ | $T_{1UV}$ | $T_{2UV}$ | $T_{3UV}$ | $T_{4UV}$ |
|---|---|---|---|---|---|
| Melem | 6,50 ±1,43 | 13,16 ±3,15 | 10,22 ±2,33 | 9,31 ±2,85 | 8,74 ± 2,36 |
| Control | 6,84 ±0,87 | 12,36 ±3,64 | 11,43 ±4,20 | 11,68 ±3,62 | 11,45 ± 3,08 |

### Table 8: statistical comparison between time (Variance analysis and Tukey test)

| Colorimetry | $T_0$ vs. $T_{1UV}$ | $T_0$ vs. $T_{2UV}$ | $T_0$ vs. $T_{3UV}$ | $T_0$ vs. $T_{4UV}$ |
|---|---|---|---|---|
| Melem | p< 0,001 | p< 0,001 | p>0,01 | p> 0,05 (n.s.) |
| Control | p< 0,001 | p< 0,001 | p< 0,001 | p< 0,001 |

A significant increase of values of parameter a*, which are indicated by skin redness marked on both areas in time $T_{2UV}$ (24 hours after the exposure to UV rays) testifies that eriteme occurred and therefore an irritation skin damage ($T_0$ vs. $T_{1UV}$ = p< 0,001).

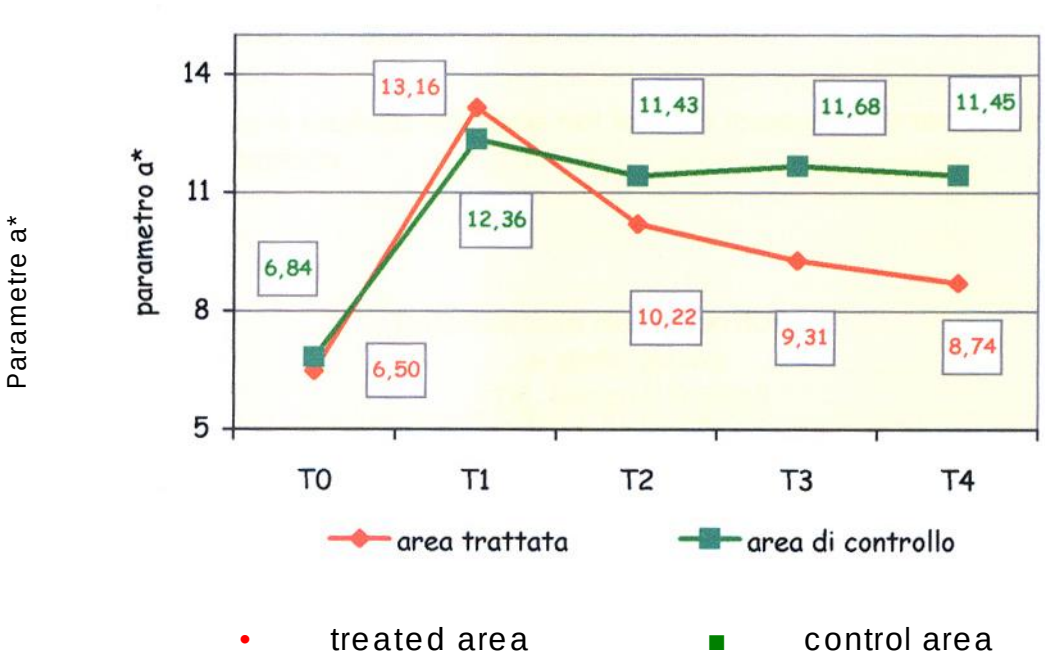
The decrease of value after this time shows a gradual return to the initial situation when the skin was not damaged.
Based on the outcome of Variance analysis and Tukey test, on the area treated with the concerned product, skin redness, statistically significant until time $T_{3UV}$ ($T_0$ vs. $T_{2sls}$ / $T_{3UV}$ = p< 0,001 and p>0,01), was alleviated 72 hours after the eriteme appearance. Actually, the comparison between the basic values $T_0$ and final time $T_{4UV}$ gives a result which is not significant ($T_0$ vs. $T_{4UV}$ = p> 0,05), showing in this way that the difference between these values is negligible.

However, on control area, the photo-induced eriteme gives a statistically significant result until the final control: $T_0$ vs. $T_{4UV}$= p< 0,001.

Table 9: statistical comparison of two segments (Variance analysis and Tukey test)

| Colorimetry | $T_{1UV}$ vs. $T_{1UV}$ | $T_{2UV}$ vs. $T_{2UV}$ | $T_{3UV}$ vs. $T_{3UV}$ | $T_{4UV}$ vs. $T_{4UV}$ |
|---|---|---|---|---|
| Melem vs. control | p>0,05 (n.s.) | p>0,05 (n.s.) | p>0,05 (n.s.) | p<0,05 |

Statistical comparison of two concerned areas has shown that 72 hours after the eriteme appearance $T_{4UV}$ values registered on control area were significantly higher than those measured on treated area (treated $T_{4UV}$ vs. control $T_{4UV}$ p <0,05). The conclusion is the following: the product has shown to be effective in alleviating photo-inducted skin redness in comparison with untreated skin.




• treated area ■ control area

Diagram 3: Diagram shows the values of parameter a* (skin redness) marked on two concerned areas in different control times.

## 8. CONCLUSIONS

For the purpose of assessing the alleviating and regenerating effect of cosmetic product MELEM, ref. ISPE 77/50/01-87/05. on the skin barrier, the sample was applied on the skin irritated by sodium lauryl sulphate in experimental purposes, and by ultraviolet radiation. The test was conducted on the volar forearm of 12 subjects comparing one untreated segment.

Based on the information obtained by the instruments it is possible to state that:

- with the application of the concerned product, the speed of skin barrier regeneration is faster in comparison to the untreated segment;
- with the application of the product an efficient alleviation of skin barrier damage caused by SLS occurred, in comparison with untreated skin;
- the product has shown to be effective in alleviating photo-inducted skin redness in comparison with untreated skin.

Responsabile del laboratorio
e della prova
Dr. Adriana Bonfigli

Laboratory and trial leader
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Il Dermatologo
Dr. Fernanda Distante

Dermatologist
Dr. Pernanda Distante
(own signature)

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