# In vitro method to evaluate the barrier properties of medical devices for cutaneous use

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### ABSTRACT

Barrier creams (BC) are marketed as cosmetic products or locally-applied medical devices to protect skin against damages induced by chemical agents or physical insults. However, the determination of the BC effectiveness is still a matter of discussion at both the clinical and the regulatory level. In this context, this work aimed at the development of a reliable, reproducible and easy-to-perform experimental protocol for the evaluation of BC performances. Preliminarily, an in vivo method based on the measurement of trans-epidermal water loss had been matter of investigation and was discarded: it required too much time and was not robust and sensitive enough. In vitro, reduction of the permeation of caffeine (used as a model of irritant), through an epidermal membrane mounted on a Franz cell or through a reconstructed 3D human epidermis model, was evaluated. Six BC among oil in water (O/W) or water in oil (W/O) creams were investigated with respect to the petrolatum, which is an efficient impermeable barrier against hydrophilic molecules. Despite minor differences, both methods could rate the effectiveness of the tested products in preventing caffeine exposure. Both methods enable to evaluate and quantify the BC effectiveness in a simple and fast manner. Their application may help regulatory agencies to prevent the marketing of ineffective products for the benefit of consumers.

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## 1. Introduction

Products used to treat or prevent a disease are classified as medicinal products or medical devices (MDs). The main difference between the two categories is related to the mechanism of action. Medicinal products exert a pharmacological, immunological or metabolic action (Directive, 2001/83/EC), while MDs achieve its principal intended purpose by a mechanical action (Directive 93/42/EEC, now Regulation (EU) 2017/745). In this context, different types of instruments, apparatus, appliances, software and materials may fulfil to the definition of Directive 93/42/EEC and now of Regulation (EU) 2017/745 and be marketed as MDs in Europe. Only recently, other type of products have started to be considered as MDs if their claimed performances can be attributed to a mechanical or physical mechanism of action. Indeed, as stated in the new regulation of the European parliament on MDs, they can also include products that are composed of substances or combinations of substances that are intended to be ingested, inhaled or administered rectally or vaginally introduced into the human body via a body orifice, or applied on skin and that are absorbed by or locally dispersed in the human body (Regulation (EU) 2017/745). Medicinal products and MDs also differ in the legislation that regulates how they are placed on the market. Medicinal products need a marketing authorization granted by regulatory agency, *e.g.* EMA or FDA, while, in the case of MDs, a CE marking for each product should be obtained. This is an assessment of quality, safety and efficacy that manufacturers must get it by notified bodies, which can be private sector organizations or a government agencies.

MDs are divided in 4 classes (I, IIa, IIb, III) with increasing risks as a function of the vulnerability of the part of the body that will enter in contact with the device, the duration of this contact, the invasivity of the device into the body, the risks for the users and the intrinsic characteristics of the device itself (Annex IX, Directive 93/42/EEC, now Annex VIII Regulation (EU) 2017/745). Usually, the biocompatibility of MDs is evaluated following the ISO 10993 (Biological Evaluation of Medical Devices) prior to clinical studies.

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**Abbreviations**

| | |
|---|---|
| BC | Barrier creams |
| O/W | oil in water emulsion |
| W/O | water in oil emulsion |
| TEWL | transepidermal water loss |
| MDs | medical devices |
| EEC | European Economic Community |
| EC | European Council |
| EMA | European Medicines Agency |
| FDA | Food and Drug Administration |
| CE | Conformité Européenne |
| ISO | International Organization for Standardization |
| OCED | Organization for Economic Co-operation and Development |
| TG | Test Guideline |
| EURL ECVAM | European Union Reference Laboratory for Alternatives to Animal Testing |
| HPLC | High Performance Liquid Chromatography |
| TEER | Trans-Epithelial-Electrical-Resistance |
| WP | White petrolatum |

This standard suggests the use of in vitro protocols in preliminary evaluations of cytotoxicity, irritation and sensitization potential as screening tool for materials and prototypes followed by in vivo tests. The standard was updated in 2009 to promote the use of "in vitro *test methods validated, reasonably and practically available, reliable and reproducible in preference to* in vivo *tests",* thus, opening to the use of new experimental models for more ethical preclinical approaches.

The possibility to identify the main mechanism of action of a product is important to properly define its regulatory classification, solving issues about borderline products, and to support product efficacy and establish its safety profile using proper positive and negative controls. Among them, the possibility to have instruments or methods useful to support product efficacy is necessary.

Barrier creams are widespread MDs for cutaneous use intended to protect the skin against damaging chemical agents or physical insults. As in most cases they act by forming a protective film against the skin damaging agent, they could be placed on the market as MDs instead of being cosmetics. Due to their action, barrier creams could be also prescribed by occupational doctors to workers. Despite the efficacy evaluation, it is worth to underline that, in vivo, the final positive effect of these products is strongly related to the proper mode of application (Wigger-Alberti et al, 1997).

The evaluation of the efficacy of barrier creams is a topic still discussed at the clinical (Mostosi and Simonart, 2016; Kresken and Klotz, 2003; Kütting and Drexler, 2003; Alvarez et al., 2001) and regulatory level. Even if some attempts were done in the past, until now there are no official methods to test the efficacy of barrier creams neither in vivo nor in vitro (Chilcott et al., 2005, 2007). Moreover, the use of animals is no more admitted, at least in the case of cosmetic products due to restrictions in the European legislation. The proposed in vivo methods involving human volunteers are generally restricted to mild-moderate irritants (Perrenoud et al., 2001) and are not suitable for the screening of many products, since long time of application is required and no significant differences among products are demonstrated (Sadhra et al., 2014; Berndt et al., 2000; Wigger-Alberti et al., 1998; Zhai and Maibach, 1996; Frosch and Kurte, 1994; Goh and Gan, 1994).

The development of in vitro methods should be therefore based on more precise, reproducible and scientific approaches to evaluate the efficacy of these products. Until now, methods based on the use of Franz diffusion cells, which give good correlation with in vivo results in skin absorption studies, have already demonstrated to be useful for the in vitro evaluation of the protective capability of barrier creams (Millerioux et al., 2009; Chilcott et al., 2002; Treffel et al., 1994).

New in vitro approaches are based on the use of reconstructed 3D human derived tissue models, which are biologically relevant models because of their similarities in tissue morphology (*e.g.*, pluri-stratified tissue) and functionality (viability and metabolism) with respect to in vivo human tissues (Bell et al., 1981) and represent a highly predictive and reproducible instrument for preclinical evaluations. These 3D models are fabricated using sophisticated technologies and quality systems, which guarantee high inter-batch reproducibility and low variability in terms of barrier properties. Thanks to their histo-morphological structure, it is possible to evaluate a product directly on the tissue models, using topical or systemic applications and products at the same concentrations and exposure conditions advised for end users leaving flexibility in experimental protocol design. Moreover, the introduction of these experimental models in the evaluation of the MDs is a very promising scientific innovation as it has already occurred in other sectors where animal testing is highly regulated (chemicals and active ingredients) or completely banned (cosmetics) and the development, validation and implementation of alternative methods are strongly promoted. *In vitro* test methods based on the use of reconstructed human epidermis have been validated or are under validation as alternative to animal testing methods for the evaluation of chemical and ingredient hazard: skin irritation (OCED TG 439), skin corrosion (OECD TG 431), eye irritation and skin sensitization (*EURL ECVAM Status Report on the Development,* 2015).

This work aimed at the development of a reliable, reproducible and easy to perform experimental protocol to be used for the evaluation/quantification of the barrier properties of MDs for topical use. An in vivo method based on the measurement of the trans-epidermal water loss (TEWL) was performed and two different in vitro methods based on Franz diffusion cell or reconstructed human epidermis model were evaluated. For in vitro method, caffeine was selected as a model considering the high number of hydrophilic molecules to which human skin can be exposed. Moreover, caffeine has been used to evaluate the permeability of different models of epidermis reconstructed in vitro used for percutaneous absorption studies. For the ability to overcome the epithelial barrier, even in the absence of damage, caffeine was used as a probe to assess the propensity of a given product to form a protective film: the reduction of the passage of caffeine through the biological model is thus considered as an index of the protective effectiveness of MD.

## 2. Materials and methods

### 2.1. Chemicals

Caffeine, Farmalabor, Italy. Acetonitrile and methanol, HPLC grade, VWR International, Italy.

The composition of the cream is as follows:

*Cream D*: dimethicone, petrolatum, cetearyl alcohol, ceteth-20, paraffinum liquidum, preservative: phenoxyethanol - methylparaben - ethylparaben - propylparaben - butylparaben, aqua.

*Cream K*: aqua, kaolin, paraffinum liquidum, petrolatum, paraffin, cetearyl alcohol, cetyl alcohol, zinc oxide, sodium lauryl sulfate, methylparaben, sodium hexametaphosphate, parfum.

*Cream Kf*: aqua, paraffinum liquidum, cera alba, lanolin, stearic

acid, octyldodecanol sodium borate, propyparaben, octyldodecyl xyloside, magnesium sulfurate, methylparaben, peg-30, dipolyhydroxystearate, quaternium 18.

*Cream* L: polynucleotides, propylene glycol, caprylyl glycol, Ethylhexylglycerin, cetearyl alcohol, dimethicone, polysorbate 60, caprylic/capric triglyceride, Gliceril stearato citrato, acrylates/vinyl isodecanoate crosspolymer, tetrasodium edta, aqua.

*Cream T*: aqua (water), paraffinum liquidum (mineral oil), octyldodecyl stearoyl stearate, polyperfluoromethylisopropyl ether, c12-c15 alkyl benzoate, cyclopentasiloxane, diphenyldimethicone, sodium carbomer, tocopheryl acetate, polyethylene, ethylhexylglycerin, lonicera japonica (honeysuckle) flower extract, disodium edta.

*Cream X*: aqua, isohexadecane, butyrospermun parkii, pentylene glycol, ethylhexyl palmitate, glycyrrhetinic acid, cera alba, peg-30 dipolyhydroxystearate, bisabolol, polyglyceril-6 polyricinoleate, tocoperyl acetate, hydrogenated castor oil, sodium hyaluronate nylon 12, butylene glycol, magnesium sulfate, piroctone olamine, allantoin, magnesium stearate, disodium edta, vitis vinifera, ascorbyl tetraisopalmitate, propyl gallate, telmesteine.

The characteristics of the seven tested products are reported in Table 1.

### 2.2. Characterization of the products: type of emulsion and percentage of water

The type of emulsion was determined on the basis of the ability of the external phase to dissolve the water soluble dye, methylene blue. A small amount of the dye was added to each cream: the dye gives color to oil in water (O/W) creams, with the appearance of a well-distributed light blue coloration; the lack of the appearance of this light blue coloration means external oil phase, that is water in oil (W/O) creams.

The percentage of water in the formulation was determined as loss of weight of a product. A sample of each cream, exactly measured, was spread on a glass support, stored in an oven at 60° until weight constancy.

### 2.3. Non-invasive in vivo methods: TEWL measurements

#### 2.3.1. Volunteers

A single application experiments was carried out on 20 healthy male and female volunteers with a mean age of $27 \pm 3$ years. Eligible volunteers satisfying the following criteria to be considered suitable for the study were selected: no alteration/damage on the skin; no pathological skin; no treatment with vasodilator drugs. Volunteers are asked: not to drink beverages containing xanthines during 2 h before the treatment; not to smoke during 1 h before the treatment; not to take contemporary drinks; not to contemporary apply other cosmetic products in treated areas.

#### 2.3.2. Method and study protocol

TEWL is measured using an accepted device (Tewameter TM 210®, Courage-khazaka electronic, Köln, Germany) with a probe consisting of an open chamber (12 mm in diameter) and mounted with sensors for determination of temperature and relative humidity. TEWL is expressed in $g/m^2/h$.

Experiments was performed in an adequate, completed closed, room with constant temperature and humidity. Temperature of the test area was held between $20 \pm 3$ °C. Humidity was held at $57\% \pm 7\%$ R.H.

Before product application, the subject remained in the test area for a period of 30 min with uncovered forearms for acclimatization.

The test product (Table 1) was applied on the skin with the help of a mask with holes of equal diameter ($0.785 \text{ cm}^2$ surface). The mask was laid on an area of left or right arm not too close to wrist and elbow, always in the same position.

The test product was exactly weighed (20 mg) and applied on the area corresponding to the mask holes, then the mask was removed and the preparation was further spread on the skin with a gloved finger during 10 s on the respective test site to help distribution. Usage of left/right forearms and sequence of product applications were randomized among the volunteers.

Basal value of hydration was measured on not treated area and served as control area.

All measurements are conducted following to the guidelines of the Standardization group of the European Society of Contact Dermatitis. Measurements were carried out following to two methods.

##### 2.3.2.1. Method 1: TEWL evolution recorded up to 5 h. 
The effects of the test products were measured at time t = 0, that is 15 min after cream application (time required for the equilibration), and after 1, 2 and 5 h after application. On each site, measurements were performed at each time. The excess of test products was not removed from the skin surface (effect of the film remained on the skin). TEWL test readings are conducted 30 s after application of the probe onto the skin, when the level is stabilized, for a duration of 90 s. The mean value over the 90 s is recorded and indicated by the instrument. Volunteers had to be in the room 30 min before measurements and could leave the room between two measurements (basal and 2 or 5 h after application).

##### 2.3.2.2. Method 2: TEWL single point. 
The effects of the test products were measured in a single point 2 h after application.

Measurements are performed as reported above.

### 2.4. In vitro experiments

As hydrophilic chemical probe to evaluate barrier properties of the creams, 1% caffeine solution was used. Caffeine was selected being a chemical reference in the OECD 428 and related Guidance

**Table 1**
List of the selected products and information given by the manufactures in the label related to function and composition.

| | Barrier cream claim | Presence of powder (dispersion) | Presence of polymer |
|---|---|---|---|
| CREAM D | no | | |
| CREAM K | Yes | + | |
| CREAM Kf | Yes | | ACRYLATE |
| CREAM L | Yes (radiation) | | |
| CREAM T | yes | | PFPE[a] AND ACRYLATE |
| CREAM X | Yes (radiation) | | |
| WHITE PETROLATUM, WP (VASELINE) | no | | |

Note:
[a] Perfluoropolyehters.

Document for the conduct of in vitro skin absorption studies. Experiments were performed over a period of 3 h. The caffeine permeation through untreated membranes was used as negative control. Barrier creams were applied on the membrane 2 h before the start of the in vitro experiments to avoid disturbance in the film formation.

### 2.4.1. Reconstructed human epidermis EPISKIN™

*2.4.1.1. Membrane.* The reconstructed skin model EPISKIN™ (EPISKIN Large) is provided by EPISKIN (Lyon, France). It is a reconstructed organotypic culture of human adult keratinocytes reproducing a multi-layered and differentiated human epidermis. Cells are grown on a collagen matrix for 13 days. Human adult keratinocytes are seeded on a dermal substitute consisting of a collagen I matrix coated with a layer of collagen IV fixed to the bottom of a plastic chamber. Epithelial differentiation is obtained by an air exposed step leading to a 3D epidermis construct (1.07 cm$^2$ surface), with basal, spinous, granular layers and a stratum corneum. Upon receipt of the EPISKIN kit (12 unit packs on day 13 of culture), the culture inserts were removed from their nutrient gel and transferred to 12-well plate previously filled with maintenance medium (2 mL/well pre-warmed at 37 °C) and incubated at 37 °C, 5% $CO_2$, saturated humidity overnight.

*2.4.1.2. Method.* Prior to experiments, the Trans-Epithelial-Electrical-Resistance (TEER) of each tissue was measured with the Millicell-ERS instrument (range 0–20 kΩ) to ensure the integrity of the barrier membrane. Epidermis samples with an electrical resistance above 10 kΩ cm$^2$ were used for experiments.

At the beginning of the experiment, 50 mg of each product (Table 1), exactly weighted, were accurately spread on 1.07 cm$^2$ epidermis with the help of a small spatula. Treated epidermis was left for 2 h at room temperature. The culture inserts were then removed and transferred to 12-well plate previously filled with 2 mL/well of saline solution (receiver compartment). Special care was taken to avoid air bubbles between the receptor fluid and the insert.

0.1 mL of caffeine solution (1% w/v) was applied on the untreated or treated epidermis as donor phase. Throughout the experiment, the inserts were incubated at 37 °C, 5% $CO_2$, saturated humidity. At predetermined times (30 min, 1 and 3 h) 2 mL of the receptor fluid was withdrawn from the receiver compartment with a micropipette (Gilson) and replaced with fresh receiver medium kept at room temperature. Sink conditions were maintained throughout the experiments. At least three replicates per test preparation were performed. The withdrawn samples were stored at 4 °C before the HPLC analysis.

### 2.4.2. Franz diffusion cell

*2.4.2.1. Membrane.* Samples were obtained from the abdominal skin of one single human donor (30–50 years old, Caucasian) who underwent cosmetic surgery and signed an informed consent for the use of the biological sample for research purposes. Epidermis samples were prepared following an internal standard procedure (Cilurzo et al., 2014).

Within 6/8 h after removal the excess fat was carefully removed from the full-thickness skin. The skin sections were cut into squares of about 2.5 × 2.5 cm with a scalpel and, after immersing the skin in water at 60 °C for 1 min, the epidermis was gently separated from the remaining tissue with forceps. Then the epidermis was left to dry in a desiccator containing silica gel and placed at 4 °C. After 24/48 h, the epidermis was removed from the desiccator, sealed in aluminium foil and frozen at −20 °C prior to use, as reported by "Guidance document for the conduct of skin absorption studies OECD series on testing and assessment; number 28" for storage condition (n. 41, page 17).

*2.4.2.2. Method.* Prior to experiments, the epidermis was thawed at room temperature. Then, the conductivity of the isolated sheets to ensure the integrity of the barrier membrane was measured; epidermis samples with an electrical resistance above 25 kΩ cm$^2$ were used for experiments (voltage: 250 mV, frequency: 100 Hz; Agilent 4263 B LCR Meter, Microlease, Italy).

At the beginning of the experiment, 100 mg of each product (Table 1), exactly weighted, were accurately spread on 4.90 cm$^2$ epidermis with the help of a small spatula. Treated epidermis was left for 2 h at room temperature and then it was mounted onto the Franz cells. 0.5 mL of caffeine solution (1% w/v) was applied on the untreated or treated epidermis as donor phase. About 3 mL of saline solution, exactly measured, was placed in the receiver compartment, to respect the sink conditions. Special care was taken to avoid air bubbles between the receptor medium and the membrane in the receptor compartment. Throughout the experiment, the receptor fluid in contact with the skin was maintained at a constant temperature of 32 ± 1 °C, with a heating circulating water bath connected to jacketed cells, under continuous stirring.

At predetermined times (30 min, 1 and 3 h) 0.2 mL samples were withdrawn from the receiver compartment with a syringe (GASTIGHT® 1001, Hamilton, USA) and replaced with fresh receiver medium kept at room temperature. Each cell was equipped with apposite syringe to withdraw and replace the receiver medium. Sink conditions were maintained throughout the experiments. At least three replicates per test preparation were performed. The withdrawn samples were stored at 4 °C before the HPLC analysis.

### 2.5. Analysis of caffeine

The analysis of the samples was performed within 24 h from the end of the experiments, using the HPLC method described below.

Caffeine concentration was determined by using a HP1100 Chemstation (Hewlett Packard, Italy) equipped with C18 reverse-phase column (Accucore XL C18, 1000 × 4,6 mm, 4 μm, Thermo Fisher Scientific Inc., USA) set at 25 °C. A sample of 20 μL was injected for an isocratic elution at 1.0 mL/min. The composition of the eluent was acetonitrile/water 0.1% v/v acetic acid (10/90 v/v) (from: Thermo Scientific Application Note: Rapid Determination of Polyphenol Antioxidants in Green Tea and Cranberry Extract Using Core Shell Columns, Pranathi P. Perati, Brian M. De Borba, and Jeffrey S. Rohrer, Thermo Fisher Scientific, Sunnyvale, CA, USA). The wavelength was set at 272 nm. Standard calibration curves for caffeine (0.5–10 μg/mL) were used. No interfering peaks from semisolid excipients were found in the chromatogram.

### 2.6. Data analysis

Parameters of the skin permeation test, namely caffeine permeated amount and flux, were determined on at least three replicates.

The amount of permeated caffeine through the skin during a sampling interval was calculated based on the receptor-phase concentration and volume of the receiver compartment.

The cumulative amount of caffeine permeating into the receptor compartment had to be plotted against time to obtain the permeation profile. The steady state flux ($J_{max}$) was determined as the slope of the linear portion of the plot ($R^2 > 0.98$).

The estimated parameters were presented as mean ± standard deviation.

### 2.7. Statistical analysis

Statistical evaluation was performed by Student T-test. Differences were considered significant at $p < 0.05$.

**Table 2**
Physical characteristics of the selected products.

|                                      | Emulsion type | Weight loss (grams of water) |
|--------------------------------------|---------------|------------------------------|
| CREAM D                              | O/W           | $55 \pm 4$                   |
| CREAM K                              | O/W           | $49 \pm 1$                   |
| CREAM Kf                             | W/O           | $23 \pm 8$                   |
| CREAM L                              | O/W           | $71 \pm 0$                   |
| CREAM T                              | O/W           | $72 \pm 1$                   |
| CREAM X                              | W/O           | $46 \pm 15$                  |
| VASELINE (WHITE PETROLATUM, WP)      | O             | —                            |

Note: O/W: oil in water; W/O: water in oil; O: oil.

## 3. Results

### 3.1. Characterization of the products: emulsion type and percentage of water

Results concerning emulsion type and percentage of water are reported in Table 2. In the group of tested products, both O/W and W/O emulsions are selected. Water content in the formulations were between 20 and 70%. The hydrophilic creams, reported as O/W, had a percentage of water usually over 50%. The lipophilic cream X (W/O) had a similar percentage of water, even if it showed an external oily phase. Only cream Kf contained a low percentage of water.

### 3.2. In vivo TEWL measurements

In vivo method was proved with a limited number of products, namely WP, cream D, cream X and Cream L.

WP was used for comparison, being known that it is an occlusive agent with moisturizing properties and useful in prevention of irritation induced by various agents (Rieger et al., 2007). Petrolatum is recommended as a standard reference substance against which protective/barrier cream may be compared; it is effective against water-soluble and water-insoluble irritants in a standardized test procedure (Wigger-Alberti and Elsner, 1997).

For the baseline measurements (Control values in Table 1 Supplementary information), no significant difference was detected for the three volunteers involved in the Method 1 measurements (TEWL evolution up to 5 h). The effect of the product application was shown as difference between control values and those measured at each time point.

TEWL values obtained after 15 min from application of WP and cream D (t = 0 in Table 1 Supplementary information) showed that these lipophilic waterproof formulations decreased water loss in comparison with control values; WP reduced TEWL values by 45% while Cream D by 15%. Decreased TEWL values could be due to a thin layer rapidly formed on the skin as already reported for silicones materials (De Paepe et al., 2014). In the case of Cream X and Cream L, TEWL values increased probably for the emulsions break and incorporated water evaporation. This behaviour was more evident for Cream L. TEWL was further measured on the same site after 1, 2 and 5 h to evaluate how long could last the protective film immediately formed (WP and cream D) or if a film could form later (Cream X and Cream L). TEWL values indicated that WP and Cream D formed a non-permeable film on the skin surface that held over 5 h, maintaining decreased TEWL values for all time point of measurements. A protective film seemed to be formed after the first hour application in the case of Cream X and Cream L: after 5 h, the percentage TEWL reduction was 21 and 9% respectively, but with a high variability among volunteers (Table 1 Supplementary information). Being TEWL values quite similar in the range 1–5 h,

the group of volunteers was enlarged to confirm this evaluation (Table 2 Supplementary information). The effect of the product application was measured only after 2 h. The occlusive behaviour of WP and cream D was confirmed, even if a high variability among volunteers was also observed.

### 3.3. In vitro experiments

#### 3.3.1. In vitro skin permeation on EPISKIN™ model

The results of caffeine quantified in the culture medium after 30 min, 1 h and 3 h are reported in Fig. 1 (Table 3 Supplementary information). Comparing results obtained in terms of caffeine permeated amounts with untreated vs treated samples, it can be observed that within 1 h application no significant differences were measured among all the samples. After 1 h, no caffeine was quantified in samples treated with WP and cream D and relative low amounts were found in all other samples, except in the case of cream T. Cream T showed a high permeated amount, not significantly different with respects to those obtained in the case of negative control. After 3 h, the barrier effect of WP was confirmed, being caffeine not quantified. The caffeine amount quantified for the untreated control, 33 $\mu g/cm^2$, corresponded to literature data in the EPISKIN™ model (Schreiber et al., 2005). Three different levels of caffeine content compared to the untreated control were underlined: WP and Cream D lower than 10 $\mu g/cm^2$, Cream X between 10 and 20 $\mu g/cm^2$ and all other products higher than 20 $\mu g/cm^2$. These levels correspond to 3 levels of caffeine permeability where higher than 20 is the more permeable; results obtained by using Cream T and Cream K are significantly different from control solution, while Cream L and Cream Kf are not.

The relative caffeine amounts at the 30 min and 1 h time points are presented in Fig. 2 (Table 3 Supplementary information). The total amount of caffeine permeated through the EPISKIN™ model after these very short time points allows to better identify products efficacy as film forming with respect to a longer period of application (all samples are significantly different from control solution; $p < 0.05$).

#### 3.3.2. Franz diffusion cell

The barrier effect of each product was evaluated by using epidermis from one single donor to avoid intra-donor variability.

The caffeine permeation profiles were linear in most cases (Fig. 3, Table 4 Supplementary information); only by using cream Kf and cream X caffeine was not detected in the receiver compartment after 30 min. In all cases, standard deviations were in the accepted order of magnitude for ex vivo skin permeability.

The degree of protection of the tested barrier creams was variable, indicating that the method could distinguish a different protective effect of the products. By using Franz cells, when WP was applied on the epidermis, protection against caffeine permeation was not complete, being after 3 h the caffeine permeated amount equivalent to 8% with respect to permeation through untreated membrane (Fig. 3, Table 4 Supplementary information). Quite good protectors were the W/O emulsions, cream X and cream Kf, and the O/W emulsions, cream D and cream K. The percentage with respect to permeation through an untreated membrane was in the range 17–36% (Fig. 3, Table 4 Supplementary information). All caffeine permeated amounts measured when epidermis was protected with the previously mentioned products were significantly different from those obtained by using caffeine solution applied directly on not treated epidermis. In the case of cream T and cream L, only a weak protection was observed; results obtained after 3 h were never significantly different from those obtained by using caffeine solution through untreated membrane.
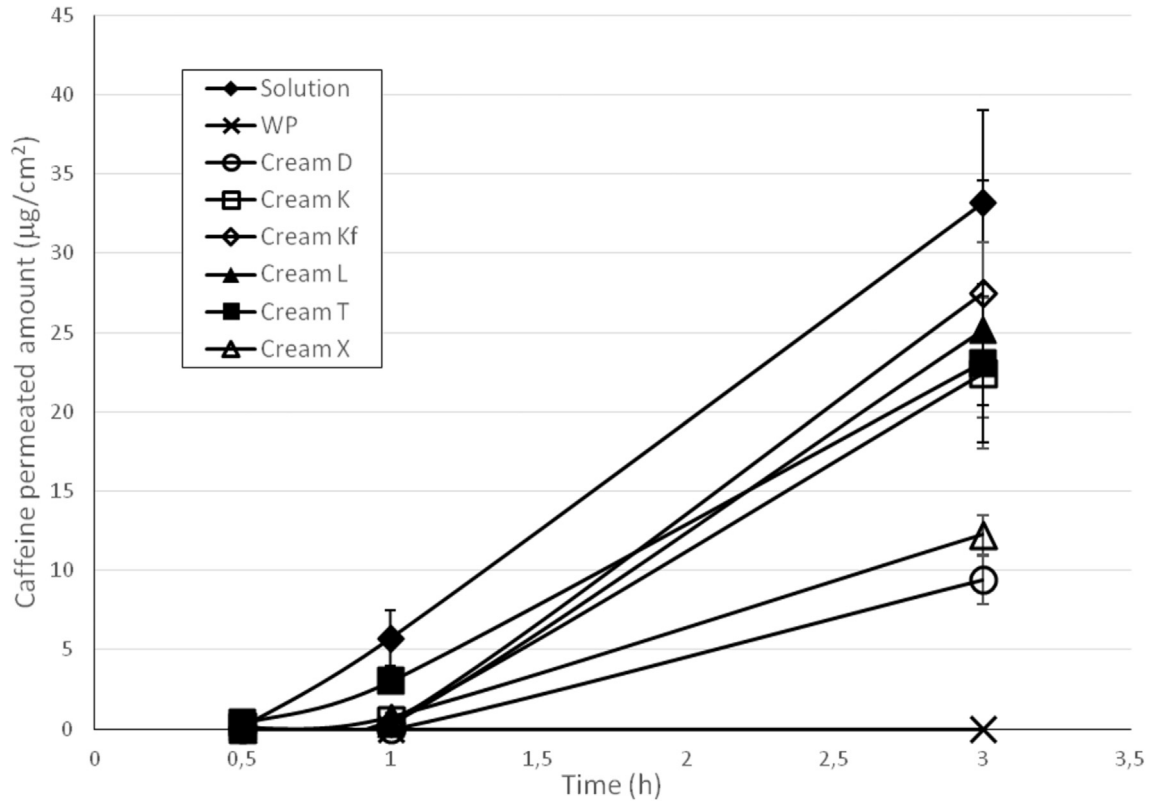
**Fig. 1.** Caffeine permeation profiles through EPISKIN™ (n = 3; mean ± s.d.).
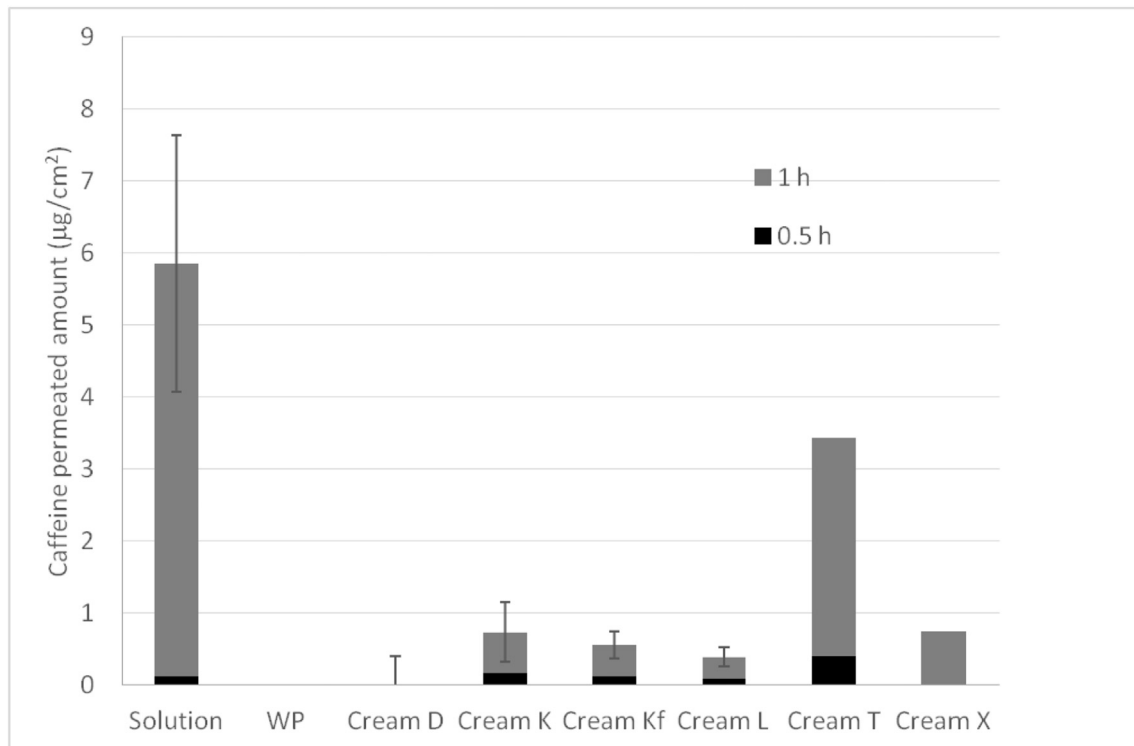


**Fig. 2.** Caffeine permeated amounts through EPISKIN™ after 0.5 and 1 h (n = 3; mean ± s.d.).
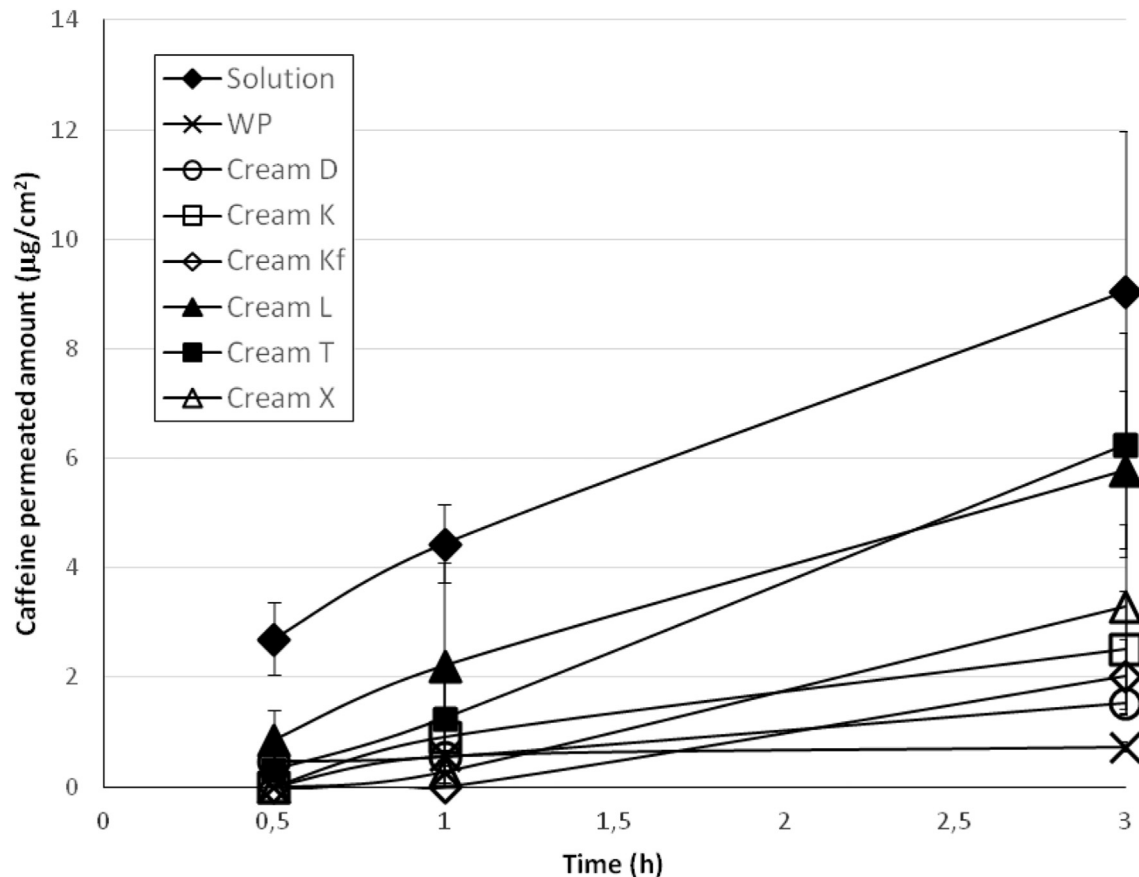
**Fig. 3.** Caffeine permeation profiles through human epidermis (n = 3; mean ± s.d.).

## 4. Discussion

BC should have the aim to prevent penetration and absorption of potential allergens and irritants into the skin. There is no indication on which components should be added in these products; therefore, composition remains a free choice of the manufacturer. Among regulatory agencies, only the FDA in the Code of Federal Regulations (21CFR347 - Code of Federal Regulations, Title 21, part 347 SKIN PROTECTANT DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE) indicates a list of ingredients considered as actives for skin protectant drug products for over-the-counter human use and specify the concentration for each ingredient. FDA describes these products as drug products that temporarily protect injured or exposed skin or mucous membrane surfaces from harmful or annoying stimuli, and may help provide relief to such surfaces. The lipophilic phase should be carefully selected. Moreover, ingredients based on film forming polymers or powders are often included in these formulations. An innovative approach based on polyamido-amine (PAMAM) dendrimer, a reactive nanoparticle, can also be used. The flux of furfural, used as a model toxicant, was decreased by PAMAM dendrimer, indicating PAMAM's protective ability against cutaneous toxicants (Moghimi et al., 2010). The dogma that O/W emulsions are primarily effective against lipophilic irritants, and W/O emulsions against hydrophilic irritants (Davidson, 1994), needs to be re-evaluated as data reported in literature not always agreed about this topic. Treffel et al. (1994) found a correlation between the percentage of protection of three dyes with different partition coefficients and water content in the formulations. Various experimental and clinical studies have been conducted to determine the efficacy of BC (Alvarez et al., 2001; Rieger et al.,

2007). A certain number of clinical studies have shown them to be beneficial, whereas other studies shown them to be ineffective or even to exacerbate skin irritation. WP was used as a positive control, being overall considered efficient as a barrier on the skin surface against penetration of a hydrophilic dye molecule (Rieger et al., 2007).

In this context, being the clinical efficacy of these creams controversial and not easy to be proved, from the regulatory point of view the need remains to have indications on safety and efficacy.

Among the previous in vivo proposals, the efficacy of the BC was evaluated either considering clinical scores, biometrics measurements, such as TEWL, and subjective opinions of subjects (Perrenoud et al., 2001; Sadhra et al., 2014) or by removing the superficial layers of the skin by skin surface biopsy and dosing dyes or other substances (Marks et al., 1989; Zhai and Maibach, 1996; Sun et al., 2000). As for in vitro methods, Franz diffusion cells have already been tested (Treffel et al., 1994; Chilcott et al., 2002; Millerioux et al., 2009), while reconstructed skin model EPIS-KIN™ was used for the first time.

In this work, the in vivo TEWL method was used for a limited number of samples, involving until 11 volunteers. Results confirmed the WP occlusive properties. Cream D showed a similar occlusive behaviour.

The possibility to have an indication on efficacy by an easier and faster in vitro method is considered desirable by regulatory agencies. In this work, we suggested a test performed by using a hydrophilic "probe" and measuring its permeation over a period of 3 h; this time is long enough to show permeation of caffeine through the membrane. Results obtained by using Franz cells agree with previous results (Treffel et al., 1994), based on a shortest
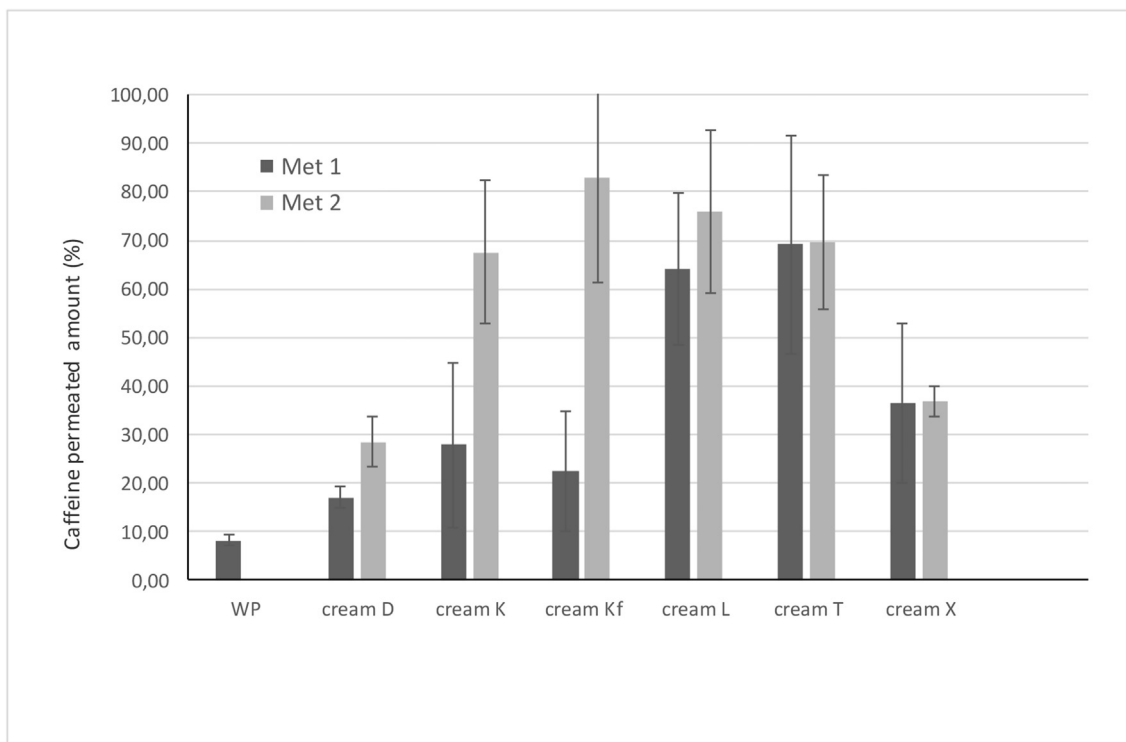
**Fig. 4.** Percentage of caffeine permeated after 3 h with respect to permeation through untreated membrane. Met 1: Franz cells; Met 2: Episkin. Data are expressed as mean ± st. dev. (n = 3).
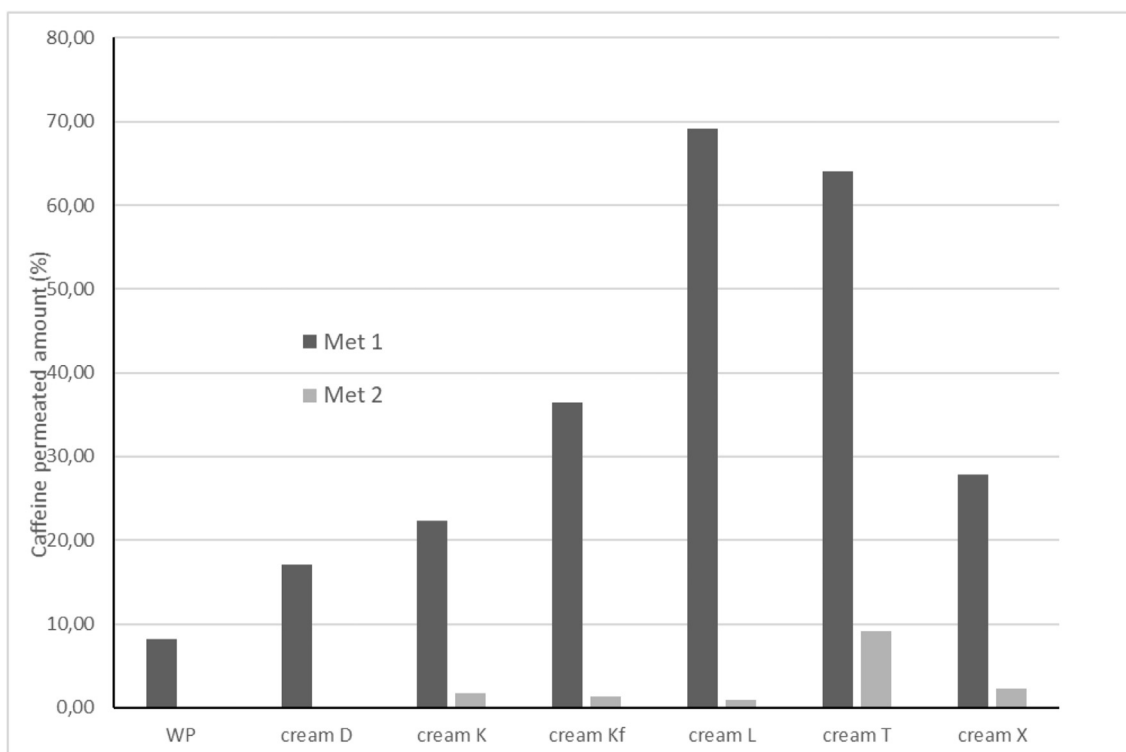


**Fig. 5.** Percentage of caffeine permeated calculated considering permeation data through untreated membrane after 3 h and treated membrane after 3 h (Met 1: Franz cells) or after 1 h (Met 2: Episkin). Data are expressed as mean (n = 3), st. dev <20%.

period, namely 30 min, and the stripping technique. It was shown that eosin, a hydrophilic probe, penetrated the stratum corneum despite the presence of a BC. In addition, the more lipophilic dye (red oil O) penetrated deeper into the stratum corneum. Moreover,

even if most of the tested products evaluated by Treffel showed a good protection after half an hour, none of them demonstrated absolute efficacy, except for WP against eosin. Cream K was used in both experimental conditions and it showed to be slightly effective against eosin or caffeine.

The Franz diffusion cell method and the 3D method could rate the effectiveness of the tested products (Fig. 4 and Fig. 5, Table 5 Supplementary information). As far as the water content is concerned, there is no real correlation, but it seemed that formulations containing more than 50% water were more permeable to caffeine. The in vivo occlusive behaviour agreed with in vitro results, being WP and cream D the most efficient among selected products.

The superimposition of results obtained by using Franz diffusion cell method after 3 h and the 3D method after 1 h seemed to be better (Fig. 5, Table 5 Supplementary information). In the case of the reconstructed skin model, it seemed more suitable to test the efficacy in a very short time, i.e. 1 h, since after 3 h from the application differences from control solution were reduced for a higher number of tested products. The Franz cell method seemed to maintain the ability to distinguish the protection effect of the tested products over the period of 3 h (permeation profiles linear for all the period) while 3D method showed a strong change in slope between 1 and 3 h (cream K, cream Kf, cream L). Differences among these tissues can explain this behaviour.

In conclusion, both in vitro method proposed in this work allowed to evaluate and quantify the efficacy of BC in a simple and fast manner. Their use could help the work of regulatory agencies and avoid the marketing of products with inadequate efficacy, for the benefit of consumers.

## Acknowledgements

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.08.007.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.08.007.

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