DELIVERY OF CAFFEINE CONTAINED IN SMART TEXTILES INTO THE SKIN

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SUMMARY

Textiles have been always considered as a "second skin" for human beings. Now, new technologies are permitting the production of smart textiles. Some fabrics may contain carrier molecules to be delivered, as a therapeutic or cosmetic compound, to the skin. They can act as "repository systems" and are capable of continually releasing small doses of active substances from the textile onto the skin.

As far as we know, no systematic works have been published to demonstrate the validity of that approach. The eventual benefits of these smart textiles must be substantiated by a suitable experimental methodology in order to get the corresponding "proof of principle".

In this paper, the different steps to be followed in the preparation of the formulation and its application on the textile have been controlled. Special emphasis has been made on the release of the active principle (caffeine) from the formulation and the textile as well as on its transdermal delivery in order to reach the target compartment of the skin. A new *in vitro* methodology on percutaneous absorption has been designed to demonstrate the delivery of encapsulated caffeine from the textile to the different skin layers (stratum corneum, epidermis and dermis).

From the results obtained in this research, maintaining a close contact between the smart textile and the skin (specially when a certain pressure is applied on the textile), it is possible to detect the presence of caffeine into the different layers of the skin in order to induce its anti-cellulite action.

INTRODUCTION

For millenniums, textile fabrics have been improved to assist the skin functions ensuring homeostasis of the whole body. Practical functions of clothing include providing the human body protection against the weather –strong sunlight, extreme heat or cold, and rain or snow – also protection against insects, noxious chemicals and contact with abrasive substances. Summarising, cloths protect against anything that might injure the naked human body. This is because textiles have been always considered as a "second skin" for human beings (1).

Now, new technologies are permitting the production of smart bioactive or biofunctional textiles. Such fabrics are able to absorb substances from the skin or can release therapeutic or cosmetic compounds to the skin. The textile industry and medicine have taken a step forward together to enriching the use of textile materials considering their interaction with the skin (2).

Percutaneous absorption is an interdisciplinary topic which is relevant to a number of widely divergent fields. The principal areas of interest are the development of: i) transdermal devices, ii) dermatological formulations and iii) safety assessment of cosmetics topically applied. Transdermal devices may be considered as one of the precursors of biofunctional textiles because they are designed to deliver a compound into the body in order to exert a therapeutic effect at a site distant from the application (3,4).

Smart or bioactive textiles are new, innovative textile products, which are extending the previous boundaries of textile applications. They can act as “repository systems” and are capable of continually release small doses of active substances from the textile onto the skin. Several active compounds have been applied on textiles using different vehicles as micro or nanocapsules in order to improve not only the fixation on the fabric but also the progressive and effective release of the active principle into the different skin layers (stratum corneum, epidermis or dermis). But, the efficacy of a given smart textile containing a specific compound must be substantiated by scientific and solid arguments in order to get the corresponding “proof of principle”.

As far as we know, no systematic works have been published to demonstrate the release of the active principle from the textile as well as the eventual pass through the stratum corneum (the essential structure of the skin barrier) and the other skin compartments.

The aim of this work has been to investigate the different steps to be followed in the preparation of several formulations containing caffeine and their application on cotton fabric. Caffeine has been selected because it is a compound commonly used in cosmetics for its stimulating activity on fat metabolism (anti-cellulite action) and as a natural antioxidant (5-7). Caffeine may be applied directly on the skin as a cosmetic formulation or incorporated in a corset in order to be delivered in a progressive way the active principle on the skin. Special emphasis has been made on the release of this active principle from the formulations and from the cotton fabric to which has been applied as well as on its transdermal delivery in order to reach the compartment target of the skin.

EXPERIMENTAL

Material

Caffeine (molecular weight: 194.19) was provided by Fluka (Fluka Chemicals, Buchs, Switzerland). Aqueous and liposomic formulations of liposomes containing caffeine (1%) were prepared by the company Lipotec Group (Spain). A cotton standard fabric (Style 400, Test Fabrics, Inc.) as described in ISO 105 F02 was used as a textile substrate to apply caffeine formulations.

A given amount of the caffeine formulations used (1% concentration) was applied on the cotton fabric in an exposure area of about 1.86 cm^2. The cotton fabric was dried at room temperature for 3 hours.

All chemicals used were of analytical grade. Methanol (HPLC grade) and distilled water were used for high-performance liquid chromatography (HPLC) analysis.

Methods

Caffeine analysis

Caffeine from the different samples was determined by HPLC using a VWR-Hitachi Elite LaChrom instrument (Darmstadt, Germany). The apparatus is equipped with a L-2130 Pump, an L-2200 Autosampler and a L-2400 UV-Vis Detector working at 271 nm. The system was operated from the software Merck EZChrom Elite v3.1.3. The column used was a LiChrocart 125-4/Lichrosorb RP-18 (5 µm) (Darmstadt, Germany). The mobile phase was a mixture of methanol and water (20:80 v:v) at 1 mL/min flow rate. The caffeine retention time was about 4.5 min.

Permeation experiments

Two experimental protocols were designed to demonstrate the release of caffeine. One, to study its release from the vehicle or the fabric and another to determine the percutaneous absorption profile of the samples containing caffeine. In both cases, permeation experiments were conducted in vertical Franz-type diffusion cells (Lara-Spiral, Courtenon, France), with an exposed surface area of 1.86 cm^2. The penetration cells consist of the upper donor (where the sample is applied) and the lower receptor chamber (filled with fluid mimicking the systemic level) separated by a membrane (nylon or skin).

For the release experiments, a nylon membrane (with a pore of 0.45 µm and a diameter of 47 mm, Millipore Co.) was used as a synthetic barrier which permits without resistance the pass of caffeine from the formulations or from the cotton fabric.

For percutaneous absorption, the skin used was obtained from unboiled back of pigs weighing about 35 Kg. After excised, the skin was dermatomed to a thickness of about 500±50µm with a Dermatome GA630 (Aesculap, Germany). Skin discs of a 2.5 cm inner diameter were prepared which fit into the penetration cells. The receptor fluid was a phosphate-buffered saline (1000-3 Sigma, USA) at pH 7.4 in distilled water with the addition of 0.04% gentamicine sulfate and 4% bovine serum albumin (4).

In this work, the permeation studies have been conducted using two levels of amount of caffeine applied on the nylon membranes or skin surface, respectively, positioned between the donor and the acceptor chambers of a Franz diffusion cell. In the case of formulations, 10µl (equivalent to about 54 $\mu g/cm^2$) were applied both on nylon membrane or skin. When a cotton fabric was impregnated with caffeine formulations, 10 or 100 µl of each formulation (aqueous or liposome based) were applied (equivalent to about 54 or 550 $\mu g/cm^2$, respectively). According to the OECD methodology used (4), the skin penetration studies were performed for 24 h.

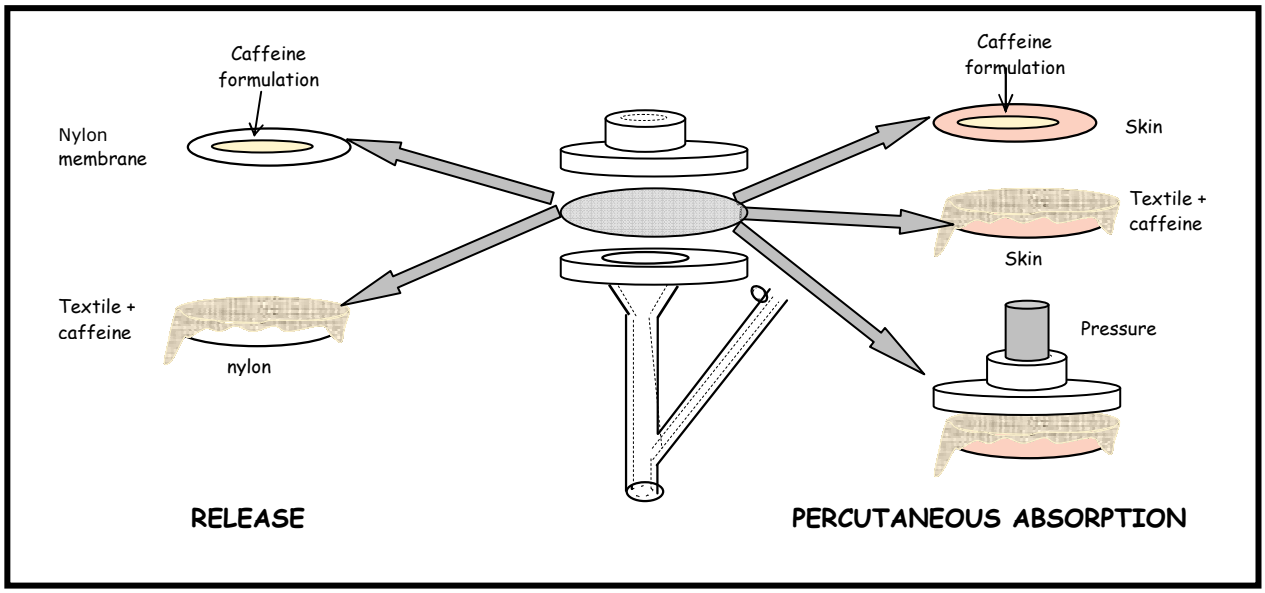

Figure 1: Schematic picture indicating the different permeation experiments performed on release and skin penetration of caffeine.

In all cases, caffeine was extracted from the different samples (formulations, cotton, nylon or skin fractions) using a methanol:water solution (1:1).

In Figure 1, the different experimental possibilities of the permeation studies are indicated both for caffeine release (from the formulation and from the fabric) and for percutaneous absorption of caffeine incorporated in the same samples. When a cotton fabric containing caffeine is applied, either on the nylon membrane or on the skin, a small amount of distilled water (about 20-40µl) was added to get a close contact between the fabric and the membrane (nylon or skin). Also, and in order to increase the contact pressure between cotton and skin, some skin permeation experiments have been carried out by applying a steel cylinder on the textile-skin substrate at a constant pressure according to standard conditions (125 g/cm^2) (8). A thin Teflon disc full of holes has been located as a rigid substrate to avoid an eventual combing effect on textile or on the skin due to the cylinder pressure (see Figure 1). With this device, the real conditions of use when a textile applied on the skin may be better simulated.

RESULTS AND DISCUSSION

Analytical determination of caffeine

As a first step, the amount of caffeine present in both formulations and fabrics has been determined. In Figure 2, a picture of the caffeine peak obtained by HPLC is shown as well as the calibration curve and the corresponding equation. Inter- and intra-day precision was checked in order to confirm the robustness of the analytical methodology used.

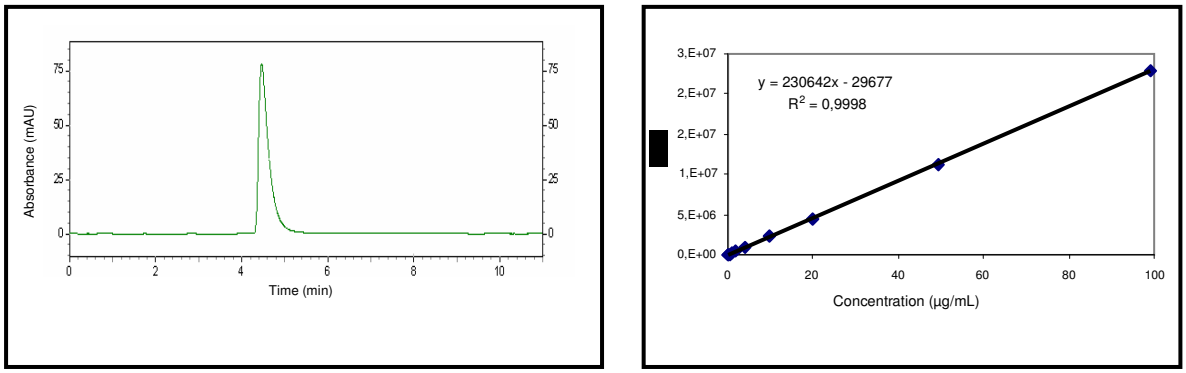

Figure 2: Analytical detection of caffeine by HPLC.

Release of caffeine from formulations and cotton

In order to know the release capacity of caffeine present in the formulations or in the same formulations applied on cotton at the same caffeine concentration (1%), several permeation studies using Franz diffusion cells through a nylon membrane have been carried at different times (from 0.5 to 5 hours).

In Figure 3, the results on membrane diffusion for both formulations, expressed as µg/cm^2, after 2 hours are indicated. Caffeine was evaluated both in the formulations, textiles andin the receptor fluid. As it can be seen, caffeine is released from formulations (aqueous or liposomic) at a very similar high extent indicating a rapid diffusion of the active principle through the membrane (40-45 µg/cm^2).

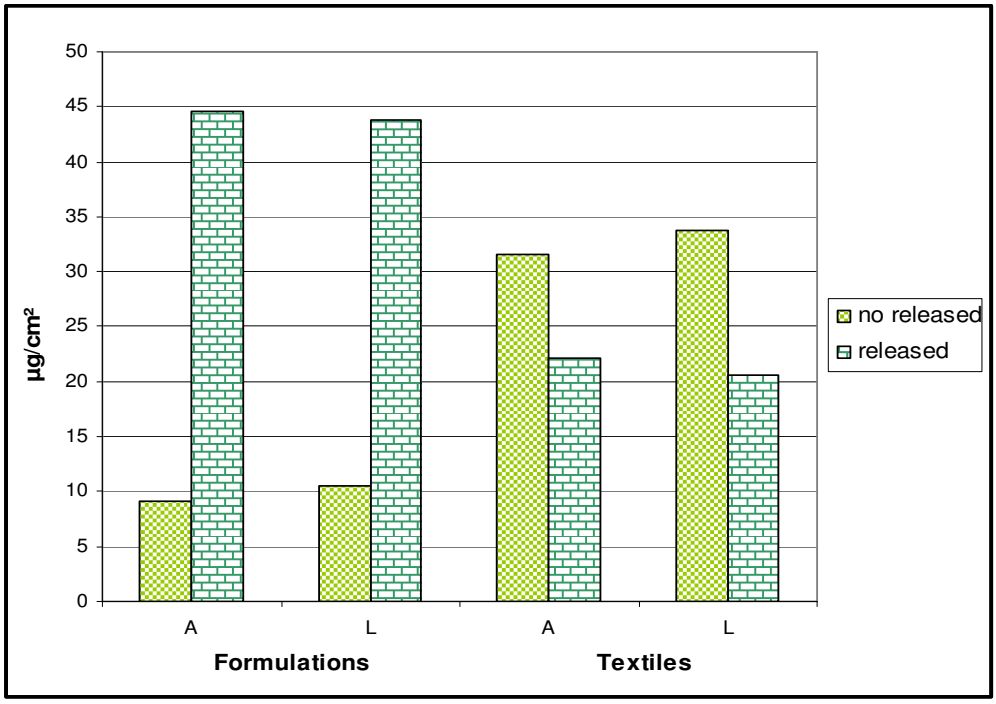

Figure 3: Release levels of caffeine from aqueous (A) and liposomic (L) formulations as well as from the cotton containing the same formulations. Amounts of caffeine applied: About 54 µg/cm^2.

However, in the case of cotton fabric containing caffeine the release values obtained are different. Only about 20-25 μg/cm^2 of the active principle was released through the membrane. Although aqueous and liposomic formulations have a similar behaviour, it seems that, when a liposome vehicle is used, the release of caffeine is slightly retarded. But, it is clear that cotton retains caffeine in the fabric acting as a reservoir of the active principle. A similar trend was observed for the other kinetic times carried out (results not shown).

In order to increase the availability of the caffeine released from the textile to the skin, the skin permeation experiments were performed with an amount of caffeine ten times higher than the one used for the free formulations.

Skin permeation studies of caffeine incorporated in free formulations or applied on cotton

In the application of smart textiles on skin at least two essential principles have to be considered. Continuous skin perspiration, the so called transpidermal water loss (TEWL), takes place, regulated by the skin barrier function mainly located at the stratum corneum, the outermost layer of the skin (9). Additionally, if some occlusion device is covering the skin, as it is the case in the most smart textiles, the TEWL is avoided in a certain amount, contributing, as a consequence, to an increase of the skin hydration and enhancing the skin permeation of a given compound topically applied (10).

Table I: Percutaneous absorption values of caffeine (expressed as μg/cm^2 and % on total amount applied) for aqueous (A) or liposomic (L) formulations as well as for cotton fabrics. Last column corresponds to the skin penetration of caffeine (aqueous solution) applied on cotton maintaining an additional pressure (cylinder).

	Formulation				**Textile**					
	A		**L**		**A**		**L**		**A + Presure**	
	μg/cm^2	**%**	μg/cm^2	**%**	μg/cm^2	**%**	μg/cm^2	**%**	μg/cm^2	%
Total applied	54.79		55.19		547.91		551.91		547.91	
Epidermis	0.32	0.58	0.11	0.20	2.64	0.48	1.21	0.22	30.82	5.63
Dermis	0.39	0.71	0.18	0.33	4.37	0.80	3.04	0.55		
Receptor Fl.	1.15	2.10	0.77	1.40	12.45	2.27	11.31	2.05	10.97	2.00
Perc. Abs. (Ep.+Der.+RF)	1.86	3.39	1.06	1.92	19.46	3.55	15.56	2.82	41.79	7.63

In Table I, the skin absorption values of caffeine (amounts detected in epidermis, dermis and receptor fluid), after an exposure time of 24 hours, for formulations and cotton fabrics containing caffeine (two levels of amount applied) are indicated. Additionally, the skin absorption value of caffeine obtained mimicking the real conditions of use (steel cylinder pressure on the skin) is also indicated. These values confirm that caffeine incorporated in a cotton fabric is able to pass through the different skin layers.

This is an important premise to demonstrate the potential efficacy of a smart textile to be applied on skin. Moreover, as it can be seen in the last column of Table I, it is possible to increase the skin bioavailability of caffeine, especially when an important occlusion of the skin is achieved.

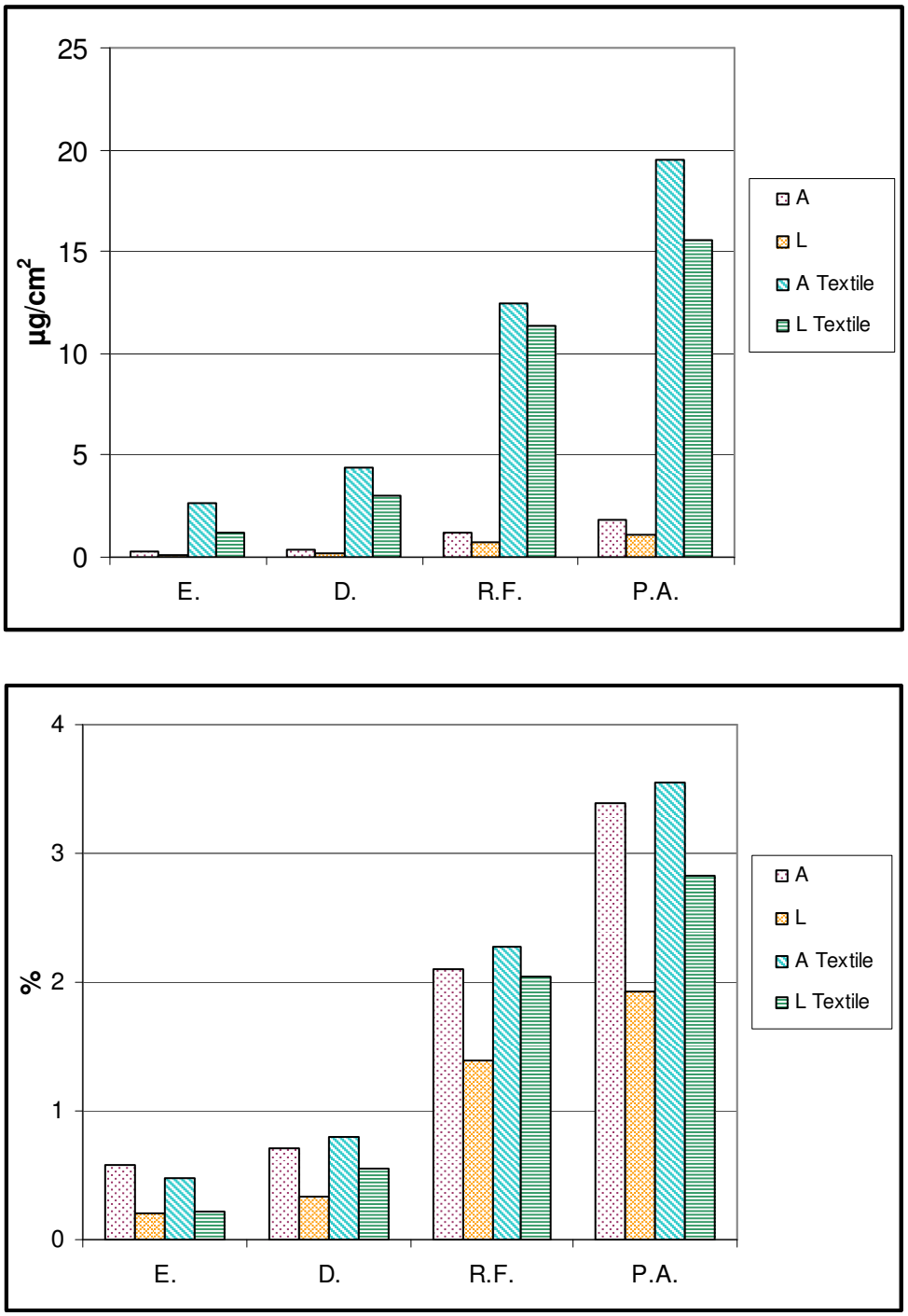

Figure 4: Caffeine content detected in Epidermis (E), Dermis (D) and in the Receptor Fluid (R.F.) as well as the global percutaneous absorption (P.A.) (expressed as µg/cm^2 and % on total amount applied) during the percutaneous absorption of formulations and textiles impregnated with that active principle.

In order to more clearly illustrate the eventual presence of caffeine in the different skin compartments, in Figure 4 the content of caffeine (expressed as µg/cm^2 and % on total amount applied) detected in the different skin compartments as well as the extent of percutaneous absorption are graphically plotted for free formulations and for cotton fabric containing caffeine. Independently of the different amounts of caffeine applied (10 µl for formulations and 100 µl for cotton), three important aspects may be considered.

First, in both cases (formulations and textiles), caffeine is able to reach the epidermis, dermis and even the receptor fluid. Second, when caffeine in an aqueous formulation is applied on cotton, the percentage of percutaneous absorption is similar to that obtained for the free formulation. Third, when a liposomic formulation is used, the percutaneous absorption decreases both for formulations and textiles. It seems that liposomes induce a certain retardation effect during the percutaneous absorption process.

The influence of the application of a pressure during the percutaneous absorption can be appreciated in Figure 5. In that Figure, are indicated the values of caffeine detected when a textile, impregnated with the same amount of an aqueous formulation of the active principle, is applied on the skin, without or with pressure. It can be seen that a close contact of the textile with the skin by applying a given pressure, promotes a higher content of caffeine located mainly in epidermis + dermis compartments.

As a consequence, the percentage of global percutaneous absorption also increases. It is clear that the skin penetration of caffeine is favoured when a pressure is applied on the textile, simulating the real conditions of use.

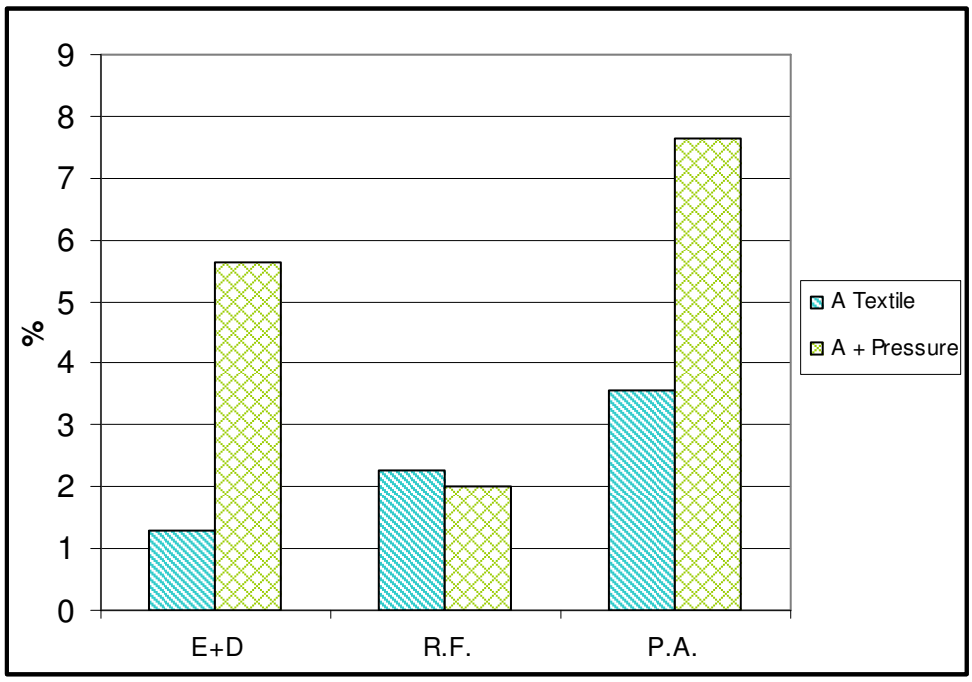

Figure 5: Percutaneous absorption (%) of textiles containing caffeine: Influence of the pressure applied on textile.

This conclusion agrees with the values reported for a bioadhesive transdermal film prepared with a polymeric materials containing caffeine (11). These results indicated that the bioadhesive film gave rise to a higher transdermal permeation compared to a commercial gel and to a saturated solution of caffeine in water. According to those authors, the precise reason for the unusually high percentage permeated from the bioadhesive film is not known, but has also been shown for other active substances, such as lidocaine (12). One possible explanation could be the formation of a saturated or supersaturated solution of caffeine in the film after its application on the skin in the presence of water.

In our opinion, the reservoir capacity of the smart textile, the close contact with the skin and the corresponding skin occlusion, are three main arguments that may explain additionally the availability of caffeine to cross the skin barrier located in the stratum corneum and to occupy the different skin compartments.

It can be concluded that, using this *in vitro* methodology, it is possible to detect the presence of caffeine in the different skin compartments. In our opinion, this is a premise to demonstrate its eventual efficacy when is applied on a textile. However, care must be taken in extrapolating the results obtained in the case of caffeine to many other different compounds applied to smart textiles in order to provide a given benefit to the skin. It is well known that, depending on many physico-chemical characteristics, a chemical compound as well as its vehicle has a different percutaneous absorption profile (13). For that reason, the demonstration of the potential targeting of a given compound requires to confirm, in a case by case basis, its presence in the skin compartments using the corresponding skin permeation methodology.

In conclusion, the use of an *in vitro* specific methodology on percutaneous absorption may be able to prove the pass of caffeine from a smart textile to the inside of the skin. Mimicking the real conditions of use (close contact and a defined pressure), the extent of skin absorption of caffeine may be enhanced.

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