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# Percutaneous release of caffeine from microemulsion, emulsion and gel dosage forms

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**Abstract**

The transport of caffeine to the hypodermis by an alcohol-free o/w microemulsion was investigated and compared with an aqueous gel and an o/w emulsion. The microemulsion was well characterized and *in vitro* diffusion measurements through pig skin having the hypodermis either kept or removed were performed in static Franz cells. The microemulsion allowed delivery of a large fraction of the caffeine in the hypodermis: 23% of caffeine reached the hypodermis after 24 h diffusion, 1.3-fold larger than from the emulsion and gel dosage forms. Half this amount was stored in the hypodermis, the other half continuing its diffusion to the receptor compartment of the Franz cell.


*Keywords:* Microemulsion; Caffeine; Percutaneous penetration; Hypodermis; Topical delivery

## 1. Introduction

Caffeine is widely used in cosmetics as an active substance because of its slimming effect. The cosmetic formulation has to be optimized such that caffeine reaches the active site in the adipocytes located in the hypodermis.

Microemulsions are new vehicles for percutaneous administration that modify drug permeation into the skin but their mechanism of action is still not made clear [1,2]. The lack of a general picture describing the action of microemulsions stems from the large variety of investigated systems, leading to apparently contradictory results, and poor characterization of the microemulsion vehicles. Several mechanisms were hypothesized [1–4]: modification of skin permeability, penetration enhancer action of high amounts of surfactant or cosurfactant, modification of bioavailability of drug because of partitioning between aqueous and oil phases and drug mobility in the vehicle.

The aim of the present study was to evaluate the benefits of a microemulsion formulation for caffeine delivery inside the skin with respect to classical emulsion and hydrogel formulations and to assess the mechanisms.

Classical *in vitro* experiments have often shown that microemulsions increased the transcutaneous transport; but this did not warrant that caffeine reached the target and stayed there a long enough time because the subcutaneous fat was eliminated from the skin sample [5–7]. Therefore, the caffeine penetration was presently measured in each skin layer for skin samples having the hypodermis either kept or removed.

## 2. Material and the methods

### 2.1. Formulations

The chemical compositions of formulations using materials of cosmetic grade are listed in Table 1. The emulsion and microemulsion contained the same lipophilic phase written in italics in Table 1.

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Table 1
Chemical composition of the investigated formulations (the components of the lipophilic phase are in italics)

| Microemulsion (ME) | % | Emulsion (E) | % | Gel (G) | % |
|---|---|---|---|---|---|
| PEG-8 caprylic/capric glycerides *LAS®, Gattefossé (Saint Priest France)* | 13.3 | PEG-6 stearate PEG-32 stearate (HLB 10) *Tefose®1500, Gattefossé (Saint Priest France)* | 5 | Carbomer *Ultrez®10, Gattefossé (Saint Priest, France)* | 0.4 |
| Polyglycerol-6 dioleate *Plurol®Oléique, Gattefossé (Saint Priest France)* | 6.67 | Stearic acid *Cooper (Melun, France)* | 3 | NaOH solution | 0.3 |
| *Isostearyl isostearate ISIS®, Gattefossé (Saint Priest, France)* | 4 | *Isostearyl isostearate ISIS®, Gattefossé (Saint Priest, France)* | 10 | Hydroxypropyl guar *Jaguar®HP 105, Saci (Paris, France)* | 0.3 |
| *Cyclomethicone DC®344, Dow Corning (Seneffe, Belgium)* | 2.3 | *Cyclomethicone DC344®, Dow Corning (Seneffe, Belgium)* | 5.75 | Glycerin *Cooper (Melun, France)* | 5 |
| *Diisopropyl adipate Ceraphyl®230, ISP (Roissy Charles de Gaulle, France)* | 1.6 | *Diisopropyl adipate Ceraphyl®230, ISP (Roissy Charles de Gaulle, France)* | 4 | Propylene glycol *Cooper (Melun, France)* | 2 |
| PPG-5 ceteth-20 *Procetyl®AWS, Croda (Trappes, France)* | 2.0 | Carbomer *Ultrez®10, Gattefossé (Saint Priest, France)* | 0.15 | Citric acid *Cooper (Melun, France)* | 0.05 |
| Phenoxyethanol and Parabens *Seppicide®HB, Seppic (Paris, France)* | 0.6 | Phenoxyethanol and Parabens *Seppicide®HB, Seppic (Paris, France)* | 0.6 | Phenoxyethanol and Parabens *Seppicide®HB, Seppic (Paris, France)* | 0.6 |
| Propylene glycol *Cooper (Melun, France)* | 2 | NaOH solution | 0.3 | | |
| | | Xanthan gum *Rhodicare®D, Saci (Paris, France)* | 0.3 | | |
| Caffeine monohydrated *Sigma (Saint Quentin Fallavier, France)* | 0.8 | Caffeine monohydrated *Sigma (Saint Quentin Fallavier, France)* | 0.8 | Caffeine monohydrated *Sigma (Saint Quentin Fallavier, France)* | 0.8 |
| Purified water | qsp 100 | Purified water | qsp 100 | Purified water | qsp 100 |
| Mean percentage (%) of caffeine as measured by HPLC ($n = 3$) | | | | | |
| $0.803 \pm 0.007$ | | $0.793 \pm 0.003$ | | $0.806 \pm 0.007$ | |
| Mean droplets diameter | | | | | |
| 15.6 nm | | $5.24 \pm 0.08$ μm | | | |

The diameters of the droplets are given in the last line.

For the preparation of emulsions, the aqueous phase (water + thickening agents + caffeine) and the oil phase (lipophilic compounds + emulsifiers) were heated at 70 °C and mixed together with a TurboTest® (Rayneri/VMI, Montaigu, France) at 1000 rpm. After the temperature reached 30 °C, the pH was adjusted at 5.5 with sodium hydroxide.

The gel was prepared by mixing all ingredients except the sodium hydroxide at room temperature with a TurboTest® at 500 rpm. Gelling was achieved by addition of sodium hydroxide up to pH 5.5.

Microemulsions were prepared at pH 5.5 by mixing the components in the same flask under stirring with a magnetic bar.

The partition coefficient of caffeine between the aqueous and lipophilic phases was measured for each vehicle using formulations containing 0.8% of caffeine but free of surfactant. The water and oil phases were mixed together with a magnetic stirrer during 24 h and equilibrated at rest during 24 h. The concentration of caffeine in each phase was measured by HPLC and it was checked that the partition coefficient was identical for the emulsion and microemulsion formulations.

### 2.2. Structural analyses

Granulometry of the emulsion (Table 1) was measured by laser diffraction using a Malvern® MasterSizerX granulometer. The droplet size distribution was obtained using the Fraunhofer optical model.

The structure of microemulsions was determined by small angle neutron scattering measurements performed on the D22 spectrometer at the Institut Laue – Langevin (ILL) European facility at Grenoble (France) [http://www.ill.fr/YellowBook/D22]. The samples contained in quartz cuvettes of 1 mm thickness were measured according to standard procedures available at the ILL and their structure was assessed from comparison with classical structural models [8]. Microemulsions have been prepared with the same chemical composition as used for percutaneous penetration experiments, but normal water was substituted by deuterated water.

### 2.3. Transepidermal water loss (TEWL)

TEWL measurements were performed in triplicate on skin pieces at the beginning of each transcutaneous

experiment and at the end of a 24 h exposure in the Franz cell using a Skin®Station (La Licorne, Meylan, France). Skin samples having TEWL levels larger than 100 g $m^{-2}$ $h^{-1}$ were discarded.

### 2.4. In vitro penetration study: Franz cell diffusion

Full-thickness pig female or male skin was obtained from the local slaughterhouse. The skin was cleaned up with tap water. The skin surface was shortly washed with 1% SDS (Sodium Dodecyl Sulfate) aqueous solution, rinsed with tap and permuted water, blotted with soft household paper and then stored at −20 °C. The skin samples having the hypodermis kept were sliced with a dermatome to a thickness of 4 mm; therefore 3 mm hypodermis was retained.

Before use, the skin samples were stored at 19 °C in a 100% relative humidity chamber. The thickness of each section was measured with a micrometer (Mitutoyo). The skin pieces were mounted between the halves of vertical Franz-type diffusion cells, the *stratum corneum* facing the donor chamber. The area available for diffusion was 2.54 $cm^2$. One gram of each formulation (i.e. 8 mg caffeine) was spread on the skin surface; this corresponded to infinite dose conditions.

The dermal side was exposed to phosphate-buffered saline (PBS, pH 7.4) receptor fluid which was continuously stirred. The diffusion cells were immersed in a water bath that maintained a constant temperature of the receptor fluid (9 mL) at 32 ± 1 °C.

The diffusion experiments were conducted in six replicates over 24 h. At each measurement time, the whole receptor compartment was emptied out, washed four times with PBS and refilled by fresh medium. The collected samples were filtered and analyzed by HPLC. After 24 h exposure, the content of the donor compartment was washed 10 times with fresh receptor liquid and collected in the same tip. The epidermis was separated from the dermis by heat treatment (45 s in hot water). After separation, the epidermis and dermis or (dermis + hypodermis) were cut into small pieces with a scalpel, collected in a vial with acetonitrile to extract caffeine, filtered and analyzed.

### 2.5. Analysis of caffeine content

Samples were analyzed for caffeine using a HPLC setup from Waters (St Quentin-en-Yvelines, France) composed of a Waters 717 injector, a Waters 600 pump, a reverse phase column XTerra® RP8 (4.6 × 250 mm–5 μm,) and a Waters 2996 photodiode array UV detector working at 271 nm wavelength. The elution with water/acetonitrile/acetic acid (85:15:1 v/v, pH 2.5) solvent at 1 mL/min flow rate and 35 °C gave a retention time of 5.0 min for caffeine. The calibration curve for quantitative analysis was linear up to 40 μg/mL.

### 2.6. Data analysis

Cumulative amounts of caffeine (g/$m^2$) permeating through the skin were corrected to account for the previous sample removal and plotted against time. Permeability constant ($P_S$) and lag time values were calculated using the pseudo steady-state slopes from plots of cumulative penetration vs time.

The mean and standard deviation (SD) of $n = 6$ determinations were calculated. Statistical comparisons were made using the Student's *t*-test (two-sample assuming equal variances) and analysis of variance (ANOVA, single factor) with the level of significance at $p \leqslant 0.05$.

## 3. Results and discussion

### 3.1. The choice of the microemulsion formulation and characterization

The composition of an o/w globular microemulsion (Table 1) was adapted from the formulation "F" reported by Kreilgaard et al. containing isostearyl isostearate as the oil phase and Plurol isostearique® and Labrasol® as surfactants [3]. The total amount of each surfactant was half that of Kreilgaard, which was possible because the isostearyl isostearate was replaced by a mixture of isostearyl isostearate, cyclomethicone and diisopropyl adipate.

Therefore, the formulations contained the same caffeine amount and the same oil phases were such that the partition coefficients of caffeine were identical in the emulsion and the microemulsion (respectively, 0.048 and 0.045).

The structure of microemulsions was assessed by small angle neutron scattering. Only the oil-in-water model of polydisperse spherical droplets [8] could fit to the experimental data, giving the mean droplet radius $R = 7.8$ nm (Table 1), the radius of the inner oil core $R_{oil} = 6.9$ nm and the variance of the size distribution $\sigma^2 = 20$ Å$^2$ ($\sigma/R < 5\%$).

### 3.2. Permeation of caffeine through the skin

The cumulative amount of caffeine (g $m^{-2}$) plotted against time showed a diffusion lag time and a linear increase (Fig. 1). The extrapolation of the linear part to the time axis gave the lag time and the slope yielded the pseudo steady-state flux $J_{SS}$ (g $m^{-2}$ $h^{-1}$) (Table 2). The permeability coefficient $P_S$ (m $s^{-1}$) was calculated as $P_S = J_{SS}/C$ ($C$ is the concentration in the donor compartment). Whatever the experiment conditions, the higher flux (and permeability) values showed that the microemulsion significantly increased the caffeine permeation rate with respect to the gel or emulsion.

Since the partition coefficient between both phases was kept constant for the emulsion and the microemulsion in the present study, the faster caffeine permeation with the microemulsion resulted from the combination of several accelerating phenomena as proposed in the literature [1,2].

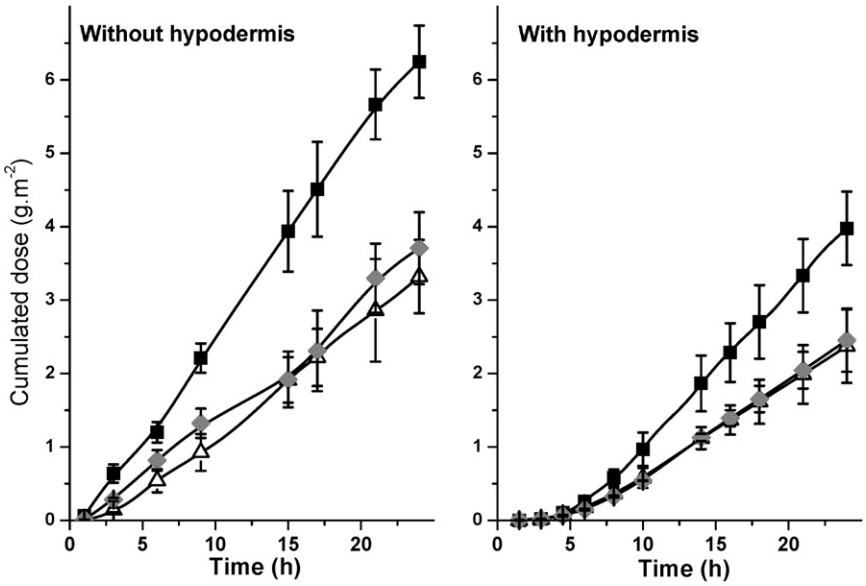


Fig. 1. Permeation of caffeine (g/m$^2$) across the skin as a function of time ($n = 6$). Microemulsion, (■); emulsion, (△); gel, (♦).

Table 2
Pseudo steady-state fluxes of caffeine (0.8% w/w) through pig skin from the three formulations (mean ± SD of $n = 6$ determinations)

| Formulation | Lag time (h) | Flux, $J_{SS}$ (g m$^{-2}$ h$^{-1}$) | Permeability coefficient, $P_S$ (m s$^{-1}$) |
|---|---|---|---|
| *Without hypodermis* | | | |
| Caffeine solution | 1.30 ± 0.01 | 0.141 ± 0.009 | $3.03 \times 10^{-10}$ |
| Microemulsion | 1.31 ± 0.18 | 0.288 ± 0.002 | $9.97 \times 10^{-9}$ |
| Emulsion | 2.78 ± 0.26 | 0.156 ± 0.008 | $5.42 \times 10^{-9}$ |
| Gel | 2.36 ± 0.20 | 0.171 ± 0.006 | $5.94 \times 10^{-9}$ |
| *With hypodermis* | | | |
| Microemulsion | 5.09 ± 0.10 | 0.210 ± 0.008 | $7.28 \times 10^{-9}$ |
| Emulsion | 4.97 ± 0.35 | 0.124 ± 0.004 | $4.31 \times 10^{-9}$ |
| Gel | 5.37 ± 0.13 | 0.131 ± 0.002 | $4.53 \times 10^{-9}$ |

One hypothesis is the penetration enhancer effect of the surfactants which could dissolve part of the intercorneocyte lipids and disrupt the *stratum corneum*. The transepidermal water loss (TEWL) before and after contacting the microemulsion is an indicator of the skin permeability change. The TEWL did not display any significant variation over the full experiment (Table 3). The microemulsion did not alter the skin barrier against water loss, suggesting that the enhancer effect of surfactant was not a dominant factor.

The lag time did not vary in accordance with the steady-state flux. A rough expectation was a short lag time in case of fast diffusion, which was the case when comparing the microemulsion and the emulsion. But the lag times of the microemulsion and reference caffeine solution were identical, whereas the flux was higher for the microemulsion. The variations of the lag time with respect to the formulation vanished when the hypodermis was present. The lag time reflects a complex sequence of events including the release of the drug from its vehicle, the reorganization of the skin barriers and the diffusion of caffeine through this time-varying medium.

Table 3
Transepidermal water loss, TEWL, of the skin samples before starting the percutaneous penetration ($t = 0$) and at the end of the experiment ($t = 24$ h) (mean ± SD of $n = 3$ determinations)

| Time, $t$ (h) | TEWL (g m$^{-2}$ h$^{-1}$) | | |
|---|---|---|---|
| | Microemulsion | Emulsion | Gel |
| 0 | 63 ± 12 | 76 ± 13 | 60 ± 9 |
| 24 | 70 ± 10 | 78 ± 10 | 79 ± 10 |

### 3.3. Caffeine distribution within the skin layers after 24 h exposure

The caffeine distribution in the different layers of the skin was measured after 24 h exposure.

The total amount that has penetrated the skin is the sum of the amounts found in the skin and receptor compartment (Table 4). In accordance with the faster permeation rate from the microemulsion, the ANOVA test marked with arrows in Fig. 2 revealed significant differences of total penetration between the microemulsion (25%) and either emulsion or gel (15–20%). The presence of the hypodermis did not significantly influence the total amount of penetrated material, suggesting that the *stratum corneum* was

Table 4
Distribution of caffeine in each skin layer after 24 h (mean ± SD of $n = 6$ determinations)

| | Caffeine amount (% of applied dose) | | | | | |
|---|---|---|---|---|---|---|
| | Microemulsion | | Emulsion | | Gel | |
| Epidermis | $0.27 \pm 0.01^{*}$ | $0.68 \pm 0.06^{**}$ | $0.37 \pm 0.05^{*}$ | $0.62 \pm 0.02^{**}$ | $0.31 \pm 0.03^{*}$ | $0.51 \pm 0.09^{**}$ |
| Dermis | $1.48 \pm 0.10^{*}$ | | $0.92 \pm 0.05^{*}$ | | $1.06 \pm 0.2^{*}$ | |
| Dermis + hypodermis | | $10.35 \pm 1.3^{**}$ | | $7.8 \pm 1.5^{**}$ | | $7.95 \pm 2.3^{**}$ |
| Receptor liquid compartment | $25.3 \pm 1.6^{*}$ | $12.4 \pm 2^{**}$ | $13.3 \pm 1.5^{*}$ | $7.8 \pm 0.8^{**}$ | $17.7 \pm 1.6^{*}$ | $7.24 \pm 2.1^{**}$ |
| Total | $27.0 \pm 1.7^{*}$ | $23.4 \pm 3^{**}$ | $14.6 \pm 1.6^{*}$ | $16 \pm 2^{**}$ | $19.1 \pm 1.7^{*}$ | $15.7 \pm 2.5^{**}$ |

* Without hypodermis.
** With 3 mm thick hypodermis.

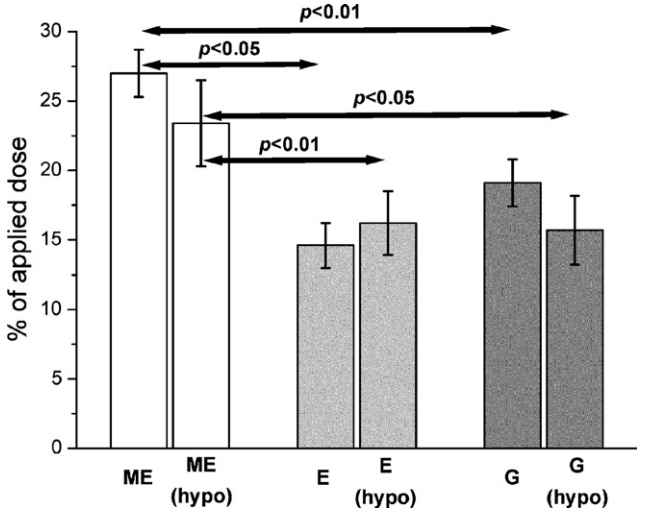


Fig. 2. Total amount of caffeine which has been delivered to the skin (epidermis + dermis + receptor fluid) after 24 h. Bars are the % of applied dose (mean of $n = 6$) and the errors bars are the standard deviations. Microemulsion, ME; gel, G; emulsion, E. "Hypo" indicates the presence of hypodermis. Arrows show the differences that were estimated significant according to the ANOVA test.

the rate limiting barrier against penetration. But the repartition of the stored caffeine within the different skin layers differed according to the presence of the hypodermis (Fig. 3).

Experiments conducted on skin samples without the hypodermis allowed estimating the potential of each vehicle to pass the *stratum corneum*. The major part of caffeine has permeated to the receptor compartment and the permeation was significantly higher from microemulsion. The same trend was observed in the dermis (1.5% for the microemulsion against 0.9% for the emulsion and gel). The caffeine amounts stored in the epidermis were not statistically different.

Experiment with full-thickness skin samples having 3 mm of hypodermis evaluated the potential of each vehicle to accumulate caffeine in the hypodermis where caffeine could exert its lipolytic effect. The repartition of caffeine in the skin compartments depended on the presence of the hypodermis. After 24 h exposure, caffeine was shared one-half in the (dermis + hypodermis) layer and one-half in the receptor compartment, independently of the type of formulation. The fraction of applied dose in the hypo-

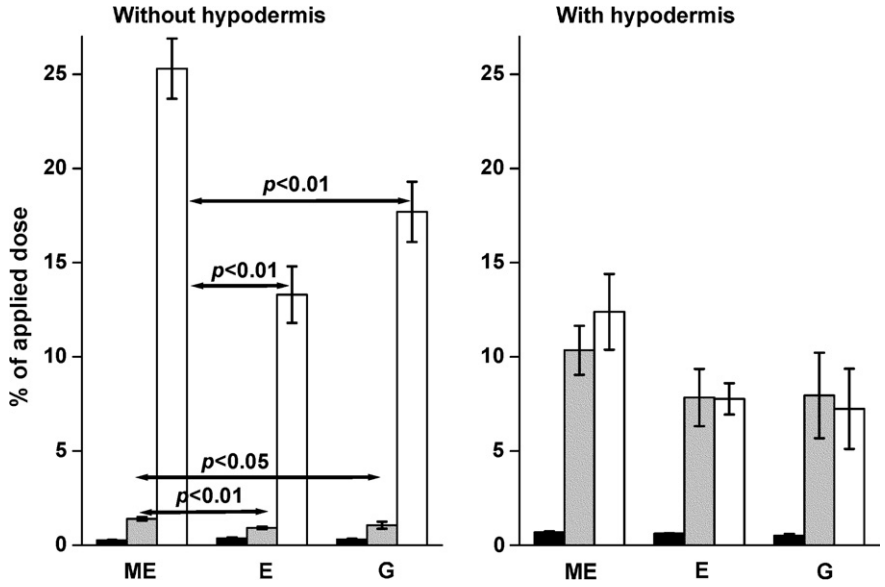


Fig. 3. Distribution of caffeine (% of applied dose) in the epidermis (black), dermis or (dermis + hypodermis) (grey) and receptor fluid (white) after 24 h. Bars are the % of applied dose (mean of $n = 6$) and the errors bars are the standard deviations. Microemulsion, ME; emulsion, E; gel, G. Arrows show the differences that were estimated significant according to the ANOVA test.

dermis and receptor compartment increased with the performance of the dosage form (microemulsion > emulsion and gel). The repartition of caffeine between both compartments was identical for every formulation.

Hypodermis offered a resistance to the drug permeation and significantly slowed down the release rates by a 1.3 factor for every vehicle. This was an obvious effect of the presence of hypodermis as a supplementary barrier in permeation experiments. More interestingly, the hypodermis lipophilic barrier retained the caffeine inside the top hydrophilic layers of the skin, namely the dermis and epidermis. The presence of the hypodermis had an influence upstream of the diffusion flux.

This study demonstrated that a significant increase in the caffeine penetration flux was obtained by applying a liquid microemulsion to the skin.

The microscopic origin of the fast release from microemulsions has been cleared up a bit. The partition of caffeine between the aqueous and oil phases cannot account for the effects since the partition coefficient was constant in the present experiments. The penetration enhancer action of the large amount of surfactant present in the microemulsion is the first mechanism that everyone thinks about but several results in the literature discard this mechanism [1,9]. Measurements of the transepidermal water loss as an indicator of alteration of the *stratum corneum* have shown that this latter barrier remained intact. Non-ionic low irritant surfactants presently used are indeed thought to keep the barrier properties of the skin.

The presence of the hypodermis beneath the dermis influenced the transport in the top layers of the skin. The hypodermis retained one-half of the caffeine which has permeated allowing it to exert its lipolytic effect.

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