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Pickering w/o emulsions: Drug release and topical delivery

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ABSTRACT

The skin absorption from Pickering emulsions as a new dosage form was investigated for the first time. Pickering emulsions are stabilized by adsorbed solid particles instead of emulsifier molecules. They are promising dosage forms that significantly differ from classical emulsions within several features. The skin permeation of a hydrophilic model penetrant (caffeine) was investigated from a w/o Pickering emulsion and compared to a w/o classical emulsion stabilized with an emulsifier. Both emulsions had the same composition and physicochemical properties in order to focus on the effect of the interfacial layer on the drug release and skin absorption processes. The highest permeation rates were obtained from the Pickering emulsion with a pseudo-steady state flux of 25 $\mu g\,cm^{-2}\,h^{-1}$, threefold higher than from a classical emulsion (9.7 $\mu g\,cm^{-2}\,h^{-1}$). After 24 h exposure, caffeine was mostly in the receptor fluid and in the dermis; cumulated amounts of caffeine were higher for the Pickering emulsion. Several physicochemical phenomena were investigated for clearing up the mechanisms of enhanced permeation from the Pickering emulsion. Among them, higher adhesion of Pickering emulsion droplets to skin surface was disclosed. The transport of caffeine adsorbed on silica particles was also considered relevant since skin stripping showed that aggregates of silica particles entered deeply the *stratum corneum*.

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1. Introduction

Pickering emulsions are attractive as new dosage forms because the classical emulsifier that stabilizes the oil droplets is replaced by solid particles (Ramsden, 1903; Pickering, 1907; Binks and Horozov, 2006; Aveyard et al., 2003). Therefore, Pickering emulsions are surfactant-free emulsions. Besides, Pickering emulsions show interesting properties that diverge from classical emulsions. The very high stabilization against coalescence allows keeping the droplet under severe conditions such as high concentrations of dispersed phase, presence of electrolytes and even they can be safely dried and redispersed (Aveyard et al., 2003). A second point is the formation of a dense shell of solid particles around the droplets that act as a barrier against materials transfer (Prestidge and Simovic, 2006). Therefore, Pickering emulsions can be viewed as capsules that could be used for a controlled delivery purpose (Simovic and Prestidge, 2007).

In spite of several claims of applications of Pickering emulsions to cosmetic formulation (Collin and Candau, 1997; Schonrock et al., 1998; Gers-Barlag and Müller, 2004), no skin absorption using Pickering emulsions as vehicles have been reported so far. We presently report an investigation of skin penetration of a hydrophilic model active substance (caffeine) encapsulated in a w/o Pickering emulsion and compare the penetration to that given by a classical emulsion stabilized by surfactant. To our knowledge, this is the first time that such evaluation of Pickering emulsions is reported.

The stability of Pickering emulsions is ensured by adsorption of colloidal particles at the oil/water interface (Binks and Horozov, 2006; Aveyard et al., 2003; Arditty et al., 2004; Kruglyakov and Nushtayeva, 2004). It is dependent on the partial wetting of particles by both phases and the silica particles give a large possibility of choice by the surface modification (Binks and Lumsdon, 2000). Since the emulsion droplets are surrounded by solid particles and their adsorption energy is high, the stability of Pickering emulsions is usually higher than for emulsifier-stabilized emulsions (Barthel et al., 2003). The silica particles adsorbed at the oil/water interface may form a densely packed and rigid shell-like structure. Under these conditions, the *in vitro* release profile of drugs from such systems could be strongly modified in comparison to emulsifier-stabilized emulsions. Simovic and Prestidge (2007) established

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correlations between the interfacial nanoparticle layer structure at the surface of emulsion droplets and release properties of a lipophilic molecule (dibutylphtalate).

The permeation of a solute from an emulsion in the skin is dependent upon a variety of physicochemical factors including the physical nature of the interfacial film, pH, the viscosity of the continuous phase, the droplet size distribution, the oil/water ratio and temperature (Hadgraft, 2001; Walters, 2002; Welin-Berger et al., 2001; Clément et al., 2000; Spernath et al., 2008).

Therefore, an attempt was made to isolate the effect of the silica layer on the release and skin absorption of caffeine from Pickering emulsions. This was achieved by formulating two identical caffeine-loaded w/o emulsions (same composition, same droplet size, same volume fraction of dispersed phase, same viscosity) differing only by the nature of the stabilizing agent. Thus, both emulsions had different oil/water interfacial layer properties.

2. Materials and methods

2.1. Materials

Hydrophobic silica HDK® H20 was a gift from Wacker Chemie (Germany). This silica contains 1.1% of carbon coming from surface modification with dimethylsilyl groups, 55% of free hydroxyl groups at the surface; it has a BET specific area of 170 $m^2\,g^{-1}$, according to the producer's data. Emulsifier Abil® EM97 (bis-PEG/PPG-14/14 Dimethicone and Cyclopentasiloxane) and Abil® Wax 9810 (C24-28 Alkyl Methicone) were from Evonik (France). Cyclomethicone oils DC®245 and DC®246 were supplied by Dow Corning (Germany). Preservative Seppicide® HB (phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben) was a gift from Seppic (France). Caffeine (HPLC grade) was purchased from Sigma–Aldrich (France). Deionized water of 18 $M\Omega\,cm^{-1}$ resistivity was used. Sodium chloride, acetonitrile (HPLC grade) and acetic acid were purchased from Carlo Erba (Italy) and used as received.

Full-thickness pig skin (1.35 ± 0.05 mm; mean ± S.E.) was used in the skin absorption experiments. The skin from the flank of three donor animals was washed and excised, the subcutaneous fatty tissue was carefully removed and the tissue was stored flat at −20 °C until use. All experiments on excised porcine skin were performed according to the recent guidelines (OECD, 2004).

2.2. Preparation of emulsions

Two w/o emulsions were prepared: a Pickering emulsion and an emulsion stabilized with an emulsifier (classical emulsion). The oil/water ratio for both emulsions was 50:50 (w/w). The compositions of the emulsions used for skin absorption studies are presented in Table 1.

2.2.1. Pickering emulsion

The type of emulsion (o/w or w/o) depends on wettability properties of particles (Aveyard et al., 2003). Water-wet particles tend to stabilize o/w emulsions and oil-wet particles tend to stabilize w/o emulsions. Therefore, the silica was first dispersed in the silicone oil using an ultrasound disperser Sonics VibraCell (BioBlock Scientific, France) at 500 W during 30 s. The oil and aqueous phases were mixed together with an UltraTurrax® device (Germany) at 22,000 rpm during 2 min (cold process) and a w/o emulsion was obtained. The silica content of emulsions was 1%.

2.2.2. Classical emulsion

The aqueous and oil phases were heated up to 70 °C. The emulsion was prepared by slow addition of the internal phase to the external phase under continuous stirring with an UltraTurrax® (Germany) at 11,000 rpm during 2 min. The emulsion was thereafter stirred with a TurboTest® (Rayneri/VMI, Montaigu, France) at 1000 rpm and maintained at 300 rpm during further 30 min until the temperature reached 25 °C.

Table 1
Composition (%) and physicochemical parameters (mean ± S.E., $n = 3$) of the Pickering emulsion and the corresponding classical emulsion.

	Pickering emulsion	Classical emulsion
Ingredient		
Hydrophobic silica HDK H20	1	–
Abil® EM 97	–	0.1
Abil® Wax 9810	–	10
Cyclomethicones DC®245:DC®246 = 1:1	48.2	39.1
Preservative	0.8	0.8
Caffeine	*0.8*	*0.8*
Water	*49.2*	*49.2*
Parameter		
Viscosity (mPa s)	550 ± 50	546 ± 50
Mean droplet size (μm)	9.7 ± 0.5	10.9 ± 0.5
Width of the size distribution (μm)	4.5 ± 0.5	4.5 ± 0.5
Caffeine content (%) measured by HPLC	0.891 ± 0.003	0.886 ± 0.005

The components of the aqueous phase are given in italics.

The w/o type of both emulsions was checked by the "dilution method" and electrical conductivity measurements. A drop of oil mixed very fast within the emulsion when it was deposited on top of the emulsion sample; conversely a drop of water felt to the bottom of the emulsion without apparent mixing, showing that oil was the continuous phase. The electrical conductivity of the emulsion (measured with a Radiometer CDM 83 conductivity meter) was very low (0.006 $\mu S\,cm^{-1}$) and close to that of pure oil, indicating again oil as the continuous phase.

2.3. Caffeine partition coefficient

The usual procedure to measure the partition coefficient of solute between the emulsions phases consists in the preparation of emulsion followed by the phases' separation by ultracentrifugation. In the case of Pickering emulsion, it was not possible to separate the phases (data not shown). Therefore, the partition coefficient P of caffeine between the aqueous and lipophilic phases was measured without stabilizing agents (silica particles for Pickering emulsion and emulsifier for classical emulsion). For each system the water and oil phases mixed with a magnetic stirrer in a closed glass flask during 24 h at 32 °C. The dispersions were equilibrated during 24 h and left 1 h at rest for phase separation. The concentration of caffeine in each phase was measured by HPLC. The log P was calculated according to $\log P = \log (C_o/C_w)$, where C_o is the caffeine concentration in the oil phase and C_w the caffeine concentration in the aqueous phase.

2.4. Particle size measurements

The size of silica particles suspended in silicone oil was measured by dynamic light scattering (NanoZS, Malvern, UK). The refractive indexes of the dispersed and dispersing phase were 1.46 for silica particles and 1.397 for silicone oil; the viscosity of the oil phase was 5.7 mPa s. Emulsion droplet size measurements were performed using a Leica® DM LM (Germany) optical microscope equipped with a video camera. The size distribution was determined using the image analysis software AnalySIS®. Pickering emulsions granulometry was also performed by small angle light scattering (MasterSizer® 2000, Malvern, UK). The refractive

indices used in the Mie optical model were: 1.46 for silica particles and 1.397 for silicone oil.

2.5. Viscosity measurements

The viscosity was measured at 20 °C using a Couette rheometer Rheomat R180 (Lamy, France) equipped with a mobile system No. 11 rotating at 200 s^{-1} speed.

2.6. TransEpidermal water loss (TEWL)

TEWL was measured using a Skin® Station (La Licorne, Meylan, France). The measurements were performed in triplicate on skin pieces just before performing the skin absorption studies (OECD, 2004). The skin samples with TEWL higher than 15 g m^{-2} h^{-1} were discarded.

2.7. In vitro release studies

Caffeine release rates were studied using the method previously described by Fokkens and de Blaey (1984) and Wissing and Muller (2002). The rate of transfer from the w/o emulsion to an aqueous phase called receptor fluid was measured. 12 mL of 0.9% NaCl aqueous solution (receptor fluid) was gently stirred with a magnetic bar rotating at 100 rpm; 1 g of emulsion was spread uniformly on the receptor fluid surface (2.54 cm^2). Stirring did not cause mixing of emulsion and receptor fluid. The full glassware device was thermostated at 32 °C. At each time of the kinetic experiment (15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 16 h, 18 h, 20 h, 22 h, 24 h), 2 mL of receptor fluid were collected and replaced by 2 mL of fresh medium. The caffeine concentration was determined by HPLC. The concentration of caffeine was such that sink conditions were fulfilled.

2.8. In vitro skin absorption studies

The *in vitro* transdermal delivery of caffeine was determined using Franz-type diffusion cells containing full-thickness porcine skin (1.35 ± 0.05 mm; mean ± S.E.) according to classical methods (Bronaugh and Maibach, 1999, 2001) and OECD (2004) guidelines. Pig skin was mounted in the two-chamber glass diffusion cell. The effective penetration area was 2.54 cm^2; the receptor compartment contained 10 mL of 0.9% NaCl aqueous solution. The solubility of caffeine was 25.82 mg mL^{-1} in water at 32 °C (Dias et al., 1999) and 25.16 mg mL^{-1} in 0.9% sodium chloride aqueous solution at 32 °C (Al-Maaieh and Flanagan, 2002). Sink conditions were thus fulfilled.

The cells were placed in a water bath at 37 °C providing a skin surface temperature of 32 °C because of heat loss. The membrane was equilibrated with the receptor fluid for 0.5 h and 1 g of emulsion (which corresponded to 3000 μg cm^{-2} of caffeine) was spread uniformly on the skin surface with a spatula. The receptor phase was withdrawn at 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 16 h, 18 h, 20 h, 22 h and 24 h exposure times and replaced with fresh medium. The collected samples were filtered and analyzed by HPLC. The replication for each experiment was $n = 6$. After 24 h exposure, the cells were dismantled and the distribution of caffeine was measured in the donor compartment and the different skin layers. The remaining emulsion in the donor compartment was recovered with a spatula, the caffeine was extracted with 0.9% sodium chloride aqueous solution, and the samples were filtered and analyzed by HPLC. The epidermis was separated from the dermis by heat treatment (45 s in water at 60 °C). After separation, the epidermis and dermis were cut into pieces with a scalpel, caffeine was extracted with 0.9% sodium chloride aqueous solution, and the samples were filtered and analyzed by HPLC.

2.9. HPLC analysis of caffeine content

The samples were analyzed for caffeine using high-pressure liquid chromatography with a reverse phase column. The HPLC set up from Waters (St. Quentin en Yvelines, France) was composed of a Waters 717 injector, a Waters 600 pump, a reverse phase column XTerra® RP8 (5 μm, 4.6 mm × 250 mm) and a Waters 2996 photodiode array UV detector working at 271 nm wavelength. The elution with water/acetonitrile/acetic acid (85:15:1) solvent at 1 mL min^{-1} flow rate and 35 °C gave a retention time of 5.5 min for caffeine. The calibration curve for quantitative analysis was linear up to 40 μg mL^{-1}.

2.10. Data analysis

The cumulated quantity of caffeine (μg cm^{-2}) permeating through the skin was plotted against time. The steady-state flux (J_{SS}) was estimated from the slope of the linear part of the permeation profile ($R^2 \geq 0.97$). The mean and standard error (S.E.) of $n = 6$ samples were calculated. Statistical analysis was performed using the Student's *t*-test, analysis of variance (ANOVA) and the differences were considered significant when $p \leq 0.05$.

2.11. Skin stripping and scanning electron microscopy (SEM) observations

Thin layers of the *stratum corneum* obtained by the skin stripping method were observed by SEM after 24 h exposure to Pickering emulsion. This was performed on three skin samples, different from those used for skin absorption studies. The skin samples were exposed to Pickering emulsion for 24 h in Franz cells. The Franz cells were dismantled, the emulsion was removed from the top of the skin by scraping, and the *stratum corneum* of the treated area was removed by 19 successive tape-stripping (Clarys et al., 2001; Weigmann et al., 2001; Jimenez et al., 2004) using D-Squame® adhesive tapes (diameter 22 mm) (Monaderm, Monaco). The stripes were stuck to a double-adhesive tape previously adhered to SEM aluminum stubs and sputter-coated with a thin gold/palladium layer using a cathodic pulverizer, Hummer II Technics (6 V, 10 mA). SEM images of all *stratum corneum* sheets were obtained using a Hitachi S800 (Japan) microscope working at 15 kV acceleration voltage ("Centre Technologique des Microstructures", CTμ, of the University of Lyon, Villeurbanne, France). SEM images of non-exposed skin were also taken as control.

Observations of silica suspensions in oil were also performed under similar conditions by directly spreading the silica suspensions on SEM aluminum stub.

2.12. Interfacial tension and contact angle measurements

The contact angles and interfacial tension were measured using a Drop Shape Analysis System DSA10 Mk2 (Krüss GmbH, Germany). The interfacial tension between deionized water and cyclomethicone oil was measured by the pendant drop method. Pictures of a pendant drop of water immersed in a cuvette containing cyclomethicone were taken and the shape of the drop was analyzed for the interfacial tension. The densities used for the calculation were 0.9982 g cm^{-3} for water, 0.958 g cm^{-3} for cyclomethicone and 0.9593 g cm^{-3} for cyclomethicone containing silica particles. The adsorption kinetics of silica particles and emulsifier molecules at cyclomethicone/water interface were measured using time-resolved pendant drop experiments. The oil contained 0.2 wt% of Abil® EM97 emulsifier or 0.1 wt% of HDK® H20 silica particles. Interfacial tension was tracked over 24 h at 20 °C. The contact angle between water, cyclomethicone and pig skin was determined by

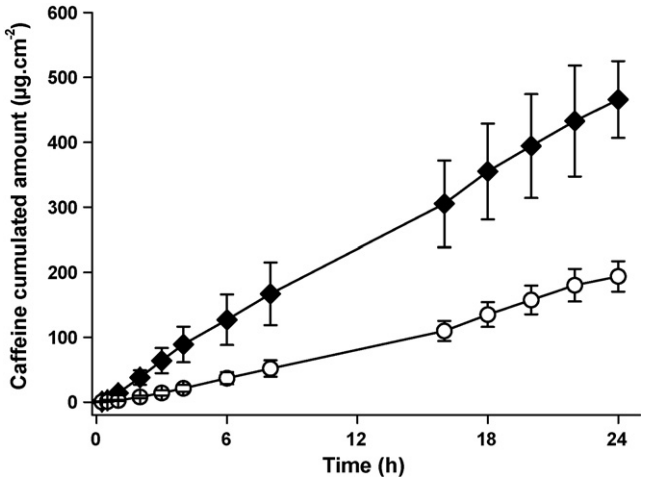

Fig. 1. Permeation profile of caffeine ($\mu g\,cm^{-2}$) from Pickering emulsion (♦, $n = 6$) and classical emulsion (○, $n = 6$) over 24 h. Each point represents the mean ± S.E. of six determinations.

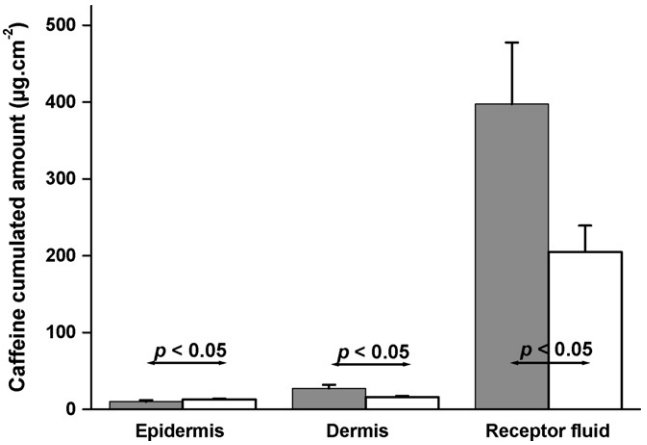

Fig. 2. Distribution of caffeine in the skin layers from both emulsions in the excised pig skin after 24 h. Grey bars: Pickering emulsion and white bars: classical emulsion. Mean ± S.E., $n = 6$.

the sessile drop method. Samples of full-thickness pig skin stuck on glass slides were immersed in the oil phase. A drop of water (volume 5–6 μL) was carefully deposited on the skin surface with a syringe. The drop shape was recorded with a high-speed framing camera and analyzed for the contact angle. The contact angle was defined as the angle between the skin surface and the tangent to the surface of the water drop. Each experiment was repeated in triplicate.

3. Results and discussion

3.1. Skin penetration experiments

Comparative experiments were performed with Pickering emulsions and classical emulsions stabilized with the Abil® EM97 surfactant. Compositions are given in Table 1. The formulations were carefully adjusted such that the properties of both emulsions were identical, in particular the droplet size and the viscosity (Table 1). Cumulated amounts of released caffeine per unit area of skin were calculated from the permeation experiments (Fig. 1). The release profiles were very classical in shape. They started with a lag time when no caffeine was detected in the receptor compartment. A linear increase of cumulated caffeine amount was observed after the lag time. Steady-state fluxes of caffeine were calculated from the slopes of the linear part of the release profiles.

The steady-state flux of caffeine was threefold higher for Pickering emulsion than for the classical emulsion (Table 2) and the lag time two times shorter for Pickering emulsion (0.5 h) compared to classical emulsion (0.92 h). Therefore, Pickering emulsion generated a significant faster release of caffeine and higher fluxes ($p < 0.05$). Consequently as indicated in Table 2, the percentage of caffeine recovered in the skin layers (receptor liquid + dermis + epidermis) after 24 h exposure was twice higher for Pickering emulsion compared to classical emulsion (14 ± 2% vs. 6.5 ± 0.7%). The distribution of caffeine in the skin layers showed that the major part of caffeine accumulated preferentially in the receptor fluid (Table 2 and Fig. 2). This result was in accordance with several studies (Bolzinger et al., 2008; Akomeah et al., 2004) showing that hydrophilic and small molecules permeated well through the skin. Even though the percentage of caffeine found in the dermis was comparatively lower, a significant difference was also noticed in this compartment (0.8 ± 0.1% for Pickering emulsion against 0.43 ± 0.03% for classical emulsion).

Table 2
Skin absorption parameters of caffeine (0.8 wt%) in Pickering and classical emulsion (mean ± S.E., $n = 6$).

	Pickering emulsion	Classical emulsion
Parameters from the permeation profile		
Lag time, *LT* (h)	0.5 ± 0.1	0.92 ± 0.08
Steady state flux, J_{SS} ($\mu g\,cm^{-2}\,h^{-1}$)	25 ± 4	9.7 ± 1.4
Permeability coefficient, k_P ($cm\,h^{-1}$)	0.0030 ± 0.0005	0.0010 ± 0.0002
Q_{abs} at 24 h ($\mu g\,cm^{-2}$)	500 ± 60	223 ± 25
Q_{abs} at 24 h (%)	13.80 ± 1.64	6.50 ± 0.68
Distribution of caffeine in skin layers after 24 h (% of applied dose)		
Epidermis	0.30 ± 0.07	0.43 ± 0.04
Dermis	0.8 ± 0.1	0.43 ± 0.03
Receptor fluid	12.7 ± 1.6	5.6 ± 0.7

Comparison with earlier works is difficult because there is not much literature data dealing with delivery systems to skin from w/o type dispersions incorporating hydrophilic penetrants which could be compared to Pickering emulsions tested in this study. Indeed, most attention has been paid to delivery with double emulsions. For example, Doucet et al. (1998) showed that w/o/w multiple emulsion gave threefold lower permeation of caffeine after 24 h than o/w emulsion, suggesting the encapsulation of caffeine in double emulsion droplets. Therefore encapsulation of caffeine inside w/o/w emulsion slowed down permeation. This is indeed quite a general feature that encapsulation provides sustained delivery. This was often observed for systems encapsulating lipophilic molecules, for example microspheres, solid lipid nanoparticles and nanocapsules (Wissing and Muller, 2002; Olivera-Martínez et al., 2005). The possible encapsulation inside Pickering emulsion droplets has often been put forwards because the solid silica particles form a rigid shell around the droplets. Sustained release has been demonstrated by Simovic and Prestidge (2007) in the case of o/w emulsions. Accelerated permeation with Pickering emulsion was measured in the present case, which was the reverse of the common expectation regarding an encapsulation effect. Therefore different mechanisms are operating. It has also been shown that another model hydrophilic solute, glucose, penetrated the skin faster for w/o/w multiple emulsion than for w/o emulsion (Ferreira et al., 1995). The authors suggested that it came from different partition coefficients between the vehicle and *stratum corneum*.

Thus, Pickering emulsions accelerated the skin absorption and permeation of the hydrophilic substance caffeine. This result is of course interesting *per se* and may deserve practical applications to

drug delivery. But the mechanism of such acceleration is also of an open question, which will be addressed in the next section.

3.2. Parameters influencing the skin penetration process

Skin absorption and permeation of caffeine involves several elementary stages that will be examined successively (Bronaugh and Maibach, 1999, 2001). It will be asked whether Pickering emulsion may influence such elementary stages. Physical chemistry experiments will be presented for either inferring the validity of our hypotheses or giving relevant information regarding the open discussion. The different possible stages are: (a) partition between aqueous and oil phases in the w/o emulsion, (b) partition between the emulsion and the skin, (c) diffusive transfer of caffeine from the water droplets to the skin (d) or direct transfer of caffeine to the skin in case of adhesion of water droplets, (e) transport of caffeine by penetration of either water droplets or silica particles inside the *stratum corneum*.

3.2.1. Partition between aqueous and oil phases

It was of prime importance that the model drug was strictly confined in the water droplets of the w/o emulsion. Therefore, the choice of dimethicone as continuous phase was motivated by the very low solubility of caffeine in silicone oil that makes the partition coefficient of caffeine between aqueous and oil phases very low. Caffeine is often used as a model water-soluble compound in skin absorption studies (Dias et al., 1999; Jimenez et al., 2004; Mourgues et al., 2004; Bolzinger et al., 2008) because of its low molecular weight and its low log P value (log $P = -0.07$ for octanol–water); moreover, it is not metabolized in the skin (Bronaugh et al., 1989). The measured log P between water and dimethicone was -2.71 ± 0.02 indicating that caffeine was restricted to the water phase of the emulsion. Therefore the full caffeine quantity was inside the water droplets. Even slight variations in the partition coefficient values would not significantly change this content because the partition coefficient is so low.

3.2.2. Partition between emulsion and skin

There are several reports showing that the type of emulsion stabilizing agent plays a crucial role on solute partitioning between emulsion and skin. Partition between the phases is an equilibrium property of the bulk phases that should not be influenced by the interfacial properties. However there are residual amounts of stabilizing agents in the bulk phases; they are in equilibrium with adsorbed materials. Such species affect the solubility properties of the solutes. This is particularly obvious in case of surfactants that self-associate as micelles, either direct micelles in water or reverse micelles in oil phase (Chevalier and Zemb, 1990). Solubilization in micelles affects the chemical potential of solutes, therefore their partition between phases. Regarding skin absorption from classical emulsion formulation, it has often been given evidence that surfactants had an enhancer role and increased skin absorption of penetrant (Ashton et al., 1992a,b). Other authors reported that surfactants acted as interfacial barriers, slowing down the transport of solutes solubilized in micelles (Bikhazi and Higuchi, 1970; Surpuriya and Higuchi, 1972). Thus, the state of the art dealing with the influence of surfactants on skin penetration is still confused. Additionally, discriminating the influences of equilibrium partition coefficient and dynamic transport processes is difficult from a single skin permeation experiment.

Regarding Pickering emulsion formulation, it is not expected that solid particles could affect the partition to the same extent as surfactants. However Cappel and Kreuter (1991) and Muller and Kreuter (1999) reported accelerated skin absorption of drugs induced by the presence of nanoparticles in the formulation and proposed the modification of vehicle/*stratum corneum* partition coefficient as a possible rationale. Thus, poly(methylmethacrylate) and poly(butylcyanoacrylate) nanoparticles mixed with methanol as model hydrophilic penetrants in saline solutions slightly increased methanol absorption through mouse skin. The penetrant was not encapsulated, but simply mixed with particles. Similar phenomena might be operative with the present Pickering emulsions that contained silica nanoparticles.

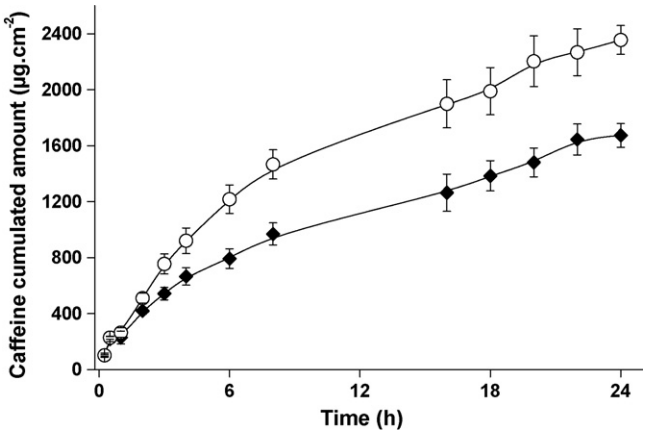

Fig. 3. Release profile of caffeine from Pickering emulsion (♦, $n = 6$) and classical emulsion (○, $n = 6$). Plot of the amount of caffeine (µg cm^{-2}) permeated from water droplets to bulk aqueous phase vs. time (h); mean ± S.E.

In the present investigation, more caffeine penetrated from Pickering emulsion than from classical emulsion so that it was presumed that the presence of emulsifier in the formulation did not enhance the skin absorption of caffeine. Caffeine transport mediated by solid particles remains a possibility that is examined in the Section 3.2.5.

3.2.3. Diffusive transfer from the water droplets

It is often said that the materials adsorbed at the water–oil interface affect the release rate. This has already been observed for many dosage forms containing surfactants (Caldero et al., 1997, 1998; Rocca et al., 1999). Therefore release of caffeine from emulsion droplets would be dependent on the nature and structure of the water–oil interface. The release of caffeine out from the water droplets was measured for Pickering and classical emulsions. Thus, the kinetics of caffeine transfer from the water droplets of the w/o emulsion to a bulk aqueous phase was measured. This method has previously been used for release studies of caffeine from suspension in mineral oil (Fokkens and de Blaey, 1984). The w/o emulsions containing caffeine were contacted with a receptor aqueous saline phase, taking care not mixing the two liquid media. It allowed avoiding the use of separation membranes which could influence the diffusion rate of solutes. The w/o emulsion was spread on the receptor fluid surface and the caffeine concentration in the aqueous phase as measured as a function of time. The viscosity of emulsions was high enough to prevent mixing with the receptor fluid, leaving the same contact area of 2.54 cm^2 during the full study. The Pickering emulsion released caffeine slower than the classical emulsion (Fig. 3). After 24 h, 45 ± 3% of caffeine was released from the Pickering emulsion and 65 ± 3% from the classical emulsion (mean of six experiments).

The release of caffeine from Pickering emulsion was considerably slowed down compared to classical emulsion, suggesting that the rigid dense shell of silica particles around the emulsion droplets acted as a barrier to interfacial diffusion. This was similar to microencapsulation inside the Pickering emulsion droplets. It has indeed been shown that silica particles assembled at oil/water

interface could slow down the release of lipophilic molecules (dibutylphthalate) from o/w emulsions (Simovic and Prestidge, 2007). The same mechanism is possible in the case of hydrophilic caffeine confined inside w/o emulsion droplets. Pickering emulsion droplets with their core/shell structure could be compared to microcapsules. The amount of silica was indeed enough for ensuring a full coverage of the interface with a dense shell of aggregated silica particles (Frelichowska et al., submitted). Incomplete coverage of the interface by particles would not allow such sustained release. Release out from the water droplets was slower for the Pickering emulsion whereas the skin absorption was faster. It is concluded that the increased skin absorption of caffeine from Pickering emulsion compared to emulsifier-stabilized emulsion does not result from a faster release from the formulation.

3.2.4. Direct caffeine transfer to the skin by adhesion of water droplets

Since caffeine was confined inside the water droplets of emulsions, direct caffeine transfer to the skin may take place if water droplets are depositing on the skin surface. Such phenomenon would be fast if the encapsulating droplets have a high affinity for the skin surface. The surface of skin is quite hydrophobic because it is covered by the lipids of the *stratum corneum*. The surface energy of skin determined by contact angle measurements (Mavon et al., 1997) is 38.1 mJ m^{-2} for forearm skin and 43.7 mJ m^{-2} for forehead skin. The adhesion energy of water droplets stabilized either by silica particles or emulsifier was calculated from the measured values of interfacial tension and contact angle on the skin surface. Adhesion energy E_{Adh} (mJ m^{-2}) of a water droplet on the skin in the presence of oil is given by the following equation (Adamson, 1990):

$$E_{Adh} = \gamma_{(s-o)} + \gamma_{(w-o)} - \gamma_{(w-s)} \quad (1)$$

where $\gamma_{(s-o)}$ is the interfacial tension between skin and oil, $\gamma_{(s-w)}$ is the interfacial tension between skin and water and $\gamma_{(o-w)}$ is the interfacial tension between oil and water.

The contact angle, θ, is given by Young's equation:

$$\cos\theta = \frac{\gamma_{(s-o)} - \gamma_{(s-w)}}{\gamma_{(w-o)}} \quad (2)$$

Thus, E_{Adh} can be calculated as:

$$E_{Adh} = \gamma_{(w-o)}(1 + \cos\theta) \quad (3)$$

Table 3 gives the interfacial tension measured by the pendant drop method between the oil and water phases of investigated emulsions. Silica particles had no interfacial activity at the oil/water interface. Similar conclusions have been reached by Okubo (1995), who observed that silica nanoparticles did not change the surface tension of water. The surfactant Abil® EM97 decreased the interfacial tension, as expected. Partial wetting was observed in every instance, the contact angles were larger than 90°, showing a better affinity of the skin surface for oil than for water. Close contact of water droplets and skin surface immersed in oil was not likely, so that direct transfer of caffeine from water droplets to skin appeared as a rate-limiting phenomenon. It was expected that the type of surface coverage of the emulsion droplets might influence such caffeine transfer. Slight but significant differences of contact angle values on the skin were noticed between a water drop stabilized with silica particles or with an emulsifier (151.4° and 143.7°, respectively) (Fig. 4).

Table 3
Interfacial tension, γ, contact angle of water with skin, θ, and calculated energy of adhesion, E_{Adh}, for the skin–cyclomethicone–water system.

Oil phase	γ (mN m^{-1})	θ (°)	E_{Adh} (mJ m^{-2})
Cyclomethicone	27 ± 1	151.20 ± 2.89	3.3
Cyclomethicone with emulsifier	1.4 ± 0.2	143.70 ± 0.82	0.27
Cyclomethicone with silica particles	27 ± 1	151.4 ± 1.3	3.3

Finally, the calculated adhesion energies of the Pickering emulsion droplets and bare water droplets were found identical. Conversely, the adhesion energy of a droplet stabilized by the surfactant was lower by a factor of 10. This effect resulted from differences of interfacial tension rather than contact angle. Thus, Pickering emulsion droplets adhered better to the skin surface than the classical emulsion. This mechanism that makes caffeine more available from Pickering emulsion in contact with the skin surface can explain the enhanced caffeine absorption that was observed in Franz cell experiments.

3.2.5. Transport by penetration of intact carrier into the stratum corneum

Many authors have discussed the possibility of the intact carrier transport through the skin (Wissing and Muller, 2002; Alvarez-Roman et al., 2004a,b,c; Rolland et al., 1993; Borm et al., 2006). Studies carried out on polymeric microspheres have shown that the size of the particles was the most relevant factor. Carriers smaller than 3 μm penetrate both by diffusion through the *stratum corneum* and by the hair follicles path. Such particles were found equally distributed inside hair follicles and in the *stratum corneum*, after exposure long enough to their aqueous suspension. Microparticles of size ranging from 3 to 10 μm only penetrate into hair follicles and particles larger than 10 μm remain on the skin surface (Rolland et al., 1993; Schaefer et al., 1990, 1994). These systems allow modulating the penetration of drugs through the *stratum corneum* and controlling the drug release at different skin sites. The mean droplet size of the present emulsions was 10 μm, much larger than the size of microcapsules that have been reported to penetrate the skin. The possible skin penetration of intact Pickering emulsion droplets should be discarded on this basis. This conclusion was drawn from literature data dealing with aqueous dosage forms however. The

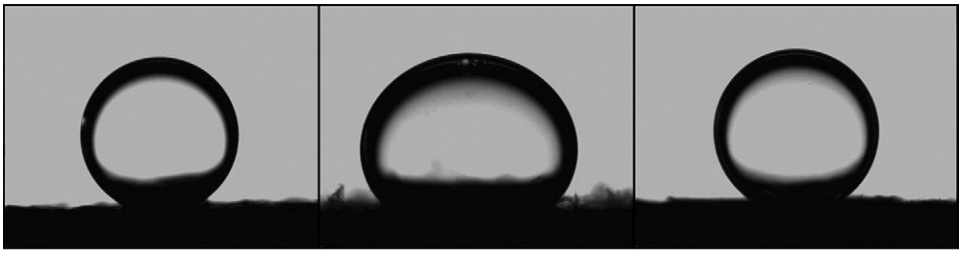

Fig. 4. Contact angles of water droplet with skin immersed in (A) cyclomethicone (151.2°); (B) cyclomethicone containing emulsifier (143.7°); (C) cyclomethicone containing silica particles (151.4°).

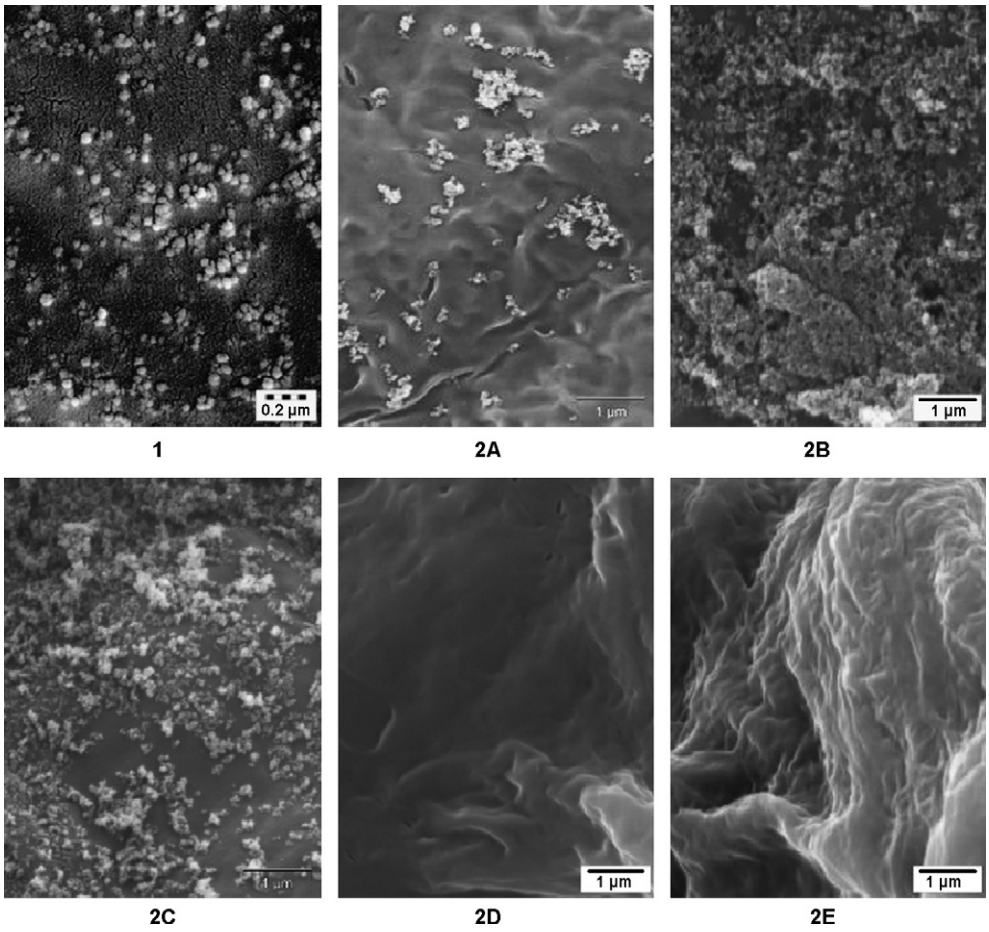

Fig. 5. (1) SEM picture of silica suspension in silicone oil. The scale bar represents 200 nm. (2A–E) SEM pictures of *stratum corneum* layers after 24 h exposure to Pickering emulsion. (2A) Skin surface; (2B) first skin strip; (2C) 10th skin strip; (2D) 15th skin strip; (2E) 19th skin strip. Scale bars represent 1 μm.

cut-off size of carriers might be quite different for particles dispersed in oil medium because the surface such particles is much more hydrophobic than particles dispersed in water medium. It is thought that hydrophobic particles may penetrate easier the *stratum corneum* through its lipidic extracellular path.

Another possible mechanism of caffeine penetration through skin is the transport by the solid particles. The mean diameter of silica particles was 100–200 nm, much smaller than the emulsion droplets. As shown in the SEM picture of a suspension of HDK H20 silica in silicone oil (Fig. 5.1), silica particles were aggregates made of elementary particles of ~20 nm diameter. Therefore not only the average diameter mattered but the full size distribution of such aggregates which extended towards small sizes of few tenth nanometers; some tiny particles, possibly elementary particles, might also be present.

Free silica particles might be present in the external phase of the Pickering emulsion if there was an excess with respect to full coverage of the water–oil interface. Breaking up of emulsion droplets at the skin surface was another possible source of free silica particles that could penetrate the skin.

Such caffeine transport mechanism implies adsorption of caffeine onto the surface of silica particles. It is indeed known that many solutes can adsorb at the surface of silica particles (Korn et al., 1980). The present silica HDK H20 bears both hydrophilic silanol groups of bare silica (55% free Si–OH ≈ 3 μmol m^{-2}) and hydrophobic groups coming from the dimethylsilyl grafts. Hydrophilic caffeine can adsorb through hydrogen bonding to the silica surface, especially in the hydrophobic environment of the *stratum corneum* lipids. Thus, particles could contribute to the transport of caffeine through the dispersing phase of the emulsion and/or through the skin.

Scanning electron microscopy observations were performed so as to assess the possible penetration of silica particles into the skin. SEM of the skin surface after 24 h exposure to Pickering emulsion showed that silica aggregates of approximately 200 nm diameter were distinctly visible (Fig. 5.2A). Consequently, the skin stripes obtained by the tape-stripping technique were also imaged by SEM. Silica aggregates were observed at the surface of corneocytes of the top stripes. Silica particles were present until the 10th tape, which roughly corresponded to about 5 μm depth in the *stratum corneum* (Fig. 5.2B–E). No significant number of silica particles could be seen in the deepest stripes of the *stratum corneum*. Skin penetration of nanoparticles is currently well documented. The present observations are in accordance with several authors (Lademann et al., 1999; Pflücker et al., 2001; Mavon et al., 2007) who found that the largest amount of nanoparticles penetrating the skin was localized in the upper layers of *stratum corneum*. The hydrophobic grafts present at the surface of the silica particles allow favorable interactions with the *stratum corneum* lipids and consequently make the penetration easier. Therefore, the silica aggregates stabilizing the Pickering emulsion penetrated the upper layers of skin and could promote the transport of caffeine. The release of caffeine adsorbed at the surface of silica particles coming from emulsion droplets breakage at the skin surface appears as a likely mechanism that would accelerate the caffeine delivery into the skin. Although not a definite proof of such caffeine transport mechanism, penetration of silica particles

in the upper skin layers demonstrated by SEM observations is to be considered operative.

Lastly, the adsorption of caffeine to silica particles by hydrogen bonding could contribute to sustained release effect. This phenomenon was suggested by Akomeah et al. (2004) to explain the epidermal retention of caffeine during permeation experiments. The carriers retained in the skin may act as microreservoirs, causing the sustained release of drug. Neither long-lasting effect nor epidermis retention was observed in the present experiments, demonstrating that such mechanism was not predominant.

4. Conclusions

The skin absorption of a hydrophilic model penetrant from w/o Pickering emulsion was significantly different of that from identical emulsifier-stabilized emulsion. Caffeine skin absorption from a Pickering emulsion was faster by a factor of 3. This was quite a surprising result, especially considering that this type of formulation looks similar to an encapsulation system. Several hypotheses were made to explain the faster diffusion from this new vehicle and complementary physical chemistry experiments were designed for assessing these hypotheses. The presence of a multilayered silica layer around the water droplets strongly limited the *in vitro* release of caffeine; therefore encapsulation was indeed operating. The enhanced skin absorption of caffeine from Pickering emulsion was possibly due to specific interactions of the formulation with the skin structures and to the penetration of nanoparticles in the formulation. These claims are supported by the following observations. The adhesion energy of Pickering emulsions water droplets was higher than for the classical surfactant-stabilized emulsion. Such improved adhesion to the skin structure allowed a faster drug release into the *stratum corneum*. Moreover, SEM observations revealed that silica aggregates entered the *stratum corneum* until the 10th tape-strip. This demonstrated the partial breakage of the emulsion droplets adhering at the skin surface and gave support to the transport of caffeine adsorbed at the surface of the silica particles through the *stratum corneum*. This comparative study of Pickering and classical emulsion gives an opportunity of deep reflection on physicochemical mechanisms of skin absorption and on vehicle parameters influence. Because of significant differences noticed between the two formulations, it is worth considering Pickering emulsions for their application either as new dosage forms for topical drug delivery or as new targeting systems in cosmetic formulations.

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