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Topical delivery of lipophilic drugs from o/w Pickering emulsions

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ABSTRACT

Surfactant-free emulsions stabilized by solid particles (Pickering emulsions) have been evaluated in the terms of skin absorption of lipophilic drugs. The behavior of three formulations: a surfactant-based emulsion, a Pickering emulsion stabilized by silica particles and a solution in triglyceride oil, were compared in order to assess the effect of the surface coating of Pickering emulsions as new dosage forms for topical application. Such comparative investigation was performed *in vitro* on excised pig skin in Franz diffusion cells with *all-trans* retinol as model lipophilic drug. Surfactant-based (classical, CE) and Pickering (PE) oil-in-water emulsions containing retinol were prepared with the same chemical composition (except the stabilizing agent: surfactant or silica particles), the same droplet size and the same viscosity. No permeation through the skin sample was observed after 24 h exposure because of the high lipophilic character of retinol. Penetration of retinol was 5-fold larger for both CE and PE than for the solution in triglyceride. The distribution of retinol inside the skin layers depended significantly on the emulsions type: the classical emulsion allowed easy diffusion through the *stratum corneum*, so that large amounts reached the viable epidermis and dermis. Conversely, high storage of retinol inside the *stratum corneum* was favored by the Pickering emulsion. The retinol content in *stratum corneum* evaluated by skin stripping, demonstrated the increased retinol accumulation from PE. Therefore Pickering emulsions are new drug penetration vehicles with specific behavior; they are well-suited either for targeting the *stratum corneum* or aimed at slow release of drug from *stratum corneum* used as a reservoir to the deeper layers of skin.

1. Introduction

Using solid-stabilized emulsions (so-called Pickering emulsions) constitute a new strategy for encapsulation and transport of drugs in pharmaceutical and cosmetic formulation. Pickering emulsions are stabilized by solid particles such as silica, clays, calcium carbonate, titanium dioxide, latex and many others (Ramsden, 1903; Pickering, 1907; Schulman and Leja, 1954; Binks and Horozov, 2006; Aveyard et al., 2003). Therefore they are surfactant-free emulsions and it is expected that such formulations behave in different ways than classical emulsions (CE) in the terms of drug delivery. The main features in comparison to classical surfactant-based emulsions are improved stability, especially at high internal phase ratio, and the easy fabrication of stable large droplets up to millimeter size (Arditty et al., 2003, 2004). The emulsion type (oil-in-water or water-in-oil) depends on the wetting properties of particles, so that hydrophobized fumed silica powders are most often used for ensuring partial wetting conditions of solid particles by both water and oil (Aveyard et al., 2003). The silica particles adsorbed at the oil/water interface form a rigid shell-like structure that coats the surface of the emulsions droplets. This latter property can strongly influence the release profile of drugs from emulsion droplets into the aqueous phase. Only Simovic and Prestidge (2007) have recently proposed the drug release profile of a model lipophilic drug *in vitro* and showed the strong influence of the stabilizing silica layer thickness and its density on the drug release profile.

The growing interest for formulations made of Pickering emulsions applied in pharmaceutical and cosmetic field is manifest through several patent applications (Schonrock et al., 1998; Gers-Barlag and Müller, 2004). Although cosmetic applications have been claimed, the skin drug absorption profiles from such vehicles have never been reported in the literature. Regarding pharmaceutical applications, dermatologic care and topical administration may benefit from the specific properties of Pickering emulsions. The aim of this report is filling the gap between the particular physicochemical properties of such emulsions and their acknowledged potential applications. Thus, the skin absorption of active

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ingredients encapsulated in Pickering emulsion droplets was investigated in this study.

Pickering emulsions of the oil-in-water (o/w) type were evaluated regarding skin absorption of retinol as a hydrophobic model drug. Such evaluation was performed by comparison with a reference classical emulsion stabilized with surfactants. In the present study three formulations were tested: an o/w Pickering emulsion stabilized by silica particles (PE), an o/w emulsifier-stabilized emulsion (classical emulsion, CE) and retinol solution in oil (SOL). The *in vitro* distribution of retinol in excised pig skin after 24 h exposure was evaluated in the horny layer by tape stripping, in the viable epidermis, in the dermis and in the receptor fluid using the static Franz diffusion cell method.

2. Materials and methods

2.1. Materials

Ingredients were kind gifts from their suppliers: Polysorbate 85 emulsifier (Montanox®85) from Seppic (France), α-Tocopherol acetate from BASF (France), Caprylic/capric triglyceride (Labrafac WL®1349) from Gattefossé (France), Polyoxyethylene (20) oleyl ether (Brij®98) from Uniqema (France); hydrophobized fumed silica HDK® HKS D from Wacker Chemie (Germany). *All-trans* retinol and phosphate buffer pH 7.4 were purchased from Sigma–Aldrich (France); analytical grade methanol was purchased from Carlo Erba (Italy). Deionized water of 18 MΩ cm^{-1} resistivity was used throughout the work.

Full-thickness pig skin (mean thickness ± S.E. = 1.35 ± 0.05 mm) was used in the skin absorption experiments. The skins of 3 donor animals were washed and excised, the subcutaneous fatty tissue was carefully removed and the skin pieces were stored flat at −20 °C until use.

2.2. Formulations

All formulations contained 0.1% retinol. Two o/w emulsions were prepared: a Pickering emulsion (PE) and an emulsion stabilized with an emulsifier (classical emulsion, CE). The oil/aqueous phases ratio for both emulsions was 10:90 (wt:wt). The compositions of PE and CE are given in Table 1. To prepare the Pickering emulsion, the silica was first dispersed in water for 2 min using an ultrasonic disperser Sonics VibraCell equipped with a 25 mm shaft working at 20 kHz, 500 W power and 80% of the full amplitude (Bioblock Scientific, France). The oil and aqueous phases of Pickering emulsion were then mixed together with an UltraTurrax® device (Germany) at 22,000 rpm for 7 min in ice bath. Emulsifier-stabilized emulsion aqueous and oil phases were mixed together with an UltraTurrax® (Germany) at 13,500 rpm for 2 min. Then the emulsion was prepared by stirring the oil and the aqueous silica dispersion with a TurboTest® (Rayneri/VMI, Montaigu, France) at 300 rpm for 30 min.

The 0.1 wt% solution of retinol in caprylic/capric triglyceride with 0.5 wt% α-tocopherol acetate (SOL) was prepared under magnetic stirring at room temperature.

All test formulations were prepared in dark conditions and stored under nitrogen. The stability of retinol in formulations was investigated for 48 h at 20 °C. All formulations were used for skin permeation studies immediately after preparation.

Table 1
Composition of the three investigated formulations (wt%) and *all-trans* retinol content measured by HPLC (mean ± S.E., $n = 3$): Pickering emulsion (PE), classical emulsion (CE) and oil solution (SOL). The components of the aqueous phase are in italics.

	PE	CE	SOL
Compositions of formulations (wt%)			
all-trans retinol	0.1	0.1	0.1
Caprylic/capric triglyceride	9.4	9.4	99.4
α-Tocopherol acetate	0.5	0.5	0.5
Polysorbate 85	–	*0.5*	–
Hydrophobic silica HDK HKS D	*7*	–	–
Water	*83*	*89.5*	–
all-trans retinol content (%)			
	0.114 ± 0.004	0.111 ± 0.001	0.111 ± 0.004

2.3. Physicochemical characterizations

Emulsions droplet size distributions were measured by small angle light scattering using a MasterSizer® 2000 (Malvern, UK). The refractive indices used for the "optical model" were 1.332 for water and 1.460 for the emulsion droplets. The average size and polydispersity index of silica particles dispersed in water was measured by means of dynamic light scattering using a NanoZS® instrument (Malvern, UK). The size and shape of silica particles were confirmed with scanning electron microscopy (SEM) observations. The samples of silica suspension in water were put on SEM aluminum stubs, left for drying and then sputter coated with a thin gold/palladium layer using a cathodic pulverizer, Hummer II Technics (6 V; 10 mA). The samples were scanned using a Hitachi S800 microscope working at 15 kV acceleration voltage at the "Centre Technologique des Microstructures" ("CTμ") at the University of Lyon (Villeurbanne, France).

The viscosity of emulsions was measured at 20 °C using a Couette rheometer Rheomat R180 (Lamy, France) equipped with a mobile system N° 11 rotating at 200 s^{-1} shear rate.

2.4. Transepidermal water loss (TEWL)

Transepidermal water loss (TEWL) was measured using a SkinStation® (La Licorne, Meylan, France). The measurements were performed in triplicate on skin pieces just before performing the skin absorption studies. The skin samples with TEWL value higher than 15 g m^{-2} h^{-1} were discarded (OECD, 2004). Complete skin stripping was also assessed by TEWL measurements; it was considered that full removal of *stratum corneum* (SC) was attained when TEWL reached 25 ± 5 g m^{-2} h^{-1}.

2.5. In vitro skin absorption studies

Full-thickness pig female or male skins (1.35 ± 0.05 mm; mean ± S.E.) were obtained from young animals sacrificed at the Laboratoire de Physiologie, Université de Lyon, France. The skin was cleaned up with tap water. The hairs were cut with an electric cutter. The skin was shortly washed with 1% sodium dodecyl sulfate aqueous solution and rinsed with tap water. Integrity of the skin samples was examined by measuring the TEWL (Skin Station, La Licorne, Meylan, France). The TEWL was measured for 1 min and skin samples with TEWL values larger than 15 g h^{-1} m^{-2} were discarded. The thickness of each skin piece (1.5 mm) was measured with a micrometer (Mitutoyo). The skin was mounted in two-chamber glass diffusion cells. The effective penetration area was 2.54 cm^2; the volume of the receiver chamber was 10 mL. The receiver solution was composed of buffer at pH 7.4 with 1.5% Brij®98 and 0.5% α-tocopherol acetate. Brij®98 was dissolved in the buffer solution at 60 °C; solution was filtered and α-tocopherol acetate was added after cooling. The solution was degassed in an ultrasonic bath with ice during 20 min.

1 g of freshly prepared formulation was spread uniformly on the skin surface. The study was carried out in occlusive conditions for 24 h in static Franz cells. At the end of the study the receptor fluid was removed and analyzed by HPLC. The Franz cells were disman-

tled and the skin surface was washed with 2 mL of receptor fluid. Then skin stripping was performed. The *stratum corneum* was separated into 21 layers using an adhesive tape D-Squame® (Monaderm, Monaco), retinol was extracted out of the strips, and extracted samples were filtered and analyzed by HPLC. The viable epidermis was separated from the dermis by heat treatment in water at 60 °C for 45 s. After separation, the epidermis and dermis were cut into pieces with a scalpel, retinol was extracted, and samples were filtered and analyzed by HPLC. Methanol with 0.5 wt% α-tocopherol acetate was used for extraction of retinol from all samples. Skin samples were immersed in extracting medium under ultrasounds in ice bath for 20 min in order to achieve full extraction.

All experiments were undertaken in the dark to avoid degradation of retinol under light exposure.

2.6. HPLC analysis of retinol content

The samples were analyzed for retinol using 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® MS C18 (3.9 mm × 150 mm, 5 μm) and a Waters 2996 photodiode array UV detector working at 325 nm wavelength. The elution with methanol/water (85:15) solvent at 1.2 mL/min flow rate and 30 °C gave a retention time of 6 min for retinol. Injection volume was 20 μL. The calibration curve for quantitative analysis was linear up to 40 μg/mL and the detection limit for retinol was 20 pg.

2.7. Data analysis

The mean and standard error of the mean (S.E.) 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 $p \leq 0.05$.

3. Results and discussion

3.1. Formulations

Two oil-in-water emulsions were obtained: a Pickering emulsion and an emulsifier-stabilized one (classical emulsion, CE) (Table 1). Rather than optimizing the formulations with respect to long-term stability, the aim of this formulation study was to select a formulation that would provide a clear distinction between the effects of interface type on the retinol transport and release properties. The formulations were therefore drastically (3 or 4 ingredients) simplified to avoid any formulation effects that would hide the main interfacial phenomena under investigation.

Special attention was paid to protecting retinol against photo-oxidative degradation. Formulations were prepared in dark bottles and stored in the dark under nitrogen atmosphere; all the work was carried out in the dark. The retinol formulations and the receptor fluid were stabilized with 0.5 wt% α-tocopherol acetate.

3.1.1. Formulation of Pickering emulsions

The stabilizing solid particles are hydrophobized at the surface by dichlorodimethylsilane grafting in order to ensure partial wetting by oil and water. According to the technical data sheet from Wacker, the HDK® HKS D fumed silica contains 0.95 wt% of carbon from elemental analysis; the surface groups comprise 71% of free hydroxyl groups and the BET specific area is 200 $m^2\ g^{-1}$. Silica was dispersed in water prior to emulsification of oil. As shown by Binks and Lumsdon (2000), the phase where the solid particles are dispersed usually becomes the continuous phase of the emulsion. The size of particles influences the minimal size of emulsion droplets:

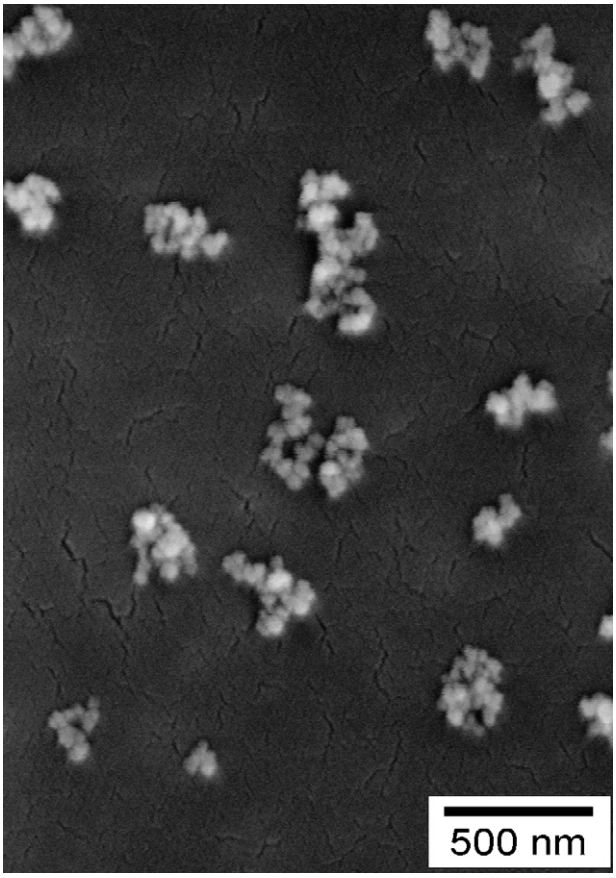

Fig. 1. SEM picture of a suspension of HDK HKS D silica in water. The primary silica particles of diameter 15–20 nm are forming aggregates of 100–200 nm size.

the emulsion drops are large in case of large stabilizing particles (Binks and Horozov, 2006). The elementary particles of fumed silica HDK® HKS D are associated as aggregates of large size reaching a few micrometers. It was useful to disperse the silica particles prior to the emulsification of oil in order to prepare a fine enough emulsion that did not cream too fast. Ultrasound dispersion of the silica suspension in water produced silica aggregates of mean diameter 140 ± 10 nm (mean ± S.E.) as measured by dynamic light scattering. The width of the particle size distribution was 50 nm. It was also confirmed by SEM (Fig. 1) that the aggregates consisted of small primary particles (about 15–20 nm diameter) associated as larger lumps.

The aqueous dispersion of silica was mixed with the emulsion oil phase with an UltraTurrax disperser. The full oil was emulsified as a milky o/w emulsion. The mean droplet size was 3 ± 1 μm as measured by small angle light scattering. The width of the droplet size distribution was 1.5 μm.

The packing of silica at the oil–water interface can be modulated by the formulation process parameters. A dense packing was aimed at by using a large amount of silica. Indeed, high silica concentration (or the presence of electrolytes) leads to dense packing at interface (Binks and Kirkland, 2002). According to the present formulation containing 7 wt% silica, full coverage of oil droplets was achieved as a dense coating made of silica aggregates. This was achieved because there was excess silica particles in the disperse emulsion phase with respect to the amount required for a dense coating of the droplet surface. Indeed, the strong affinity of silica particles for the oil–water interface leads to maximum coverage. Therefore the presence of excess silica in the aqueous phase indicates a dense coverage (Horozov et al., 2007; Frelichowska et al., submitted). Such excess silica was detected by careful analysis of the

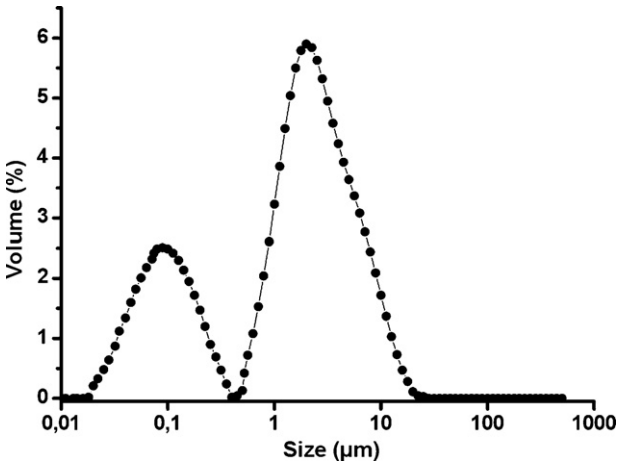

Fig. 2. Size distribution of the Pickering emulsion as measured by light scattering. The population of particles below 1 μm diameter corresponds to non-adsorbed silica dispersed in the aqueous phase; the larger size population corresponds to the oil droplets.

particle size distribution measured by small angle light scattering (Fig. 2): the population of particles smaller than 1 μm corresponded to aggregated silica in excess in the aqueous phase (Frelichowska et al., submitted). The presence of excess silica is an indirect indication showing that the coating of the interface is dense, thus preventing excess silica particles to adsorb.

3.1.2. Formulation of classical emulsions

The emulsifier-stabilized emulsion (classical emulsion, CE) was prepared such that the oil content, mean droplet size and emulsion viscosity were the same as those of the Pickering emulsion (Table 1). An emulsifier (Polysorbate 85, HLB = 11.0) allowing o/w emulsions was chosen. The amount of emulsifier was adjusted in order to obtain the same droplet size distribution as for the Pickering emulsion (3 ± 1 μm). The viscosity of the classical emulsion was similar to that of the Pickering emulsion (6 ± 2 mPa s) to avoid the influence of viscosity on the skin absorption profile (Eros et al., 2003). The physicochemical properties of both emulsions are given in Table 2.

3.1.3. Choice of drug

It was important to select a model drug, which would be fully encapsulated inside the droplets. Retinol was chosen as a model lipophilic active ingredient. Its highly lipophilic character (log *P* = 5.68) guarantees the presence of retinol exclusively in the oil phase (dispersed phase) of the emulsion. It was not intended to disclose a formulation that would provide a definite beneficial effect regarding the action of retinol, which is mainly located in the basal cells of epidermis and dermis (Fisher and Voorhees, 1996; Vahlquist et al., 1982). Retinol accelerates the renewal processes of skin and increases collagen synthesis. In topical formulations it is often used at 0.1% concentration.

The stability of retinol is a difficult issue since it is degraded by oxygen, acids, metals, UV and visible light and temperature (De Ritter, 1961; Manan et al., 1995; Sorg et al., 1999). In this study, α-tocopherol was added and all experiments were done in the dark to protect retinol from oxidation. All vehicles were used for skin absorption studies immediately after their preparation.

Table 2
Physicochemical characterizations of the Pickering emulsion (PE) and the corresponding classical emulsion (CE). Mean ± S.E., *n* = 3.

Parameter	PE	CE
Viscosity at 20 °C (mPa s)	6 ± 2	5 ± 2
Mean droplet size (μm)	3 ± 1	3 ± 1
pH	5.8 ± 0.1	5.7 ± 0.1

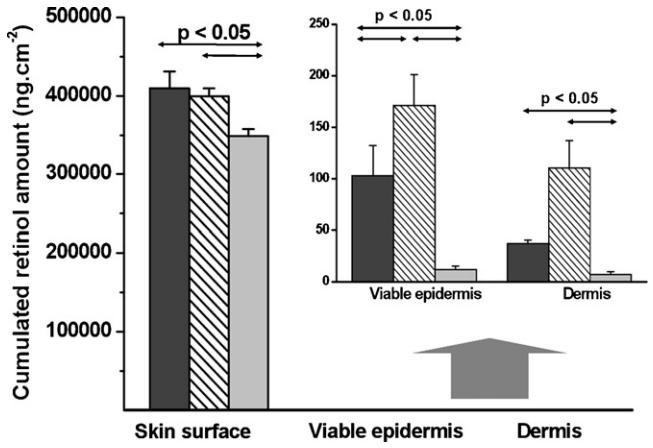

Fig. 3. Effects of formulation type on skin distribution of retinol in the excised pig skin after 24 h. ■: PE; ▨: CE; □: SOL. Mean ± S.E., *n* = 6. Values for viable epidermis and dermis are shown expanded by a factor of ~1000.

3.2. Total skin absorption

Retinol absorption was measured *in vitro* on porcine skin. Pig skin has been shown to be the most relevant animal model for human skin (Bronaugh et al., 1982; Marti-Mestres et al., 2007). Figs. 3 and 4 and Table 3 illustrate the penetration of retinol into excised full-thickness pig skin. No retinol was detected in the receptor fluid. Retinol penetrates the skin very poorly in general (Gollnick and Dummler, 1997). It has been found very often that lipophilic drugs penetrate the skin less than hydrophilic ones (El Hussein et al., 2007), because they have a high affinity for the horny layer. Log *P* is often used to predict the uptake into the *stratum corneum* (Kitagawa et al., 1997) and the permeation profile through the relationship given by Potts and Guy (1992). Log *P* = 5.68 gives a permeability coefficient (log K_p) of −7.5. Also the poor solubility of retinol in water (0.06 μM at pH 7.3 (Szuts and Harosi, 1991) impedes its transport through the epidermis and dermis.

The total amount of retinol absorbed in the full skin after 24 h (Table 3) was the highest for the classical and Pickering emulsions (0.071% and 0.068% of applied dose, respectively). The difference between the two emulsions was not significant according to the *t*-

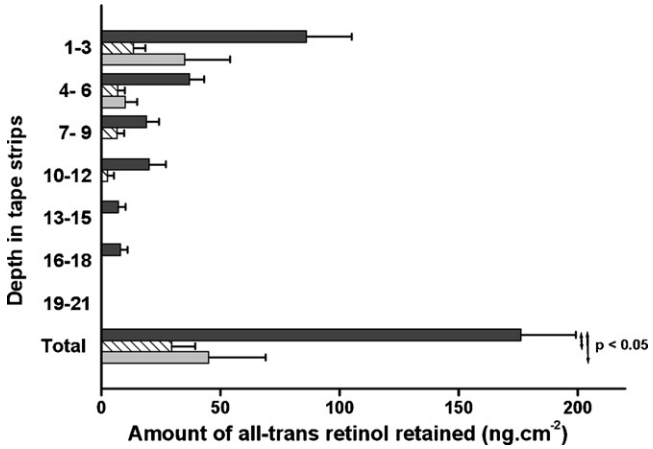

Fig. 4. Retinol distribution in *stratum corneum* as assessed by skin stripping. ■: PE; ▨: CE; □: SOL. Mean ± S.E., *n* = 6.

Table 3
Distribution of retinol in skin layers after 24 h exposure. Amounts are given in ng cm^{-2} and in percentage of absorbed dose (%). Mean ± S.E., $n = 6$.

	SOL		CE		PE	
	ng cm^{-2}	%	ng cm^{-2}	%	ng cm^{-2}	%
Stratum corneum	45 ± 24	70	30 ± 10	10	176 ± 23	56
Viable epidermis	12 ± 3	19	170 ± 30	55	103 ± 29	33
Dermis	7 ± 3	11	110 ± 27	35	37 ± 4	11
Total	64	100	310	100	316	100

test. The uptake from retinol solution was 5-fold lower (0.014% of applied dose) and significantly different from PE and CE.

3.3. Distribution of retinol within the skin layers

3.3.1. Distribution within stratum corneum, viable epidermis and dermis

The full figures are reported in Fig. 3 and the corresponding values in Table 3. Retinol mainly remained inside the *stratum corneum* from the lipophilic solution (SOL): 70% of the penetrated amount was retained in the *stratum corneum* after 24 h exposure; 19% was found in the epidermis and finally 11% reached the dermis. Conversely, the CE did not retain so much retinol in the *stratum corneum* (11% of the penetrated amount). Therefore larger amounts reached the epidermis (55%) and dermis (35%). The classical emulsion accelerated the transport through the *stratum corneum*. The distribution in skin layers was quite different for the Pickering emulsion since retinol was mainly retained in the *stratum corneum* (56% of the penetrated amount). The fraction of the penetrated amount retained in the *stratum corneum* was the same for the Pickering emulsion and the solution; but the actual amount was much higher for the Pickering emulsion because the total absorption was much higher (5-fold). The remaining fraction of absorbed retinol reached the epidermis (33%) and dermis (11%). Therefore, there were quite large (significant according to the *t*-test) differences between the emulsions and solution and between the two types of emulsions. The main features were:

- Skin absorption from emulsions was larger than from solution.
- Surfactant-based emulsion allowed retinol to go across the *stratum corneum* quite easily whereas Pickering emulsion favored storage of retinol inside the *stratum corneum*.

Regarding possible applications to cosmetic formulation or pharmaceutical delivery, emulsions are superior to solutions because they allow higher skin absorption. The two tested emulsions showed quite different behavior and could be used for quite different purposes. The classical emulsion allows penetration to the hydrophilic internal layers of skin. On the contrary, the target of Pickering emulsion is the *stratum corneum*.

3.3.2. Distribution inside stratum corneum

The tape stripping technique was used to evaluate the accumulation of drugs inside the *stratum corneum*. The amount of recovered substance with respect to strip number (related to depth) gives the penetration profile of investigated substance (Ricci et al., 2005; Wissing and Muller, 2003; Jimenez et al., 2004). The accumulation of drug in the keratinous structures and/or lipids of *stratum corneum* is predominantly governed by the lipophilic character of applied agents (Hagedorn-Leweke and Lippold, 1998).

Fig. 4 shows the penetration profiles of retinol from the three formulations (EP, EC and SOL). The major results showed that PE promoted significantly the retinol accumulation in the *stratum corneum* compared to the two other formulations. Moreover retinol was found in all *stratum corneum* sheets compared to the solution and CE which were mainly recovered in the upper layers of the *stratum corneum*. Statistical analysis of these results showed significant differences between PE and CE, PE and SOL but no significant difference between SOL and EC.

To summarize, the retinol penetration profile in the skin was significantly different for both PE and CE emulsions. Retinol is preferentially accumulated in the *stratum corneum* when encapsulated in the rigid droplets of PE. CE favored permeation towards the viable skin.

3.4. General discussion

The Pickering emulsion caused large skin absorption of retinol and the storage of retinol inside the *stratum corneum*, whereas a classical emulsion allowed quite an easy passage of retinol through the *stratum corneum*. The two emulsions that caused large skin absorption were quite different with respect to the distribution of retinol within the skin layers. Two aspects are discussed in the following. Firstly a brief discussion on the possible microscopic mechanisms responsible of such different behaviors of PE and CE is presented. It is difficult to specify the origin of such differences on the basis of skin penetration experiments only, so that this discussion is short for proposing ideas and not very speculative. Secondly the specificities of Pickering emulsions regarding skin absorption are detailed with comparison to the behavior of other dosage forms described in the literature. The objective is to present clear information with respect to the utility and indications of Pickering emulsions as a new dosage form for topical applications.

3.4.1. Discussion on mechanisms

3.4.1.1. The role of surfactant and particles presence in the skin transport. While the Pickering emulsions are “emulsifier-free”, the potential penetration enhancement effect of surfactant is avoided. Conversely, the presence of emulsifier in a classical emulsion can modify the absorption process of retinol into the skin. Polysorbates indeed display penetration enhancer properties (Cappel and Kreuter, 1991; Lopez et al., 2000; Nokhodchi et al., 2003). But surfactants (including polysorbates) can also decrease the permeation of lipophilic drugs because of the decrease of thermodynamic activity of drug solubilized in the micelles (Cappel and Kreuter, 1991). The actual influence of surfactants is manifold and it is also not obvious that surfactant used as emulsifier be as active as free surfactant used as penetration enhancer because it is adsorbed to oil droplets.

On the other hand, nanometric silica particles penetrate the *stratum corneum* up to approximately half its thickness during 24 h exposure (Frelichowska et al., 2009). It is not excluded that the silica particles may act as a vehicle for the transport of retinol deeper in the skin, due to adsorption of molecules on its surface (Korn et al., 1980).

3.4.1.2. The influence of vehicle size and stability. The droplet size has a definite effect on the penetration in biological structures. Thus, Schaefer et al. (1989, 1990) showed that microspheres smaller than 3 μm distributed randomly into hair follicles and in the SC; microparticles ranging from 3 to 10 μm penetrated through the hair follicles and particles larger than 10 μm did not penetrate and

remained on the skin surface. In the present study, both emulsions (PE and CE) had the same mean drop size (3 μm), so intact emulsion droplets might have penetrated the *stratum corneum*. The larger accumulation of retinol in SC observed for PE suggests the retention of this type of encapsulation system inside the external skin layers. Indeed the enhanced stability of Pickering emulsions with respect to classical makes possible the penetration of intact oil droplets inside the SC lipidic medium in between the corneocytes. In contrast, intact droplets of classical emulsion probably do not penetrate the skin; the CE droplets are presumably broken on the skin surface. The solid particles at the oil/water interface may act as a physical barrier against early drug release inside the outermost layers of SC in the same way as for micrometer-sized polymer carriers (microparticles, microcapsules). The droplets of PE in this study are surrounded by solid (silica) particles providing a kind of rigid shell around the drops. Thus, that kind of structure can be viewed as an encapsulation system like microcapsules, allowing transport and providing sustained release (Simovic and Prestidge, 2007).

3.4.2. The influence of particle rigidity

Lastly, oil droplets are soft particles whereas polymer microcapsules are hard particles. The rigidity of the silica shell around the oil droplet provides mechanical resistance; this gives supplementary stability with respect to oil droplets of classical emulsions (Aveyard et al., 2003). It is thought that classical emulsion droplets break at the surface of skin and release their content into the *stratum corneum*. Conversely intact PE droplet may penetrate deeper the *stratum corneum*. The oil droplets of Pickering emulsions resist droplet breaking but can nevertheless deform since they are filled with liquid oil. Deeper penetration of PE droplets may result from this special property of Pickering emulsions. The benefit of carrier deformability for drug transport through the *stratum corneum* was initially postulated for liposomes (Bouwstra and Honeywell-Nguyen, 2002). It has been presumed that flexible liposomes could penetrate in between the corneocytes, whereas rigid liposomes made of lipids in their gel-state could not enter the *stratum corneum* as intact carriers because they could not adapt their shape to the geometrical confinement in the inter-corneocyte lipid medium. Highly flexible liposomes have been designed accordingly (Cevc, 1996; Cevc and Blume, 1998; Honeywell-Nguyen et al., 2002). It is however not established that liposomes could penetrate the skin as intact carriers; this is still a matter of debate. On the same footing, Olvera-Martínez et al. (2005) suggested that the size AND the rigidity of carriers had both significant effects on the skin penetration of octylmethoxycinnamate (OMC) from a nanoemulsion, nanocapsules and an o/w emulsion (size = 2.9 μm). Classical emulsion and nanoemulsion penetrated to the same depth and similar retinol amounts were detected. Nanocapsules penetrated deeper and a higher retinol amount was detected in the deep layers of *stratum corneum*. On the other hand, the total quantity of retinol permeated was the highest for nanoemulsion, less for classical emulsion and the lowest for nanocapsules, suggesting that the release from nanocapsules was slow.

3.4.3. Specificities of Pickering emulsions as topical dosage form

3.4.3.1. Dosage forms that show similar features as Pickering emulsions. Various nano- and micro-sized carriers have been tested as topical or transdermal drug delivery systems. Drug absorption largely varies from limited penetration (solid particles made of polymer or lipids, soft liquid particles (emulsions), liposomes) to high permeation rates with concentrated surfactant formulations (micelles, microemulsions) and solutions containing penetration enhancers (Bolzinger et al., 2008). Few of them cause retention of lipophilic drugs in the *stratum corneum* in the same way as the present Pickering emulsions do: polymer microparticles (including microcapsules) and solid lipid nanoparticles (SLN). These systems can modulate the penetration of drugs through the *stratum corneum* and modify the drug release at different skin sites due to two main characteristics: specific interactions of intact carrier with the skin and rigidity of the carrier. Pickering emulsions are additional possible carriers that have their own specificities. Among known dosage forms, Pickering emulsions are closer to polymer particles of the same size. But it is not obvious that the storage mechanism in the *stratum corneum* is the same.

The most relevant report on skin delivery of retinol from polymer particles was given by Failloux et al. (2004). They studied the release and storage of retinol in skin from alginate microcapsules (10 μm) and an o/w emulsion (20 μm). Confocal Raman spectroscopy of human biopsies showed that intact capsules passed to the epidermis and were stored longer than emulsion droplets. Additionally, the slow motion of the particles could be monitored in time-resolved observations. *In vitro* experiments on hairless mice skin pieces showed a larger permeation of retinol from emulsion after 24 h exposure. The retinol stored inside the skin during 24 h exposure was slowly released for a supplementary 24 h delay without exposure. Larger amounts of retinol were released from microcapsules than from emulsions, suggesting that the amounts of retinol stored inside the skin during exposure was larger. Encapsulated retinol was maintained longer in the skin compared to classical o/w emulsion. The core–shell structure thus ensured the reservoir system, able to deliver retinol for longer periods of time. The close similarity with the present work suggests that the storage mechanism for solid-stabilized emulsion in the external layers of skin is similar to that observed for microparticles by Failloux et al. (2004).

The accumulation of the acyclovir encapsulated in PLGA microparticles was observed in the most external skin layers (de Jalón et al., 2001). The possible explanation given by the authors was the occlusive effect caused by the presence of microparticles, which could form an adherent film at the skin surface, therefore reducing the transepidermal water loss and favoring the drug penetration. In the deep skin layers, sustained drug delivery was observed during 90 h. Total absorbed quantity of drug was less for microspheres than for free acyclovir suspension. The occlusion mechanism was not proven and has only been proposed by analogy with the behavior of SLN explained below.

An *in vivo* study by Alvarez-Román et al. (2001, 2004) disclosed that polycaprolactone nanocapsules loaded with OMC better protected skin against UV-induced erythema than free OMC. Additionally, in OMC encapsulated in nanoparticles remained in the *stratum corneum*. As possible explanations, better adhesion and tighter coverage of the skin surface coming from the high specific surface of nanometric particles was proposed. OMC having $\log P = 5.96$ (Brinon et al., 1999) is similar to retinol in terms of hydrophobic character. A complementary investigation by Jimenez et al. (2004) gave a comparison performed *in vitro* between the same polycaprolactone nanocapsules, and o/w or w/o emulsions loaded with OMC. The total OMC quantity penetrating through the skin was less for nanocapsules than for both emulsions. In particular, OMC did not reach the viable epidermis when encapsulated in nanocapsules. The sustained accumulation of encapsulated OMC in the *stratum corneum* was obviously the cause of the higher UV blocker action.

SLN also cause higher penetration of lipophilic drugs in the SC (Wissing and Muller, 2002, 2003; Jenning et al., 2000; Muller et al., 2002). The occlusive effect of accumulated SLN at the skin surface was proposed as the origin of penetration enhancement because it maintained a higher hydration level of the skin. The behavior of Pickering emulsions on skin is quite different to that of SLN. The high stability of PE droplets keeps the oil inside the droplets and prevents any manifestation of the occlusive property of the oil.

3.4.3.2. Potential indications of Pickering emulsions. Regarding highly lipophilic molecules, Pickering emulsions promote high storage in the *stratum corneum*. The practical utility of such behavior is either preventing skin penetration of molecules that should be retained at the skin surface, *e.g.* sunscreens, or using the *stratum corneum* as a reservoir for slow release of the drug to the deeper skin layers. One indication of Pickering emulsions, regarding pharmaceutical application, is sustained release targeted to the viable epidermis, from drug storage in the deeper layers of *stratum corneum*, in the same way as it has been claimed for microparticles. It has indeed been reported that polymeric microparticles act as drug micro-reservoirs in the horny layer (Rolland et al., 1993). For instance, de Jalón et al. (2001) reported sustained delivery of acyclovir to the basal epidermis from PLGA microparticles of 5 μm diameter (compared to drug suspension). Basal epidermis is the site of action of acyclovir against the Herpes virus; it is also the site of action of retinol.

There is a need for making the skin penetration path clear: either inter-corneocyte (SC lipids) or through hair follicles. The intra-corneocyte path is ruled out for lipophilic drugs. According to Schaefer et al. (1989, 1990), the droplet size allows both paths. The surface chemistry of the particles may also have a decisive influence. The hair follicle penetration mechanism proposed by Lademann et al. (2007) obviously depends at lot on surface properties of particles. As Pickering emulsions are quite different to polymer capsules, with respect to the surface chemistry, there is an opportunity to select the specific path for skin penetration by selection of the carrier type (PE, SLN or polymer capsule) and its surface properties.

4. Conclusions

Pickering emulsions are possibly useful vehicles for topical application of lipophilic dugs. They exhibit significant differences in skin absorption profile of the model lipophilic drug, *all-trans* retinol, compared to an emulsifier-stabilized emulsion of the same droplet size. The main feature is an increased accumulation of drug in *stratum corneum* for PE compared with CE and SOL. The ability of PE to accumulate may be due to the lack of flexibility of the droplets, compared to emulsifier-stabilized droplets. Better understanding of the mechanisms would require complementary physicochemical investigations of the interactions of PE droplets with the *stratum corneum* components. Further studies are also needed to evaluate PE stabilized with different kind of particles and containing different model drugs. As the PE formulation is able to retain lipophilic ingredients in the outer layer of skin, this kind of formulation can be potentially used to target drugs to the skin surface. Such a problem is also addressed in the field of sunscreen formulations where the organic UV-filter should stay at the surface of the *stratum corneum* and not penetrate in the deeper layers of the skin (Fernandez et al., 2000a,b; Alvarez-Román et al., 2001, 2004).

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