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Vehicle effects on *in vitro* release and skin permeation of octylmethoxycinnamate from microemulsions

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ABSTRACT

The high content of surfactants is one of the major limits to microemulsions (MEs) use in pharmaceutical and cosmetic field. In this work MEs with low surfactant content were prepared by the phase inversion temperature (PIT) method using different oil phases and emulsifiers. The effects of vehicle composition on *in vitro* release and skin permeation of octylmethoxycinnamte (OMC), one of the most used UVB filter, was evaluated. These MEs showed droplet sizes in the range 32–77 nm and a single peak in size distribution. MEs prepared using the most lipophilic lipids (decyl oleate or cetyl stearyl isononanoate) showed the lowest stability. *In vitro* release and skin permeation profiles were affected by both lipophilicty and structure of the lipid used as internal phase and the formulation that released the lowest amount of OMC provided the lowest active compound skin permeation. It is noteworthy that no OMC release and skin permeation were observed using oleth-20/glyceryl oleate as emulsifiers. Furthermore, a skin permeation enhancement effect was observed depending on the vehicle components. The results of this work suggest that PIT MEs could provide controlled skin drug delivery by choosing proper associations of oil phase lipids and emulsifiers.

1. Introduction

The formidable barrier properties of the skin prevent the permeation of therapeutically effective amounts of a large number of drugs (Cevc and Vierl, 2010). In recent years, many strategies have been investigated to improve drug efficacy after topical administration. Among these strategies, the use of microemulsions (MEs) is regarded as a promising approach to enhance release and skin permeation of both hydrophilic and hydrophobic drugs (Schmalfub et al., 1997; Kreilgaard, 2002; Philip et al., 2003; Sintov and Shapiro, 2004; Huang et al., 2008). Microemulsions consist of an aqueous and an organic phase to which a suitable emulsifier system is added to ensure the lowering of interfacial tension required to obtain a stable formulation. Compared to conventional emulsions, MEs are characterized by droplet size in the submicron range, transparency, thermodynamic stability, low viscosity and ease of manufacturing (Azeem et al., 2009).

However, MEs pharmaceutical and cosmetic application is generally limited by their high content of surfactants (from 25% up to 80%) that could be irritant for the skin (Sirotti et al., 2002; Peltola et al., 2003; Izquierdo et al., 2005; El Maghraby, 2008).

In the last decade, the phase inversion temperature (PIT) method has been proposed as a new procedure to obtain oil-in-water MEs containing percentages of non ionic surfactants as low as those used to prepare O/W emulsions (Diec et al., 2001), thus greatly improving MEs skin tolerability.

Although many studies have been performed on a large variety of lipids and surfactants to determine their influence on the properties of topical microemulsions such as droplet size, drug loading, phase behavior and system stability (Warisnoicharoen et al., 2000; Li et al., 2005; Djekic and Primorac, 2008; El Maghraby, 2008), to date the effects of vehicle composition on drug release and skin permeation from PIT MEs have not been thoroughly investigated. In a previous work, we pointed out that PIT MEs provided different drug release profiles depending on oil phase lipophilicity, drug partition coefficients and type of surfactant used (Montenegro et al., 2006). Furthermore, the results of this study suggested that these MEs could allow to achieve controlled drug delivery by choosing proper combinations of oil phase lipids and emulsifier systems. However, since non ionic surfactants are known to act as skin penetration modifiers (Lopez et al., 2000) drug skin permeation after topical administration of these MEs may depend on surfactant enhancement effects, in addition to drug release profile from the vehicle.

Therefore, in the present study, we evaluated the relationship between type of oil phase and surfactants used and *in vitro* drug release and skin permeation from the resulting oil-in-water MEs prepared by the PIT method. Six lipids, with different lipophilic-

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Table 1
Composition (%, w/w) of microemulsions 1–6 prepared with different oil phases.

Compound	Formulation					
	1	2	3	4	5	6
Isoceteth-20	5.5	5.5	5.5	5.5	5.5	5.5
Glyceryl oleate	2.2	2.2	2.2	2.2	2.2	2.2
Octyl dodecanol	5.0	–	–	–	–	–
Cetyl stearyl isononanoate	–	5.0	–	–	–	–
Decyl oleate	–	–	5.0	–	–	–
Isopropyl myristate	–	–	–	5.0	–	–
Isopropyl palmitate	–	–	–	–	5.0	–
Triglyceride caprylic/capric	–	–	–	–	–	5.0
Water[a]	86.3	86.3	86.3	86.3	86.3	86.3
OMC	1.0	1.0	1.0	1.0	1.0	1.0

[a] Water containing 0.35% (w/w) imidazolidinyl urea and 0.05% (w/w) Kathon CG was used.

ity and commonly used in pharmaceutical and cosmetic field (octyl dodecanol, decyl oleate, cetyl stearyl isononanoate, isopropyl myristate, isopropyl palmitate, triglyceride caprylic–capric), were used as oil phase to prepare MEs containing a low amount of a surfactant mixture (isoceteth-20/glyceryl oleate, 5.5 and 2.2.%, w/w, respectively), selected on the basis of previous studies of ours (Montenegro et al., 2006). Octylmethoxycinnamate (OMC), one of the most widely used lipophilic UVB filter, was added as model drug to evaluate the effects of MEs composition on its *in vitro* release and skin permeation. Then, the oil phase (triglyceride caprylic–capric) that provided the formulation with the best physico-chemical properties and the most interesting *in vitro* release and skin permeation results was used to prepare MEs containing low percentages of different emulsifier systems and their influence on OMC *in vitro* release and skin permeation was evaluated.

2. Materials and methods

2.1. Materials

Polyoxyethylene-20-cetyl ether (Brij 58, ceteth-20) was supplied by Fluka (Milan, Italy). Polyoxyethylene-20-isohexadecyl ether (Arlasolve 200 L, isoceteth-20) was a kind gift of Bregaglio (Milan, Italy). Glyceryl oleate (Tegin O) was obtained from Th. Goldschmidt Ag (Milan, Italy). Polyoxyethylene-20-oleyl ether (Brij 98, oleth-20) was purchased from Sigma (Milan, Italy). Glyceryl isostearate (Peceol isostearique) was a kind gift of Gattefossè (Milan, Italy). Cetyl stearyl isononanoate (Tegosoft CI), glyceryl monostearate (GMS), isopropyl myristate (IPM), isopropyl palmitate (IPP), triglyceride caprylic–capric (Tegosoft CT) and imidazolidinyl urea (Kemipur 100) were bought from A.C.E.F. (Milan, Italy). Methylisothiazolinone and isothiazolinone (Kathon CG) were a kind gift of Sinerga (Italy). Octyl dodecanol (Eutanol G) and decyl oleate (Tegosoft DO) were a kind gift of Cognis Care Chemicals (Como, Italy). Octylmethoxycinnamate (OMC) was kindly supplied by Basf (Ludwigshafen, Germany). Regenerated cellulose membranes (Spectra/Por CE; Mol. Wet. Cut off 3000) were supplied by Spectrum (Los Angeles, CA).

Acetonitrile and water used in the HPLC procedures were of LC grade and were bought from Merck (Milan, Italy). All other reagents were of analytical grade.

2.2. Preparation of o/w microemulsions

Microemulsions were prepared using the phase inversion temperature (PIT) method (Diec et al., 2001). The composition of formulations 1–6 and 6A–6H is shown in Tables 1 and 2, respectively. Octanol/water partition coefficients (Log *P*) of lipids used to obtain MEs 1–6 were obtained from Advanced Chemistry Development (ACD) software Solaris V4.67 and were: isopropyl myristate: 7.432 ± 0.212; isopropyl palmitate: 8.495 ± 0.213; octyl dodecanol: 9.195 ± 0.213; triglyceride caprylic/capric: 10.853 ± 0.311; decyl oleate 12.942 ± 0.322; cetyl stearyl isononanoate 15.591 ± 0.323.

The aqueous and the oil phases were separately heated at 80–90 °C, then the aqueous phase was added drop by drop to the oil phase, at constant temperature and under continuous stirring. Then, the formulation was cooled to room temperature under slow agitation. At the phase inversion temperature the turbid mixture turned into a clear "bluish-white" o/w microemulsion. The phase inversion temperature was determined using a Crison 525 conductivity meter (Symphony VWR, Italia) which measured an electric conductivity change when the inversion from w/o to o/w microemulsion occurred.

2.3. Transmission electron microscopy (TEM)

For negative-staining electron microscopy, 5 μl of ME dispersions were placed on a 200-mesh formvar copper grid (TAAB Laboratories Equipment, Berks, UK), allowed to be adsorbed. Then the surplus was removed by filter paper. A drop of 2% (w/v) aqueous

Table 2
Composition (%, w/w) of microemulsions 6A–6H containing different emulsifier systems.

Compound	Formulation							
	6A	6B	6C	6D	6E	6F	6G	6H
Isoceteth-20	5.5	6.0	–	–	–	–	–	–
Ceteth-20	–	–	5.5	5.5	5.5	–	–	–
Oleth-20	–	–	–	–	–	5.5	6.0	5.5
Glyceryl oleate	–	–	2.2	–	–	2.2	–	–
Glyceryl isostearate	2.4	–	–	2.4	–	–	2.4	–
Gliceryl monostearate	–	2.6	–	–	2.6	–	–	2.6
Triglyceride caprylic/capric	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Water[a]	86.1	85.4	86.3	86.1	85.9	86.3	85.6	85.9
OMC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[a] Water contained 0.35% (w/w) imidazolidinyl urea and 0.05% (w/w) Kathon CG.

solution of uranyl acetate was added over 2 min. After the removal of the surplus, the sample was dried at room condition before imaging the ME with a transmission electron microscope (model JEM, 2010, Jeol, Peabody, MA, USA) operating at an acceleration voltage of 200 kV.

2.4. Photon correlation spectroscopy (PCS)

The droplet sizes of the MEs being tested were determined using a Zetamaster S (Malvern Instruments, Malvern, UK), at 20 °C, by scattering light at 90°. The instrument performed particle sizing by means of a 4 mW laser diode operating at 670 nm. The mean diameter and the polidispersity index values were the averages of results obtained for two separate preparations.

2.5. Stability tests

Samples of MEs 1–6 and 6A–6H were stored in airtight jars, in the dark and kept at room temperature or at 37 °C for two months for stability tests. Droplet size, polidispersity index and pH value of each sample were measured at time intervals. A pH-meter CRISON, mod. Basic 20 (Milan, Italy) was used to measure pH values.

2.6. In vitro release experiments

OMC release rates from all prepared MEs were measured through cellulose membranes using Franz-type diffusion cells (LGA, Berkeley, CA). This method has been previously reported as a suitable technique for evaluating drug release from topical formulations (Shah et al., 1989).

The cellulose membranes were moistened by immersion in water for 1 h at room temperature before being mounted in Franz-type diffusion cells. The diffusion surface area was 0.75 cm^2 and the receiving chamber volume was 4.5 ml. The receiving phase consisted of a water/ethanol (50/50, v/v) solution for ensuring pseudo-sink conditions (Touitou and Fabin, 1988) and was constantly stirred at 700 rpm and thermostated at 35 °C to maintain the membrane surface at 32 °C. 500 μl of each ME were applied on the membrane surface and the experiments were run for 24 h. At intervals (0, 3, 6, 9, 24 h), 200 μl of the receptor phase were withdrawn and replaced with an equal volume of receiving solution equilibrated to the experimental temperature (35 °C). Samples of the receptor phase were analyzed by the HPLC method described below to determine their active compound content. At the end of the experiments, samples of the ME applied on the membrane surface were withdrawn and analyzed to determine ME droplet sizes and polidispersity indexes. Each experiment was performed in triplicate.

2.7. In vitro skin permeation experiments

Samples of adult human skin (mean age 38 ± 8 years) were obtained from breast reduction surgery. Subcutaneous fat was carefully trimmed and the stratum corneum and the epidermis (SCE) were separated from the dermis as described by Kligman and Christophers (1963). SCE membranes were used to evaluate the skin permeation of the tested MEs since the dermis *in vitro* can act as an artificial barrier to the penetration of lipophilic compounds (Bronaugh and Stewart, 1984). SCE membranes were dried in a desiccator at approximately 25% R.H. and stored at 4 °C until use, according with the procedure described by Swarbrick et al. (1982). Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in the same Franz type-diffusion cells and using the same experimental conditions described above. To ensure sink conditions, a mixture water/ethanol (50/50, v/v) was used as receptor fluid. As reported in the OECD Guidelines 428 (2004), this receiving phase does not significantly affect the integrity of the skin. Each formulation (500 μl) was applied to the skin surface and the experiments were run for 24 h. At intervals (0, 3, 6, 9, 24 h), samples (200 μl) of the receptor phase were withdrawn and replaced with an equal volume of receiving solution pre-equilibrated to 35 °C. Samples of the receptor phase were analyzed with the HPLC method described below to determine the amount of OMC permeated.

Each experiment was performed in duplicate on three different skin donors.

2.8. High performance liquid chromatography (HPLC) analyses

The HPLC system consisted of a Varian ProStar model 230 (Varian, Milan, Italy) with an auto-sampler Varian model 410 and a Galaxie software for data elaboration. The chromatographic analyses were performed using a Waters Simmetry, 4.6 cm × 15 cm reverse phase column (C_{18}). The mobile phase consisted of an acetonitrile/water mixture (80:20, v/v). All the analyses were carried out at room temperature at a flow rate of 1.0 ml/min. 20 μl of each sample were injected and the column effluent was monitored continuously at 310 nm. The amount of OMC in each sample was calculated by reporting the peak area of a sample on a standard calibration curve that was built up by relating known concentrations of OMC with the respective peak areas. No interference of the other formulation components was observed. The sensitivity of the HPLC method was 0.1 μg/ml.

2.9. Data analysis

Active compound flux ($\mu g\,cm^{-2}\,h^{-1}$) through cellulose membrane or through the skin was calculated by plotting the cumulative amount of compound released or permeated against time and dividing the slope of the steady-state portion of the graphs by the area through which diffusion took place. The lag time was determined from the *x*-intercept values of the regression lines.

Results were expressed as the mean ± S.D. and Student's *t*-test was used to evaluate the significance of the difference between mean values. Values of $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Effects of oil phase lipophilicity (MEs 1–6)

3.1.1. Morphology and stability of MEs 1–6

MEs 1–6 showed droplet sizes in the range 32–49 nm and a single peak in size distribution, with no substantial difference upon OMC addition (Table 3). As reported in the literature (Ghosh and Murthy, 2006), the oil phase affects droplet curvature radius depending on its ability to penetrate and to swell the tail group region of the surfactant layer. Short chain oils penetrate this region to a greater extent compared to long chain alkanes and therefore they swell this region to a greater extent leading to an increased negative curvature. Our findings suggest that there is no correlation between lipid lipophilicity and droplet size. Although these lipids show different physico-chemical properties they seem to be able to penetrate the tail group region of the surfactant layer at the same extent, probably due to hydrophobic interactions between lipid and surfactant at the interfacial layer that did not lead to a change of droplet curvature radius.

TEM analysis (Fig. 1) confirmed PCS results, showing dispersed and homogeneous systems, with droplet size in the nano-size range, for all the MEs under investigation. As shown in Table 3, PIT values were similar for unloaded MEs 1–5 while ME 6 showed a slightly higher value. Furthermore, the addition of OMC to MEs

Table 3
Phase inversion temperature (PIT), droplet size (Size ± S.D.) and polidispersity index (Poly ± S.D.) of MEs 1–6 and 6A–6H with and without OMC.

ME	OMC % (w/w)	PIT	Size ± S.D. (nm)	Poly ± S.D.
1	–	76	45.6 ± 0.6	0.478 ± 0.025
	1%	82	45.5 ± 0.7	0.095 ± 0.003
2	–	78	32.7 ± 0.1	0.169 ± 0.042
	1%	85	35.3 ± 0.5	0.101 ± 0.015
3	–	78	49.0 ± 0.7	0.498 ± 0.028
	1%	85	47.4 ± 0.6	0.515 ± 0.034
4	–	78	36.0 ± 0.2	0.315 ± 0.010
	1%	85	45.9 ± 0.4	0.259 ± 0.008
5	–	76	41.4 ± 0.5	0.330 ± 0.009
	1%	80	44.1 ± 0.3	0.280 ± 0.011
6	–	81	38.7 ± 0.2	0.307 ± 0.009
	1%	88	44.8 ± 0.3	0.195 ± 0.002
6A	–	80	49.3 ± 0.7	0.487 ± 0.026
	1%	85	37.0 ± 0.2	0.225 ± 0.005
6B	–	80	76.4 ± 1.0	0.502 ± 0.059
	1%	85	–	–
6C	–	80	46.0 ± 0.6	0.469 ± 0.038
	1%	85	29.5 ± 0.6	0.301 ± 0.012
6D	–	80	65.5 ± 1.2	0.499 ± 0.031
	1%	85	41.8 ± 0.8	0.512 ± 0.026
6E	–	80	77.4 ± 0.9	0.309 ± 0.022
	1%	85	38.9 ± 1.1	0.405 ± 0.011
6F	–	80	68.1 ± 0.2	0.407 ± 0.007
	1%	85	28.9 ± 0.2	0.489 ± 0.032
6G	–	80	53.9 ± 0.3	0.389 ± 0.012
	1%	85	27.8 ± 0.7	0.280 ± 0.008
6H	–	80	57.9 ± 0.9	0.401 ± 0.022
	1%	85	43.1 ± 0.9	0.216 ± 0.012

1–6 caused a significant increase of PIT values compared to the corresponding unloaded MEs. Since higher PIT values are regarded as predictive of a greater stability for emulsified systems (Izquierdo et al., 2005; Foster et al., 1990), we would have expected a better stability for unloaded ME 6 and OMC loaded MEs 1–6. Izquierdo et al. (2005) reported that there is a correlation between the HLB temperature and the surfactant HLB: the HLB temperature increases with the increase of surfactant HLB. Therefore, it could be expected that emulsions containing surfactants showing similar HLB have similar PIT values and similar stability. However, these authors performed their experiments using different surfactant concentrations and mixing ratio but the same oil phase.

In our experiments we used surfactants with similar HLB values (isoceth-20 15.5; ceteth-20 15.7; oleth-20 15.3) but we used different oil phases. After 2 months of storage at room temperature and 37 °C, loaded and unloaded MEs 1, 4, 5 and 6 did not show any significant change in their droplet size, while formulations 2 and 3 showed an increase after 2 months of storage at 37 °C (Table 4). Stability of ME 2 was lower than that of ME 3 since droplet sizes of loaded or unloaded ME 2 showed about a 75% increase while droplet size increase was only about 25% (unloaded)—35% (loaded) for ME 3. As MEs 2 and 3 were prepared using the most lipophilic lipids among those selected, these stability data suggest that both the oil phase lipophilicity and the different structures of the lipid used as internal phase could influence formulation stability at high temperature. Since in ME 3 both the lipid (decyl oleate) and the cosurfactant (glyceryl oleate) have a linear unsaturated acyl chain (oleic chain), a better interaction between the acyl chains of lipid and cosurfactant could be expected compared to microemulsion 2 (containing a branched lipid), thus contributing to increase ME 3 stability compared to microemulsion 2. Therefore, the results of our stability studies suggest that lipophilicity and structure of the oil phase may play an important role in determining microemulsion stability, in addition to surfactant HLB.

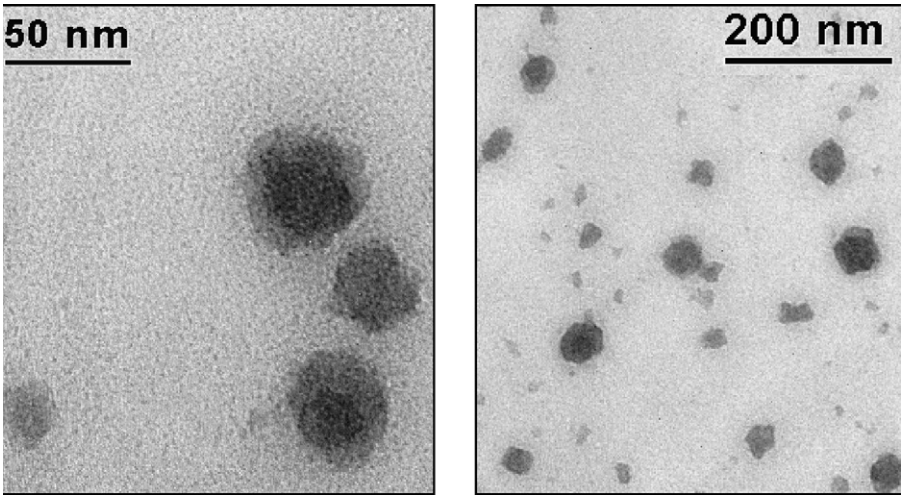

Fig. 1. Transmission electron microscopy (TEM) of PIT microemulsions.

Table 4
Stability data (Size ± S.D.) of microemulsions (MEs) 1–6 unloaded and loaded with 1% of OMC at 24 h and after storage for 2 months at room temperature (R.T.) and 37 °C.

MEs	OMC % (w/w)	Size ± S.D. (nm) after 24 h	Size ± S.D. (nm) after storage for 2 months at R.T.	Size ± S.D. (nm) after storage for 2 months at 37 °C
1	–	45.6 ± 0.6	44.5 ± 0.9	41.6 ± 1.0
	1%	45.5 ± 0.7	47.5 ± 0.8	45.9 ± 0.7
2	–	32.7 ± 0.1	33.9 ± 0.3	65.4 ± 0.5
	1%	35.3 ± 0.5	39.6 ± 0.5	69.4 ± 0.6
3	–	49.0 ± 0.7	35.6 ± 0.8	61.9 ± 1.0
	1%	47.4 ± 0.6	46.8 ± 0.7	62.8 ± 1.2
4	–	36.0 ± 0.2	36.9 ± 0.5	37.5 ± 0.7
	1%	45.9 ± 0.4	49.5 ± 0.7	48.3 ± 0.6
5	–	41.4 ± 0.5	36.5 ± 0.5	39.5 ± 0.5
	1%	44.1 ± 0.3	45.6 ± 0.3	46.1 ± 0.7
6	–	38.7 ± 0.2	35.2 ± 0.2	37.5 ± 0.5
	1%	44.8 ± 0.3	42.7 ± 0.5	45.6 ± 0.5

Loaded and unloaded MEs 1–6 showed pH values ranging from 4.5 to 5.9 with no significant change upon storage at R.T. and 37 °C (data not shown).

3.1.2. *In vitro* release and skin permeation of OMC from MEs 1–6

In vitro release and skin permeation experiments were carried out using the infinite dose technique, i.e. applying a large amount of formulation (500 μl) on the membrane surface. The use of an infinite dosing in *in vitro* release and skin permeation studies avoids active compound depletion from the donor compartment during the experiment, thus ensuring a constant driving force for the release process and allowing the achievement of steady-state conditions. The cumulative amount of OMC released after 24 h and its release rate (flux through a cellulose membrane) from MEs 1–6 decreased in the following order: 4 > 5 > 1 ≈ 3 > 2 > 6 (Table 5). Significantly longer lag times were observed to achieve steady-state conditions from MEs 4 and 5 while the greatest lag time value was obtained for ME 6 whose OMC release was the lowest among MEs 1–6. When two lipids that differs in their structure only for a methylene group (IPM and IPP) were used as oil phase, release parameters seem to be dependent mainly on lipid lipophilicity. The high lag time values observed for these MEs suggest the existence a sort of barrier that needs to be overcome for OMC release to take place. The existence of a lipophilic barrier to drug release from oil droplets of O/W MEs has already been postulated by other authors (Trotta, 1999). Apart from ME 6, a relationship, although not linear, was observed between lipid lipophilicity and amount of active compound released at the end of the experiment. A similar trend has been already observed in a previous work of ours (Montenegro et al., 2006). In particular, ME 4, containing the least lipophilic lipid (isopropyl myristate), provided the greatest amount of OMC released after 24 h, but the lowest OMC release was obtained from ME 6 whose oil phase (triglyceride caprylic/capric) had an intermediate Log *P* value. This suggests that, in addition to lipophilicity, the structure of the lipid used as oil phase may play an important role in determining the release rate of the active molecule from PIT microemulsion. Since the lipid used to prepare ME 6 is a mixture of triglycerides with different chain lengths, its structure is significantly different from that of the other lipids that are branched or linear esters or alcohols. The different structure of triglyceride caprylic/capric could involve stronger interactions between OMC and oil phase and/or a steric hindrance that could lead to an overall decrease of OMC release from this ME. Furthermore, owing to its medium length acyl chains, a better intercalation of this lipid between the surfactant and co-surfactant molecules could be postulated due to hydrophobic interactions. Therefore, a closer packing of the surfactant layer that would decrease OMC diffusion out of the droplets could be expected.

Lipophilicity and structure of the lipid used as internal phase of PIT MEs affected not only OMC release, but also its *in vitro* skin permeation profile. Results of OMC *in vitro* skin permeation experiments from MEs 1–6 are reported in Table 6. The cumulative amount of OMC permeated after 24 h and its flux through excised human skin from MEs 1–6 decreased in the order: 1 > 4 > 3 ≈ 5 ≈ 2 > 6. By comparing in *vitro* release results with *in vitro* skin permeation data, we observed that the amount of OMC released from the vehicle was greater than that permeated through the skin from the same formulation for all the MEs tested, apart from ME 6. This suggests that the rate-limiting step in OMC skin permeation process was its permeation through the skin rather than its release from the vehicle. As regards ME 6, OMC release rate was equal to its flux through the skin, thus indicating that OMC skin permeation from this formulation was limited by its release from the vehicle. Therefore, in our experiments, the formulation that released the lowest amount of OMC provided the lowest active compound skin permeation. As shown in Table 6, OMC skin permeation from ME 1 was about three-fold higher that that obtained from ME 3, although OMC release from these formulations was similar. These results could be due a penetration enhancer effect of the vehicle components. As reported in the literature (Williams and Barry, 2004), substances like alcohols and

Table 5
Cumulative amount released after 24 h (Q_{24} ± S.D.), Flux ± S.D. and lag time of OMC from micromulsions (MEs) 1–6 and 6A–6E.

MEs	Q_{24} ± S.D. (μg cm^{-2})	Flux ± S.D. (μg cm^{-2} h^{-1})	Lag time (h)
1	259.22 ± 16.21	15.57 ± 1.22	2.30
2	39.29 ± 3.51	5.52 ± 0.85	1.18
3	248.32 ± 14.06	14.81 ± 1.12	2.72
4	600.00 ± 46.26	45.96 ± 4.04	6.79
5	544.65 ± 43.62	41.81 ± 3.71	6.87
6	2.15 ± 0.18	0.12 ± 0.01	0.01
6A	1.97 ± 0.08	0.11 ± 0.01	0.08
6C	2.95 ± 0.16	0.17 ± 0.02	0.10
6D	2.70 ± 0.19	0.15 ± 0.01	0.38
6E	11.80 ± 0.67	0.67 ± 0.08	0.01

Table 6
Cumulative amount permeated through excised human skin after 24 h (Q_{24} ± S.D.), Flux ± S.D. and lag time of OMC from microemulsions (MEs) 1–6 and 6A–6E.

MEs	Q_{24} ± S.D. (μg cm^{-2})	Flux ± S.D. (μg cm^{-2} h^{-1})	Lag time (h)
1	44.09 ± 8.83	2.43 ± 0.46	0.62
2	10.58 ± 3.25	0.57 ± 0.16	0.96
3	12.9 ± 2.31	0.69 ± 0.13	1.04
4	20.71 ± 5.04	1.13 ± 0.31	0.29
5	12.81 ± 3.21	0.67 ± 0.22	0.76
6	1.95 ± 0.50	0.11 ± 0.03	0.51
6A	1.65 ± 0.31	0.09 ± 0.02	0.22
6C	2.71 ± 0.25	0.16 ± 0.02	0.83
6D	2.55 ± 0.66	0.15 ± 0.03	0.23
6E	9.89 ± 1.98	0.57 ± 0.11	0.41

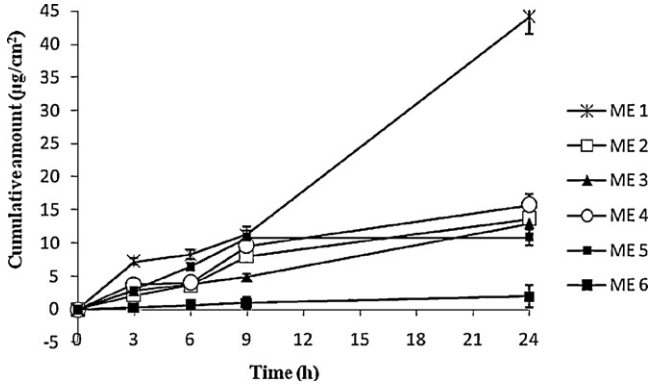

Fig. 2. *In vitro* permeation of OMC through human skin from MEs 1–6.

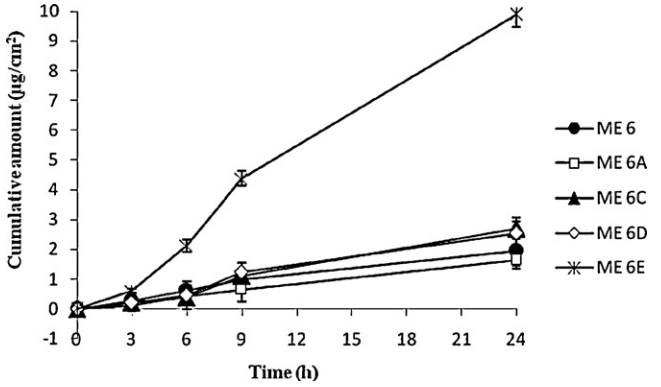

Fig. 3. *In vitro* permeation of OMC through human skin from MEs 6A–6E.

esters could act as penetration modifiers, thus improving drug skin permeation. The presence of these substances in our formulations could have altered the skin barrier properties, thus explaining the different amounts of OMC permeated from vehicles that provided the same OMC release. OMC skin permeation data from ME 2, 3 and 5 were similar, apparently. However, plotting the cumulative amount of OMC permeated against time, different skin permeation profiles were obtained (Fig. 2). OMC skin permeation from MEs 1–4 showed a biphasic trend: a low permeation up to 6 h from the beginning of the experiment followed by a faster OMC skin permeation. ME 6 provided a low but constant OMC skin permeation during 24 h while a plateau 9 h after the beginning of the experiment was observed evaluating OMC skin permeation from ME 5. These different profiles could be due to a different ability of the components of the vehicle to act as skin penetration modifiers. However, as regards ME 6 no enhancement effect could be pointed out since OMC skin permeation was limited by its release from the vehicle.

3.2. Effects of different emulsifier systems (MEs 6A–6H)

3.2.1. Morphology and stability

For a sunscreen agent to be safe and effective, it should remain on the skin surface after its topical application, showing a skin permeation as low as possible.

Since the ME prepared using triglyceride caprylic–capric as oil phase provided the lowest OMC skin permeation, we used this lipid to study the effect of different emulsifier systems on the physico-chemical and technological properties of the resulting PIT microemulsions. Preliminary experiments showed that different amounts of surfactant/cosurfactant mixtures were required to obtain stable MEs with 5% lipid phase (Table 2). In Table 3, the mean droplet sizes of MEs 6A–6H loaded and unloaded with OMC are reported. All unloaded MEs showed mean droplet sizes ranging from 46 to 77 nm and a single peak in size distribution. The addition of 1% OMC significantly reduced the droplet sizes of MEs prepared with ceteth-20 and oleth-20 as surfactants (MEs 6C–6H). A different behavior was observed for MEs containing isoceteth-20 as surfactant (ME 6, 6A, 6B). Upon addition of OMC to ME 6, 6A, 6B, we observed a small increase of droplet size when glyceryl oleate was used as cosurfactant, a reduction for ME 6A containing glyceryl isostearate while the association isoceteth-20/gliceryl monostearate did not allow us to obtain a formulation in the field of existence of the microemulsion due to an increase over 150 nm of droplet size. These results suggest that different interactions of the active compound with the surfactant/cosurfactant layer could take place at the droplet interface and affect the emulsifier system's properties leading to change of the field of existence of the microemulsion, as reported in previous works (Ghosh and Murthy, 2006). A reduction of flexibility of the surfactant/co-surfactant layer could explain these results since more rigid surfactant films are believed to reduce the range of existence of MEs (Azeem et al., 2009). OMC-loaded and unloaded MEs 6A–6H showed pH values in the range 4.6–5.8 (data not shown). PIT values were similar for unloaded ME 6–6H. The addition of OMC caused the same increase of PIT values for all the prepared MEs. Therefore, a better stability would have been expected for OMC-loaded ME 6–6H. However, stability tests performed for 2 months at room temperature and 37 °C did not show any significant change of pH, droplet size and polidispersity index for loaded and unloaded MEs 6A–6H (data not shown).

3.2.2. Influence of surfactants on in vitro release and skin permeation

Results of OMC *in vitro* release experiments from MEs 6A–6H are reported in Table 6. OMC release from ME 6B was not determined because the addition of OMC to ME 6B turned out an emulsion. ME 6, 6A, 6C and 6D released similar amounts of OMC while ME 6E provided a significantly higher OMC release. On the contrary, MEs prepared using oleth-20 as surfactant and different co-surfactants (6F, 6G and 6H) did not provide any active compound release. These results suggest that both the type of surfactant and co-surfactant used to obtain a stable ME may play an important role in determining OMC release from the formulation.

These findings could be due to the different lipophilicity of the surfactants and co-surfactants used to prepare MEs 6–6H. Log *P* values (calculated by Advanced Chemistry Development ACD/LogPDB software vers. 11.01) of surfactants increased in the order: isoceteth-20 (2.33) < ceteth-20 (2.48) < oleth-20 (3.09) while Log *P* values of co-surfactants were in the following order: glyceryl oleate (6.68) < glyceryl isostearate (6.93) < glyceryl monostearate (7.09). *In vitro* release data showed that the most lipophilic surfactant did not provide any OMC release regardless of the type of co-surfactant used to obtain the MEs under investigation. However, lipophilicity cannot be regarded as the key parameter in determining OMC release from MEs 6–6H since the highest OMC release was observed from ME 6E containing the most lipophilic co-surfactant and a surfactant with an intermediate Log *P* value. Therefore, in addition to lipophilicity, the structure of surfactant and co-surfactant could play an important role in determining ME ability to release the loaded active compound by affecting the packing of the interfacial layer and, hence, active compound release from the oil droplets.

As expected, no OMC skin permeation was observed from ME 6F–6H. OMC skin permeation profiles from MEs 6, 6A, 6C, 6D were similar while ME 6E showed a greater OMC flux through the skin (Fig. 3). The comparison of *in vitro* release results with skin permeation data pointed out that OMC skin permeation from MEs 6–6E was limited by its release from the vehicle and no penetra-

tion enhancement effect was observed, regardless of the emulsifier system used. These results suggest that both the type of surfactant and co-surfactant used to obtain PIT MEs may play an important role in determining OMC *in vitro* release and skin permeation.

4. Conclusions

PIT MEs provided different OMC *in vitro* release and skin permeation profiles, depending both on lipid lipophilicity and structure and on type of surfactant used. It is noteworthy that no OMC *in vitro* release and skin permeation were observed when oleth-20/glyceryl oleate was used as emulsifier system. Therefore PIT MEs could be a useful tool to improve safety and effectiveness of active compounds that should remain on the skin surface after their topical application, showing a skin permeation as low as possible.

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