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Influence of encapsulation on the in vitro percutaneous absorption of octyl methoxycinnamate

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Abstract

The purpose of this study was to evaluate the in vitro transdermal permeation and skin accumulation of one ultraviolet (UV) absorber—octyl methoxycinnamate (OMC)—through pig skin and to determine the quantity of OMC in the skin surface and different pig skin layers (stratum corneum, viable epidermis, dermis, and receptor fluid). Four cases have been considered: the application of oil-in-water (O/W) and water-in-oil (W/O) emulsions containing the same filter free and encapsulated in nanocapsules (NC). The influence of the carrier on the percutaneous penetration was studied.

Data showed that UV absorber exhibited increases in skin accumulation when is formulated in emulsions in free form. Skin accumulation of OMC-free in the emulsions was significantly ($P < 0.05$) greater than that of OMC-encapsulated for all formulations investigated. OMC-free skin accumulation ranged from $127.8 \pm 22.8\,\mu g/cm^2$ (O/W emulsion) to $172.1 \pm 12.9\,\mu g/cm^2$ (W/O emulsion). OMC-encapsulated skin accumulation ranged from $50.3 \pm 13.1\,\mu g/cm^2$ to $43.0 \pm 6.5\,\mu g/cm^2$ at NC–O/W and NC–W/O, respectively. No significant differences were found in the transdermal permeation of cinnamate for any of the formulations tested. The results of this study demonstrate that the inclusion of OMC-encapsulated in sunscreen formulations decreases the skin accumulation of the cinnamate since the in vitro release mechanism of OMC-nanocapsules is governed by hydrophobicity and crystallinity of the polymer and by the high lipophilicity of the drug. The crystallinity of the polymer have the ability of reflecting and scattering UV radiation on their own thus leading to photoprotection without the need for molecular sunscreens.

Keywords: Sunscreen; Octyl methoxycinnamate (OMC); Nanocapsules (NC); Percutaneous penetration; Stripping technique

1. Introduction

On certain aspects, sun exhibition can be beneficial (Vitamin D synthesis). However, it can have serious consequences on skin such as actinic aging or something worse: sunburn, photoaging, and skin cancers of different types according to the proportion and exhibition length (Berset et al., 1996; Vanquerp et al., 1999). Thus, it is very important to use sunscreen agents in order to protect oneself from the effects of UV (Godwin et al., 2002). Health agencies world-wide also recommend the use of sunscreens as a means of lowering the risk of developing skin cancer.

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UV filters are also incorporated in sunscreens and into many everyday-use cosmetics such as moisturizers and lipsticks (Benson, 2000) in order to protect the skin from damage through UV radiation (Bredholt et al., 1998; Straub, 2002).

Sunscreen preparations are usually applied superficially to large skin areas. Therefore, effectiveness implies that sunscreen filters adhere to skin like a protective film. They should have a high affinity for the stratum corneum. The UV filters are designed to remain on the uppermost layers of the skin; penetration through the skin is low (Jiang et al., 1997). For sunscreens to be effective, the UV absorbers must remain in the outermost regions of the skin (Lu et al., 1999). An ideal sunscreen product should exhibit high skin accumulation of UV absorbers with minimal permeation to the systemic circulation (Gupta et al., 1999; Potard et al., 1999).

At present, research as far as sunscreens are concerned moves towards the conception of new cosmetic formulas, which have a total innocuity, a small capacity to overcome the skin barrier, a good substantiality, and an important remanence.

Colloidal drug carriers, including submicron emulsions, nanospheres, nanocapsules, liposomes, and lipid complexes, have been attracting increasing interest in recent years, as vehicles for the topical administration of lipophilic drugs (Allémann et al., 1993; Santos-Magalhaes et al., 1995). Concretely, the nanocapsules (NC) are introduced as the new generation of carriers for cosmetics, especially for UV blockers for the use on human skin and/or hair.

The aim of our work was to establish a comparison between the skin permeation of one common liposoluble sunscreen, a derivative of the cinnamic acid—octyl methoxycinnamate (OMC), from different emulsions: encapsulated sunscreen—poly(ϵ-caprolactone) nanocapsules and free. In this study, we investigated the influence of the carrier NC on in vitro percutaneous absorption of the OMC compared to a same O/W and W/O emulsions. An in vitro technique was used to allow accurate quantification of the amount of sunscreen which penetrated into and remained within the skin and a stripping method performed during the time of contact (3 and 24 h) with the pig skin.

2. Materials and methods

2.1. Basic data

Octyl methoxycinnamate is an organic UV-B filter that was originally developed in the 1950s and has been one of the most widely used sunscreens for decades. OMC is a cinnamic acid derivative that has a methoxy group on the *para* position and is esterified with 2-ethylhexanol; the chemical name is 2-ethylhexyl-3-(4-methoxyphenyl)-2-propenoate (Chisvert et al., 2001).

2.2. Chemicals

The poly-caprolactone (PCL) was supplied by Sigma–Aldrich Chimie, ref.: 44,074-4 (St. Quentin-Fallavier, France). The molecular weight was 80,000 (Aldrich data). Montanox® 80 (Polysorbate 80: POE-20 sorbitan monooleate) was obtained from Seppic (Paris, France). Acetone (Rectapur® grade), was purchased from Riedel de Haën (St. Quentin-Fallavier, France). Escalol® 557 (octyl methoxycinnamate) was furnished by ISP France (Roissy CDG, France; product code: 50098). All solvents used in this study were high performance liquid chromatography (HPLC) grade.

Phosphate buffered saline was supplied from Sigma (St. Quentin-Fallavier, France) and Oramix® BG 14 (butyl glucoside) was furnished by Seppic (Paris).

2.3. Preparation and characterization of OMC-NC

OMC-loaded PCL NC were prepared by the interfacial polymer deposition procedure (Fessi et al., 1989). Seven hundred and twenty milligrams of PCL were dissolved in 90 ml of acetone at 50 °C. Thereafter, a specified quantity of Escalol® 557 (OMC) (1500 mg) were added to the acetone solution. The resulting organic solution was injected under moderate magnetic stirring (350 rpm) into 120 ml of distilled water containing a non-ionic surfactant—Montanox® 80—used as stabilizing agent. The aqueous phase immediately turns milky with bluish opalescence as a result of the formation of a nanocapsule suspension (NC-S). This operation was repeated four times. These suspensions were mixed. The acetone, which rapidly diffused towards the aqueous phase, was then removed under

reduced pressure at 35–40 °C (Rotavapor® RE-140, Büchi, Switzerland) for approximately 120 min. Finally, the nanocapsule suspension was concentrated to the desired final volume by remove water under the same conditions.

The mean size of the NC-S was measured with a Coulter Nano-sizer® (Coulter Electronics, Margency, France). These measurements were carried out with laser-light scattering calibrated with a standard latex nanoparticle suspension (300 nm). An aliquot of nanocapsule suspension was resuspended in distilled water. Measurements were then performed at an angle of 90° to the incident light, and data were collected over a period of 90 s.

OMC content in the NC—*encapsulation efficiency*—was calculated by the difference between the total and free estimated drug concentrations. The extraction of OMC from NC was performed with acetonitrile (MeCN) under vortex—Top-Mix® Agitator—for 1 min and ultrasonic agitation—Bransonic® 220—for 20 min. Following to complete dissolution of the polymer for 3 h, a sample aliquot was diluted with acetonitrile to a theoretical concentration of 10 μg/ml. Finally, an aliquot (10 μl) of this solution was injected into the HPLC system. Non-incorporated OMC was isolated by the combination of different ultrafiltration and centrifugation procedures, and measured in the ultrafiltrate. Free OMC was measured in the clear supernatant following separation of nanocapsules from aqueous medium by centrifugation of 5 ml colloidal suspension at 19,000 rpm for 30 min (Hettich Universal 2S). Thereafter, 10 μl of this solution were analysed by the HPLC method as described in the following. The OMC entrapped in NC was expressed as the percentage of active principle incorporated in the NC relative to the total amount of OMC in the medium (Ferranti et al., 1999). The *yield* was expressed as the ratio between the actual active principle in the suspension and the theoretical amount.

2.4. Vehicles

The sunscreen agent—free and encapsulated—were first incorporated in an oil-in-water emulsion (O/W)—PEG-5 Glyceryl Stearate from ICI Surfactants, mineral oil from Codex, C12-15 Alkyl Benzoate from Croda Chemicals Ltd., Caprylic/Capric Triglyceride from Stearinerie Dubois, Stearic Acid from Cooper, Cetyl Alcohol from Cooper, Phenoxyethanol, Methylparaben, Ethylparaben, Propylparaben, and Butylparaben from Seppic, glycerin from Laserson and Sabetay and distilled water—then in a water-in-oil emulsion (W/O)—Polyglyceryl-4 Isostearate, Cetyl Dimethicone Copolyol, and Hexyl Laurate from Goldschmidt, mineral oil from Codex, C12-15 Alkyl Benzoate from Croda Chemicals Ltd., Caprylic/Capric Triglyceride from Stearinerie Dubois, hydrogenated castor oil from Henkel, sodium chloride, Phenoxyethanol, Methylparaben, Ethylparaben, Propylparaben, and Butylparaben from Seppic, glycerin from Laserson and Sabetay and distilled water.

Two different emulsions (O/W and W/O) were formulated under the following conditions. Water and other components were heated separately at 75 °C. Under agitation at 650 rpm with an homogenizer (Turbotest Type 33/300, Rayneri) the aqueous phase was added to the other components in 1 min and cooled to room temperature over 60 min. The sunscreen concentrations were held constant (5%) for all emulsions. The OMC-NC-emulsions were formed under constant agitation at room temperature for ensure the NC integrity, and were stored at 8 ± 1 °C before further assays were made.

Four sunscreen emulsions were used: (1) OMC-NC-O/W emulsion, (2) OMC-NC-W/O emulsion which contained NC-S with a total content of OMC of 5%, (3) OMC-O/W emulsion, and (4) OMC-W/O emulsion with OMC-free at 5%.

2.5. Skin preparation

Pig skin used for these experiments was obtained from the flank of female pigs (2 months old and 30 kg in weight) of the races Landras and Pietrain. Human skin would be the obvious choice but is not always readily available. Pig skin is used because it shares essential permeation characteristics with human skin. An internationally validated and accepted animal-free test method (SCCNFP/0119/99) has been available, where the pig skin model constitutes an excellent model to predict percutaneous penetration in humans. So, we consider that the pig skin is a very attractive model for in vitro percutaneous absorption studies in order to mimic human skin.

Skin was collected as soon possible after that the animals may have been sacrificed. The skin was cleaned and shaven, and the subcutaneous fat was removed completely. The skin surface (epidermal side) is cleaned with the aid of a cotton impregnated in 0.5 ml of a sodium lauryl sulphate (1%, w/w; 1 g sodium lauryl sulphate/99 g water) aqueous solution. The remaining fat in the dermal side is entirely removed by a cotton impregnated in ether. The skin is washed five times with bidistilled water and is dried very well with a clean and dry cotton. This rapid wash did not affect to the skin integrity. The integrity of the skin samples was examined by a physical method, transepidermal water loss (TEWL) (Tewameter® TM 210) for damage or diseased conditions. Before the measurement, the probe was positioned on the skin for 2 min to allow stabilization of its sensor. The TEWL was measured for 1 min. The skin in which the barrier was disrupted was not used in the study.

The full skin thickness was measured using dial thickness gauge (Mitutoyo, Japan, 0.01–10 mm). Only intact skin discs with an effective permeation area of 2.54 cm^2 were kept and sealed then stored at −20 °C until use (Kurul and Hekimoglu, 2001).

2.6. Diffusion cells preparation

The skin samples were mounted in static diffusion cells—in a modified Franz diffusion cells (Franz, 1975) with a surface of 2.54 cm^2 and a receptor volume of 10 ml in such a way that the dermal side of the skin was exposed to the receptor fluid and the *stratum corneum* was exposed to the air (non-occlusive conditions). Receptor fluid must not adversely affect the barrier properties of the skin or the physicochemical properties of OMC. The receptor fluid (pH 7.4) consisted of a phosphate buffered saline solution containing sodium chloride (NaCl) 120 mM/l, potassium chloride (KCl) 2.7 mM/l, and phosphate buffer 10 mM/l, containing 4% (w/w) Oramix® BG 14. The solubility of the OMC in the receptor fluid was checked prior to beginning the experiments. OMC under these conditions is readily soluble in the receptor fluid.

Products were applied at a finite dose of 8 mg/cm^2 on the skin using a curved spatula in a manner in which the emulsion film covered the entire skin surface with an uniform and homogeneous spreadability over the whole skin area, but without determining its thickness. The exact amount applied was calculated by weight difference. The cell was filled with 10 ml of receptor fluid through the lateral inlet using a burette. Visible air bubbles, between the bottom side of the skin and the receptor fluid, can be eliminated through the lateral inlet.

The diffusion cells were placed in a thermostated water bath Clifton (Lyon, France) with horizontal agitation (speed of 100 strokes per minute) assuring the homogeneity of receptor fluid and a mean skin temperature of 32.0 ± 0.1 °C. Eight replicates were used for the study.

2.7. In vitro distribution of OMC in the flank pig skin layers

In order to determine the NC integrity in the different skin layers (OMC-NC-O/W and OMC-NC-W/O emulsions), the quantity of OMC-encapsulated and free was studied in receptor fluid, OMC-encapsulated and MeCN, OMC-free, respectively.

After an exposure time of 3 and 24 h, the receptor liquid and skin were removed, and the sunscreen product remaining on the skin surface was withdrawn with a round top spatula. The remaining product in the skin surface was dissolved with MeCN or receptor liquid in order to determine the quantity of OMC and each sample was shaken with a vortex mixer—Top-Mix® Agitator for 1 min and ultrason—Bransonic® 220 for 20 min. Subsequently, an aliquot of this last solution was dissolved in MeCN to determine the quantity of encapsulated filter remaining in the skin surface. Levels of OMC were analyzed by HPLC.

The dried skin samples were mounted onto a mechanical support to separate superficial layers of the *stratum corneum*, by the procedure known as 'tape stripping' (Clarys et al., 2001; Weigmann et al., 2001). For this purpose, the stratum corneum of the treated area was removed by 19 successive tape-strippings using D-Squame™ ($\phi = 22$ mm) (Monaderm, Monaco) and afterwards peeled it off with the part of the *stratum corneum* adhered to it. Strippings were applied under controlled conditions. It can not be said with the accuracy that with 19 accomplished strippings may have been eliminated thoroughly the stratum corneum of the dermis and viable epidermis. For them, would have to be checked visually with the aid of to stereomicro-

scope. However, bibliography (SCCNFP/0119/99) for each skin preparation, indicates that stratum corneum will be eliminated by tape-stripping (from 10 to 20 strips). We consider that the first strip permitted us to get back the quality of filter which had not penetrated into the stratum corneum. In order to determine the remanence of a substance after some time of application, the first strip was taken into account. The first strip was pooled and studied separately and the 18 following strippings were pooled of three in three and deposited in a vessel with an adequate solvent. Then, we determined the sunscreen concentration in these solutions.

After eliminating *stratum corneum* from skin samples by the tape-stripping procedure, the viable epidermis was separated from the dermis with dissection after immersing in water at 60 °C for 45 s. Previous studies proved that in time and temperature selected conditions, water could not extract any quantity of OMC-substance strongly lipophylic. The viable epidermis and dermis samples were separately digested in the solvent.

2.8. Analysis of OMC

For the quantification of OMC in NC suspension, emulsions, skin surface and various skin layers—stratum corneum, viable epidermis, dermis and receptor fluid—have been developed and validated by a direct, very sensitive, simple, and rapid high-performance liquid chromatographic (HPLC) analytical method.

The HPLC system (Waters, USA) consisted of a solvent delivery pump (Waters 515) equipped with a 10 μl autosample loop injector (Waters 717). Samples were chromatographed using a Xterra RP 18, 5 μm bead size, 150 mm × 3.9 mm column (Waters, USA) thermostated at 30 °C. The mobile phase, filtered through a 0.45 μm filter prior to use, consisted of a gradient elution: 0–5 min: mixture of acetonitrile and water (85:15, v/v); 5–15 min: with increasing acetonitrile concentration from 85 to 100 at a flow-rate of 1.0 ml/min. The column effluent was monitored using a UV detector set at 310 nm (Waters 996 Photodiode Array). The data recording system consisted of a personal computer with system Millenium 32 software (Waters).

A standard stock solution of OMC (20 μg/ml) was prepared by dissolving the filter in acetonitrile. The calibration curve was prepared with acetonitrile solutions of OMC at concentrations ranging from 1 to 20 μg/ml. The standard curves were linear ($r = 0.999$). The validation results were established for three injections per concentration and seven concentrations. The repeatability and the reproducibility of the method were expressed by the relative standard deviation at a concentration of 10 μg/ml OMC ($n = 8$); the values were 0.30 and 0.45%, respectively. The detection limit was calculated to be 0.2 fg. The unknown concentrations were determined by using the standard curve as reference.

2.9. Statistical analysis

Prismas™ software was used to performed statistical analysis on the data. Difference in the concentrations of sunscreen agent in receptor fluid, dermis, epidermis, stratum corneum, and skin surface for each of the formulations was compared by ANOVA test. A $P < 0.05$ was considered statistically significant (Brand and Mueller, 2002).

3. Results and discussion

3.1. Nanocapsules

Octyl methoxycinnamate poly(ε-caprolactone) nanocapsules were prepared by an interfacial deposition technique. The advantage of this method is the instantaneous, simple, efficient, and reproducible formation of small nanocapsules exhibiting a high drug loading capacity. The absence of any aggregate or sediment at low OMC concentrations attested to good association of polymer with the oil. The first stage in the preparation of OMC-NC using the solvent evaporation method is the formation of an emulsion. The encapsulating material is dissolved in organic solvent to form the oil phase. The second stage, the evaporation of solvent, is more important. With the evaporation of organic solvent, the oil phase is made thicker. It is very easy to coacervate when the collisions occur. Experimental conditions selected to obtain OMC-loaded NC of 374 nm resulted in to high entrapment percentage (97.52%) and yield (82.95%).

3.2. In vitro experiments

The profiles that involved the OMC-NC-formulation showed a modest burst released. This can be explained by the high hydrophobicity and crystallinity of the caprolactone polymer and by the high lipophilicity of the drug, preventing diffusion from the OMC-NC-emulsions into the skin. In addition, the slow diffusion rate of OMC from the nanocapsules, can establish that the OMC was entirely encapsulated and not adsorbed at the external surface of the nanocapsules.

The results presented in Table 1 demonstrated the in vitro percutaneous absorption of OMC through pig skin for the four studied formulations and to the times of 3 and 24 h.

After a 3 and 24 h exposure (Table 1), OMC remained primarily on the skin surface (SS) 89.989 ±5.108 % and 83.177 ± 4.117% (NC-O/W), 82.993 ± 1.828% and 81.954 ± 3.301% (NC-W/O), 57.831 ± 6.772% and 59.981 ± 5.420% (O/W), 50.835 ± 5.001% and 48.203 ± 6.803% (W/O) of the applied dose, respectively, were recovered from the sunscreen product remaining on the skin surface. The NC emulsions form a film on the skin and fix the NC within the film. A one-way analysis of variance was performed and the *P*-value of the *F*-test is less than 0.05, there is a statistically significant difference between the emulsions ($P < 0.05$).

Firstly, we have studied the behaviour of OMC in stratum corneum (SC) for the four emulsions. Tape-stripping method is a useful technique for selectivity in removing the skin's outermost layer, the stratum corneum. The amounts recovered range from 27.288 to 163,098 μg/cm^2 of OMC. The percentages found at level of the stratum corneum (Table 1) as a function of applied dose and time were 8.321 ± 4.448% and 15.572 ± 4.300% (NC-O/W); 16.338 ± 1.844% and 17.555 ± 2.965% (NC-W/O); 40.497 ± 6.549% and 36.591 ± 5.254% (O/W); 45.812 ± 4.828% and 46.393 ± 6.336% (W/O), respectively. The greatest concentration of OMC in stratum corneum was obtained with O/W and W/O emulsions. The lowest levels were found with OMC-NC emulsions. The results indicate that the diffusion coefficients are greater in the case of the OMC-free emulsions: the partition coefficients SC/vehicle OMC was increased (Treffel and Gabard, 1996). The octanol–water partition coefficient ($P_{o/w}$) for OMC is $\log P_{o/w} = 5.96$. Octanol may mimic the solvent properties of the stratum corneum. Increase in $P_{o/w}$ only leads to increased penetration from a aqueous system. If a lipophylic vehicle is used, flux may decrease as $P_{o/w}$ is increased (Roberts et al., 2002). So, an increase in the $P_{o/w}$ value leads to a larger transfer of permeant from the vehicle into the stratum corneum. However, at high values of $P_{o/w}$, the transfer from stratum corneum to the underlying aqueous tissues becomes less favourable and transport through these hydrophilic tissues could control the rate of the overall process. These results confirm that under the operating conditions of our study OMC has a high affinity to stratum corneum. OMC-free confirmed its high affinity for the stratum corneum due to its capacity to form a reservoir within the lipid phases of this compartment. This reservoir effect was linked to its physico-chemical properties. This property is particularly important for sunscreens because it has been suggested that the amount of sunscreen inside the stratum corneum may be directly related to its sun protection value. In our study, the *P*-value was less than 0.05, thus there is a statistically significant difference between the medians at 95% confidence level.

Fig. 1 shows the distribution of OMC in stratum corneum as a function of stripping number from different emulsions. After 3 h and for the first strip, 44 and 38% of OMC remained at the level of the stratum corneum for O/W and W/O emulsions, respectively, against only 30 and 27% for NC-O/W and NC-W/O emulsions, respectively. After 24 h, OMC-free was still more remanent than the OMC-encapsulated (37 and 38% against 21 and 20%). Application of the UV filter in the emulsion (O/W) resulted in higher stratum corneum UV filter concentrations compared with application in emulsion (W/O).

The total skin (TS) content (Table 1) for OMC-free emulsions (42.169 ± 6.772% and 40.019 ± 5.420% (O/W) and 49.165 ± 5.001% and 51.797 ± 6.803% (W/O)) was larger than that obtained for OMC-encapsulated emulsions (10.011 ± 5.108% and 16.823 ± 4.117% (NC-O/W) and 17.007 ± 1.828% and 18.046 ± 3.301% (NC-W/O)). Total skin accumulation of OMC-free was significantly ($P < 0.05$) greater than that of OMC-encapsulated for all formulations investigated. However, accomplishing the statistic analysis of the different layers separately, were found significant differences ($P < 0.05$) between the quantity of OMC found in the stratum corneum,

Table 1
In vitro skin distribution of OMC (mean (%) ± S.D., $n = 8$)

	NC-O/W		NC-W/O		O/W		W/O	
	3 h	24 h	3 h	24 h	3 h	24 h	3 h	24 h
SS[a]	89.989 ± 5.108	83.177 ± 4.117	82.993 ± 1.828	81.954 ± 3.301	57.831 ± 6.772	59.981 ± 5.420	50.835 ± 5.001	48.203 ± 6.803
SC[b]	8.321 ± 4.448	15.572 ± 4.300	16.338 ± 1.844	17.555 ± 2.965	40.497 ± 6.549	36.591 ± 5.254	45.812 ± 4.828	46.393 ± 6.336
VE[c]	0.789 ± 0.898	0.274 ± 0.217	0.668 ± 0.467	0.320 ± 0.320	0.999 ± 0.232	2.283 ± 0.786	2.468 ± 0.695	3.718 ± 1.211
E[d]	9.110 ± 5.161	15.846 ± 4.439	17.007 ± 1.828	17.874 ± 3.132	41.496 ± 6.745	38.873 ± 5.410	48.280 ± 5.144	50.111 ± 6.808
D[e]	0.885 ± 0.622	0.924 ± 1.188	0.000 ± 0.000	0.084 ± 0.121	0.674 ± 0.038	1.146 ± 0.191	0.885 ± 0.270	1.686 ± 0.283
RF[f]	0.016 ± 0.028	0.053 ± 0.091	0.000 ± 0.000	0.087 ± 0.149	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
TP[g]	0.901 ± 0.638	0.977 ± 1.157	0.000 ± 0.000	0.171 ± 0.267	0.674 ± 0.038	1.146 ± 0.191	0.885 ± 0.270	1.686 ± 0.283
TS[h]	10.011 ± 5.108	16.823 ± 4.117	17.007 ± 1.828	18.046 ± 3.301	42.169 ± 6.772	40.019 ± 5.420	49.165 ± 5.001	51.797 ± 6.803
Recovery	89.770 ± 10.663	81.699 ± 12.827	64.275 ± 10.375	65.375 ± 8.807	94.164 ± 7.440	81.747 ± 5.004	89.979 ± 7.916	87.397 ± 7.297

[a] SS: surface skin.
[b] SC: stratum corneum.
[c] VE: viable epidermis.
[d] E: SC + VE (epidermis: stratum corneum + viable epidermis).
[e] D: dermis.
[f] RF: receptor fluid.
[g] TP: D + RF (transepidermal penetration: dermis + receptor fluid).
[h] TS: SC + VE + D + RF (total skin: stratum corneum + viable epidermis + dermis + receptor fluid).

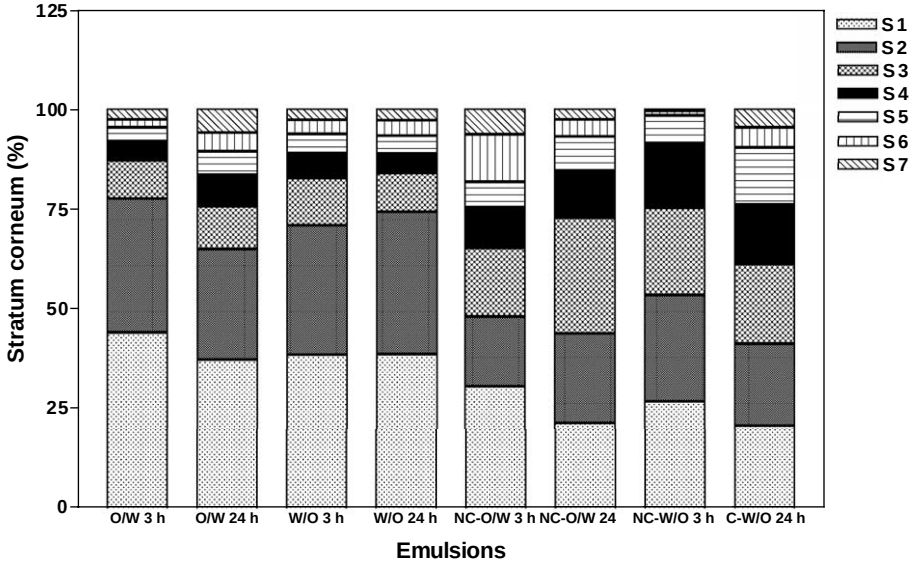

Fig. 1. Distribution in stratum corneum (SC) of OMC as a function to the number of strips.

viable epidermis and dermis for the different formulations, and, however, the accumulation at level of the receptor fluid did not result significantly ($P > 0.05$).

Fig. 2 show the distribution of OMC in the different pig skin layers ($\mu g/cm^2$) from different emulsions. After 3 and 24 h, the highest concentration of OMC in pig skin was obtained with W/O where the amount was $174.782 \pm 30.606\ \mu g/cm^2$ and $172.076 \pm 12.890\ \mu g/cm^2$. High concentrations were also obtained with O/W emulsion ($155.051 \pm 32.668\ \mu g/cm^2$ and $127.813 \pm 22.832\ \mu g/cm^2$). We did not find any difference between the two NC-emulsions (32,886 ±

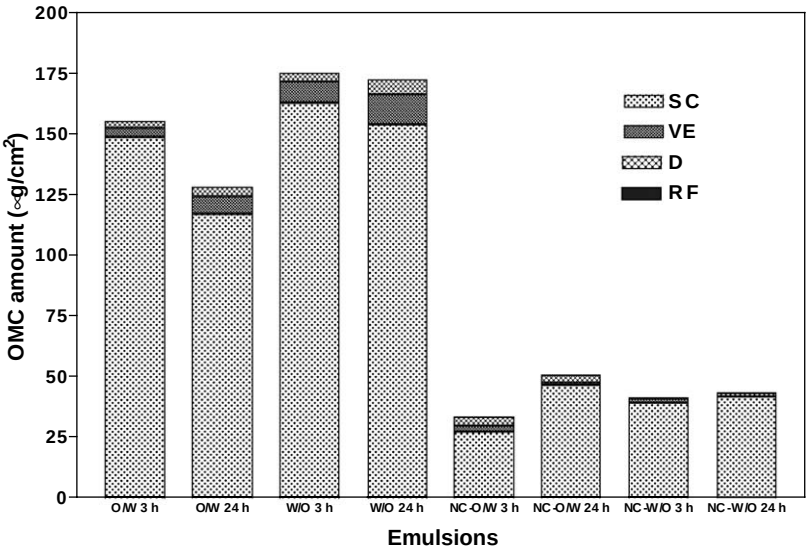

Fig. 2. Skin distribution of OMC.

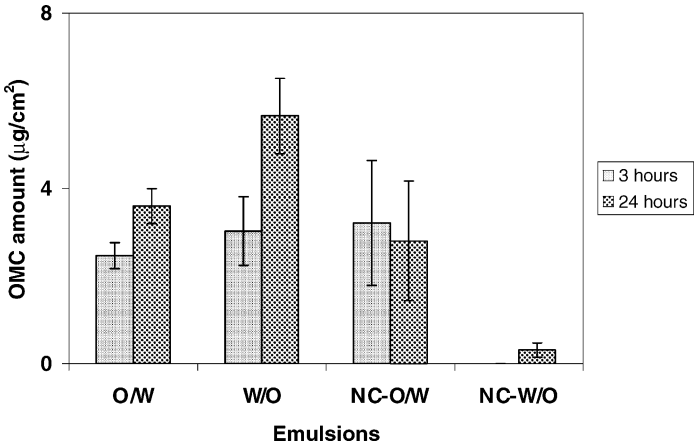

Fig. 3. Transepidermal penetration (TP) of OMC (mean ± S.D., $n = 8$).

17,183 μg/cm^2 and 50,325 ± 13,106 μg/cm^2 (O/W) and 41,025 ± 5445 μg/cm^2 and 43,049 ± 6510 μg/cm^2 (W/O)).

From Fig. 2, it is possible to visualize the distribution of OMC in the different skin layers (μg/cm^2) according to the time. It can be observed that the majority distribution is at stratum corneum and viable epidermis level. Despite the highly lipophilic nature of OMC, SC is the rate limiting skin layer for penetration. Penetration and SC retention were formulation dependent. The ratio of SC content to the amount penetrated is a useful tool for evaluating sunscreen penetration. OMC-encapsulated are produced important increases in the skin accumulation of the UV absorber cinnamate without a concomitant increase in their transdermal penetration that suggests the formation of an intracutaneous depot. It is expected that increased skin accumulation of UV absorbers will increase substantivity of sunscreens and provide longer lasting photoprotection.

Transepidermal penetration (TP) represented by the percentage of OMC in the dermis and receptor fluid (Table 1) was low for all formulations (0.901±0.638% and 0.977 ± 1.157% (NC-O/W), 0.000 ± 0.000% and 0.171 ± 0.267% (NC-W/O), 0.674 ± 0.038% and 1.146 ± 0.191% (O/W) and 0.885 ± 0.270% and 1.686±0.283% (W/O)). Nevertheless, the transepidermal penetration of OMC in O/W and W/O emulsions was slightly higher than that of OMC-NC emulsions. Their percentage in the receptor fluid is zero or very low (0.016 ± 0.028 and 0.053 ± 0.091 (NC-O/W), 0.087 ± 0.149 (NC-W/O)). Fig. 3 shown the transepidermal penetration of OMC (μg/cm^2) from different emulsions. Significant differences ($P < 0.05$) were found in the transdermal permeation of OMC for the formulations tested. This low values, may be explain with the dermis as a permeation-limiting factor.

The transcutaneous fluxes and the apparent skin diffusivity of OMC appeared to be similar for the OMC-free and the OMC-encapsulated emulsions. Consequently, the nanocapsule vehicle does not appear to greatly influence the overall transcutaneous permeation process for OMC. Each layer of skin (stratum corneum, viable epidermis, and dermis) presents a specific diffusional resistance for a drug. The vehicle may affect the diffusional resistance in the stratum corneum. But this effect could be negligible if the diffusional resistance in dermis is very high. So the overall barrier function could be determined by a single high diffusional resistance. The unaffected structure of dermis is certainly the higher transport resistance layer for OMC (Brinon et al., 1999).

4. Conclusions

Our studies showed clearly that incorporation of OMC in NC decreased the release compared to the same emulsion. NC are able to provide a sustained release carrier system, therefore the sunscreen remains longer on the surface of the skin were it is intended to act.

It may be also concluded that cutaneously applied free OMC is able to penetrate the skin but in this case the major part remains in the surface of the skin. The reservoir function of the stratum corneum for OMC was assessed by stripping method, and confirmed with the levels found in pig skin by in vitro technique.

From the results obtained in the study it can be deduced that the use of NC-emulsions decreases the penetration of OMC in pig skin when compared with the same W/O and O/W emulsions. The results achieved here show the importance of encapsulation effect on the skin penetration of OMC. The two emulsions, the NC-O/W and NC-W/O emulsions restrain the concentration of this UV-filter in the skin, whereas O/W and W/O emulsions allow a greater penetration. NC-emulsions are novel vehicle-type dispersion and can be used as drug carriers. The effect observed in this study also shows that occlusion with oily vehicles does increase the skin penetration of free OMC.

We believe that topical application of a NC-OMC emulsions may be more effective (synergetic effect) due probably at the NC-film formation on the skin surface. In conclusion, the results of this study emphasize the potential of sunscreen nanocapsules as new skin drug delivery systems.

Finally, the encapsulation suppresses the differences observed in the formulation of the OMC as of the emulsions O/W and W/O. Only they intervene the nanocapsules due to the presence of the polymer. The vehicle of the nanocapsules (the emulsion) does not participate in the phenomenon of absorption–diffusion of the OMC.

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