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Benzophenone-3 entrapped in solid lipid microspheres: Formulation and *in vitro* skin evaluation

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ABSTRACT

Solid lipid microspheres (SLM), lipid-in-water formulations made from oil-and-wax mixtures, were studied concerning feasibility. SLMs were then loaded with a benzophenone-3, water insoluble UVAB-filter intended for dermal application. Microspheres were prepared by dispersion with homogenisers and investigated by polarizing micrography and scanning electron micrography. For the selected formulations, investigations on percutaneous penetration of B-3 capacity were performed *"in vitro"* using Franz cells. Microspheres, 5–50 μm in size, and a spherical shape were obtained from several mixtures. B-3 was added and the loading capacity of this drug in the SLM was obtained for a maximum of 5% when the lipophilic phase was 18%. The lipophilic mixture with non-ionic surfactants in the selected formulation of lipid microspheres has a favorable effect on size. The selected formulation is also cosmetically adapted and it is composed of physiological and biodegradable lipids. B-3 was released and penetrated into skin more quickly and in greater quantity than in SLM form, from vehicles containing free B-3. This work has shown that SLM is an excellent carrier for lipophilic sunscreens like B-3 in order to decrease the release and penetration rate of this UV absorber compared with B-3 in oily solution.

1. Introduction

Sunscreens are extremely complicated products in terms of formulation, because their scientific, commercial, and cosmetic considerations are strongly linked with a divergent necessity. Demands made on sunscreens are many and varied. They must be efficient, substantive and safe formulations with a high-quality texture and leave a good impression on the skin after application. One of the most important properties of a UV-filter is its absorption spectrum. Obtaining full spectrum protection in a sunscreen formulation requires the inclusion of several components which, in combination, absorb throughout the solar spectrum from 290 to 400 nm. Most manufacturers produce products in which organic chemical sunscreens, photoprotectants are added and frequently physical blockers can be combined to obtain sunscreens claiming an SPF (sun protection factor) of 15 or higher. Like in the United States, UV-filters are closely controlled in the European community. UV-filters are selected in Annex VII to be formulated and their maximum levels are notified in the Cosmetic Directive (78/768/CEE). The classical formulations used for protection against solar radiations are sunscreen creams, lotions, oily preparations and, more recently, sprays. To obtain non-greasy formulations which can be quickly and easily applied to the skin, the new tendency is to use a weak concentration of oily phase or to incorporate special oily components like silicones, but these types of formulations are limiting factors for solubilisation of poorly water-soluble UV-filters.

Some of the more recent formulations are oil-free lotions with alcohol as the main ingredient, in order to obtain refreshing sprays and achieve adequate solubilisation of UV-filters, even though this solvent is not appropriate to the field. Some recent formulations are oil-free preparations limiting the greasy impression after application.

To enhance the refreshing sensation and simplicity of application, sprays are now often used. To achieve adequate solubilisation of UV-filters products, even though this solvent is not appropriate to this field; ethyl alcohol is used as a major ingredient. Propylene and butylene glycols are also added to these types of formulation, but it is well known that ethyl alcohol and all these ingredients increase skin penetration. Lipid spheres or lipospheres are alternative formulations called SLNs (solid lipid nanospheres) or SLMs (solid lipid microspheres), their classification depending on the particle size. These formulations have emerged as an appropriate carrier system for sunscreen agents. Various production methods and the influence of preparation parameters have been studied by several authors for SLMs (Albertini et al., 2009) and SLNs (Schäfer-Korting et al., 2007; Trotta et al., 2003; Pardeike et al., 2009), and the techniques used to produce SLNs are based on the melt dispersion technique, the solvent evaporation technique, high-pressure

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homogenisation, dilution of microemulsion and, more recently, on the spray congealing technique. Last decade, solid lipid nano and microsphere and nanostructured lipid carriers (NLC) were studied as drug carriers, especially for lipophilic compounds, in pharmaceutical and cosmetically fields. Solid lipid spheres were used for prolonged release, as shown by Wissing et al. (2000a,b) for insect repellents and perfume (Wissing et al., 2000a,b). The small size of nanosystems can increase the amount of drug penetrating into the skin, but little data exists in the field of percutaneous penetration.

On the other hand, with a fluorescent probe in different types of nanoparticle systems such as solid lipid nanoparticles and nanostructured lipid carriers, Lombardi Borgia et al. (2005) demonstrated that drug targeting appears to be more strictly related to the mode of interaction between drug-and-particle than penetration enhancement. Very recently, the same team (Küchler et al., 2009) compared percutaneous penetration of nile red in SLMs with a new system a dendritic core-multishell (CMS). They concluded that nanotransporters can favour the penetration of a model dye into the skin, even more than SLNs which may reflect size effects. Due to their composition, these particles have limited drug loading but present an excellent potential to protect drugs from photo-degradation. In the sunscreen formulation area, solid lipid microparticles are used to enhance sunscreen photostability, for example, they are loaded with UV-filter agents like octyl-dimethylaminobenzoate (Tursilli et al., 2007) or butylmethoxydibenzoylmethane (Iannuccelli et al., 2006).

The use of solid lipid microspheres as drug carriers seems a very interesting system to attain controlled drug release in sunscreen formulations and the aim of this study was to formulate structured SLMs with natural and well-tolerated ingredients in order to entrap a sufficient concentration of B-3 and to investigate its possible limited percutaneous penetration. Another clear advantage of SLMs is the fact that lipid matrix can be made from natural and physiological and/or bio-mimetic lipids, which enhance the safety of formulations and their biodegradability. In a previous work (Larroque et al., 2003) we highlighted the major role of nature and concentration of oily ingredients on formulation results. The feasibility of preparing SLMs using surfactants and, in this study, several oily and waxy ingredients was investigated with the melt dispersion technique. To obtain the blend for the particle matrix, waxes were mixed with oils at a ratio ranging from 50:50 to 90:10. Due to the characteristics required for sunscreen formulations, microsystems were preferred to nanosystems here, in order to minimize percutaneous penetration. All selected formulations obtained must not only be cosmetically adapted, but also smooth to the touch, with a non-greasy feeling. In order to determine the influence of SLMs on skin, absorption studies were conducted *in vitro*. SLMs and oily solution were tested in parallel using pig skin membranes to demonstrate the possible retention of B-3 in the SLM formulation.

During the last decade sunscreens were heavily applied before any exposure to sunlight, even on young children, but some formulations with skin absorption enhancer are not adapted. In these conditions, large amounts of sunscreens are applied several fold on a large area (1.7 m^2) and can enter through skin the bloodstream. The aim of our work was to produce SLM with natural compounds as carriers for B-3 in order to decrease the release and penetration rate and amount of this UV-filter in skin layers and systemic compartment.

2. Materials and methods

2.1. Chemicals

Benzophenone-3 (2-hydroxy-4-methoxybenzophenone, B-3) was obtained from Merck (Eusolex®4360), and used without further purification introduced in its formulation. B-3 is a UVA-B filter with a MW of 228.26, a log *P* of 3.7 and a melting point of 62–65 °C (SCCP/1069/06, 2006a). Chemical products used to define SLMs were: glyceryl stearate (Cutina GMS, Cognis, France), glyceryl laurate (Monomuls 90-O-18®, Cognis, France), cholesterol (HCG 1658®, Prod'hyg, France), alkyl polyglucosides (Plantacare 818®, Cognis, France), palmitate sucrose ester (sucrose palmitate P-1570®, Rioto, France), refined olive oil (codex grade, Bertin, France), sodium hyaluronate (AAV 9052®, HTL, France), cetyl alcohol (Sippol C16®, Cognis, France), dicaprylyl carbonate (Cetiol CC®, Cognis, France). Chemical products used for the *in vitro* experimentation were: bidistilled water, methanol (Carlo Erba Reagents, France), propylene glycol (Prolabo, France), ethanol (Carlo Erba Reagents, France), water (Ecotainer®, Aqua B.Braun, Belgium), and physiologic serum (Versol® NaCl 0.9%, Aguettant, France). All reagents used for chromatography were of analytical-reagent grade. Ultra high-quality water was obtained from Alpha-Q systems (Millipore, France).

2.2. Preparation of formulations

2.2.1. Liquid vehicle

B-3 is solubilised in an oily solution, a mixture of Cetiol CC, ethyl alcohol and propylene glycol (50/25/25, w/w/w).

2.2.2. SLM preparation

Microspheres were prepared by dispersion of lipid mixture with homogeniser in the presence of non-ionic surfactants. In order to become entrapped in lipid microspheres, B-3 was first dissolved in the oily mixture which was melted and maintained at 75 °C until B-3 was completely dissolved. To produce microspheres, the aqueous phase heated at 75 °C was then added to the oily phase under agitation at 8000 rpm with a homogenizer (Ultraturax, IKA) for duration of 30 s. A cold water bath was then added and maintained at 4–5 °C for 5 min more. The wax enveloping B-3 was solidified during this step. Ingredients were preferred for their physiological properties like cetyl alcohol or sodium hyaluronate. Others ingredients for their good compatibility with skin for example olive oil or monoglycerides. For surfactants we have selected two types of compounds, alky polyglucosides and sucrose esters for their mildness and their irritant limited properties.

2.3. Texture profile analysis (TPA)

Hardness and adhesiveness of waxy and oily mixtures were determined by TPA using a TA XT2 texturometer (Stable Micro Systems Ltd., Surrey, UK). The tests were undertaken at 22 ± 2 °C at a rate of 1 mm/s to a distance of 4 mm with a 2 mm cylindrical probe.

2.4. Microscopic analysis of SLMs

Lipid microspheres were investigated under polarizing microscope with various magnifications. Anisotropic texture was revealed by polarized light microscopy (Axiostar plus, Zeiss). Effects of lipid composition on the morphology of lipid microspheres were shown by scanning electron micrograph of dried SLM formulations (ESEM®, environmental scanning electron microscopy). All analyses were carried out in a room with a temperature of 25 ± 2 °C.

2.5. In vitro skin permeation experiments

2.5.1. Diffusion cells

For this investigation, static Franz glass diffusion cells were used (Laboratory Legallais, France). These cells consist of donor and receptor chambers between which a piece of whole porcine skin is positioned. About 5 μl of vehicle per cm^2 were applied in donor compartment which was sealed after that from the atmosphere

with Parafilm® a plastic film (in order to minimize evaporation). The membrane was mounted over the diffusion cells and cells were equilibrated for 1/2 h, before removing air bubbles. The area of cells was around 1 cm^2 and receptor volume ~9 ml, accurately measured for each cell. The cells were kept in a water bath at constant temperature, 37 °C (Polystat CC1, Huber). The receptor chamber contents were continuously shaken using magnetic stirrers (600 rpm, Variomag Electronicrührer Poly15, Germany). The receptor fluid was a sterile saline solution (Versol® NaCl 0.9%) for experiments carried out from 0 to 24 h. Gentamycin 1% (gentalline, Schering-Pough) was added for experiments carried out from 48 h (Marti-Mestres et al., 2007), and under these conditions, B-3 is readily soluble in the receptor fluid (4.7 μg ml^{-1}, $n = 6$) (Fernandez et al., 2000a,b).

2.5.2. Skin preparation

Pigs' ear skins (three different donors) were obtained from freshly killed animals at a local slaughterhouse (Pezenas, France). Ears were removed post-sacrifice before the carcass was exposed to the high-temperature cleaning procedure, and the ears were then kept in an isotherm container at 4 °C. Full-thickness porcine skins were measured with thickness gauges (Mitutoyo Ehy 331, Japan) and samples were immediately frozen to 20 °C (for a duration of 6 weeks maximum) and thawed just prior to use (Fernandez et al., 2000a,b). Skin integrity was checked by visual observations and transepidermal water loss (TEWL) with a TM 210 Tewameter (Courage – Khasaka, Germany). TEWL measurements were performed on porcine skin pieces at the beginning of each experiment and samples with TEWL levels of over 10 g m^{-2} h^{-1} were discarded.

2.5.3. Skin absorption procedure and data collection

The formulation was maintained in contact with the pigskin during for 24 and 48 h. with occlusion. A first series of eight experiments was conducted with the aim of obtaining kinetic parameters.

Receptor fluid sampling time: After a period of 4, 8, 16, 24, 34 and 48 h, the entire receptor volumes were withdrawn and replaced by the mixture of saline solution. Six experiments were then conducted over a 24-h period, and B-3 was determined in each compartment.

Product excess on the skin (*dislodgeable dose*): The skin's surface was washed and excess formulation removed by washing with 10 ml of methanol. After washing, the pooled solutions were analyzed using HPLC-UV. The stratum corneum (SC) was recovered using 15 D-squame® (Cuderm) strips which were applied with a pressure of 220 g cm^{-2}. All strips were placed in a vial with 2 ml methanol and a magnetic bar. The vials were left on the magnetic agitator for 24 h. After this time, each sample was analyzed on HPLC after filtration. To separate the epidermis from the dermis, the skin was first put into aluminium foil, then into occlusive film (Parafilm®), and finally into a water bath at 60 °C for 2.5 min. After separation, the epidermis and dermis were cut into small pieces and collected in different vials (Al haushey et al., 2007) with 2 ml of methanol and a magnetic bar. The vials were left on the magnetic agitator for 24 h. After this time, each sample was analyzed using HPLC-UV after filtration with a 0.22 μm Millipore filter.

2.6. High performance liquid chromatography assay

B-3 was quantified by HPLC with fluorescence detection. The chromatographic system (Hewlett-Packard 1050 system) was equipped with a 1050 quaternary pump, and a variable wavelength diode UV 6000 LP detector. Since the concentration of B-3 in the skin and in the receptor fluid can be very low due to only slight penetration, the analytical method used to determine the amounts of studied compounds must be sensitive enough. The compounds were analyzed using a reversed-phase C_{18} column (Nucleosil, Macherey-Nagel, 5 μm, 250 mm × 3 mm). The system was set at 40 °C. B-3 was eluted using a mobile phase consisting of methanol/water (80:20) at a flow-rate of 0.8 ml/min. According to the absorbance spectrum, the detection wavelength was adjusted to its UV maximum wavelength of 287 nm.

2.7. Data analysis

The cumulative amount of B-3 (μg/cm^2) permeating per unit of skin surface area was plotted against time (hours). The skin flow-rate was determined from Fick's law of diffusion

$$J_s = \frac{dQ}{A.dt} \quad (1)$$

In which J_s is the steady-state skin flow-rate in μg cm^{-2} h^{-1}; dQ is the change in the amount of B-3 passing through the skin into the receptor compartment in μg; A is the active diffusion area in cm^2; and dt is the change in time. The flow-rate was obtained from the slope of the linear region of the plot. The permeability coefficient (K_p) was calculated as

$$K_p = \frac{J_s}{C} \quad (2)$$

in which C is the drug concentration of donor formulation.

To compare the influence of two systems upon B-3 uptake, 6 independent experiments were performed using the skin of 3 donator pigs. Statistical comparisons were made using Student's t-test with $P < 0.05$ as the minimal level of significance. All computations were carried out using Statgraphic statistical software (Statgraphics Plus, 2001).

3. Results and discussion

3.1. SLMs preparation

As demonstrated in previous work (Larroque et al., 2003), textural properties of lipid's mixtures (wax and oil) must be studied before formulation of SLMs by texture profile analysis in order to select the right composition. Some of lipid's mixtures could be too hard or too soft. If SLM are too hard; spreadness of formulations on skin is disagreeable for consumer. On the opposite, if the lipid's mixtures are too soft, the stability at 50 °C of the formulations could be not assure. Two parameters were calculated from TPA: the "maximum force peak" in the TPA profile represents the wax hardness and "adhesiveness" corresponds to the negative area of the curve during retraction of the probe. The selected mixtures of oily components for SLMs presented a force of 550–750 g s^{-1} with a minimum negative surface, an example is presented in Fig. 1.

SLMs were then prepared according to the melt dispersion technique. The most common method of preparing drug-loaded SLMs is to dissolve the drug in the oil phase at 75 °C, and then homogenise the mixture until the drug crystals are dissolved. The aqueous phase was incorporated into the oil phase with the dispersed B-3 under rapid homogenisation with an ultraturax. The homogenisation was considered to be a crucial step which affected the particle size of the SLMs, but the choice of lipid matrix plays the major role on the morphology and size of the particles. In a set of experiments, mixtures of lipids consisting of refined olive oil with several alternative waxy ingredients were tested with several internal proportions. Finally, sodium hyaluronate, one of the natural moisturizing factors (NMFs) found in the skin, was added as an additional ingredient to increase the sensory perception in the sense of improving texture to give it a non-greasy feeling, and increasing viscosity.

As a general consideration in formulation, solid lipid particles are considered as satisfactory when they are spherical in shape, 1–50 μm in size and, with regard to hardness, they must not be too hard so that the particles can be spread easily over the skin.

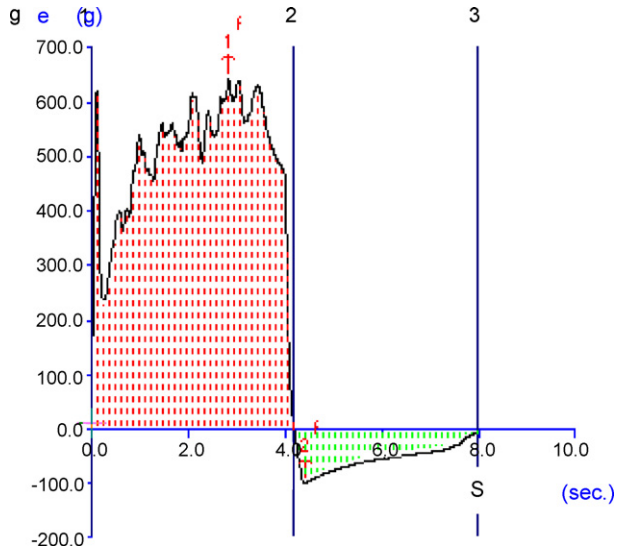

Fig. 1. Force–time curve obtained for the selected waxy mixture as measured by a stable micro-system texture analyser.

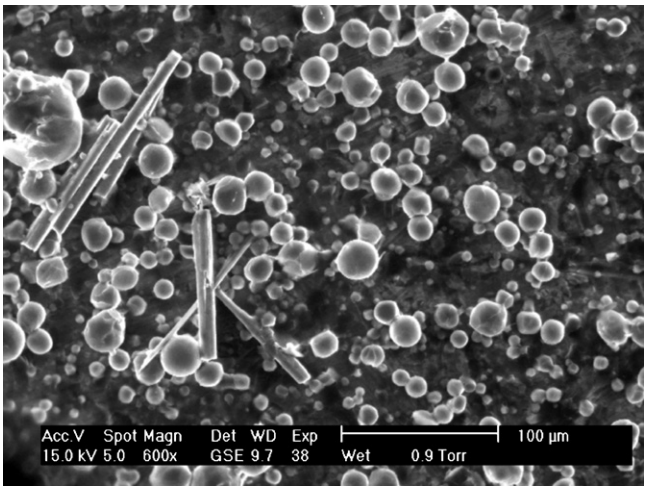

Fig. 2. ESEM®, environmental scanning electron microscopy, SLMs with 15% of oily phase (olive oil, cetyl alcohol) containing 5% of B-3, with crystals of drug.

In order to obtain the spherical shape, two types of mild non-ionic surfactants (alkyl polyglucoside and sucrose ester) were tested in small quantity (0.5%). The addition of an emulsifier leads to variable effects on the size. At this concentration, sugar ester has a good impact on the particles' spherical shape. On the other hand, alkyl polyglucoside was rejected due to its foaming activity. Mixtures of olive oil with Cutina GSM, cholesterol and Monomuls waxes produced solid lipid microspheres without sufficient characteristics and were also rejected. In each case, at a low concentration of 0.5%, sodium hyaluronate acted to increase the viscosity and stabilise formulations.

A major disadvantage of SLMs is the frequently low loading capacity, which is generally limited to about 10–15% of the amount of lipids. We first selected stable formulations with 10% of lipids in which B-3 was incorporated at 5%, corresponding to 15% lipid-drug particles. In order to obtain qualitative information on drug encapsulation, formulations were analyzed by scanning electron micrography in order to visualise possible crystals of B-3. In these conditions, crystallisation of the UV-filter was found (as shown in Fig. 2).

A concentration of 10% of lipids was too limited and it has been necessary to increase the oily part to 18%. The final formulation of SLMs was composed of 23% of total oily phase with a composition of cetyl alcohol/olive oil/B-3 and an aqueous phase composed of sodium hyaluronate/sugar ester/water (see Table 1).

The surface micro-morphology of spheres was investigated by changing the amount of emulsifiers: alkyl polyglucoside and sucrose ester. With sucrose ester the surface morphology of spheres can be observed clearly. Investigations by mean of electron microscopy showed the spherical shape of lipospheres and photographs are reported in Fig. 3.

With the selected formulation, no B-3 crystals were observed (crystals generated outside solid particles). Therefore, in the right experimental conditions, SLMs can entrap B-3 at a concentration of 5%. Due to the melting points of cetyl alcohol (49 °C), B-3 (62–65 °C) and the high concentration of solid compounds in lipid matrix, the blends obtained were solid at ambient temperature and the particles were structured as lipid carriers. Particles were observed with a polarized microscope (Fig. 4) in order to visualise the crystalline structure. The major advantage of this structure is to avoid or minimize the expulsion of the active compound (B-3) during storage.

3.2. Comparison of absorption of B-3 from SLMs and oily solution

An external calibration of B-3 was used to validate the analytical method in terms of linearity, precision, accuracy and limits of detection and quantification. The detection limit was calculated as the concentration that led to a signal three times the noise level, the quantification limit as 10 times the noise level. Characteristics concerning the validation terms for all products are given in Table 2. The linearity of the validation plot from 0.5 to 20 mg l^{-1} was tested and r^2 was 0.998. The limits of detection or quantification were low enough to appreciate the amount of product contained in each sample.

The hypothesis that the delivery of poorly water-soluble drug into and across skin would be limited by solid lipid microparticles was tested in comparison with an oily solution. In each case, a 10 μl dose of 5% B-3 was applied to the skin membrane. This dose is higher than the recommended dose used in sunscreen products. An SLM

Table 1
Composition and preparation of selected SLM.

Ingredients	Quantity express in %	Operating conditions
Lipophilic phase		
Cetyl alcohol	10.8	Wax and oil were first mixed and melted and then benzophenone-3 was added at 75 °C
Olive oil	7.2	
Benzophenone-3	5	
Hydrophilic phase		
Sugar ester	0.5	Sodium hyaluronate and sugar ester were solubilised in water and this phase was then heated at 75 °C
Sodium hyaluronate	0.0025	
Water (freshly distilled)	q.s.p 100	

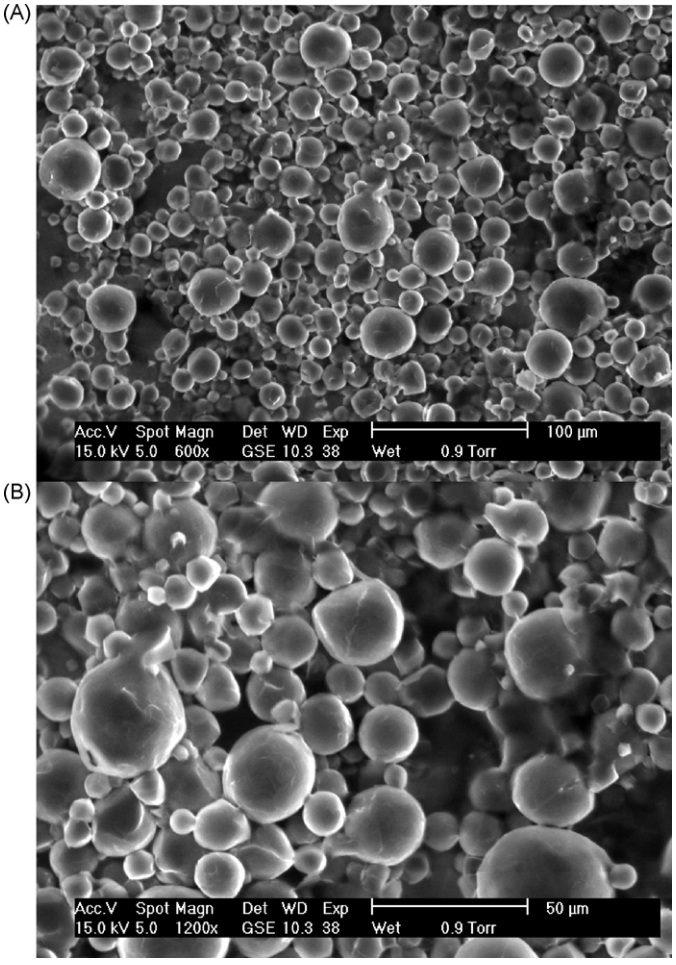

Fig. 3. ESEM®, environmental scanning electron microscopy of SLMs with 23% of oily-waxy phase (containing 5% of B-3), without crystal drug. SLMs produced by melt dispersion. A and B at two different magnitudes.

system containing 5% B-3 in a mixture of Cetiol CC, ethyl alcohol and propylene glycol (70/25/5, w/w/w) was selected as a formulation model and typical plots of permeation of B-3 were obtained and reported in Fig. 5.

Drug penetration into the skin is often rather poor for many drugs and particularly for high lipophilic or hydrophilic drugs, or

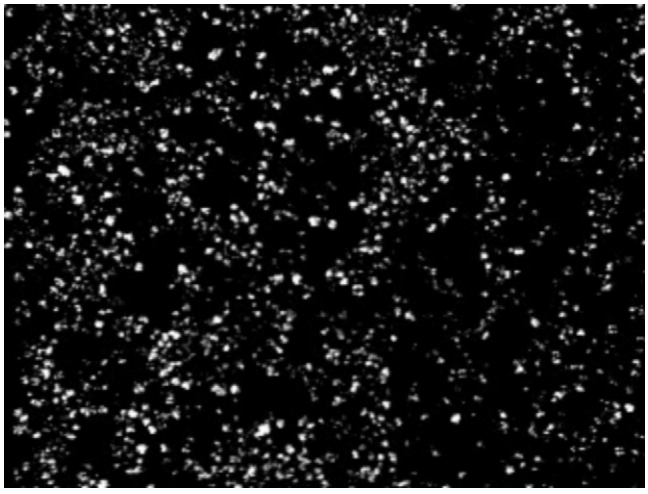

Fig. 4. Polarised micrograph. SLMs produced by melt dispersion, crystal structure.

Table 2
Parameters of calibration curves obtained by HPLC, limit of detection (LOD), and limit of quantification (LOQ) of B-3.

INCI name	Benzophenone-3 (B-3)
Level, n	5
Correlation coefficient, r^2	0.998
CV (%)	0.17
Limit of detection (mg/l)	0.17
Limit of quantification (mg/l)	0.56

with a high molecular mass (>500 Da). B-3 with a log P of 3.7 and an MW of 228.25 g mol^{-1}, is a good candidate for skin absorption. In this work, the steady-state flow-rate of B-3 in oily solution was found to be 0.254 µg cm^{-2} and when B-3 was formulated in microsphere particles it was not possible to evaluate the flow-rate: levels in receptor fluids were always below the LOD (Table 3).

The thickness of whole porcine skin was found to be 1.03 ± 0.14 and 1.11 ± 0.02 mm for experiments with SLM and oily solution respectively. A compartmental study at 1 day after application was made and the distribution of B-3 in each layer of skin is shown in Table 4. The epidermis (except for the stratum corneum, the amounts found in stratum corneum are not considered to be dermally absorbed) and dermis are considered as a sink, therefore the amounts found in these tissues are considered as absorbed and are added to those found in the receptor fluid (SCCP, 2006a,b) found for SLMs and the oily vehicle were 20.08 and 30.20 µg cm^{-2} respectively after a 24-h exposure. A t-test was run, and there is a statistically significant difference between the means of the two samples at the 95% confidence level ($P = 0.009$). The variances of the two samples are equal because there is no statistically significant difference in the standard deviation at the 95% confidence level. Cochran's C test = 0.63 with $P = 0.56$. It can be said that the total amount of B-3 absorbed and recovered after 24 h exposure was 1.5 times greater in the skin with the oily vehicle than observed with the SLM formulation.

The mass balance of the applied dose in each case was determined. The overall recovery of the test substance within the adequate range and percentage of applied dose absorbed in both cases are reported in Fig. 6.

Similar results were obtained by Yener et al. (2003). In their work they compared several formulations with another UV-filter, octyl methoxycinnamate (OMC). The OCM penetrated into rat skin more quickly and in greater amount from vehicles containing free OMC than when in SLM form. In this case systemic compartment cannot be compared, with a log $P_{o/w} = 5.96$ there is no systemic absorption for OCM in all conditions.

More recently in the pharmaceutical field, Puglia et al. (2008), demonstrated that the lipid particles were able to reduce drug pen-

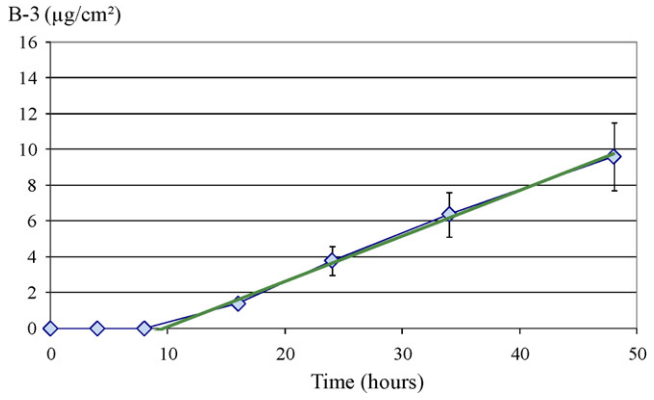

Fig. 5. Permeation of B-3 (µg cm^{-2}) through the skin as a function of time, using excised, full-thickness pig-ear skin. Data are presented as means ± SD ($n = 14$).

Table 3
Skin absorption parameters of B-3 through pig skin from the two formulations ($n = 14$ determinations for B-3 in oily solution). The first 6 complete experiments were carried out from 0 to 24 h and 8 supplementary experiments were then made from 0 to 48 h to obtain the curve.

	% of drug	Samples	Flow, J ($\mu g\,cm^{-2}\,h^{-1}$)	K_p ($\times 10^4$ cm h^{-1})	Lag time (h)	24 h cumulated dose ($\mu g\,cm^{-2}$)
B-3 in oily solution	5%	14	0.254 ± 0.016	0.051	9.56	3.803 ± 0.794
B-3 in SLMs	5%	6	–	–	–	<LOD
Control	–	2	–	–		<LOD

Table 4
Distribution data in each skin compartment for B-3 following the application of oily vehicles and SLM formulations.

B-3 in each compartment ($\mu g\,cm^{-2}$)	Product excess on the skin	Stratum corneum (on adhesive strips)	Epidermis	Dermis	Quantities in receptor fluid at 24 h	Total non-absorbed ($\mu g\,cm^{-2}$)	Total absorbed ($\mu g\,cm^{-2}$)
Oily vehicle							
Mean ($n = 6$)	250.43 ±46.79	9.89 ±2.55	3.31 ±0.78	23.22 ±6.67	3.67 ±0.92	260.31 ±45.80	30.20 ±6.18
SLMs							
Mean ($n = 6$)	269.24 ±24.51	7.93 ±7.21	4.09 ±2.35	15.98 ±5.05	<LOD	277.17 ±24.33	20.07** ±4.71
Control							
Mean ($n = 2$)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Results are expressed as means ± S.D.
** $P < 0.01$, compared with B-3 in an oily vehicle.

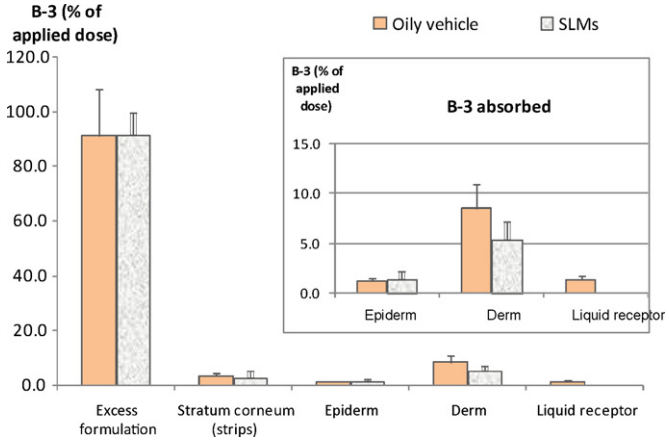

Fig. 6. Distribution of B-3 in the skin layers from both formulations. 24-h distribution of B-3 applied to skin in the diffusion cells expressed as a percentage of applied dose (mean ± SD of $n = 6$).

etration for ketoprofen and naproxen, simultaneously increasing permeation and accumulation in the horny layer. If required, the inclusion of a substance in SLN drug absorption can be modulated and can induce epidermal targeting, especially when the drug is located on the SLN particles' surface (Stecova et al., 2007). Despite the fact that lipid particles have been studied intensively, there is little data with the objective of limiting skin penetration. We investigated only those SLMs which were >1 μm in size, because solid nanoparticles (<20 nm) applied topically might penetrate deeply into the epidermis, maybe reaching the dermis and finally the systemic circulation, acting as enhancers for drug delivery.

A targeting effect in the skin layer was seen in this work on solid lipid particles. The solubility of the drug in SLMs will influence the partition coefficient of the drug between the formulation and the skin, in turn affecting the penetration rate of the drug. The interpretation, on the basis of the theory of limited absorption seems to be a pronounced attachment of the B-3 in the lipid-matrice.

4. Conclusions

This work has shown that the selecting the lipid forming the core of lipospheres should be considered as the most important factor when determining drug-incorporation efficacy. A limited delivery of drugs on the skin's surface or in the upper layers of skin is a major challenge in sunscreen formulations and we have confirmed that SLMs are excellent carriers for lipophilic UV-filters. B-3 was successfully encapsulated in SLMs in order to decrease the release, penetration rate and amount of this UV absorber compared to B-3 in solution in an oily vehicle. The oily vehicle and SLMs demonstrated different behaviour and B-3 penetrated the horny layer more efficiently when applied as an oily vehicle. Lipid-based microspheres appear to be an ideal formulation candidate to use in sunscreen preparations. For sunscreen products it is important for the active ingredient not to be systemically absorbed and the main advantage in this field is how much remains and the limited penetration of the drug in the skin when the drug is entrapped in SLMs.

References

Albertini, B., Mezzena, M., Passerini, N., Rodriguez, L., Scalia, S., 2009. Evaluation of spray congealing as technique for the preparation of highly loaded solid lipid microparticles containing the sunscreen agent, avobenzone. J. Pharm. Sci. 98, 2759–2769.
Al haushey, L., Bolzinger, M.A., Bordes, C., Gauvrit, J.Y., Briançon, S., 2007. Improvement of a bovine serum albumin microencapsulation process by screening design. Int. J. Pharm. 344, 16–25.
Fernandez, C., Marti-Mestres, G., Mestres, J.P., Maillols, H., 2000a. LC analysis of benzophenone-3 in pigskin and in saline solution. II. Application to determination of 'in vitro' and 'in vivo' skin penetration from solvents, coarse and submicron emulsions. J. Pharm. Biomed. Anal. 24, 155–165.
Fernandez, C., Marti-Mestres, G., Mestres, J.P., Maillols, H., 2000b. LC analysis of benzophenone-3 in pigskin and in saline solution. Application to determination of in vitro skin penetration. J. Pharm. Biomed. 22, 393–402.
Iannuccelli, V., Sala, N., Tursilli, R., Coppi, G., Scalia, S., 2006. Influence of liposphere preparation on butylmethoxydibenzoylmethane photostability. Eur. J. Pharm. Biopharm. 63, 140–145.
Küchler, S., Radowski, M.R., Blaschke, T., Dathe, M., Plendl, J., Haag, R., Schäfer-Korting, M., Kramer, K.D., 2009. Nanoparticles for skin penetration enhancement – a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. Eur. J. Pharm. Biopharm. 71, 243–250.
Larroque, M., Mestres, J.P., Garreau, H., Marti-Mestres, G., 2003. Lipid microspheres in sunscreen formulations. In: Skin and Formulation, Paris, October 23–24, p. 16.
Lombardi Borgia, S., Regehly, M., Sivaramakrishnan, R., Mehnert, W., Korting, H.C., Danker, K., Röder, B., Kramer, K.D., Schäfer-Korting, M., 2005. Lipid nanoparticles for skin penetration enhancement—correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. J. Control. Release 110, 151–163.
Marti-Mestres, G., Mestres, J.P., Bres, J., Martin, S., Ramos, J., Vian, L., 2007. The "in vitro" percutaneous penetration of three antioxidant compounds. Int. J. Pharm. 331, 139–144.
Pardeike, J., Hommoss, A., Müller, R.H., 2009. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int. J. Pharm. 366, 170–184.

Puglia, C., Blasi, P., Rizza, L., Schoubben, A., Bonina, F., Rossi, C., Ricci, M., 2008. Lipid nanoparticles for prolonged topical delivery: an in vitro and in vivo investigation. Int. J. Pharm. 357, 295–304.

Schäfer-Korting, M., Mehnert, W., Korting, H.C., 2007. Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv. Drug Deliv. Rev. 59, 427–443.

SCCP, 2006a. Opinion on benzophenone-3. Colipa n° S38 adopted by the Scientific Committee on Consumer Products during the 10th Plenary. European Commission, SCCP/1069/06. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_078.pdf.

SCCP, 2006b. Opinion for Basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients. adopted by the Scientific Committee on Consumer Products during the 7th Plenary. European Commission, SCCP/0970/06. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_03.pdf.

Statgraphics Plus, 2001. Manugistics, Inc., Maryland, USA.

Stecova, J., Mehnert, W., Blaschke, T., Kleuser, B., Sivaramakrishnan, R., Zouboulis, C.C., Seltmann, H., Korting, H.C., Kramer, K.D., Schäfer-Korting, M., 2007. Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. Pharm. Res. 24, 991–1000.

Trotta, M., Debernardi, F., Caputto, O., 2003. Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique. Int. J. Pharm. 257, 153–160.

Tursilli, R., Piel, G., Delattre, L., Scalia, S., 2007. Solid lipid microparticles containing the sunscreen agent, octyl-dimethylaminobenzoate: effect of the vehicle. Eur. J. Pharm. Biopharm. 66, 483–487.

Wissing, S.A., Mäder, K., Müller, R.H., 2000a. Prolonged efficacy of the insect repellent lemon oil by incorporation into solid lipid nanoparticles (SLNTM). In: Proceeding in the 3rd World Meeting of Pharmacy, Biopharmacy and Pharmaceutical Technology, Berlin.

Wissing, S.A., Mäder, K., Müller, R.H., 2000b. Solid lipid nanoparticles (SLN) as a novel carrier system offereing prolonged release of perfume Allure (Chanel). In: Int. Symp. Control. Release Bioact. Mater., vol. 27, pp. 311–312.

Yener, G., Incegül, T., Yener, T.N., 2003. Importance of using solid lipid microspheres as carriers for UV filters on the example octyl methoxy cinnamate. Int. J. Pharm. 258, 203–207.