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Solid lipid nanoparticles (SLN) as carriers for the topical delivery of econazole nitrate: in-vitro characterization, ex-vivo and in-vivo studies

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Abstract

Solid lipid nanoparticles (SLN) designed for topical administration of econazole nitrate (ECN), were prepared by o/w high-shear homogenization method using different ratios of lipid and drug (5:1 and 10:1). SLN were characterized in terms of particle size, morphology, encapsulation efficiency and crystalline structure. After incorporation of SLN into hydrogels, rheological measurements were performed, and ex-vivo drug permeation tests were carried out using porcine stratum corneum (SC). In-vivo study of percutaneous absorption of ECN as a function of application time and composition of gels was carried out by tape-stripping technique. Penetration tests of the drug from a conventional gel were performed as comparison. High-shear homogenization method resulted in a good technique for preparation of ECN-loaded SLN. Particles had a mean diameter of about 150 nm and a regular shape and smooth surface. The encapsulation efficiency values were about 100%. Ex-vivo tests showed that SLN were able to control the drug release through the SC; the release rate depended upon the lipid content on the nanoparticles. In-vivo studies demonstrated that SLN promoted a rapid penetration of ECN through the SC after 1 h and improved the diffusion of the drug in the deeper skin layers after 3 h of application compared with the reference gel.

Introduction

Solid lipid nanoparticles (SLN) have been extensively proposed as novel topical carrier systems for cosmetics (Muller et al 2002; Wissing & Müller 2003) and drugs (Jenning & Gohla 2001). The carrier is composed of physiological and well-tolerated lipids of low toxicity (Muller et al 1997). SLN offer protection of labile compounds against chemical degradation (Jenning & Gohla 2001), controlled release of the active ingredients (zur Mühlen & Mehnert 1998; zur Mühlen et al 1998), prolonged residence time of sunscreen agents in the stratum corneum (Wissing & Müller 2001a, b) and targeting of drug to the upper layers of the skin (Jenning et al 2000a).

The stratum corneum is the target organ of anti-mycotic treatment, and the improvement of local bioavailability leads to enhanced efficacy of the applied formulation (Alberti et al 2001). Recently, SLN and nanostructured lipid carriers (NLC) have been investigated as topical particulate carriers for different imidazole antifungal agents (Souto & Muller 2006). Because of high lipophilicity, these drugs are, in fact, good candidates for SLN encapsulation. On the other hand, the small size of lipid particles ensures close contact to the stratum corneum and an occlusive effect that can be exploited to enhance the drug penetration into the skin (Jenning et al 1999; Souto et al 2004). Besides, the solid lipid matrix offers the possibility of controlled drug release (Jenning et al 2000b; Wissing et al 2001; Müller et al 2002).

SLN incorporating clotrimazole (Souto et al 2004) and ketoconazole (Souto & Muller 2005), prepared by high-pressure homogenization, have been already investigated in terms of physicochemical stability, occlusion properties and in-vitro drug release using cellulose acetate membrane. However, until now, the effect of SLN on penetration of antifungal agents using experimental animal models and in human stratum corneum has not been investigated.

The aim of this study was to develop topical gels containing SLN dispersions loaded with econazole nitrate. Precirol ATO was selected as the lipid material of SLN. The SLN were prepared by an oil-in-water (o/w) high-shear homogenization method. Nanoparticles were characterized in terms of particle size, morphology, encapsulation efficiency and crystalline structure. The influence of the SLN on ex-vivo drug skin permeation through porcine stratum corneum was evaluated and compared with a conventional gel. Then, the in-vivo study to investigate the percutaneous absorption of econazole nitrate as a function of application duration and composition of gels was carried out by tape-stripping method.

Materials and Methods

Materials

Econazole nitrate was kindly provided by Erregierre SpA (Bergamo, Italy) and glycerol palmitostearate (Precirol ATO 5) was from Gattefossé (Cedex, France); Tween 80 (polyoxyethylene-sorbitan-monooleate) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and hydroxypropylmethylcellulose (HPMC K100M) from Dow Chemicals (Midland, USA). Methanol (CHROMASOLV for HPLC, gradient grade) and $NH_4H_2PO_4$ were obtained from Rielden-de Haën AG (Seelze, Germany). Polytetrafluoroethylene (PTFE) syringe filters (13 mm diameter, 0.2 μm porosity) were supplied by Alltech Italia Srl (Milano, Italy).

Preparation of SLN

SLN dispersions with different ratios of lipid:drug (5:1 and 10:1) were prepared by oil-in-water emulsification technique. Table 1 reports the composition of the prepared SLN dispersions.

Econazole nitrate was added to the lipid material (Precirol ATO 5) previously melted at 80°C. The hot lipid phase was slowly dispersed under stirring in a surfactant solution containing Tween 80 at the same temperature, and the mixture was homogenized by high-shear homogenization (Silverson L4R mixer, Crami, Italy), emulsifying at 6200 rev min^{-1} for 5 min.

The obtained nanoemulsion recrystallized upon rapid cooling down at room temperature, under magnetic stirring, and consequently the formation of an SLN dispersion occurred.

An aqueous dispersion of Precirol without ECN was prepared to obtain drug empty nanoparticles, used as comparison. Each preparation was carried out in triplicate.

SLN spray-drying

SLN powders were obtained by spraying the SLN aqueous original dispersions with a spray-dryer (Mini Buchi B-191; Buchi Laboratorius-Technik AG, Flawil, Switzerland) using a standard 0.5-mm nozzle. The SLN dispersions were fed to the nozzle with a peristaltic pump, atomized by the force of compressed air and blown together with heated air to the chamber where the solvent in the droplets was evaporated. The dried nanoparticles were harvested from the apparatus collector and kept under vacuum for 48 h. The process conditions of the spray-drying process were as follows: inlet temperature 90 °C; outlet temperature 48 °C; spray-pressure about 2 atm and spray-rate of feed 5 mL min^{-1}. The total volume of feed-solution sprayed for the preparation of each batch of nanoparticles was always 100 mL.

Table 1 Composition of SLN dispersions (% w/w)

Batch	PCR	Tween 80	ECN	Water
SLN 0	5.0	2.5	–	92.5
SLN 1	5.0	2.5	1.0	91.5
SLN 2	10.0	2.5	1.0	86.5

Characterization of SLN

Particle size determination

Particle size and size distribution measurements of the SLN suspended in the original dispersions were performed using photon correlation spectroscopy (PCS). A Coulter N5 submicron particle sizer (Beckman Coulter, Miami, FL) was used to determine the mean particle diameter and the polydispersity index (PI) as a measure of the width of the distribution. Size analyses were carried out on blank and drug-loaded nanoparticles; before each determination, all samples were sonicated for 10 s and properly diluted with MilliQ water (Millipore, USA) to ensure light-scattering intensity within the required range of the instrument (between 5×10^4 and 1×10^6 counts s^{-1}). The diluent was filtered with a 0.2-μm filter to remove any impurities that could affect the scattering of the light. The mean diameter of the particles was calculated in unimodal using the following conditions: fluid refractive index 1.333; temperature 25°C; viscosity 0.890 centipoises; angle of measurement 90°; sample time 3.0 μs and sample run time 300 s.

Scanning electron microscopy (SEM)

The morphology (shape and surface characteristics) of spray-dried SLN was studied by scanning electron microscopy (SEM) (model DSM 962; Carl Zeiss Inc., Germany). Samples of nanoparticles (3–5 mg) were placed on a double-sided tape, which had been previously secured on aluminium stubs. The samples were then analysed at 20 kV acceleration voltage after gold sputtering, under an argon atmosphere.

Yields of production

The yields of production were calculated as the weight percentage of the final product after drying, with respect to the initial total amount of solid materials used for the preparations.

Encapsulation efficiency

The determination of the ECN incorporated into SLN was carried out on each batch of drug-loaded SLN. A weighed amount of spray-dried drug loaded SLN (50 mg) was dissolved in methanol (10 mL) under stirring at 80°C for 10 min and then cooled to room temperature to preferentially precipitate the lipid. The suspension was centrifuged at 300 rev min^{-1} for 5 min and 50 μL of supernatant was diluited to 5 mL. The drug content in the solution was measured after filtration (PTFE syringe filters 13 mm, 0.2 μm porosity) by HPLC

analysis and calculated as a percentage with respect to the theoretical amount of ECN.

Determination of ECN was performed by a Varian Prosta 210 liquid chromatography system equipped with a DAD detector Varian 330 (Varian Deutschland GmbH, Dramstad, Germany) by the modified method of Piemi et al (1999). The chromatographic separation was performed using a spherisorb octyl 5 μm RP-C8 Waters column, 250 × 4.6 mm (Supelco, Milano, Italy). Twenty microlitres of extract samples or calibration standards were directly injected into the column and eluted with methanol–0.05 M $NH_4H_2PO_4$ (85:15 v/v). The column flow-rate was set at 1.0 mL min^{-1} and at room temperature. Detection was carried out by monitoring the absorbance at 200 nm. The elution period was 8 min. The retention time of ECN was about 5.8 min. At the end of each working day, the column was washed with the zero-time solvent mixture. Mobile phases were filtered by a cellulose acetate membrane filter and degassed before their use. The calibration curves were found to be linear in the range of 0.5–20.0 μg mL^{-1} (y = 784 808.83x + 171 123.1; R^2 = 0.9992).

The results were expressed as the mean of three replicates for each batch.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analyses were performed on spray-dried particles using a DSC Q100 V9.0 instrument (TA Instrument, New Castle, USA). The instrument was calibrated with indium (calibration standard, purity 99.999%) for melting point and heat of fusion. A heating rate of 10°C min^{-1} was employed in the range 0–250°C. Analysis was performed under an Ar purge (50 mL min^{-1}). Standard aluminium sample pans were used. A sample of about 4–6 mg was taken for analysis. An empty pan was used as reference.

The thermal analyses were carried out on ECN, on drug-free and drug-loaded SLN, and on a physical mixture of ECN and drug-free SLN.

The experiments were carried out in triplicate.

Preparation and characterization of hydrogels

To prepare the hydrogel formulations, SLN 1 and SLN 2 dispersions (100 g) were added to a gelling agent (HPMC K100M, 2.0% w/w) under magnetic stirring at room temperature and mixed at approximately 1000 rev min^{-1} for 15 min to yield gels (named SLN 1 gel and SLN 2 gel, respectively) containing a final concentration of 1.0% w/w ECN. A gel containing only ECN pure drug (ECN gel) was prepared as reference formulation.

The gel formulations were visually inspected for their colour, homogeneity, consistency, spreadability and phase separation. The pH values of 1% aqueous solutions of the investigated gels were measured by a pH meter (Hanna Instruments, model 8417, Milano, Italy).

Viscosity determinations of the prepared gels were carried out using a rotational viscometer (Viscotester VT 181; Haake, Karlsruhe, Germany) equipped with a sensor system E500 (diameter 8 mm, length 18 mm). About 40 g of formulations were heated at 25 ± 1°C in a measuring cylinder and viscosity of the gels was performed at a rotational speed of 2712 s^{-1}.

The average of three readings was used to calculate the viscosity.

The physical stability of SLN in the original dispersions and after their incorporation into hydrogels was monitored by particle size examination to investigate the possible aggregation of particles.

The stability was determined immediately after preparation of SLN gels.

For the PCS analysis the SLN dispersion and gel formulations were diluted with water and sonicated for 2–5 min; the mean particle size of SLN was performed in triplicate by PCS method as previously described.

Preparation of skin

Porcine and human skin have similar surface lipids, barrier thickness and morphological aspects and thus excised porcine skin is considered to be useful for estimation of ex-vivo human skin penetration behaviour (Gupta et al 1999; Jacobi et al 2005).

Adult pig ears were obtained from a local slaughterhouse. The ears were removed from the carcass before the steam cleaning process. Any ears that were obviously damaged were deemed unsuitable and discarded. Hairs on both the surfaces of ears were removed using a hair clipper. Shaved ears were immersed in water held at 60°C for 2 min. The stratum corneum was gently peeled off from both the surfaces of the ears and stored at −20°C in aluminium foil until used.

Ex-vivo drug permeation studies

Every time before permeation studies, the frozen skin was allowed to thaw and then hydrated for 30 min at 32°C in the receptor medium.

The stratum corneum was cut and mounted on the bottom of a cylindrical plastic support connected to a drive shaft of the dissolution apparatus (Erweka DT 70; Erweka GmbH, Heusenstamm, Germany) as previously reported (Gavini et al 2005a). The stratum corneum was clamped to the support by a stainless-steel ring and then gels were uniformly spread out on the surface of the skin. The system was then inserted into the vessel containing a methanol–water (70:30 v/v) solution (200 mL) as receptor medium, keeping the dermis side in contact just with fluid.

The composition of the receptor medium was chosen because ECN is well soluble in it, while the SLN remain intact in the medium. The rotational speed was set at 25 rev min^{-1} and the temperature for the receptor compartment was set at 32°C.

Samples (1.0 mL) were withdrawn at different intervals (1, 2, 3, 5, 7, 9 and 24 h) and the amount of drug permeated through stratum corneum was measured by HPLC and calculated, referring to the calibration curve prepared in methanol (standard solutions in the range of 0.5–20 mg L^{-1}). An equal volume of fresh medium was added after each sampling.

The permeation studies were carried out on gels containing SLN 1 and SLN 2 and on ECN gel chosen as reference formulation.

Each result represents the mean value of three experiments.

In-vivo experiments

After receiving local ethics committee approval and written informed consent, five healthy subjects (Caucasians, females, aged 26 ± 6 years, with no history of dermatological disorders) were recruited for this study.

The anatomical site chosen was the inner forearm. The subjects refrained from any cosmetic treatment of the test areas for at least 12 h leading up to the experiment. Their forearm was wiped with 1.0 mL ethanol 96% and 1.0 mL water and dried before application. The vehicles (100 mg of gel) were applied to the skin surface, and were spread uniformly over the whole area. An open circular cell, fixed with adhesive tape, delimited this area (2.83 cm^2). Formulations were applied for either 1 h or 3 h.

After the application times, the excessive substance on the applied area was quickly removed and weighed. The treated area was lightly cleaned with a cotton swab and rinsed twice with 1.0 mL ethanol, followed by 1.0 mL distilled water and finally lightly dried with a cotton swab. Then stratum corneum of the treated area was removed by seven successive tape strips using wax adhesive tapes (Depilzero; Sara Lee HBC, Italy). Each strip was obtained in a controlled way: a 1-kg rubber weight was rolled over it 10 times and the direction of stripping was alternated.

For each formulation and subject, the first strip was discarded and the following six were collected and extracted with 10 mL of methanol, stirring for 2 min at 40°C. The solution was evaporated to dryness at 40 °C and the residue was reconstituted in 500 μL of methanol; then samples were filtered through 0.2-μm PTFE filters (Alltech Italia Srl, Milano, Italy) and analysed by HPLC.

The analytical method was modified with respect to previously described conditions using a mobile phase of methanol–0.05 M $NH_4H_2PO_4$ (77:23 v/v) and an elution period of 15 min.

Under these conditions, the retention time of ECN was about 10.5 min. Comparison of the chromatograms of extracted blank tapes and tapes + stratum corneum did not reveal any interfering peaks with ECN. Linearity of the calibration curve was obtained in a concentration range of 1.0–50.0 μg mL^{-1} (y = 854 525.54x + 52 288.8; R^2 = 0.9997). The efficiency of extraction from the strips was validated for recovery that resulted as being in the range of 95.5 ± 1.7% of ECN (n = 3).

The results were expressed as the mean of five subjects.

Statistical analysis

Analysis of viscosity, ex-vivo and in-vivo data was performed using the Kruskal–Wallis test (GraphPad Prism, version 2.01; GraphPad Software Incorporated). In all cases, individual differences between treatments were evaluated using a non-parametric post-hoc test (Dunn's test). $P < 0.05$ denoted statistically significant difference.

Results and Discussion

Preparation and characterization of SLN

The high-shear homogenization method, previously proposed for the preparation of solid lipid microparticles containing juniper oil (Gavini et al 2005b), appears to be also a suitable and rapid technique for the production of SLN containing ECN. It is a one-step process that simply involves the preparation of an o/w emulsion.

As reported in Table 2 all formulations had a mean diameter of about 150 nm. Moreover, SLN 0 was characterized by the lowest polydispersity index (PI = 0.14) with respect to SLN 1 and SLN 2 formulations that showed PI values below 0.4, indicating a broad distribution (Donini et al 2002).

SEM photomicrographs of SLN 0 and SLN 2 (chosen as an example) are shown in Figure 1. Drug-free nanoparticles had a regular shape and smooth surface (Figure 1A).

The presence of the drug (1% w/w) (SLN 1) did not cause considerable modifications of particle morphology; in all cases no free drug crystals appeared (data not reported). The formulation SLN 2, containing 10% (w/w) Precirol, was characterized by partially aggregated particles (Figure 1B).

The encapsulation efficiency values were in the range 97–102%, independently of lipid-to-ECN-weight ratios employed (Table 2). According to Souto et al (2004), this result can probably be attributed to the high affinity of the lipophilic drug for the lipidic material.

Yields of production obtained were always relatively high and were in the range 75–97%.

Figure 2 shows the results of DSC analyses of ECN (raw material), drug-free nanoparticles (SLN 0), physical mixture of SLN 0 added to pure drug, and drug-loaded SLN batches (SLN 1 and SLN 2). The DSC curves indicated that ECN (curve a) exhibits a single endothermic peak at 165°C, in agreement with the melting point, followed by a large and irregular exotherm (Godefroi et al 1969).

The DSC scan of SLN 0 empty nanoparticles (curve b) showed a melting peak of lipid material at 62.12°C. The physical mixture of drug and SLN 0 (curve c) and drug-loaded SLN 1 (curve d) and SLN 2 (curve e) formulations displayed similar thermograms. In all cases, the presence of Precirol endothermic peak and the disappearance of the ECN melting endotherm were observed. However, the curve c exhibite a weak broad exotherm between 155 and 170°C,

Table 2 Mean diameter, polydispersity index, drug content, encapsulation efficiency and yield of production of SLN

Batch	Mean diameter (nm)	Polydispersity index	Drug content (w/w)		Encapsulation efficiency (%)	Yield of production (%)
			Theoretical	Actual		
SLN 0	143.3 ± 6.22	0.14 ± 0.02	—	—	—	96.6 ± 0.32
SLN 1	139.6 ± 12.67	0.36 ± 0.02	11.8 ± 0.15	11.4 ± 0.17	96.5 ± 1.43	87.8 ± 0.67
SLN 2	154.3 ± 4.93	0.34 ± 0.03	7.41 ± 0.14	7.59 ± 0.23	102.4 ± 3.05	74.8 ± 0.16

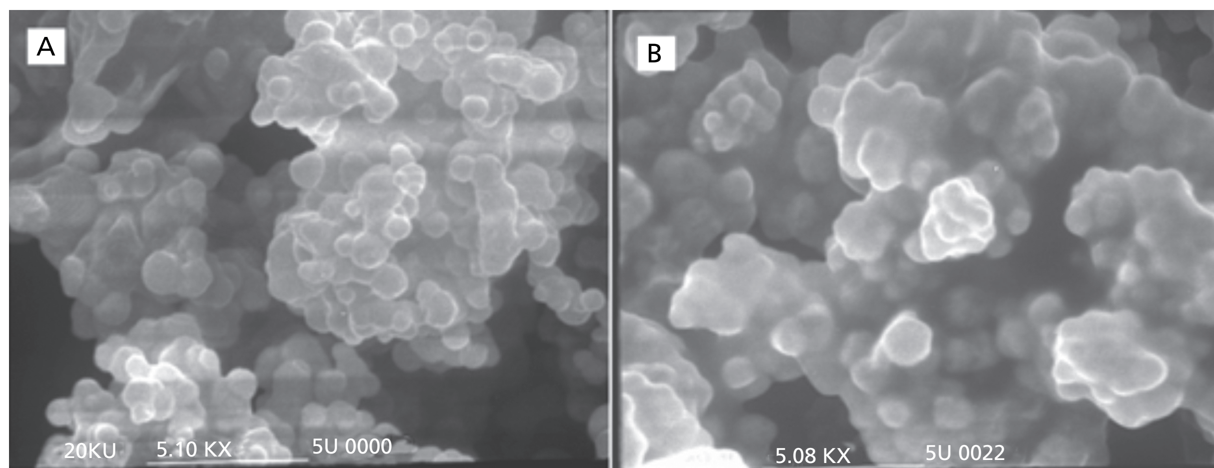

Figure 1 SEM photographs of SLN 0 (A) and SLN 2 (B). Magnification: 5000×.

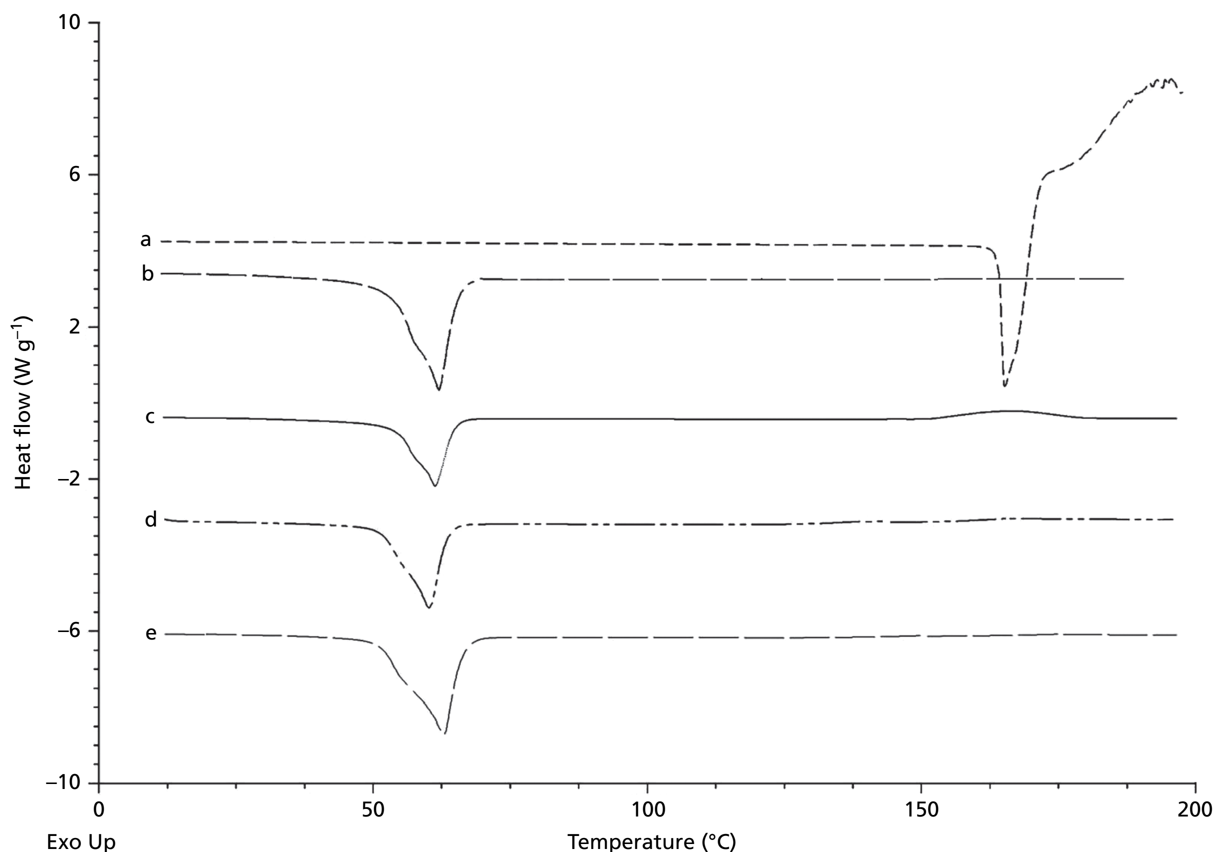

Figure 2 DSC thermographs of ECN pure (a), empty nanoparticles (SLN 0) (b), physical mixture of SLN 0 and ECN (c), SLN 1 (d) and SLN 2 (e).

probably due to dissolution of drug crystals into the molten lipid. Therefore, the differences in shape of the calorimetric curves of drug-loaded SLN could be attributed to either the drug amorphization or its dissolution in the molten carrier during DSC scan. Moreover, the drug could act as an impurity by inducing a small decrease of the lipid peak temperature that appears to be more evident in the case of SLN 1 (from about 62 to 60.17°C).

As reported by Muller et al (2002), SLN can be easily formulated in topical products through their incorporation into existing products or by addition of a viscosity agent to SLN in aqueous dispersion or by direct production of highly

concentrated dispersions. In this study, the SLN dispersions are formulated in hydrogels for topical application using the second approach mentioned; a gel containing only ECN pure drug and HPMC as gelling agent (ECN gel) was used as reference. The ECN gel appeared opalescent with a smooth and homogeneous appearance immediately after preparation but a separation of drug from the gel phase was observed after a short time (5–6 h). On the other hand, gels containing SLN 1 and SLN 2 were characterized by a white colour, good stability and properties of consistence and spreadability, regardless of the concentration of lipid used.

It is known that the viscosity of topical gels is an important physical parameter which affects the rate of drug release; in general, an increase in viscosity of vehicles would cause a more rigid structure and decrease the drug release rate (Wang et al 2001; Pose-Vilarnovo et al 2004). To investigate the possible influence of formulation rheological properties on ECN release, the viscosity of prepared gels containing SLN was evaluated and compared with gel reference.

The ECN gel was characterized by a slightly reduced viscosity (8136 ± 542.4 mPa s); the incorporation of nanoparticles into hydrogels did not produce significant changes ($P > 0.05$) in the viscosity values, which resulted in 8407 ± 271.2 mPa s for SLN 1 and 9085 ± 591.1 mPa s for SLN 2 gels, respectively. These results suggest that the drug release from gels, following application to the skin, is not influenced by the rheological properties of the formulations.

The physical stability of SLN dispersions before and after their incorporation into hydrogels was monitored by particle size examination to investigate the possible aggregation of particles due to incompatibility or interactions between the gel-forming ingredients. The analysis of samples showed that the incorporation of SLN into hydrogel did not affect the average sizes of drug-loaded nanoparticles, which remained substantially unchanged (155.8 ± 5.6 nm for SLN 1 and 163.5 ± 0.9 nm for SLN 2, respectively).

Ex-vivo drug permeation studies

To evaluate the influence of SLN composition on the ECN permeation through the pig skin, ex-vivo permeation studies were carried out over 24 h. This test allowed determination of the concentration of drug permeated from formulation and dissolved in the acceptor medium. The ECN gel, containing the drug not incorporated into SLN, at the same concentration (1% w/w), was used as reference.

The cumulative amount of drug permeated (μg cm^{-2}) through the stratum corneum was plotted against time (Figure 3).

The permeation profiles obtained show that the release rate of ECN depends on the composition of gels. The formulation without SLN (ECN gel) was characterized by a more rapid and irregular permeation profile, compared with the gels containing the drug encapsulated into nanoparticles (SLN 1 and SLN 2 gels). The cumulative amount of drug released from ECN gel within the first hour was negligible; the drug permeated through stratum corneum ranged from about 33 μg cm^{-2} after 2 h to 125 μg cm^{-2} at the end of the test.

The drug encapsulation into SLN decreased the permeation through the skin but also reduced the variability in the

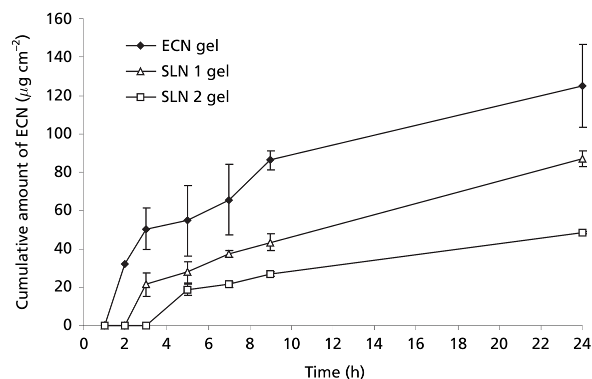

Figure 3 Ex-vivo permeation profiles of ECN from gels through stratum corneum. Data are means ± s.d., n = 3.

release profiles, due to the controlled drug diffusion from the solid crystalline lipid.

After the lag time of 1.19 h and 1.61 h, obtained for SLN 1 and SLN 2 gels, respectively, the release profile of ECN from these formulations results was linear with square root of time as indicated by the excellent correlation coefficients obtained ($R^2 = 0.9955$ for SLN 1 gel and $R^2 = 0.9944$ for SLN 2 gel). Besides, for SLN 1 gel, containing 5% (w/w) of lipid, the penetration rate increased (i.e. 43.3 ± 4.4 μg cm^{-2} of drug permeated after 9 h) compared with SLN 2, containing 10% (w/w) of Precirol (26.8 ± 1.0 μg cm^{-2}).

Statistical analysis showed that the total amount of drug released from ECN gel through stratum corneum after 24 h (124.72 ± 21.6 μg cm^{-2}) was significantly different ($P < 0.05$) when compared with SLN 2 gel (48.46 ± 0.8 μg cm^{-2}). On the other hand, no significant differences were found from the comparison between ECN and SLN 1 gels and between formulations containing SLN at different lipid-to-drug ratio ($P > 0.05$).

According to literature on models for the incorporation of active compounds into SLN, this behaviour could be due to the formation of drug-enriched core surrounded by a practically drug-free lipid shell (Muller et al 2002; Souto et al 2004). The higher lipid content in SLN 2 formulations leads to the increased diffusional distance, which determines a significantly slower drug release (Wissing et al 2004). Additionally, the decrease in the amount of drug permeated with an increase in the lipid concentration into SLN can be explained through the formation of a lipid film on top of the stratum corneum through which the ECN must diffuse before partitioning into stratum corneum, according to Muller et al (2002). They reported that the adhesion of particles on the skin surface leads, after water evaporation, to particle fusion and film formation. This effect is particularly exploited when the active agent localization in the skin surface is desired, such as sunscreen application (Wissing & Müller 2001a, b).

In-vivo experiments

Tape stripping is a useful and minimally invasive technique for studying the in-vivo penetration, distribution and dermatopharmacokinetics of topically applied cosmetic products and

drugs within the stratum corneum (Weigmann et al 1999; Surber et al 2001). In this study, the in-vivo penetration of ECN into human stratum corneum as a function of application duration and composition of gels was investigated. The extraction method of drug from tapes stripped allows the recovery of the ECN freely dissolved and ECN encapsulated into SLN.

Figure 4 shows the cumulative amount of ECN penetrated into the skin out of control gel (ECN gel) and gels containing SLN 1 and SLN 2 after two different application times (1 h and 3 h). After 1 h of application, the amount of drug penetrated into stratum corneum from ECN gel ($1.56 \pm 0.3\,\mu g\ cm^{-2}$) was significantly lower ($P < 0.05$) than that from SLN 1 gel ($2.32 \pm 0.3\,\mu g\ cm^{-2}$), while it was not significantly different ($P > 0.05$) when compared with SLN 2 gel ($2.01 \pm 0.3\,\mu g\ cm^{-2}$). On the contrary, after 3 h, the drug recovered into stratum corneum for ECN gel ($1.84 \pm 0.2\,\mu g\ cm^{-2}$) was significantly higher ($P < 0.01$) than for the gel containing SLN 2, with 10% of lipid material ($0.90 \pm 0.3\,\mu g\ cm^{-2}$). No significant differences were detected between formulations containing solid lipid nanoparticles for both considered application times.

The results obtained suggest that the amount of ECN recovered into the upper skin layers from SLN gels, with respect to formulation containing pure drug, depends on the application time and on the composition of particles.

After a shorter period of application, the improvement of drug penetration observed from SLN 1 gel could be due to the smaller size of lipid particles compared with the drug not encapsulated in reference gel (dvs about 10 μm); in fact, nanoparticles may penetrate easily into follicular openings and stick tightly to the stratum corneum, leading to a localization in the upper skin (Jenning et al 2000a).

When SLN 1 gel was applied for a longer time, the quantity of drug recovered was not different from that following application of ECN gel. According to Wissing et al (2001a), the result can be explained by considering that the adhesion of particles and the consequent formation of a lipidic film on the skin surface might determine a reduction of the penetration rate of the drug into the stratum corneum. Moreover, the drug penetrated within the first hour can diffuse in deeper skin layers during 3 h.

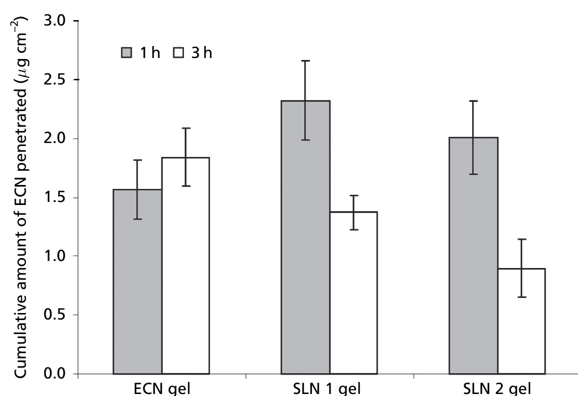

Figure 4 Cumulative amount of ECN penetrated through human stratum corneum from prepared gels, after 1 h and 3 h of application time. Data are means ± s.d., n = 5.

In the case of SLN 2 gel, with higher lipid content, the effect produced from the film lipid layer on the skin surface appears to be more evident, particularly after 3 h of application. This finding is in agreement with Jenning et al (2000a), who reported an improvement of retinyl palmitate penetration in the upper skin layers following a short SLN treatment and a reduction for prolonged application with respect to conventional formulations.

Conclusions

High-shear homogenization appears to be a suitable method for the preparation of SLN-containing ECN characterized by high encapsulation efficiencies and good yields of production. After incorporation into hydrogels, SLN are able to provide a controlled release of ECN through porcine stratum corneum compared with a conventional gel. In-vivo results suggest that SLN with lower lipid concentration improve the ECN penetration rate into upper skin after a short period of application and could be useful for site-specific delivery of drugs to the skin.

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