JPP
Journal of
Pharmacy and Pharmacology

JPP 2009, 61: 559–567
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Received September 26, 2008
Accepted February 04, 2009
DOI 10.1211/jpp/61.05.0003
ISSN 0022-3573

Research Paper

Evaluation of solid lipid microparticles produced by spray congealing for topical application of econazole nitrate

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Abstract

Objectives The aims of this study were to evaluate the suitability of the spray congealing technique to produce solid lipid microparticles (SLMs) for topical administration and to study the skin permeation of a drug from SLMs compared with solid lipid nanoparticles (SLNs).

Methods Econazole nitrate was used as model drug and Precirol ATO 5 as the lipidic carrier. SLMs and SLNs were both prepared at 5 : 1, 10 : 1 and 12.5 : 1 lipid : drug weight ratios and characterised in terms of particle size, morphology, encapsulation efficiency and chemical analysis of the particle surface. SLMs and SLNs were also incorporated into HPMC K 100M hydrogels for ex-vivo drug permeation tests using porcine epidermis.

Key findings SLMs had particle sizes of 18–45 μm, while SLNs showed a mean diameter of 130–270 nm. The encapsulation efficiency was 80–100%. Permeation profiles of econazole nitrate were influenced by both particle size (significant difference until 9 h) and the amount of lipid.

Conclusions The results confirm the usefulness of SLNs as carriers for topical administration and suggest the potential of SLMs for the delivery of drugs to the skin.

Keywords econazole nitrate; skin delivery; solid lipid microparticles; solid lipid nanoparticles; spray congealing

Introduction

In recent years, solid lipid micro- and nanoparticles (SLMs and SLNs) have emerged as attractive carriers for topical application in the pharmaceutical and in cosmetic fields because of their advantages over polymeric systems.[1–3]

SLNs have been proposed to enhance the percutaneous absorption of drugs[4] and allow drug targeting to the skin.[5–8] Improved dermal absorption of active pharmaceutical ingredients loaded into lipid carriers may result from increases in the surface contact of drug and corneocytes, skin occlusion, rapid and steady release, and surfactant effects.[9] It is well demonstrated that, after application to the skin, the small particles can make close contact with superficial junctions of corneocytes on the outermost skin layers because of a pronounced adhesive effect.[10] Adherence of lipid nanoparticles to the skin leads to the formation of a film and subsequently to an occlusion effect.[11] The first model for film formation by SLNs on the skin was developed by Müller and Dingler[12] and the occlusion effect has been widely demonstrated in in-vitro models[13] and after application *in vivo*.[14]

The main approaches used for the production of finely dispersed lipid nanoparticles include high-pressure homogenisation,[15] microemulsions,[16] solvent emulsification–evaporation or –diffusion,[17,18] water/oil/water double-emulsion method[19] and high-speed stirring and/or ultrasonication.[20]

SLMs have been less extensively investigated for skin application compared with SLNs but have recently been shown to be potentially useful for both topical and transdermal drug delivery.[21–25]

SLMs can be prepared by different processes such as solvent evaporation, melt dispersion, hot and cold homogenisation, spray drying and spray congealing.[26] Spray congealing has

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attracted increasing attention in recent years because this technique does not require the use of organic or aqueous solvents and hence it is environmentally friendly and less time consuming than other methods; moreover the process can be easily employed on an industrial scale. Spray congealing, also called spray chilling, involves the atomisation of a fluid (solution or suspension of the active pharmaceutical ingredient in a melted carrier) into an environment maintained at a temperature below the carrier melting point. The atomisation leads to the formation of melted droplets, which then solidify upon cooling, producing the final microparticles.[27] Ultrasonic atomisers[28–30] and, more recently,[31] a wide pneumatic nozzle have been proposed as new atomisers, allowing the preparation of non-aggregated and spherical microparticles with high encapsulation efficiency suitable for oral administration. Until now, spray congealing has not been considered as a production technique for SLMs for skin application.

The aims of this study were to evaluate the feasibility of producing SLMs suitable for topical administration using the spray congealing technique and to study the skin permeation of a drug from the SLMs compared with SLNs. Econazole nitrate (ECN) was used as a model drug and Precirol ATO 5 as the lipidic carrier; microparticles and nanoparticles were both prepared at three different lipid : drug weight ratios (5 : 1, 10 : 1 and 12.5 : 1). The particles obtained were characterised in terms of particle size, morphology, encapsulation efficiency and chemical analyses of their surface.

After incorporation of micro- and nanoparticles into hydrogels, the ex-vivo permeation studies of the ECN from formulations through porcine skin were then carried out and compared with the non-encapsulated ECN.

Materials and Methods

Materials

ECN (molecular weight 444.7, log P 5.61) was kindly provided by Erregierre SpA (Bergamo, Italy) and glyceryl palmitostearate (Precirol ATO 5) from Gattefossé (Cedex, France). PEG sorbitan monooleate (Tween 80) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and hydroxypropylmethylcellulose (HPMC K100M) from Dow Chemicals (Midland, MI, USA). Methanol (Chromasolv for HPLC, gradient grade) and $NH_4H_2PO_4$ were obtained from Rielden-de Haën AG (Seelze, Germany). Other chemicals were of HPLC or analytical grade.

Preparation of solid lipid microparticles (SLM)

SLMs were produced by the spray congealing process using three different ratios of Precirol ATO 5 : ECN (5 : 1, 10 : 1 and 12.5 : 1% w/w) and are designated SLM 1, SLM 2 and SLM 3, respectively. Precirol ATO 5 was heated at 10°C above the melting point. The drug was then added to the molten carrier and magnetically stirred to obtain a suspension, which was then loaded into a thermostatted feeding chamber placed above the wide pneumatic nozzle, which is an innovative external-mix two-fluid atomiser described in detail in a previous paper.[31] The main differences between the wide pneumatic nozzle and commercial two-fluid atomisers are the following: the internal diameter of the orifice is bigger than usual, being 4.5 mm, and it works in an unusual configuration (Figure 1). The molten fluid is delivered to the orifice from A (molten fluid inlet) by the Venturi effect, while the atomisation air is delivered from B (air inlet), in a radial direction with respect to the molten fluid. Atomisation occurs in C, where the air input converges with the molten fluid. Another important aspect of the wide pneumatic nozzle is that the whole device is heated by two resistors connected to an inverter (not shown in the figure); in this way, the atomisation air is heated inside the nozzle and inlet air at ambient temperature can be used. Two operating parameters can be set: pressure of the air and temperature of the device. In preliminary studies atomisation was carried out varying the air pressure from 2 to 3 bar and the nozzle temperature from 85 to 120°C. Thereafter, microparticles were obtained with the air pressure set at 2.5 bar and the nozzle temperature at 100°C. Atomisation leads to the formation of melted droplets which then solidify during the fall in the chamber at room temperature, producing the final microparticles, which were collected and stored in a vacuum desiccator at room temperature. Each SLM formulation was prepared in triplicate.

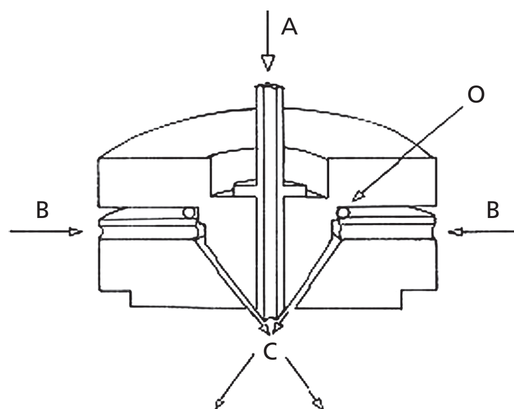

Figure 1 Schematic representation of the wide pneumatic nozzle: A, fluid feed (molten fluid inlet); B, air inlet; C, atomisation of fluid; O, O-ring

Preparation of solid lipid nanoparticles

Preparation of SLN 1 and SLN 2 dispersions (lipid : drug ratio of 5 : 1, 10 : 1% w/w respectively) by modification of the high-shear homogenisation method has been reported previously by Sanna and colleagues.[8] In the current work the SLN 3 dispersion was produced using the same ratio of lipid : drug as for SLM 3 (12.5 : 1% w/w). Briefly, the drug was added to the lipid material (Precirol ATO 5) previously melted at 80°C. The hot lipid phase was then slowly dispersed under stirring in a surfactant (Tween 80, 2.5% w/w) solution at the same temperature, and the mixture was homogenised using a Silverson L4R mixer (Crami, Italy), at 6200 rpm for 5 min. The nanoemulsion obtained was solidified by rapid cooling at −5°C with magnetic stirring to give the SLN dispersions. Each SLN formulation was prepared in triplicate.

Lyophilisation of solid lipid nanoparticles

A weighed amount of SLN aqueous dispersion was frozen overnight at –80°C and then lyophilised using a 5Pascal LIO 5P apparatus (Cinquepascal srl, Milano, Italy). The freeze-drying process was carried out at –54.5°C under vacuum (0.909 mbar) for 8 h and the SLN powders were collected for successive experiments.

Characterisation of particles

Particle size analysis

The particle size of the SLMs was measured by laser diffraction (Coulter LS 100 Q Laser Sizer, Beckman Coulter, Miami, FL, USA) after dispersion of microparticles in a surfactant aqueous solution. The average particle size was expressed as the volume–surface diameter, d_{vs} (μm). The particle size distribution was expressed in terms of the SPAN index, calculated from the following equation: SPAN = $(d_{90} - d_{10}) / d_{50}$, where d_{10}, d_{50} and d_{90} are the diameter sizes and the value is the percentage of particles smaller than that size, a high SPAN value indicating a wide size distribution.[32]

Particle size analysis of the original SLN 3 dispersion was performed by photon correlation spectroscopy (PCS) (Coulter N5 submicron particle sizer, Beckman Coulter). PCS gives the mean particle size and the polydispersity index (PI) as a measure of the width of the distribution. Prior to analysis, each sample was diluted with distilled water to achieve an appropriate concentration of particles. All measurements were done in triplicate and data were expressed as means ± SD.

Drug content

Samples (50 mg) of SLMs or lyophilised SLNs were dissolved in methanol (10 ml) under stirring at 80°C and then slowly cooled to room temperature to precipitate the lipid. After centrifugation (3000 rpm for 5 min), an aliquot of supernatant was diluted 100 times with methanol. The drug content in the solution was determined by HPLC after filtration, using a Varian Prosta 210 liquid chromatography system equipped with a Varian 330 diode array detector (Varian Deutschland GmbH, Dramstad, Germany) using an Apherisorb RP-C8 column (5 μm, 250 × 4.6 mm; Supelco, Milano, Italy). The mobile phase was methanol and 0.05 M $NH_4H_2PO_4$ (85 : 15 v/v), delivered at a flow rate of 1.0 ml/min. Detection was at 200 nm. The elution period was 8 min and the retention time of ECN was about 5.8 min.[8] Calibration curves were linear in the range 0.5–20 μg/ml. The results are expressed as the mean of three replicates for each batch of SLMs or SLNs.

The encapsulation efficiency was calculated as a percentage with respect to the theoretical amount of ECN used for preparation of the particles.

Scanning electron microscopy

The shape and surface characteristics of SLMs and lyophilised SLNs were determined by scanning electron microscopy (SEM). Samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards, Milano, Italy) and examined using an ESEM-FEI Quanta 200 SEM (FEI Company, Hillsboro, OR, USA) at an accelerating voltage of 25 kV using the secondary electron technique.

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) analyses were performed in an ultra-high-vacuum chamber working at a base pressure of about 7×10^{-10} Torr, equipped with a conventional Mg-anode X-ray source (Leybold-Heraeus EA11 SCD, Cologne, Germany) (hv = 1253.6 eV) and a double-pass cylindrical mirror analyser. The samples were prepared by pressing a suitable amount of SLNs and SLMs onto pure tantalum foil (99.999% purity, Goodfellow, Huntingdon, UK). XPS measurements were carried out on ECN (raw material) and on ECN-loaded SLNs and SLMs.

Preparation of hydrogels

Two per cent gelling agent (HPMC K100M) was added to the original SLN dispersion of SLN 3 and to SLM batches 1, 2 and 3 dispersed in a surfactant aqueous solution (Tween 80, 2.5% w/w as used for the preparation of the original dispersions of SLN 1–3) in a beaker and gently stirred at room temperature for 15 min to yield gels. A gel containing non-encapsulated ECN was prepared as the reference formulation. The final preparations contained 1% ECN (w/w).

Ex-vivo permeation studies

Pig skin is frequently used as a model membrane for permeation studies because the stratum corneum is similar in thickness to the human membrane and it has similar permeability properties to human skin, showing an analogous penetration for topically applied compounds.[33–35]

Ears from adult domestic pigs 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 discarded. The ears were washed with water and dried using soft tissue. Hairs were removed and the skin was heated in distilled water at 60°C for 2 min, and the epidermis gently peeled off and used for the permeation studies.[36] The epidermal membrane was chosen for these studies because of the lipophilic nature of the permeant, ECN and because it most accurately represents the in-vivo situation and hence is the tissue of choice for most ex-vivo permeation studies.[36]

Permeation studies were carried out on gels containing SLMs or SLNs and on non-encapsulated ECN gel.

The epidermis was cut and clamped by means of a plastic ring to the bottom of a support consisting of a plastic tube (height 1.91 cm, diameter 2.28 cm).[37,38] A weighed amount of each gel (about 200 mg) was spread uniformly on the surface of the skin (area = 4.08 cm^2). The cylindrical support was connected to the drive shaft of the dissolution apparatus (Erweka DT 70, Erweka GmbH, Heusenstamm, Germany). The system was then inserted into the vessel containing the receptor medium, so that the dermis side touched the surface of the fluid, taking care not to trap air under the membrane. The working conditions were 200 ml methanol/water (50 : 50 v/v) as receptor medium, 32°C and 25 rpm. The composition of the receptor medium was chosen because ECN dissolved readily in it whereas the solid lipid particles did not. Alcohols are commonly used as a co-solvent with water for receptor solutions for poorly soluble permeants.

The amount of ECN permeated through the epidermis at different times (1, 2, 3, 4, 5, 6, 7, 8 and 24 h) was determined by HPLC analysis using the method described above. Linear regression analysis of the permeation data was performed by plotting the cumulative amount of ECN determined in the receptor solution against the square root of time (in h). From these plots, the drug permeation rate (corresponding to the slope) and the lag time (obtained by extrapolating the linear portion of the curve to the abscissa) were determined for each formulation.

Statistical analysis

The statistical significance of ex-vivo permeation data was tested using the Kruskal–Wallis test. Individual differences between formulations were evaluated using Dunn's test as a non-parametric post-hoc test, using GraphPad Prism, version 2.01 (GraphPad Software Inc. La Jolla, CA, USA). The differences were considered to be statistically significant when P was less than 0.05.

Results

Preparation and characterisation of micro- and nanoparticles

Preliminary experiments in which the operating parameters of the atomiser were varied (air pressure from 2 to 3 bar and nozzle temperature from 85 to 120°C) were performed to determine the parameters required to produce micro-particles with a particle size in the range 10–50 μm, which is considered optimal for topical applications.[22] Satisfactory particle size was obtained using an air pressure of 2.5 bar and the nozzle temperature at 100°C. In fact, as shown in Table 1, the d_{vs} varied between 18 and 45 μm for all the SLM samples. In addition, yields were in the range 80–92% and the encapsulation efficiencies were 103.2, 83.4 and 104.9% for SLM 1, SLM 2 and SLM 3, respectively.

For comparison, SLN 3, which had the same composition as SLM 3, were produced by the high-shear homogenisation method used by Sanna and colleagues[8] for the preparation of SLN 1 and SLN 2 ECN-loaded nanoparticles, which had the same lipid : drug ratio as SLM 1 and SLM 2. The yields of SLN were in the range 75–88%, and the encapsulation efficiency varied from 97 to 102%. PCS data (Table 1) showed that SLN formulations were characterised by a mean diameter varying from 135 to 270 nm; the PI data indicated a narrow and unimodal distribution[39] for SLN 1 and SLN 2, while with increased amount of lipid, as in SLN 3, the PI value increased to 0.8.

The shape and morphology of both SLNs and SLMs were studied by SEM. Figure 2 shows photomicrographs of the lyophilised SLN 1, SLN 2 and SLN 3 formulations, revealing that the nanoparticles were aggregated and fused. SEM analysis of SLM 1, 2 and 3 at low magnification (Figure 3a, c and e) showed non-aggregated microparticles with a regular and spherical shape. Higher magnification of the biggest microspheres (Figure 3b, d and f) revealed that the surfaces of the SLMs were not completely smooth and had some surface irregularities.

Figure 4 shows the XPS results for ECN (raw material) and drug-loaded SLNs and SLMs. The spectrum of the ECN molecule (curve 1) exhibited the Cl and N peaks at about 210 and 410 eV, respectively, in addition to the O and C peaks. By contrast, the curves for SLM 1, SLM 2 (curves 2 and 3), SLN 1 and SLN 2 (curves 4 and 5) are characterised by the disappearance of Cl and N peaks, suggesting the absence of drug molecules on the surface of the particles. It can therefore be reasonably hypothesised that SLN 3 and SLM 3, having lower drug content, would also be characterised by the complete encapsulation of ECN.

Ex-vivo permeation studies

Figure 5 shows the amount of ECN that permeated through the porcine skin from SLM and SLN gels against the square root of time; Table 2 gives the Higuchi's rate constant lag time and the total amount of ECN permeated from different gels after 24 h.

The non-encapsulated ECN is able to permeate the epidermis. The cumulative amount of drug released from ECN gel within the first hour is negligible (lag time 60 min); the total amount permeated through the epidermis ranged from about 32 μg/cm^2 after 2 h to 124 μg/cm^2 at the end of the test (24 h), with a release rate of 25.34 μg/cm per h$^{1/2}$ (Figure 5).

Permeation profiles and permeation parameters show that all SLM gels are characterised by a similar release rate of ECN (24–28 μg/cm per h$^{1/2}$) and the same total amount of drug permeated after 24 h. However, SLM 1 and SLM 2 gels have a very short lag time (about 12 min) while SLM 3, containing the highest amount of lipid, is characterised by a longer lag time (Table 2).

The release rate of ECN from gels containing SLN 1, 2 or 3 showed that the cumulative amount permeated after 24 h

Table 1 Particle size characterisation of the solid lipid microparticles (SLMs) and nanoparticles (SLNs)

Formulation	d_{vs} (μm)	SPAN index	Mean diameter (nm)	Polydispersity index
SLM 1	18.0 ± 3.17	1.27 ± 0.02		
SLM 2	44.7 ± 5.16	1.13 ± 0.04		
SLM 3	31.6 ± 3.17	0.6 ± 0.07		
SLN 1			135.8 ± 15.37[a]	0.268 ± 0.08[a]
SLN 2			156.3 ± 8.43[a]	0.286 ± 0.07[a]
SLN 3			271.9 ± 14.64	0.818 ± 0.09

SLN and SLM formulations 1, 2 and 3 have lipid : drug ratios of 5 : 1, 10 : 1 and 12.5 : 1 (% w/w), respectively. d_{vs}, volume–surface diameter. Values are means ± SD (n = 3). [a]Data from Sanna *et al.*[8]

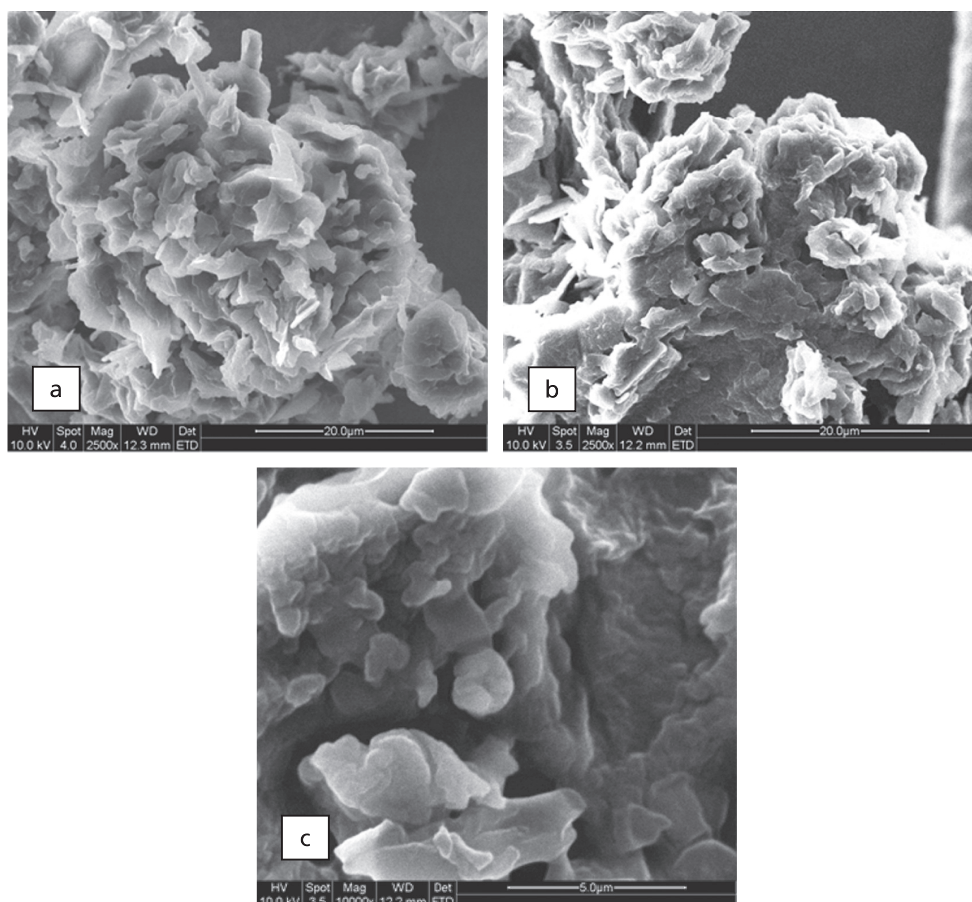

Figure 2 Scanning electron micrographs of the solid lipid nanoparticle formulations SLN 1 (a), SLN 2 (b) and SLN 3 (c)

was not significantly different regardless of the amount of lipid used. The lag times were between 60 and 97 min for all SLN gels.

Discussion

Preparation and characterisation of micro- and nanoparticles

The spray congealing process for the production of SLMs suitable for topical administration was evaluated; in particular the wide pneumatic nozzle, recently successfully employed to produce both propafenone-hydrochloride- and vitamin-E-loaded lipid microspheres,[31] was used as the atomiser.

The results demonstrated that the spray congealing technique using the wide pneumatic nozzle is a suitable technology for the production of ECN-loaded lipid microparticles potentially useful for skin delivery. Selection of appropriate manufacturing parameters made it possible to obtain particles with a diameter suitable for topical administration. All SLM formulations had low SPAN indexes, demonstrating that the spray congealing technique is able to produce microparticles characterised by a narrow size distribution independently of the lipid : drug ratio. Furthermore, the SLMs were obtained with good yields and satisfactory encapsulation efficiencies.

With regard to SLNs, the results confirmed that the high-shear homogenisation method is also suitable for the preparation of SLN 3, as nanoparticles were obtained with good yields and encapsulation efficiency. The high encapsulation efficiency values reflect the affinity of the lipophilic drug for the lipidic material,[40] as well as the lipid compounds chosen. It is widely reported in the literature that higher encapsulation efficiencies can be obtained using mixtures of acylglycerols because of the formation of voids and vacancies within the lipid matrix of the particles.[3] The particle size of SLN can be influenced by the lipid : drug weight ratios used ($P < 0.05$): increasing the amount of lipid, as in SLN 3, increased the mean diameter and the PI value. The high lipid content presents an obstacle to the dispersion of nanoparticles and makes their aggregation easy, giving a dispersion characterised by big particle size and no unimodal distribution.

SEM results revealed that the lipid : drug ratio used did not affect the morphology of either SLNs or SLMs. The surface of SLMs had some irregularities, which could be attributed to ECN crystals on the microparticle surface or be caused by the rapid solidification of Precirol during the spray congealing process. To clarify this aspect, XPS analysis was performed on samples at higher drug content. XPS is a spectroscopic surface

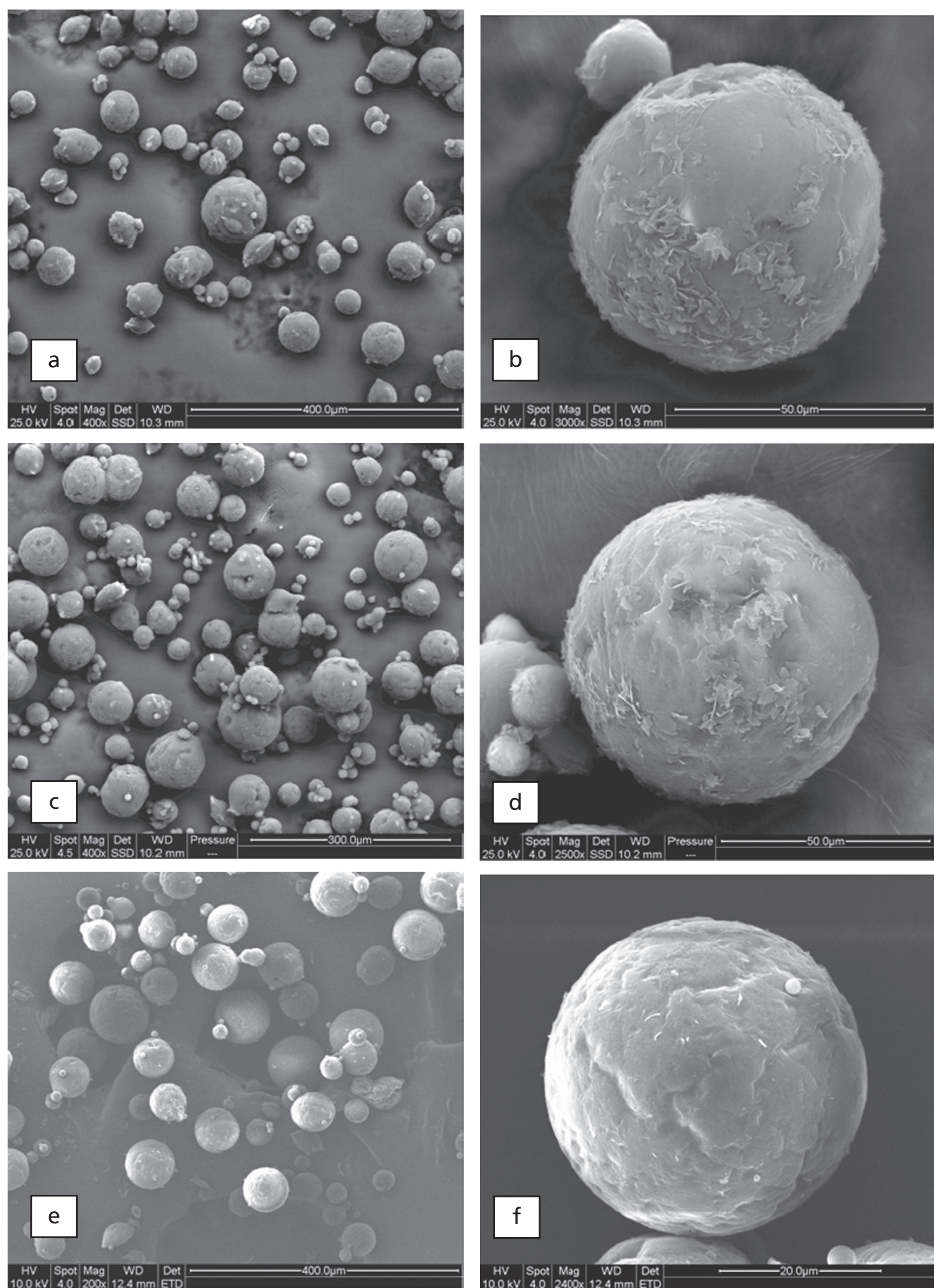

Figure 3 Scanning electron micrographs of the solid lipid microparticle formulations SLM 1 (a and b), SLM 2 (c and d) and SLM 3 (e and f) at low (a, c, e) and high (b, d, f) magnification

chemical analysis used to estimate the elemental composition, chemical state and electronic state of the elements on the surface of a material (up to 10 nm).[30] XPS uses a beam of X-rays to irradiate the material while simultaneously measuring the kinetic energy and the number of electrons that escape from the surface of the material being analysed. Each element produces a characteristic set of XPS peaks at characteristic binding energy values that directly identify each element. Thus, information on drug distribution in the particles, present on the surface or encapsulated within the SLMs and SLNs can be obtianed. Since ECN is the unique component having Cl and N atoms in the structure, its exact location in SLMs and SLNs can be detected from XPS analysis. The results show that the irregularities on the microparticle surface are not due to the presence of drug crystals on or close to the external layer of the microparticles. Thus, the spray congealing technique and high-shear homogenisation process promote efficient incorporation of the drug into the micro- and nanoparticles. This can be

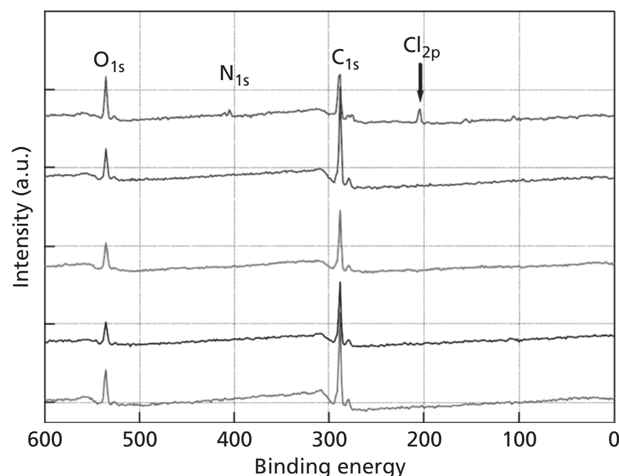

Figure 4 X-ray photoelectron spectroscopy photoemission spectra of the econazole nitrate raw material (a), SLM 1 (b), SLM 2 (c), SLN 1 (d) and SLN 2 (e). Solid lipid nanoparticle (SLN) and microparticle (SLM) formulations 1 and 2 have lipid : drug ratios of 5 : 1 and 10 : 1 (% w/w), respectively.

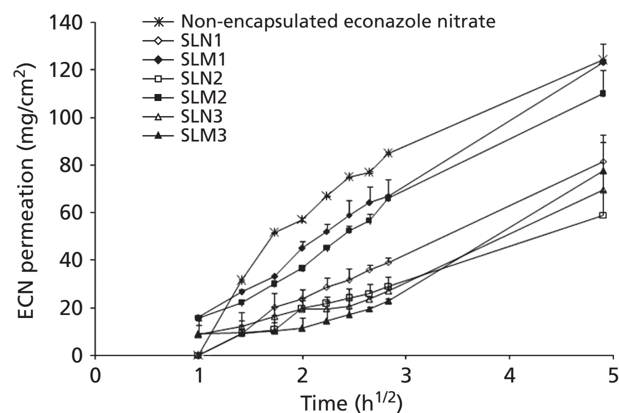

Figure 5 Cumulative amount of econazole nitrate (ECN) permeating through porcine epidermis. Gels contained non-encapsulated econazole nitrate, SLN/SLM 1, SLN/SLM 2 and SLN/SLM 3. SLN, solid lipid nanoparticle; SLM, solid lipid microparticle.

attributed to the high affinity of ECN for the lipid matrix as well as the use of a suitable amount of lipid substance sufficient to entrap the drug into micro- and nanoparticles.

Ex-vivo permeation studies

In order to evaluate the potential of SLM for topical administration and to compare the permeation ability of ECN from SLMs and SLNs, ex-vivo permeation studies were performed after the incorporation of SLNs (in original dispersions) and SLMs into a gel base which does not induce dissolution of lipid particles. For comparison, a gel containing the non-encapsulated drug was also tested.

Considering the permeation data obtained from SLMs, drug permeation appears to be influenced only by high lipid : drug ratios. In fact, increasing the lipid content delays and decreases the drug release from the SLM 3 formulation compared with both SLM 1 in the range 3–9 h ($P < 0.01$) and SLM 2 after 7 and 9 h ($P < 0.05$). Furthermore, the permeation profile of SLM 3 is significantly different ($P < 0.01$) from the permeation behaviour of non-encapsulated ECN gel at 2–9 h. This profile may be due to the higher amount of lipid, which increases the diffusional layer that delays the drug release from formulation and decreases the total amount of ECN recovered compared with SLM 1 and SLM 2 gels.

Comparison of permeation results for SLN 1–3 and non-encapsulated drug shows that SLN 2 and SLN 3 delayed ECN permeation through the skin ($P < 0.05$ and $P < 0.01$ after 9 h; $P < 0.001$ and $P < 0.05$ after 24 h, for SLN 2 and SLN 3, respectively). These results also demonstrate that permeation of ECN from SLN gels depends on the formulation: a low lipid content results in permeation of more ECN. This finding agrees with a proposed structural model for the incorporation of active compound into SLNs consisting of a drug-enriched core enclosed in an outer shell enriched with lipid.[41] Thus, the slower drug permeation from SLN 2 and SLN 3 gels may result from the increased diffusional distance due to the higher lipid content,[19] as well as the higher affinity of the drug for the lipidic matrix.

Finally, comparison of the gels containing the particles with the same composition (SLM 1 vs SLN 1; SLM 2 vs SLN 2; SLM 3 vs SLN 3) shows that both the drug release

Table 2 Release rate, lag time and cumulative amount of econazole nitrate (ECN) permeated after 24 h through epidermis from gels

Formulation	Release rate (μg/cm^2 per h$^{1/2}$)	R^2 Higuchi model	Lag time (min)	Cumulative amount of ECN permeated after 24 h	
				(μg/cm^2)	% of applied dose
ECN	25.35 ± 0.12	0.935	60.0 ± 1.10	124.2 ± 0.12	25.34
SLM 1	27.62 ± 1.37	0.996	11.6 ± 3.25	121.43 ± 7.13	25.09
SLM 2	25.02 ± 2.37	0.988	12.7 ± 1.54	108.53 ± 11.64	22.44
SLM 3	23.74 ± 1.90	0.967	138 ± 7.29	77.62 ± 2.99	15.83
SLN 1	20.42 ± 1.23	0.995	71.4 ± 1.18[a]	80.72 ± 8.68[a]	16.63
SLN 2	14.67 ± 1.43	0.994	96.6 ± 2.28[a]	48.46 ± 0.80[a]	12.04
SLN 3	16.57 ± 1.02	0.952	60.0 ± 3.83	69.49 ± 0.52	14.18

SLN and SLM formulations 1, 2 and 3 have lipid : drug ratios of 5 : 1, 10 : 1 and 12.5 : 1 (% w/w), respectively. Data are means ± SD ($n = 5$). [a]Data from Sanna *et al.*[8].

rate and cumulative amount of ECN permeated after 24 h were not significantly different. This result was unexpected, as data from the literature demonstrated that the methods and the production conditions of micro- and nanoparticles (spray congealing process and high-shear homogenisation) can lead to different incorporation of drug inside the particles,[3,41] which influences the release of ECN from the systems. However, the SLMs and SLNs show different adhesive forces between the surface of the stratum corneum and particles as a function of the particle size. It is known that nanoparticles create a monolayered lipid film of smaller interparticle pores on the skin, with a consequent occlusion effect that is higher than with lipid microparticles; the film formation increases skin hydration.[3] This effect depends on the particle size and influences the ability of the formulation to control drug release. Thus, the strict adhesion between nanoparticles could fix the drug into a formed film and thus decrease the diffusion through the stratum corneum, thus leading to localisation of drug on the skin surface.[41] On the other hand, the lower adhesion to the skin and the larger pores between the microparticles can improve the accumulation of drug released from microparticles on the skin surface, allowing more rapid permeation.

Conclusions

The results show that the spray congealing technique using the wide pneumatic nozzle enables the production of ECN-loaded SLMs with a diameter suitable for topical administration. Moreover, the results confirm that high-shear homogenisation is a good method for the preparation of SLNs containing ECN. Both techniques were characterised by good production yields and high encapsulation efficiencies. The ex-vivo permeation studies show that the permeation profiles of ECN through epidermis are influenced both by the particle size (significant difference until 9 h) and the amount of lipid. Thus, the results confirm the usefulness of SLN as carriers for topical administration and suggest the potential of SLM for the controlled delivery of drugs to the skin.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to discolse.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Lippacher A *et al*. Liquid and semisolid SLN™ dispersions for topical application: rheological characterization. *Eur J Pharm Biopharm* 2004; 58: 561–567.
2. Müller RH *et al*. Nanostructured lipid carriers (NLC) in cosmetic dermal products. *Adv Drug Deliv Rev* 2007; 59: 522–530.
3. Souto EB *et al*. Topical delivery of oily actives using solid lipid particles. *Pharmaceut Technol Europe* 2007; 19(12).
4. Jenning V *et al*. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *Eur J Pharm Biopharm* 2000; 49: 211–218.
5. Jenning V *et al*. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. *J Control Release* 2000; 66: 115–126.
6. Santos Maia C *et al*. Drug targeting by solid lipid nanoparticles for dermal use. *J Drug Target* 2002; 10: 489–495.
7. Lombardi Borgia S *et al*. Lipid nanoparticles for skin penetration enhancement – correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. *J Control Release* 2005; 110: 151–163.
8. Sanna V *et al*. Solid lipid nanoparticles (SLN) as carriers for the topical delivery of econazole nitrate: in-vitro characterization, ex-vivo and in-vivo studies. *J Pharm Pharmacol* 2007; 59: 1057–1064.
9. Schafer-Korting M *et al*. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Adv Drug Deliv Rev* 2007; 59: 427–443.
10. Cevc G. Lipid vesicles and other colloids as drug carriers on the skin. *Adv Drug Deliv Rev* 2004; 56: 675–711.
11. Alvarez-Roman R *et al*. Enhancement of topical delivery from biodegradable nanoparticles. *Pharm Res* 2004; 21: 1818–1825.
12. Müller RH, Dingler A. The next generation after the liposomes: solid lipid nanoparticles (SLN, Lipopearls) as dermal carrier in cosmetics. *Eurocosmetics* 1998; 7–8: 19–26.
13. de Vringer T, de Ronde HA. Preparation and structure of a water-in-oil cream containing lipid nanoparticles. *J Pharm Sci* 1995; 84: 466–472.
14. Sivaramakrishnan R *et al*. Glucocorticoid entrapment into lipid carriers – characterisation by parelectric spectroscopy and influence on dermal uptake. *J Control Release* 2004; 97: 493–502.
15. Siekmann B, Westesen K. Melt-homogenized solid lipid nanoparticles stabilized by the nontonic surfactant tyloxapol 1. Preparation and particle size determination. *Pharm Pharmacol Lett* 1994; 3: 194–197.
16. Gasco MR. Solid lipid nanospheres from warm microemulsions. *Pharm Technol Eur* 1997; 9: 52–58.
17. Hu FQ *et al*. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. *Int J Pharm* 2002; 239: 121–128.
18. Trotta M *et al*. Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique. *Int J Pharm* 2003; 257: 153–160.
19. Wissing SA *et al*. Solid lipid nanoparticles for parenteral drug delivery. *Adv Drug Deliv Rev* 2004; 56: 1257–1272.
20. Hou D *et al*. The production and characteristics of solid lipid nanoparticles (SLNs). *Biomaterials* 2003; 24: 1781–1785.
21. Gavini E *et al*. Solid lipid microparticles (SLM) containing juniper oil as anti-acne topical carriers: preliminary studies. *Pharm Dev Technol* 2005; 10: 479–487.
22. Iannuccelli V *et al*. Influence of liposphere preparation on butyl-methoxydibenzoylmethane photostability. *Eur J Pharm Biopharm* 2006; 63: 140–145.
23. El-Kamel AH *et al*. Testosterone solid lipid microparticles for transdermal drug delivery. Formulation and physicochemical characterization. *J Microencapsul* 2007; 24: 457–475.
24. Scalia S *et al*. Influence of solid lipid microparticle carriers on skin penetration of the sunscreen agent, 4-methylbenzylidene camphor. *J Pharm Pharmacol* 2007; 59: 1621–1627.
25. Tursilli R *et al*. Solid lipid microparticles containing the sunscreen agent, octyl-dimethylaminobenzoate: effect of the vehicle. *Eur J Pharm Biopharm* 2007; 66: 483–487.

26. Jaspart S *et al*. Solid lipid microparticles: formulation, preparation, characterization, drug release and applications. *Expert Opin Drug Deliv* 2005; 2: 75–87.
27. Killeen MJ. Spray drying and spray congealing of pharmaceutics. In: Swarbrick J, Boylan J, eds. *Encyclopedia of Pharmaceutical Technology*. New York: Marcel Dekker, 1996: 207–221.
28. Rodriguez L *et al*. Description and preliminary evaluation of a new ultrasonic atomizer for spray-congealing processes. *Int J Pharm* 1999; 183: 133–143.
29. Passerini N *et al*. Characterization of carbamazepine-Gelucire 50/13 microparticles prepared by a spray-congealing process using ultrasounds. *J Pharm Sci* 2002; 91: 699–707.
30. Passerini N *et al*. Controlled release of verapamil hydrochloride from waxy microparticles prepared by spray congealing. *J Control Release* 2003; 88: 263–275.
31. Albertini B *et al*. New spray congealing atomizer for the microencapsulation of highly concentrated solid and liquid substances. *Eur J Pharm Biopharm* 2008; 69: 348–357.
32. Dubey RR, Parikh RH. Studies of PLGA microspheres. *Pharm Tech Eur* 2004; 16: 23–34.
33. Simon GA, Maibach HI. The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations – an overview. *Skin Pharmacol Appl Skin Physiol* 2000; 13: 229–234.
34. Schmook FP *et al*. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. *Int J Pharm* 2001; 215: 51–56.
35. Williams A. Experimental design. In: *Transdermal and Topical Drug Delivery – From Theory to Clinical Practice*. London: Pharmaceutical Press, 2003: 51–82.
36. Davies DJ *et al*. Multi-species assessment of electrical resistance as a skin integrity marker for in vitro percutaneous absorption studies. *Toxicol in Vitro* 2004; 18: 351–358.
37. Gavini E *et al*. Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: in-vitro/ex-vivo studies. *J Pharm Pharmacol* 2005; 57: 287–294.
38. Gavini E *et al*. Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for nasal administration of metoclopramide. *Eur J Pharm Biopharm* 2008; 68: 245–252.
39. Donini C *et al*. Preparation of poly(methacrylic acid-g-poly (ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications. *Int J Pharm* 2002; 245: 83–91.
40. Souto EB *et al*. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *Int J Pharm* 2004; 278: 71–77.
41. Müller RH *et al*. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev* 2002; 54: S131–155.