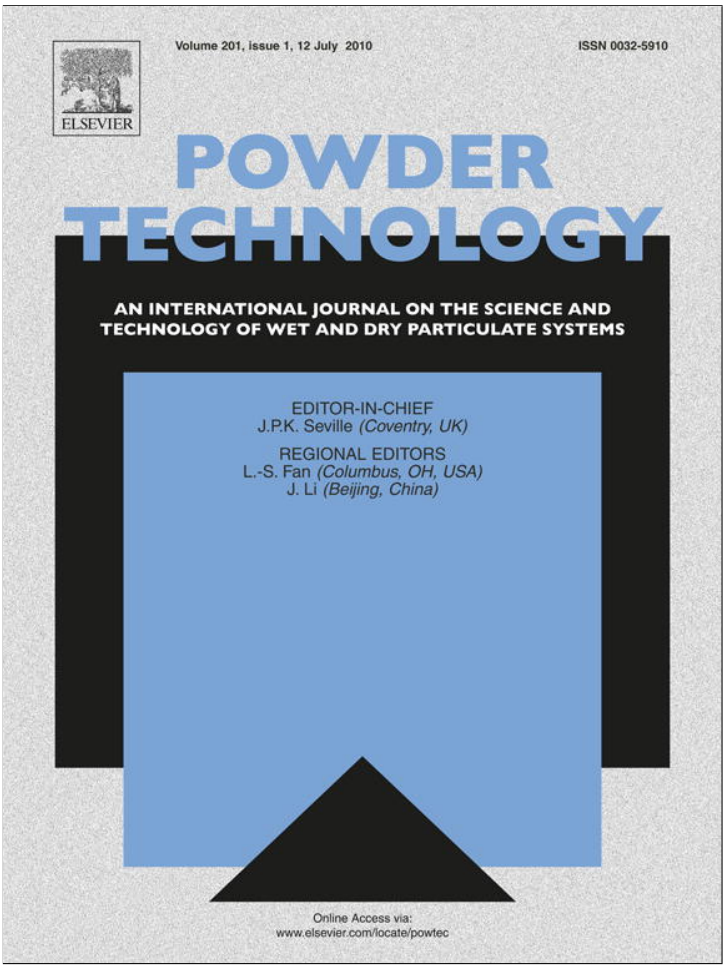

Powder Technology 201 (2010) 32–36

Contents lists available at ScienceDirect

Powder Technology

journal homepage: www.elsevier.com/locate/powtec

Effect of lipid nanoparticles containing fatty alcohols having different chain length on the *ex vivo* skin permeability of Econazole nitrate

Vanna Sanna [a,*], Giuseppe Caria [b], Alberto Mariani [b]

[a] Prion DGN c/o Porto Conte Research Centre Loc. Tramariglio, 07041, Alghero, Sassari, Italy
[b] Department of Chemistry and local INSTM unit, University of Sassari, 07100, Sassari, Italy

ARTICLE INFO

Article history:
Received 2 November 2009
Accepted 24 February 2010
Available online 1 March 2010

Keywords:
Lipid nanoparticles
Fatty alcohols
Skin permeation

ABSTRACT

The aim of this study was to investigate the effect of saturated fatty alcohols having different chain length (C_{12}–C_{18}), formulated into Precirol-based lipid nanoparticles, on the *ex vivo* skin permeability of Econazole nitrate.
Nanoparticles were prepared by o/w high shear homogenization using a mixture of Precirol and fatty alcohol as the lipid phase. A formulation containing only Precirol was used as a comparison.
Lipid nanoparticles were characterized in terms of particle size, encapsulation efficiency and crystalline structure. After incorporation into hydrogels, *ex vivo* drug permeation tests were carried out through porcine stratum corneum.
The particles had a mean diameter below 200 nm and the encapsulation efficiency ranged from 95 to 98%. *Ex vivo* permeation results demonstrated that the drug flux from formulations containing fatty alcohols increased as the alcohol chain length increased.
The analysis of results revealed that the effect of fatty alcohols on the Econazole nitrate permeation is structure-dependent, and associated with an increase of the permeability coefficients that can improve the interaction between alcohols and skin lipids.

1. Introduction

The stratum corneum (SC) is commonly known as the principal physical barrier to most substances that come in contact with the skin [1]. The success of dermatological products depends on the ability of the drug to overcome the barrier properties and to penetrate through skin in sufficient quantities to achieve its desired therapeutic effect [2]. Thus, significant effort has been devoted to developing strategies in order to promote the transport of drugs across the SC [3]. These approaches encompass both particulate carrier systems and penetration enhancers [4,5].

During the past several years, there was an increasing interest in investigating the lipid based systems, such as Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), for topical application of cosmetic and active pharmaceutical products [6–8]. The small size of nanoparticles ensures a close contact between the drug and the corneocytes and, after water evaporation, produces the formation of an adhesive layer occluding the skin surface [9]. The resulting hydration of the SC may be also related to a reduction of corneocyte packing and the widening of the inter-corneocyte gaps can facilitate drug penetration into deeper skin strata [10,11].

In a previous work, we demonstrated that SLN based on Precirol® ATO (PCR) are able to control the release of Econazole nitrate (ECN) and to promote *in vivo* a rapid skin penetration of drug [12]. In addition, long chain fatty esters such as isopropyl myristate and isopropyl palmitate were successfully incorporated into nanoparticles to improve the *ex vivo* skin permeation of ECN [13].

Fatty esters and alcohols are well-established permeation enhancers that are commonly incorporated in semisolid vehicles to increase permeation of drugs across the SC [14,15]. Their penetration-enhancing effects are due to the reduction of skin resistance as a permeation barrier, attributed to an intercalation into the structured lipids of the SC and the disturbance of the lipid packing order [16].

The aim of this study was to prepare SLN containing Precirol® ATO and fatty alcohols (with different length chain, C_{12}–C_{18}) as lipid phase, loaded with ECN, and to evaluate the effects of various alcohols on nanoparticle characteristics and on *ex vivo* drug permeation.

2. Experimental

2.1. Materials

Econazole nitrate (ECN) was kindly provided from Erregierre SpA (Bergamo, Italy) and glycerol palmitostearate (Precirol® ATO 5, PCR) from Gattefossé (Cedex, France); Tween 80 and Myristyl alcohol (purity 98%) were purchased from Sigma-Aldrich (Steinheim, Germany); Lauryl

* Corresponding author. Tel./fax: +39 079998624.
E-mail address: vsanna@uniss.it (V. Sanna).

0032-5910/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.powtec.2010.02.035

alcohol (purity 99%) was supplied by Acros (Geel, Belgium), Cetyl alcohol (purity 90%) by Azienda Chimica Farmaceutica Spa (Piacenza, Italy) and Stearyl alcohol (purity 95%) by RES Pharma (Milano, Italy); hydroxypropylmethylcellulose (HPMC K100M) was obtained from Dow Chemicals (Midland, USA), methanol and $NH_4H_2PO_4$ from Rielden-de Haën AG (Seelze, Germany). All other solvents and chemicals were of analytical grade.

2.2. Preparation of nanoparticles

Suspensions of lipid nanoparticles were formulated in order to contain 5 g of total lipid matrix, 1 g of ECN, 2.5 g of surfactant (Tween 80), and distilled water to 100 g.

ECN-loaded nanoparticles were produced by the high shear homogenization method, as previously reported [12,13], by using the mixture of different fatty alcohols and Precirol as the lipid phase (Table 1). A formulation containing PCR and ECN, without fatty alcohol, was used as a reference.

Fatty alcohol and drug were added to the melted Precirol® and the mixture was dispersed under stirring in an aqueous surfactant solution of identical temperature (80 °C) and homogenized (Silverson L4R mixer, Crami, Italy) at 6200 rpm for 5 min. Then, the nanoemulsions were cooled to room temperature till nanoparticle dispersions were obtained.

The nanoparticles were then centrifuged and washed twice with deionized water to remove residues and the unloaded ECN from the surface of nanoparticles.

The recovered solid was resuspended in water to a final volume of 100 ml and a weighed amount of dispersion was frozen below −80 °C in a deep-freezer (Dairei Co., Ltd. Tokyo, Japan) and kept at this temperature for 24 h; eventually, it was lyophilized at −54.5 °C under vacuum (0.909 mbar) for 8 h using a 5Pascal LIO 5P apparatus (Cinquepascal srl, Milano, Italy). Then the lipid powders were collected for next experiments.

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.

2.3. Gels enriched with lipid nanoparticles

For the preparation of hydrogels, a gelling agent (HPMC, 2% w/w) was added to the nanoparticle dispersion and the resulting mixture was stirred to yield gels containing 1% (w/w) of ECN. The lipid nanoparticles loaded ECN hydrogels were stored at room temperature until use.

2.4. Characterization of nanoparticles

2.4.1. Particle size analysis

Particle size and size distribution measurements were performed using photon correlation spectroscopy (PCS). A Coulter N5 submicron particle sizer (Beckman Coulter, Miami, FL, USA) was used to determine the mean particle diameter and the polydispersity index (PI) as a measure for the width of the particle size distribution. Prior to analysis, each sample of particles and gels was diluted with bidistilled water until the appropriate concentration of particles was reached (between 5×10^4 and 1×10^6 counts s^{-1}) and sonicated for 10 s.

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 ms and sample run time 300 s.

Table 1
Fatty alcohols used for the preparation of nanoparticles.

Nanoparticle batch	Fatty alcohol	
	Type (abbr.)	Chain length (C atoms)
A1	Lauryl alcohol (AL)	12
A2	Myristyl alcohol (AM)	14
A3	Cetyl alcohol (AC)	16
A4	Stearyl alcohol (AS)	18

2.4.2. Drug encapsulation efficiency

Samples (50 mg) of freeze-dried nanoparticles were dissolved in methanol (10 ml) under stirring at 80 °C and then slowly cooled to room temperature to preferentially precipitate the lipid. After centrifugation at 3000 rpm for 5 min, aliquot of supernatant was diluted 100 times with methanol. The drug content in the solution was analyzed by HPLC and the entrapment efficiency was calculated as a percentage with respect to the amount of drug added during nanoparticle preparation as previously described [13].

2.4.3. HPLC analysis

The HPLC analysis was carried out using the modified method previously reported [12,13]. The equipment consisted of a Varian Prostar 210 HPLC system including an autosampler Varian 410 and a diode array detector Varian 330 (Varian Deutschland GmbH, Dramstad, Germany). The chromatographic separation was performed at room temperature using a spherisorb 5 μm RP-C8 column (250 mm × 4.6 mm, Supelco, Milano, Italy) with a flow rate of 1.0 ml/min. Twenty microlitres of samples or calibration standards was injected into the column and eluted with a solution of methanol and 0.05 M $NH_4H_2PO_4$ (85:15, v/v). Detection was carried out by monitoring the absorbance signals at 200 nm. The elution period was 8 min and the retention time of ECN was about 5.8 min.

2.5. Differential Scanning Calorimetry (DSC)

The crystalline structure of the lipid nanoparticles was determined by DSC analysis using a DSC Q100 calorimeter (TA Instrument, New Castle, USA). The instrument was calibrated for temperature and enthalpy using the melt of indium ($\Delta H = 28.4$ J/g). Samples of 4–6 mg were accurately weighed into aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 10 °C/min at different temperature range and performed under an Ar purge (50 ml/min).

The thermal measurements were carried out on physical mixture of ECN-PCR-fatty alcohols, and on drug loaded nanoparticles (A1–A4).

2.6. Ex vivo skin permeation studies

The *ex vivo* permeation studies of ECN from gel formulations were carried out through porcine SC for 8 h by using the modified method and conditions previously reported [13,17,18].

The SC was cut and mounted on the bottom of a support (height 1.91 cm, diameter 2.28 cm), and a weighed amount of gels (about 200 mg) was uniformly arranged on the surface of the skin. The cylindrical support was connected to a drive shaft of the dissolution apparatus (Erweka DT 70, Erweka GmbH, Germany). The system was then inserted into the vessel containing the receptor medium, so that the dermis side touched the surface of the fluid. The following working conditions were used: 200 ml of methanol/water (70/30 v/v) solution as receptor medium, 32 °C and 25 rpm. The composition of the receptor medium was chosen because ECN was well soluble in it, while the lipid nanoparticles did not dissolve in this medium; alcohols are commonly used as co-solvents with water as receptor solution for poorly soluble permeants [18].

The cumulative amounts of ECN permeated were plotted as a function of time. The permeation rate of ECN at steady-state (J) through skin was estimated from the slope of the linear region of the plots.

The permeation rate of ECN at steady-state (J) through skin was estimated from the slope of the linear region of the plots.

The permeability coefficient (K_p) was calculated from the following equation:

$$K_p = J / C$$

where J is the flux (μg/cm^2/h) and C the concentration of ECN in the receptor medium (μg/cm^3).

2.7. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) (Origin®, version 7.0 SR0, OriginLab Corporation, USA). Individual differences were evaluated using a nonparametric post hoc test (Tukey's test) and considered statistically significant when $P<0.05$.

3. Results and discussion

3.1. Preparation and characterization of nanoparticles

The high shear homogenization is a one-step process that simply involves the preparation of an oil in water emulsion. This technique, previously employed for the formulation of solid lipid micro- and nanoparticles [12,13,19], was here successfully applied to prepare nanoparticles based on Precirol and different fatty alcohols having different chain length (C_{12}–C_{18}), loaded with ECN.

As shown in Table 2, the encapsulation efficiency values of formulations were found to be in the range of 95–99% and no direct relationship between the lipid phase composition of nanoparticles and drug loading was evident. This result can be attributed to the high affinity of the lipophilic drug toward the lipid material [19]. In addition, the presence of the fatty alcohol long chain can lead to the creation of a less ordered solid lipid matrix and leaves enough space to accommodate drug molecules [20–23].

Moreover, yields of production obtained were always high and included in the range of 88 to 92%.

The comparison of mean diameter and PI of lipid nanoparticles in original dispersion and after incorporation into hydrogel was reported in Table 3.

The control formulation, containing only Precirol and ECN, was characterized by an average diameter of 144 nm, significantly lower ($P<0.05$) with respect to batches A1 and A2 containing PCR and AL and AM alcohols (with 12 and 14 carbon atoms), respectively. The use of fatty alcohols AC and AS (A3 and A4 formulations), at longer chain, produced a decrease of particle dimensions that resulted to be of 156 and 164 nm, respectively. Besides, all formulations were characterized by PI ranging from 0.3 to 0.4 and the PI values decreased with the increase of chain length of the alcohol employed. In all cases, the nanoparticle dispersions exhibit a unimodal particle size distribution, typical of monodispersed systems [19].

The incorporation of nanoparticles into hydrogels leads to the increase of mean diameter and PI values that can be attributed to the presence of the gelling agent that obstacles a good dispersion of nanoparticles and makes easy their aggregation.

Table 2
Drug content, encapsulation efficiency, and yield of production of lipid nanoparticles. Data expressed as mean ($n=3$) ± Standard Deviation (S.D.).

Batch	Drug content (%)	Encapsulation efficiency (%)	Yield of production (%)
Control	11.40 ± 0.2[a]	96.50 ± 1.4[a]	87.78 ± 0.7[a]
A1	11.55 ± 0.3	98.24 ± 2.7	88.03 ± 1.9
A2	11.62 ± 0.3	98.85 ± 2.9	92.18 ± 2.4
A3	11.54 ± 0.2	98.12 ± 1.6	90.89 ± 0.1
A4	11.16 ± 0.2	94.94 ± 1.7	88.97 ± 1.3

[a] Data reported from reference [12].

Table 3
Mean diameter and polydispersity index of lipid nanoparticles before and after incorporation into hydrogels. Data expressed as mean ($n=5$) ± Standard Deviation (S.D.).

Batch	Nanoparticles		Gel	
	Mean diameter (nm)	Polydispersity index	Mean diameter (nm)	Polydispersity index
Control	143.6 ± 0.4[a]	0.27 ± 0.02[a]	206.7 ± 6.3	0.30 ± 0.02
A1	179.3 ± 16.8	0.39 ± 0.05	208.7 ± 26.9	0.48 ± 0.01
A2	194.4 ± 1.9	0.36 ± 0.04	290.4 ± 22.5	0.53 ± 0.03
A3	155.8 ± 5.6	0.34 ± 0.07	383.63 ± 35.6	0.46 ± 0.08
A4	163.5 ± 0.95	0.30 ± 0.01	213.8 ± 42.2	0.37 ± 0.02

[a] Data reported from reference [12].

3.2. Differential Scanning Calorimetry (DSC)

In order to determine their degree of crystallinity, the lipid nanoparticles were investigated by DSC analysis. Fig. 1 reports the comparison between the thermal measurements performed on ECN (raw material), on PCR and on the prepared lipid nanoparticles.

The DSC scan of ECN exhibited a single endothermic peak at 165 °C, corresponding to its melting point, followed by an exothermal peak due to the beginning of a degradation process. The thermograms of lipid nanoparticles showed the disappearance of the ECN melting endotherm due to dissolution of the drug in the molten lipid during DSC scan.

Besides, the DSC curves were characterized by the shift of the PCR and fatty alcohols melting endotherms to lower temperatures that resulted more evident for drug loaded nanoparticles containing fatty alcohols. In particular, the endothermic peaks of AL and AM alcohols, and PCR decrease of about 7–8 °C (for A1 and A2 batches), while the AC and AS alcohols (A3 and A4 batches) melt simultaneously to PCR by forming a unique and broad peak.

This finding can be attributed to the reduced particle size and the increased specific surface area that led to a decrease in the melting enthalpy compared with the heat flow through the larger crystals of ingredients not formulated in nanoparticles [24].

Additionally, the depression of the melting points of A1–A4 batches can be ascribed to the interactions between lipids and incorporated drug molecules that induced an increase of the lattice defects.

3.3. Ex-vivo permeation studies

The effect of various fatty alcohols on the cumulative amount of ECN diffused across porcine SC as a function of time was investigated.

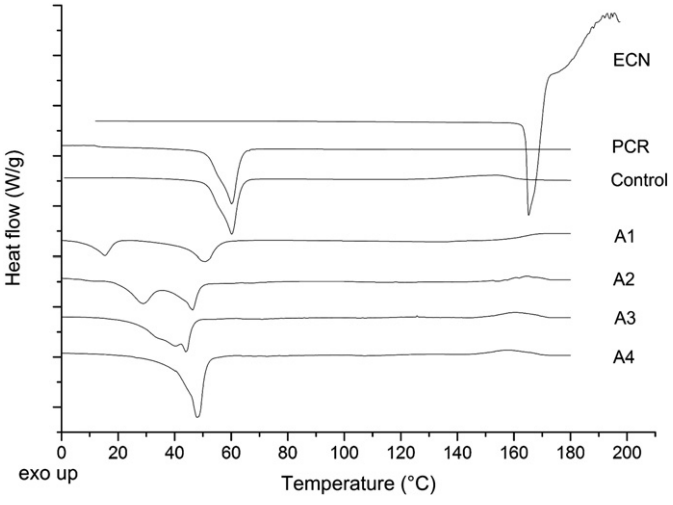

Fig. 1. DSC curves of pure drug (ECN), Precirol (PCR), Control and A1–A4 loaded nanoparticles.

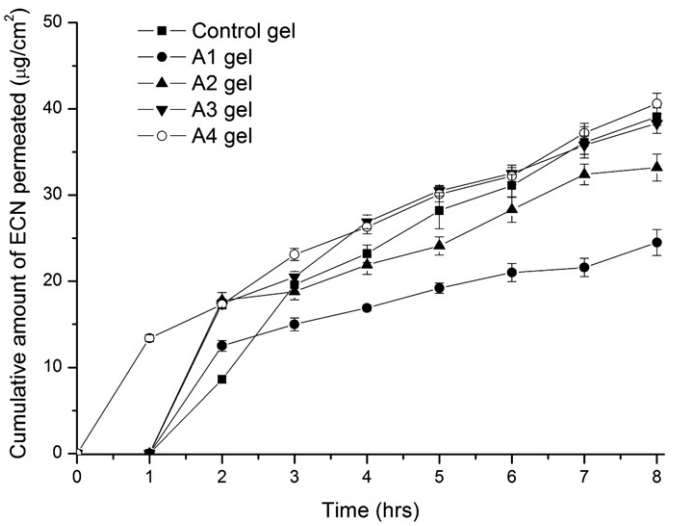

Fig. 2. *Ex vivo* skin permeation profiles of ECN from gels containing lipid nanoparticles (Control and A1–A4). Data expressed as mean ($n=5$) ± S.D.

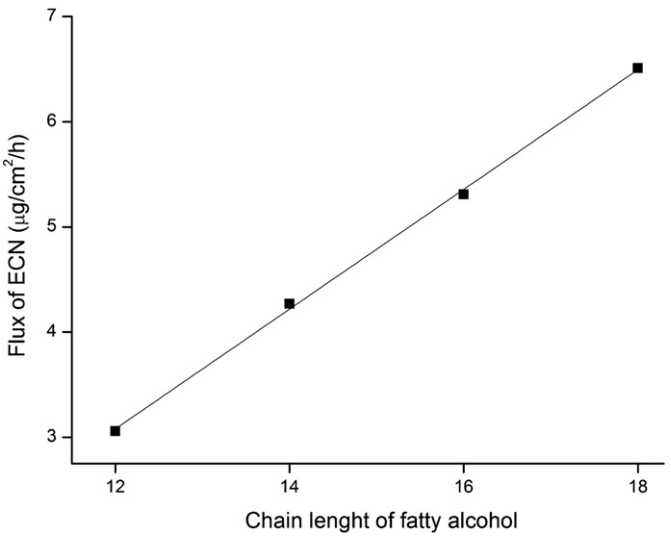

Fig. 3. Plot of flux values against the length chain of fatty alcohols.

The gel formulations were characterized by similar permeation profiles and the release rate of drug was related to the chain length of the fatty alcohol used (Fig. 2). Control and A1–A3 gels released ECN at the second hour of test only, while about 13 μg/cm^2 of ECN was permeated from A4 formulation within the first hour.

Besides, for all formulations the plot of the amount of ECN released as a function of time showed a linear relationship as confirmed by the good correlation coefficients obtained ($R^2=0.974$–0.992), thus indicating that ECN permeation followed pseudo-first order kinetics (data not reported). Moreover, at the end of the test, the amount of ECN permeated from Control gel (39.1 μg/cm^2) resulted almost equivalent to A3 and A4 gels and significantly higher ($P<0.05$) with respect to A1 and A2 gels (24.46 and 34.38 μg/cm^2, respectively).

In Table 4, the J and K_p values calculated for tested gel formulations are listed.

Statistical analysis showed that the drug flux of ECN from Control gel, containing only Precirol as lipid phase, was significantly larger than that of A1 formulation, while not significantly different with respect to A2–A4 gels.

Regarding A1–A4 formulations, the increase of fatty alcohols chain length from C_{12} to C_{18} is associated to an increase of flux values. Formulation A1 containing AL alcohol (with 12 carbon atoms) showed a significantly lower flux ($P<0.05$) compared to A3–A4 gels containing alcohols with longer chain length.

In particular, as depicted in Fig. 3, an excellent linear relationship ($R^2=0.999$) was obtained when the flux values of ECN were plotted against the chain length of fatty alcohol used.

Similarly, the K_p values of Control gel was found to be significantly larger if compared with A1 and A2 formulations. Moreover, statistical differences ($P<0.05$) between gels containing different alcohols (A1–A4) were found. Besides, the analysis of K_p values of gels containing different alcohols showed a similar linear correlation ($R^2=0.986$; data not reported).

Table 4
The comparison of permeation data for ECN from tested gels. Data expressed as mean ($n=5$) ± S.D.

Batch	J (μg/cm²/h)	$K_p \cdot 10^{-5}$ (cm/s)
Control	5.31 ± 0.13[a]	14.75 ± 0.73[a]
A1	3.06 ± 0.44	8.50 ± 0.46
A2	4.27 ± 0.55	11.86 ± 0.24
A3	5.01 ± 0.59	13.92 ± 0.82
A4	6.51 ± 0.41	18.08 ± 0.08

[a] Data reported from reference [12].

The mechanism by which fatty alcohols increase skin permeability appears to involve disruption of the densely packed lipids which fill the extracellular spaces of the SC [25].

Their effect as penetration enhancers, after addition to semisolid vehicles, have been studied on a variety of drugs [26,27], and, in some cases, a parabolic relationship with the alkyl chain length was observed, with C_{10} and C_{12} being the most effective [28]. As an explanation of this behavior, it has been proposed that alcohols with a certain chain length possess an optimal balance between partition coefficient or solubility parameter and affinity to skin.

The linear relationship observed in this study for saturated alcohols from 12 to 18 C atoms can be ascribed to the strong interaction between the fatty alcohols and Precirol as well as to the incorporation into nanoparticles that can modify the affinity of alcohols toward lipids in SC.

Furthermore, it is known that the permeability coefficients of n-alkanols increased linearly as the chain length increases [29,30]. Thus, the increase of the enhancement effect of lower alcohols as a result of the increase in the alkyl chain length may be attributed also to the increased permeation of alcohols through the skin.

4. Conclusions

In conclusion, nanoparticles based on Precirol and fatty alcohols having different length chain can be easily produced using the high shear homogenization method.

The enhancement effect of fatty alcohols on ECN skin permeation is strongly related to their chain length. Among the tested fatty alcohols, the Cetyl and Stearyl ones (having 16 and 18 C atoms, respectively) were found to be the most efficient penetration enhancers, with potential application in skin delivery of the drug.

Acknowledgments

The authors thank the Dipartimento di Scienze del Farmaco, University of Sassari for facilities. VS is grateful to Master & Back Program of Regione Autonoma della Sardegna.

References

[1] F. Yamashita, M. Hashida, Mechanistic and empirical modelling of skin permeation of drugs, Adv. Drug Deliv. Rev. 55 (2003) 1185–1199.
[2] B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery, Eur. J. Pharm. Sci. 14 (2001) 101–114.

[3] H.A.E. Benson, Transdermal drug delivery: penetration enhancement techniques, Curr. Drug Deliv. 2 (2005) 23–33.
[4] M. Schäfer-Korting, W. Mehnert, H.C. Korting, Lipid nanoparticles for improved topical application of drugs for skin diseases, Adv. Drug Deliv. Rev. 59 (2007) 427–443.
[5] A.F. El-Kattan, C.S. Asbill, B.B. Michniak, The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems, Int. J. Pharm. 198 (2000) 179–189.
[6] R.H. Muller, M. Radtke, S.A. Wissing, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv. Drug Deliv. Rev. 54 (2002) S131–S155.
[7] S. Wissing, R.H. Muller, L. Manthei, C. Mayer, Structural characterization of Q10-loaded solid lipid nanoparticles by NMR spectroscopy, Pharm. Res. 21 (2004) 400–405.
[8] C.S. Maia, W. Mehnert, M. Schafer-Korting, Solid lipid nanoparticles as drug carriers for topical glucocorticoids, Int. J. Pharm. 196 (2000) 165–167.
[9] H. Zhai, H.I. Maibach, Effects of skin occlusion on percutaneous absorption: an overview, Skin Pharmacol. Appl. Skin Physiol. 14 (2001) 1–10.
[10] R.H. Muller, M. Radtke, S.A. Wissing, Nanostructured lipid matrices for improved microencapsulation of drugs, Int. J. Pharm. 242 (2002) 121–128.
[11] E.B. Souto, S.A. Wissing, C.M. Barbosa, R.H. Muller, Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery, Int. J. Pharm. 278 (2004) 71–77.
[12] V. Sanna, E. Gavini, M. Cossu, G. Rassu, P. Giunchedi, 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. 59 (2007) 1057–1064.
[13] V. Sanna, A. Mariani, G. Caria, M. Sechi, Synthesis and evaluation of different fatty acid esters formulated into Precirol® ATO-based lipid nanoparticles as vehicles for topical delivery, Chem. Pharm. Bull. 57 (2009) 680–684.
[14] S. Lombardi Borgia, M. Regehly, R. Sivaramakrishnan, W. Mehnert, H.C. Korting, K. Danker, B. Röder, K.D. Kramer, M. Schäfer-Korting, 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 (2005) 151–163.
[15] M. Fujii, N. Hori, K. Shiozawa, K. Wakabayashi, E. Kawahara, M. Matsumoto, Effect of fatty acid Esters on permeation of ketoprofen through hairless rat skin, Int. J. Pharm. 205 (2000) 117–125.
[16] H. Trommer, R.H. Neubert, Overcoming the stratum corneum: the modulation of skin penetration, Skin Pharmacol. Physiol. 19 (2006) 106–121.
[17] E. Gavini, G. Rassu, V. Sanna, M. Cossu, P. Giunchedi, Mucoadhesive microspheres for nasal administration of an antiemetic drug, Metoclopramide: in vitro/ex vivo studies, J. Pharm. Pharmacol. 57 (2005) 287–294.
[18] N. Passerini, E. Gavini, B. Albertini, G. Rassu, M. Di Sabatino, V. Sanna, P. Giunchedi, L. Rodriguez, Evaluation of solid lipid microparticles produced by spray congealing for Econazole Nitrate topical application, J. Pharm. Pharmacol. 61 (2009) 559–567.
[19] V. Sanna, N. Kirschvink, P. Gustin, E. Gavini, I. Roland, L. Delattre, B. Evrard, Preparation and *in vivo* toxicity study of solid lipid microparticles as carrier for pulmonary administration, AAPS PharmSciTech 5 (2004) art. 27.
[20] H.K. Vaddi, P.C. Ho, Y.W. Chan, S.W. Cha, Terpenes in ethanol: haloperidol permeation and partition through human skin and stratum corneum change, J. Control. Release 8 (2002) 121–133.
[21] C. Donini, D.N. Robinson, P. Colombo, F. Giordano, N.A. Peppas, Preparation of poly (methacrylic acid-g-poly(ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications, Int. J. Pharm. 245 (2002) 83–91.
[22] E.B. Souto, S.A. Wissing, C.M. Barbosa, R.H. Muller, Evaluation of the physical stability of SLN and NLC before and after incorporation into hydrogel formulations, Eur. J. Pharm. Biopharm. 58 (2004) 83–90.
[23] V. Jenning, A.F. Thunemann, S.H. Gohla, Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids, Int. J. Pharm. 199 (2000) 167–177.
[24] F.Q. Hu, S.P. Jiang, Y.Z. Du, H. Yuan, Y.Q. Ye, S. Zeng, Preparation and characteristics of monostearin nanostructured lipid carriers, Int. J. Pharm. 314 (2006) 83–89.
[25] H. Bunjes, M.H.J. Koch, K. Westesen, Effect of particle size on colloidal solid triglycerides, Langmuir 16 (2000) 5234–5241.
[26] S. Andega, N. Kanikkannan, M. Singh, Comparison of the effect of fatty alcohols on the permeation of melatonin between porcine and human skin, J. Control. Release 77 (2001) 17–25.
[27] C. Lee, T. Uchida, E. Noguchi, N. Kim, S. Goto, Skin permeation enhancement of Tegafur by ethanol-panasate 800 or ethanol–water bynary vehicle and combined effect of fatty acids and fatty alcohol, J. Pharm. Sci. 82 (1993) 1155–1159.
[28] K.B. Sloan, H.D. Beall, H.E. Taylor, Transdermal delivery of theophilline from alcohol vehicles, Int. J. Pharm. 171 (1998) 185–193.
[29] B. Aungst, N. Rogers, E. Shefter, Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactant, sulfoxides, and amides, Int. J. Pharm. 33 (1986) 225–234.
[30] N. Kanikkannan, K. Kandimalla, S.S. Lamba, M. Singh, Structure–activity relationship of chemical penetration enhancers in transdermal drug delivery, Curr. Med. Chem. 6 (1999) 593–608.