Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
International Journal of Pharmaceutics 341 (2007) 215–220
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers

Marta P. Alves [a,b], Ana L. Scarrone [b], Marcos Santos [b], Adriana R. Pohlmann [a,c], Sílvia S. Guterres [a,*]

[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, CEP 90610-000, Porto Alegre, RS, Brazil*
[b] *Centro Universitário Franciscano, UNIFRA, Rua dos Andradas 1614, CEP 97010032, Santa Maria, RS, Brazil*
[c] *Departamento de Química Orgânica, Instituto de Química, Universidade Federal de Rio Grande do Sul, CP 15003, CEP 91501-970, Porto Alegre, RS, Brazil*

Received 29 October 2006; received in revised form 3 March 2007; accepted 25 March 2007
Available online 30 March 2007

Abstract

The objective of this work was to study the in vitro skin penetration of a drug model (nimesulide) from semi-solid topical formulations containing nanospheres, nanocapsules or nanoemulsion. Nanoprecipitation, interfacial deposition and spontaneous emulsification methods were used to prepare the nanostructured suspension. The hydrodynamic diameters were 252 nm for the nanoemulsion, 277 nm for the nanocapsules and 202 nm for the nanospheres containing nimesulide. The different nanocarrier systems were incorporated in the hydrophilic gels and their ability of delivering the drug into the human skin were investigated using stripping technique and Franz-type diffusion cells. The amount of nimesulide released into the stratum corneum (SC) from the gel containing nanocapsules (GNM-NC) and the gel containing nanospheres (GNM-NS) was similar. On the other hand, for the gel containing nanoemulsion (GNM-NE), the nimesulide was not quantified in SC, but it has been directly permeated for the dermis. The penetration of the nimesulide using the gel containing nanocapsules (GNM-NC) was larger in the deeper skin than using the gel containing nanospheres (GNM-NS) or the one containing nanoemulsion (GNM-NE). The gels containing nanocarriers (GNM-NC, GNM-NS and GNM-NE) were able to release the drug in the viable layer of the skin, comparing to a non-particulated nimesulide-loaded formulation at the same concentration.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Nimesulide; Nanoemulsion; Nanocapsules; Nanospheres; Nanoparticles; Skin penetration

1. Introduction

Nanoparticles and microparticles have been increasingly investigated to achieve targeted and sustained release of drugs (Couvreur et al., 2002; Schaffazick et al., 2003; Jalón et al., 2001a). These systems have been extensively studied for oral and parenteral administration, and they are also useful to deliver drugs into the skin. Besides, some investigations have shown that the nanoparticles and liposomes present the tendency to interact with inflamed tissues. Additionally, nanometric systems present an enormous surface area, which makes them suitable for important pharmaceutic and cosmetic applications, such as topical formulations of lipophilic encapsulated drugs for a homogeneous release (Bouchemal et al., 2004).

These carriers present some advantages for topical application since sustained release is important to supply the skin with the drug over a prolonged period of time (Jalón et al., 2001b). The mechanism of action of nanoparticles can be attributed to their association with the skin surface. The small particle size ensures close contact with the stratum corneum. That is why the amount of encapsulated agent penetrating into the viable skin facilitates drug transport by changing the vehicle/stratum corneum partition coefficient (Jenning et al., 2000a; Alvarez-Román et al., 2004). Studies have been conducted in order to explain the mechanism in which the nanocarriers are able to increase the release of some drugs in the skin (Lopez et al., 2000). For instance, vitamin A was released

* Corresponding author at: Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, CEP 90610-000, Porto Alegre, RS, Brazil.
Tel.: +55 51 33165500; fax: +55 51 33165437.
E-mail address: nanoc@farmacia.ufrgs.br (S.S. Guterres).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.03.031

from solid–lipid–nanoparticles into the upper skin layers, but did not penetrate into the deeper skin strata (Jenning et al., 2000b). Quantification of octyl methoxycinnamate (OMC) in the skin using tape-stripping technique has showed that its nanoencapsulation increased 3.4-folds of the level of OMC into the stratum corneum. The confocal images showed that the fluorescence profile observed in the skin after application of Nile Red-loaded nanoparticles was different from that visualized after application of Nile Red dissolved in propylene glycol. The modified distribution of Nile Red-loaded nanoparticles was due, at least in part, to its altered thermodynamic activity (relative to that in propylene glycol) that resulted in a partition coefficient in the SC increased (Alvarez-Román et al., 2004).

Taking into account those considerations, the objective of this work was to evaluate the ability of nanocapsules, nanospheres and nanoemulsion in modulating the skin penetration of a drug model, the nimesulide. Nimesulide (4-nitro-2-phenoxymethanesulfonamide) is an anti-inflammatory non-steroidal drug, which selectively inhibits cyclooxygenase-2. It is widely used for the treatment of rheumatoid arthritis and inflammatory conditions (Rabasseda, 1997; Bernareggi, 1998). This analgesic presents a very low solubility in water (0.01 mg/mL), an octanol–water partition ($\log P$) of 2.60 and a $\mathrm{p}K_\mathrm{a}$ value of 6.46 (Gupta et al., 1996; Piel et al., 1997; Fallavena and Schapoval, 1997). Semi-solid topical formulations containing nimesulide-loaded nanocapsules, nimesulide-loaded nanospheres or nimesulide-loaded nanoemulsion were evaluated using Franz diffusion cells and a tape-stripping technique in order to investigate if these formulations would be able to modify the distribution of nimesulide in the different strata of the full-thickness human skin. The influence of the nanocarriers was compared to a similar gel formulation containing the free drug in the absence of nanocarriers. Additionally, the flux and permeability coefficient of the gel containing nimesulide-loaded nanocapsules, using heat-separated human skin membrane, were also evaluated. As far as we know, this is the first report consecrated to compare the ability of different nanocarriers in modulating the penetration of a lipophilic drug in the human skin.

2. Materials and methods

2.1. Materials

Nimesulide was obtained from Henrifarma and poly(ε-caprolactone) (M_W = 80,000) from Aldrich (Strasbourg, France). Caprylic/capric triglyceride, sorbitan monostearate, polysorbate 80, methylparaben, propylparaben, sorbitol and triethanolamine were supplied by Delaware (Porto Alegre, Brazil). Carbopol 940® was acquired from B.F. Goodrich (São Paulo, Brazil). All other chemicals and solvents used were of pharmaceutical grade. All reagents were used as received.

2.2. Preparation and characterization of nanocapsules, nanospheres and nanoemulsion (Alves et al., 2005)

Formulations containing nimesulide were prepared by nanoprecipitation and interfacial deposition (nanocapsules and nanospheres, respectively) (Fessi et al., 1988; Schaffazick et al., 2005; Cruz et al., 2006) and spontaneous emulsification (nanoemulsion). Briefly, an acetone solution containing triglycerides, nimesulide, sorbitan monostearate and poly(ε-caprolactone) was added into an aqueous solution containing polysorbate 80. Acetone was removed and the suspension concentrated by evaporation (bath at 40 °C) under reduced pressure (4 bar) and the final formulation was adjusted to 100 mL. Nanosphere suspensions were prepared omitting the oil and nanoemulsion omitting the poly(ε-caprolactone). Formulations were made in triplicate. The particle sizes (z-average) and polydispersity were determined at room temperature (20 °C) using a Zetasizer® (Nano series, Malvern Instruments, UK). Samples were diluted 500-folds in MilliQ® water.

2.3. Preparation of gels containing nimesulide-loaded nanocapsules, nanospheres, nanoemulsions, nimesulide or control gel

Carbopol 940® gels containing nimesulide-loaded nanocapsules (GNM-NC), nanospheres (GNM-NS), and nanoemulsion (GNM-NE) were developed and characterized as previously described (Alves et al., 2005). The gels were constituted of the Carbopol 940® (0.2 g), methylparaben (0.2 g), propylparaben (0.1 g), sorbitol (5.0 mL) and triethanolamine (0.2 mL). A similar gel (GC-NM) formulation containing nimesulide (0.13 g), sorbitan monostearate (0.76 g) and polysorbate 80 (0.76 g), but using water in the place of the nanocarrier formulation was also prepared for comparison. In parallel, a gel (GC) with neither nimesulide nor nanocarriers was also prepared as control. All formulations were prepared in triplicate.

2.3.1. pH determination

The pH values of the suspensions, nanoemulsions and of the gels containing nanocarriers were determined directly in the samples (Micronal B374 potentiometer).

2.3.2. Quantification of nimesulide in the gels

The content of nimesulide in the gels was determined by HPLC after extraction. Approximately 1.0 g of each formulation was accurately weighed and placed in a 50 mL volumetric flask. Acetonitrile was added and the flask was heated at 65 °C until the gel has been completely dissolved. The solution was cooled to room temperature and properly diluted with acetonitrile. After filtration through a 0.45 μm hydrophilic membrane (Durepore®), the solutions were injected (10 μL). Accuracy was 93.4% (Alves et al., 2005).

2.4. High-performance liquid chromatography (HPLC) analysis

The samples were analyzed by HPLC. The system consisted of an SPD-10A Shimadzu detector, LC-10AD Shimadzu pump, SIL-10A Shimadzu injector and Lichrospher® 100 RP-18 (5 μm) column provided by Merck. The mobile phase consisted of acetonitrile/water (55:45, v/v). The flow-rate was 1.0 mL min^{-1}; the temperature was 20 °C and the detection

at 230 nm. The HPLC method for nimesulide quantification (Nagoji et al., 2002), either in the skin or in the receptor compartment has been previously validated. The detector response was found to be linear at the concentration range from 0.8 μg/mL to 40.0 μg/mL ($r > 0.999$). The nimesulide was detected with a retention time of 5.2 min. Accuracy and precision values have been calculated and they were below 2% (R.S.D.). The limit of quantification of the method was 0.24 μg/mL. The selectivity of the assay was confirmed by the individual analysis of blank samples from the skin layers and from the receptor compartment of the diffusion cells (United States Pharmacopoeia, 2006).

2.5. Human skin penetration experiments

2.5.1. Preparation and storage of skin samples

Skin samples were obtained from a female patient, who had undergone abdominal plastic surgery. Immediately after excision the subcutaneous fatty tissue of the abdominal region was removed using a scalpel. The skin was wrapped in aluminum foil and stored in polyethylene bags at −20 °C until use.

2.5.2. Dermis–epidermis separation

The flux and permeation coefficient of the nimesulide from the gel containing drug-loaded nanocapsules were determined using heat-separated human skin membrane. Individual portions of skin were immersed in water at 60 °C for 55 s. The epidermis (stratum corneum and viable epidermis) was carefully removed from the dermis using forceps (Heard et al., 2003).The flux (J_{ss}) was determined as the angular coefficient of a curve obtained by plotting the cumulative amount of the permeated drug versus time. The permeability coefficient (k_p) was calculated using the following equation:

$$k_p = \frac{J_{ss}}{c} \quad (1)$$

where c is the initial concentration of drug in the vehicle applied to the donor phase.

2.5.3. Diffusion cells preparation

Prior the use, the skin specimens were defrosted and the full-thickness human skin or epidermis were then placed on Franz-type diffusion cells, with nominal diffusion area of 3.14 cm^2 and a receptor volume contained 6 mL of phosphate buffer solution (pH 7.4) containing 10% polyethylene glycol 400 (Babu et al., 2003). An infinite dose of nimesulide (1 g of gel) of each formulation: nimesulide-loaded nanocapsules (GNM-NC), nimesulide-loaded nanospheres (GNM-NS), nimesulide-loaded nanoemulsion (GNM-NE), nimesulide gel (GC-NM) or control gel (GC), were placed with syringe on the skin surface in the donor chamber. At given time intervals (2 h, 4 h, 6 h, 8 h, 12 h, and 24 h) aliquots of 500 μL were collected and the same volume of fresh phosphate buffer solution (pH 7.4) was replaced. The receptor medium was maintained at 37 ± 1 °C and magnetically stirred. The blank vehicles without either nimesulide or nanocarriers (control gel, GC) served as reference in the analytical measurements. Five replicates were used for each formulation.

Additionally, the gel containing nanocapsules (GNM-NC) was tested in heat-separated epidermal membranes, which were placed upon filter paper supports, mounted in Franz-type diffusion cells. The receptor phase consisted of phosphate-buffered (pH 7.4) containing 10% polyethylene glycol 400 (Babu et al., 2003).

2.5.4. Horizontal skin sectioning and nimesulide extraction

After 24 h of the permeation experiments, the skin was removed and rinsed with distilled water. This procedure was repeated three times and the drug content in the donor compartment, stratum corneum, epidermis/dermis or receptive solution was analyzed according to the procedure described in Section 2.5.

2.5.4.1. Determination of nimesulide concentration in the stratum corneum and epidermis/dermis. The determination of the drug in the stratum corneum and in epidermis/dermis has been carried out as previously described (Touitou et al., 1998; Lboutounne et al., 2002; Abdulmajed and Heard, 2004; Verma and Fahr, 2004). Consecutive layers of stratum corneum (SC) were removed, adhering to an adhesive (Scotch 3M-19 × 40 mm) tape in the exposed area to the product. The stratum corneum was removed using 15 pieces of adhesive tape for each formulation. The tapes were weighted before and after the extraction of the layers of the skin and the amount of active substance kept in the tapes was extracted with acetonitrile at 60 °C for 60 min and vortexed four times for 30 s. The remaining tissue was cut into small pieces and placed in another vial. The drug was extracted with 2 mL of acetonitrile, ultrasonicated for 60 min and vortexed four times for 30 s. Samples were filtered through membranes (0.45 μm Durepore®) and were analyzed according procedure described in Section 2.5.

2.5.4.2. Determination of the thickness of the SC. For determination of the thickness of the SC, the adhesive tapes were accurately weighed before and after stripping. The layer thickness was calculated according to the following equation (Michel et al., 1992; Verma et al., 2003):

$$T = \frac{d}{ap} \quad (2)$$

where T represents the thickness of SC removed (mm); d the differences in strip weight after and before stripping (μg); a the area of the strip (mm^2); p is the density of the SC ($p = 1 \times 10^3/1 \times 10^9\ \mu m^3$) (Michel et al., 1992).

2.6. Statistical analysis

All the results are expressed as the mean value ± the standard deviation of the mean and statistically analyzed using variance analysis (ANOVA). Results presenting $p < 0.05$ were considered statistically different. The comparisons among the averages were performed using Tukey's test.

3. Results and discussion

Formulations showed pH values from 5.1 to 5.3 and diameters were 277, 202 and 253 nm for NC, NS and NE, respectively. The polydispersity values were below 0.25 indicating narrow size distributions of the particles and, consequently, good homogeneity of these systems (Guterres et al., 1995; Calvo et al., 1996; Müller et al., 2001; Milão et al., 2003; Alves et al., 2005).

Hydrogel formulations showed final nimesulide concentrations of 1300 μg/cm^3 (nanocapsules: GNM-NC), 1230 μg/cm^3 (nanospheres: GNM-NS) and 1270 μg/cm^3 (nanoemulsion: GNM-NE). The similar gel formulation (GC-NM) prepared using water in the place of nanocarrier suspension for comparison presented 1300 μg/cm^3 of free nimesulide. Furthermore, all gel formulations presented pH values close to 7.0, which is adequate for the cutaneous administration.

The ability of the different drug-loaded nanocarriers incorporated in the hydrophilic gels (GNM-NC, GNM-NS, and GNM-NE) in delivering the drug into the human skin was investigated, using the skin stripping technique and Franz-type diffusion cells (Franz, 1975). These studies were accomplished with the objective of evaluating the influence of the composition and the type of structure of the different nanocarriers on the nimesulide penetration in human skin.

The drug was detected in the stratum corneum for the gel containing nimesulide-loaded nanocapsules (GNM-NC) (1.29 ± 0.52 μg/cm^2) (Fig. 1) and the gel containing nimesulide-loaded nanospheres (GNM-NS) (1.18 ± 0.28 μg/cm^2) (Fig. 1). On the other hand, for the gel containing nimesulide-loaded nanoemulsion (GNM-NE), no drug was detected in this layer. The formulations prepared with poly(ε-caprolactone) (GNM-NC and GNM-NS) were the only that presented retention in the stratum corneum. This behavior suggests a higher affinity of the polymeric formulations (GNM-NC and GNM-NS) with the horny layer. The stratum corneum is considered to be the diffusional barrier of mammalian skin for water and the most solutes (Verma and Fahr, 2004). This external layer of the skin provides the principal barrier to the percutaneous permeation of topically applied substances (Maghraby et al., 2000).

The presence of nimesulide in the deeper skin was significantly higher ($p < 0.05$) for the gel containing drug-loaded nanocapsules (GNM-NC) (5.09 ± 1.22 μg/cm^2) than for the gels containing drug-loaded nanospheres (GNM-NS) (2.93 ± 0.41 μg/cm^2) or drug-loaded nanoemulsion (GNM-NE) (3.46 ± 0.84 μg/cm^2) (Fig. 1). For the gels GNM-NE and GNM-NS the deeper skin presented statistically similar amounts of nimesulide ($p > 0.05$). The gel containing nanocapsules (GNM-NC) presented a larger penetration of nimesulide in stratum corneum and in the deeper skin (Table 1). The differences of this formulation in relation to the nanoemulsion and to the nanospheres are the presence of the polymer in the former and the oil in the latter.

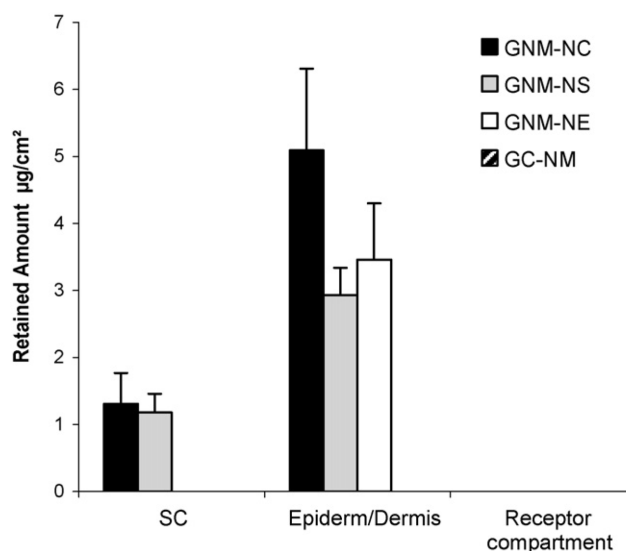

Fig. 1. Distribution of nimesulide in the SC (thickness assumed to be equal to 14.8 μm) and deeper skin (epidermis/dermis) following topical administration of gel containing nimesulide-loaded nanocapsules (GNM-NC), nanospheres (GNM-NS), nanoemulsion (GNM-NE) and gel containing nimesulide (GC-NM).

The drug was not detected in the receptor compartment solution for none of the tested formulations (Fig. 1). This result is in agreement with previous studies, which reported that particulated drug carriers (microparticles and nanoparticles) improve the drug residence in the skin without increasing transdermal transport (Asbill and Michniak, 2000; Lboutounne et al., 2002; Alvarez-Román et al., 2004). Additionally, the high specific area of the carrier facilitates the contact of the encapsulated molecules with the stratum corneum (Jenning et al., 2000b; Maia et al., 2000; Alvarez-Román et al., 2004). The function of these particles is to deliver an ingredient to the upper layer of the skin, and in optimal applications to prolong the time in which this

Table 1
Amount of nimesulide delivered from different formulations into the strata of human abdominal skin using a Franz-type diffusion cell in vitro after 24 h (mean ± S.D., $n = 5$)

Formulations	Total skin[a] (μg/cm^2)	Drug dose applied onto SC (μg/cm^2)	Surface skin after 24 h (μg/cm^2)
GNM-NC	6.40 ± 1.54 a	414.0	347.1 ± 29.53 b
GNM-NS	4.10 ± 0.65 b	391.7	356.1 ± 11.35 b
GNM-NE	3.46 ± 0.67 b	404.4	373.2 ± 30.83 b
GC-NM	–	414.0	424.2 ± 20.32 a

GNM-NC, gel containing nimesulide-loaded nanocapsule; GNM-NS, gel containing nimesulide-loaded nanosphere; GNM-NE, gel containing nimesulide-loaded nanoemulsion; GC-NM, gel containing nimesulide. The averages signed with different letters (a and b) (inside of the same column) were considered statistically different in agreement with Tukey's test ($p < 0.05$).

[a] Total skin: stratum corneum + deeper skin.

Table 2
Assessment by tape stripping of stratum corneum (SC) for nimesulide penetration into human skin (mean ± S.D., $n = 5$)

Formulations	Drug absorbed by SC[a] (μg/cm^2)	Drug concentration in SC[b] (μg/cm^3)	Applied drug dose absorbed[c] (%)	$K_{SC/veh}$[d]
GNM-NC	1.29 ± 0.46	871.6 ± 309.7	0.31 ± 0.11	0.67 ± 0.24
GNM-NS	1.18 ± 0.28	797.3 ± 187.0	0.30 ± 0.07	0.65 ± 0.15
GNM-NE	Not detected	–	–	–
GC-NM	Not detected	–	–	–

GNM-NS, gel containing nimesulide-loaded nanosphere; GNM-NE, gel containing nimesulide-loaded nanoemulsion; GNM-NC, gel containing nimesulide-loaded nanocapsule; GC-NM, gel containing nimesulide.

[a] Amount of drug (nimesulide) absorbed into the SC after 24 h exposure treatment.

[b] Concentration of drug in the SC after 24 h exposure. Calculated as the ratio of the amount of nimesulide (μg/cm^2) in the SC to the SC thickness assumed to be equal to 14.8 μm.

[c] Applied drug dose absorbed calculated as the ratio of the amount of nimesulide (μg/cm^2) in the SC to the nimesulide dose applied.

[d] Partition coefficient ($K_{SC/veh}$) of the nimesulide between the SC and the formulation: calculated as the ratio of the nimesulide concentration in the SC (footnote b) to nimesulide concentration in the vehicle (NC-1300 μg/cm^3; NS-1230 μg/cm^3).

ingredient remains on the skin (Magdassi, 1997; Jenning et al., 2000b; Barratt, 2000; Alvarez-Román et al., 2001; Lboutounne et al., 2002).

Table 1 shows the concentration of the drug applied on the surface of the skin (donor compartment) for each formulation. These formulations were maintained in contact with the skin for 24 h, and nimesulide was quantified at the end of the experiments. The concentration of the nimesulide in the skin surface after 24 h for the gels containing nanocapsules (GNM-NC), nanospheres (GNM-NS), nanoemulsion (GNM-NE) and gel without nanocarriers (GC-NM) were 347.1 μg/cm^2, 356.1 μg/cm^2 and 373.2 μg/cm^2, and 424.2 μg/cm^2, respectively (Table 1).

The results showed that 100% of the drug was retained in the gel without nanocarriers (GC-NM), indicating that the nimesulide was not diffused from the vehicle to the skin surface in the formulation without nanocarriers. The modified distribution of nimesulide when delivered via nanoparticles is probably due to alterations in the thermodynamic activity, increasing the drug diffusion through the vehicle. The ability of a drug in a topical formulation to permeate the skin and to exert its effect depends on two consecutive events. The drug must first diffuse out from the vehicle to the skin surface and then it must permeate this barrier. When a drug molecule diffuses from an aqueous donor phase through a lipophilic membrane to a receptor phase it experiences two types of resistance: the resistance of diffusion in the aqueous phase and the resistance of diffusion through the membrane. Both steps are dependent upon the physicochemical properties of the drug, vehicle and barrier (Nishihata et al., 1988; Kriwet and Müller-Goymann, 1995).

The apparent partition coefficient ($K_{SC/veh}$) of nimesulide was determined alter successive tape stripping of the stratum corneum (Table 2). The nimesulide concentration after 24 h of treatment was calculated for a thickness of approximately 14.8 μm of the horny layer. The apparent partition coefficients ($K_{SC/veh}$) of nimesulide between stratum corneum and the gel did not show significant difference ($p > 0.05$) between the GNM-NC (1300 μg/cm^3) and the GNM-NS (1230 μg/cm^3).

Since the gel containing nimesulide-loaded poly(ε-caprolactone) nanocapsules (GNM-NC) showed the higher drug penetration in the skin compared to the nanospheres and the nanoemulsion, it was used for determining the flux and the permeability coefficient of nimesulide.

The gel containing nimesulide-loaded nanocapsules (GNM-NC) demonstrated favorable skin penetration characteristics. The flux (J_{ss}) and permeability coefficients (k_p) were 0.39 μg/cm^2/h and 9.42×10^{-4} cm/h, respectively. The results showed that nimesulide permeated the heat-separated human skin more easily than the whole skin. The analyses of in vitro skin absorption of lipophilic compounds, based on receptor fluid levels only, should be treated with extreme caution. All information on the fate of the drug in the skin is required to demonstrate whether it remained in the stratum corneum and/or in deeper layers of the skin (Sandt et al., 2004; Wilkinson et al., 2006).

4. Conclusions

The gels containing nimesulide-loaded nanocarriers were able to promote the drug penetration in the stratum corneum and/or in the layer of viable skin compared to a nimesulide-loaded non-particulated formulation. The gel containing nimesulide-loaded nanocapsules (GNM-NC) and the gel containing nimesulide-loaded nanospheres (GNM-NS) presented significant similar drug penetration into the stratum corneum. On the other hand, the gel containing nimesulide-loaded nanoemulsion (GNM-NE) showed no drug in the stratum corneum, but the drug has permeated directly for the dermis. The gel containing nanocapsules (GNM-NC) showed the higher penetration of nimesulide in the skin as compared to the nimesulide-loaded nanospheres and nimesulide-loaded nanoemulsion. Briefly, for the first time, this comparative study demonstrated the influence of the presence of the polymer and of the type of nanocarrier (matricial, vesicular or emulsion) on the penetration of a drug through the human skin.

Acknowledgements

The authors thank FAPERGS, CNPq/Brasília and Rede de Nanobiotecnologia CNPq/MCT e Rede de Nanocosméticos CNPq/MCT.

References

Abdulmajed, K., Heard, C.M., 2004. Topical delivery of retinyl ascorbate co-drug. 1. Synthesis, penetration into and permeation across human skin. Int. J. Pharm. 280, 113–124.

Alvarez-Román, R., Barré, G., Guy, R.H., Fessi, H., 2001. Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection. Eur. J. Pharm. Biopharm. 52, 191–195.

Alvarez-Román, R., Naik, A., Kalia, Y.N., Guy, R.H., Fessi, H., 2004. Enhancement of topical delivery from biodegradable nanoparticles. Pharm. Res. 21, 1818–1824.

Alves, P.M., Pohlmann, A.R., Guterres, S.S., 2005. Semisolid topical formulations containing nimesulide-loaded nanocapsules, nanospheres or nanoemulsion: development and rheological characterization. Pharmazie 60, 900–904.

Asbill, C.S., Michniak, B.B., 2000. Percutaneous penetration enhancers: local versus transdermal activity. Pharm. Sci. Technol. To. 3, 36–41.

Babu, R.J., Kanikkannan, N., Kikwai, L., Ortega, C., Andega, S., Ball, K., Yim, S., Singh, M., 2003. The influence of various methods of cold storage of skin on the permeation of melatonin and nimesulide. J. Control. Rel. 86, 49–57.

Barratt, G.M., 2000. Therapeutic applications of colloidal drug carriers. PSTT 3, 163–171.

Bernareggi, A., 1998. Clinical pharmacokinetics of nimesulide. Clin. Pharmacokinet. 35, 247–274.

Bouchemal, K., Briançon, S., Perrier, E., Fessi, H., Bonnet, I., Zydowicz, N., 2004. Synthesis and characterization of polyurethane and poly(ether urethane) nanocapsules using a new technique of interfacial polycondensation combined to spontaneous emulsification. Int. J. Pharm., 89–100.

Calvo, P., Alonso, M.J., Vila-Jato, J.L., Robinson, J.R., 1996. Improved ocular bioavailability of indomethacin by novel ocular drug carriers. J. Pharm. Pharmacol. 48, 1147–1152.

Couvreur, P., Barratt, G., Fattal, E., Legrand, P., Vaulthier, C., 2002. Nanocapsule technology. A review. Crit. Rev. Ther. Drug Carrier Syst. 19, 99–134.

Cruz, L., Soares, L.U., Costa, T.D., Mezzalira, G., da Silveira, N.P., Guterres, S.S., Pohlmann, A.R., 2006. Diffusion and mathematical modeling of release profiles from nanocarriers. Int. J. Pharm. 313, 198–205.

Fallavena, P.R.B., Schapoval, E.E.S., 1997. pK_a determination of nimesulide in methanol-water mixtures by potentiometric titrations. Int. J. Pharm. 158, 109–112.

Fessi, H., Puisieux, F., Devissaguet, J., 1988. Procédé de préparation des systémes collïdaux dispersibles d'une substance sous forme de nanocapsules. European Patent, 0274961 A1.

Franz, T.J., 1975. Percutaneous absorption on the relevance of in vitro data. J. Invest. Dermatol. 64, 190–195.

Gupta, S.K., Prakash, J., Awor, L., Joshi, S., Velpandian, T., Sengupta, S., 1996. Anti-inflammatory activity of topical nimesulide gel in various experimental models. Inflamm. Res. 45, 590–592.

Guterres, S.S., Fessi, H., Barratt, G., Puisieux, F., Devissaguet, J., 1995. Poly(DL-lactide) nanocapsules containing diclofenac. I. Formulation and stability study. Int. J. Pharm. 113, 57–63.

Heard, C.M., Monk, B.V., Modley, A.J., 2003. Binding of primaquine to epidermal membranes and keratin. Int. J. Pharm. 257, 237–244.

Jalón, E.G., Blanco-Príeto, M.J., Ygartua, P., Santoyo, S., 2001a. Topical application of acyclovir-loaded microparticles: quantification of the drug in porcine skin layers. J. Control. Rel. 75, 191–197.

Jalón, E.G., Blanco-Príeto, M.J., Ygartua, P., Santoyo, S., 2001b. PLGA microparticles: possible vehicles for topical drug delivery. Int. J. Pharm. 226, 181–184.

Jenning, V., Gysler, A., Schäfer-Korting, M., Gohla, S., 2000b. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur. J. Pharm. Sci. 49, 211–218.

Jenning, V., Schäfer-Korting, M., Gohla, S., 2000a. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J. Control. Rel. 66, 115–126.

Kriwet, K., Müller-Goymann, C.C., 1995. Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum. Int. J. Pharm. 125, 231–242.

Lboutounne, H., Chaulet, J., Ploton, C., Falson, F., Pirot, F., 2002. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(ε-caprolactone) nanocapsule encapsulated form and as a digluconate. J. Control. Rel. 82, 319–334.

Lopez, R., Collett, J.H., Bentley, M.V.L.B., 2000. Influence of cyclodextrin complexation on the in vitro permeation and skin metabolism of dexamethasone. Int. J. Pharm. 200, 127–132.

Magdassi, S., 1997. Delivery systems in cosmetics. Colloids Surf. 123/124, 671.

Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2000. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int. J. Pharm. 196, 63–74.

Maia, C.S., Mehnert, W., Schäfer-Korting, M., 2000. Solid lipid nanoparticles as drug carriers for topical glucocorticoids. Int. J. Pharm. 196, 165–167.

Michel, C., Purmann, T., Mentrup, E., Seiller, E., Kreuter, J., 1992. Effect of liposomes on percutaneous penetration of lipophilic materials. Int. J. Pharm. 84, 93–105.

Milão, D., Knorst, M.T., Guterres, S.S., 2003. Hydrophilic gel containing nanocapsules of diclofenac: development, stability study and physico-chemical characterization. Pharmazie 58, 325–329.

Müller, C.R., Schaffazick, S.R., Pohlmann, A.R., de Lucca Freitas, L., Pesce da Silveira, N., Dalla Costa, T., Guterres, S.S., 2001. Spray-dried diclofenac-loaded poly(epsilon-caprolactone) nanocapsules and nanospheres. Preparation and physicochemical characterization. Pharmazie 56, 864–867.

Nagoji, D.E.V., Panda, J., Rao, S.S., Patro, S.S., 2002. Determination of nimesulide in dosage formulations by reverse phase high performance liquid chromatography. Asian J. Chem. 14, 339–343.

Nishihata, T., Kamada, A., Sakai, K., Takahahi, K., Matsumoto, K., Shinozaki, K., Tabat, Y., Keigami, M., Miyagi, T., Tatsumi, N., 1988. Percutaneous absorption of diclofenac in rats and humans: aqueous gel formulation. Int. J. Pharm. 46, 1–7.

Piel, G., Pirotte, B., Delneuville, I., Neven, P., Llabres, G., Delarge, J., Delattre, I., 1997. Study of the influence of both cyclodextrins and L-lysine on the aqueous solubility of nimesulide; isolation and characterization of nimesulide-L-lysine-cyclodextrin complexes. J. Pharm. Sci. 86, 475–479.

Rabasseda, X., 1997. Safety profile of nimesulide: ten years of clinical experience. Drugs Today 33, 41–50.

Sandt, J.J.M., Burgsteden, J.A., Cage, S., et al., 2004. In vitro predictions of skin absorption of caffeine, testosterone and benzoic acid: a multi-centre comparison study. Regul. Toxicol. Pharmacol. 30, 181–271.

Schaffazick, S.R., Guterres, S.S., Freitas, L.L., Pohlmann, A.R., 2003. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. Quím. Nova 26, 726–737.

Schaffazick, S.R., Pohlmann, A.R., de Cordova, C.A., Creczynski-Pasa, T.B., Guterres, S.S., 2005. Protective properties of melatonin-loaded nanoparticles against lipid peroxidation. Int. J. Pharm. 289, 209–213.

Touitou, E., Meidan, V.M., Horwitz, E., 1998. Methods for quantitative determination of drug localized in the skin. J. Control. Rel. 56, 7–21.

United States Pharmacopoeia, 2006. Validation of Compendial Methods, 29th ed. United States Pharmacopoeial Convention, Rockville.

Verma, D.D., Fahr, A., 2004. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. J. Control. Rel. 97, 55–66.

Verma, D.D., Verma, S., Blume, G., Fahr, A., 2003. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur. J. Pharm. Biopharm. 55, 271–277.

Wilkinson, S.C., Maas, W.J.M., Nielsen, J.B., Greaves, L.C., Van de Sandt, J.J., Williams, F.M., 2006. Interactions of skin thickness and physicochemical properties of test compounds in percutaneous penetration studies. Int. Arch. Occup. Environ. Health 25, 1–9.