

## ***In vitro* skin penetration of clobetasol from lipid nanoparticles: drug extraction and quantitation in different skin layers**

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Clobetasol propionate (CP) is a potent topical corticosteroid that causes several cutaneous and systemic side effects. In the present work, CP was encapsulated in nanostructured lipid carriers (NLCs) to increase drug retention in the outer skin layers and improve the safety of topical therapy. NLCs were prepared using a microemulsion technique with a mixture of lecithin, taurodeoxycholate, stearic acid, and oleic acid. *In vitro* penetration studies were performed in a modified Franz-type diffusion cell, and porcine ears were used as a model of human skin. A simple and sensitive liquid chromatographic method was developed and validated for clobetasol determination in different skin layers. NLCs presented uniform size distribution, high zeta potential and entrapment efficiency values (> 98%). The analytical procedure was validated according to FDA guidelines. Clobetasol recoveries from skin samples were higher than 85%, with no interference of skin components and NLC ingredients. In experiments, after 6 h, a higher drug accumulation in the stratum corneum arising from NLCs compared to aqueous CP solution was observed. Thus, the NLCs demonstrated high potential for targeting CP to the skin and ensuring drug accumulation in the stratum corneum.

**Uniterms:** Clobetasol propionate/*in vitro* skin penetration. Nanostructured lipid carriers/*in vitro* study.

Propionato de clobetasol (CP) é um potente corticóide tópico, que apresenta vários efeitos adversos cutâneos e sistêmicos. No presente trabalho, CP foi encapsulado em carreadores lipídicos nanoestruturados (NLCs) visando aumentar a retenção do fármaco nas camadas superficiais da pele e a segurança da terapia tópica. NLCs foram preparados usando a técnica de diluição de microemulsão com mistura de lecitina, taurodesoxicolato, ácido esteárico e ácido oléico. Estudos de penetração *in vitro* foram realizados em células de difusão de Franz modificadas usando pele de orelha de porco como modelo de pele humana. Um método simples e sensível de cromatografia líquida foi desenvolvido e validado para a determinação de clobetasol nas diferentes camadas da pele. NLCs apresentaram distribuição de tamanho uniforme e valores elevados de potencial zeta e eficiência de encapsulação (>98%). O procedimento analítico foi validado de acordo com as diretrizes do FDA. A recuperação de clobetasol a partir das amostras de pele foi maior que 85%, sem interferência dos componentes da pele e dos excipientes das NLCs. Após 6 horas de experimento observou-se maior acúmulo do fármaco a partir das NLCs comparado à solução aquosa de CP. Dessa forma, as NLCs mostraram elevado potencial para direcionar o CP para a pele, pois elas possibilitaram o acúmulo do fármaco no estrato córneo.

**Unitermos:** Propionato de clobetasol/penetração cutânea *in vitro*. Carreadores lipídicos nanoestruturados/ estudo *in vitro*.

### **INTRODUCTION**

Corticosteroids have been successfully employed for the treatment of several inflammatory skin conditions.

Their clinical efficacy is associated with drug skin retention; however, they can permeate the stratum corneum, reaching deeper skin layers, which may cause local and systemic side effects (Zhang, Smith, 2011). Clobetasol propionate (CP) is a superpotent topical corticosteroid (USA class I as per Stoughton–Cornell classification) (Horn *et al.*, 2010). Skin atrophy, skin infections, and hypothalamic-pituitary-adrenal (HPA) axis suppression

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are some of the possible side effects of topical corticosteroids, which are related to the clinical potency of the drug (Horn *et al.*, 2010).

Improvements in the safety of topical corticosteroid therapy may be achieved by drug nanoencapsulation, which can increase the drug's accumulation in the skin while minimizing deep drug permeation and adverse systemic effects (Senyigit *et al.*, 2010). CP has been nanoencapsulated in lecithin/chitosan nanoparticles (Senyigit *et al.*, 2010), polymer-coated lipid nanocapsules (Fontana *et al.*, 2011), liposomes (Rao, Murthy, 2005), and lipid nanoparticles (Kalariya *et al.*, 2005; Hu *et al.*, 2006).

In the present work, CP was encapsulated in nanostructured lipid carriers (NLCs) to increase drug retention in the outer skin layers and avoid systemic absorption. NLCs are the second generation of lipid nanoparticles prepared with blends of solid lipids and oils (Pardeike, Homoss, Muller, 2009). These particles were developed to increase loading capacity and minimize expulsion of active compounds during storage, which are limitations of the first generation of lipid nanoparticles. NLCs have attracted researchers' attention due to their small size, high drug loading, and biocompatible and biodegradable lipid composition. NLCs were produced in this case using a simple microemulsion technique, which was previously employed by our research group to produce lipid nanoparticles containing the cytotoxic drug topotecan (Souza *et al.*, 2011). It is important to note that several papers have reported that NLCs have enormous potential to improve skin penetration and the retention of drugs in the skin (Pardeike, Homoss, Muller, 2009).

Thus, the purpose of this work was to develop clobetasol-loaded NLCs to improve the accumulation of the drug in the outer skin layers. We also developed an extraction procedure and analytical method that is able to quantify CP in both viable and non-viable skin with no interference from skin components or NLC ingredients.

## MATERIAL AND METHODS

### Reagents

Clobetasol propionate (min. 98%) was purchased from Sigma-Aldrich (São Paulo, Brazil). Stearic acid (mp 58.77 °C, Vetec, Brazil), oleic acid (Sigma-Aldrich, Brazil), soy lecithin (Lipoid S100, Germany), and sodium taurodeoxycholate (Sigma-Aldrich, Brazil) were used for the preparation of the nanostructured lipid nanoparticles. Acetonitrile was purchased from J. T. Baker (Phillipsburg, USA). Water was purified using a Milli-Q system (Millipore, Billerica, USA) with a 0.22 µm pore end filter.

### Production and characterization of CP-loaded NLCs

CP-loaded NLCs were prepared using a microemulsion technique. A mixture of lecithin, taurodeoxycholate, stearic acid, and oleic acid was heated, and CP was added to the melted material. Then, 250 µL of distilled water was added to the mixture under magnetic stirring, and a thermodynamically stable microemulsion system was formed. The microemulsion was dispersed into cold water (2–4 °C) under vigorous stirring (13,400 rpm for 10 min, IKA T25 Ultra-Turrax, Germany) using a 1:20 ratio (microemulsion:water) to form an NLC dispersion. The dispersion had a total lipid content of 2% (w/v), stabilized by 1.25% (w/v) of surfactant (lecithin: taurodeoxycholate, 4:1 ratio). The stearic acid: oleic acid ratio in the NLCs was 3:1 (Souza *et al.*, 2011). The CP in the formulation comprised 4% (w/w) of the lipid content.

Dynamic light scattering was used to assess the mean particle size and polydispersity index (PDI) of the NLC dispersions. Samples were diluted (1:100) with purified water and analyzed using a Zetasizer Nano S (Malvern Instruments, UK). Zeta potential was determined from electrophoretic mobility using a Zetasizer Nano ZS (Malvern Instruments, UK).

Encapsulation efficiency (EE) was determined by the ultrafiltration technique (eq. 1). To isolate the entrapped CP from free drug, 1 mL of freshly prepared NLC dispersion was subjected to ultrafiltration (Vivaspin 2<sup>®</sup>, Sartorius, USA) for 60 min at 4,000 g at 4 °C. Filtrate containing the free drug was withdrawn for HPLC analysis as described below. All analyses were performed in triplicate. The total amount of drug incorporated in the NLC was determined by lipid disruption with acetonitrile followed by HPLC quantification. Drug loading and drug recovery were calculated according eq. 2 and 3, respectively.

$$EE\% \left( \frac{w}{w} \right) = \frac{\text{amount of CP in the formulation} - \text{amount of free drug}}{\text{Amount of CP in the formulation}} \times 100 \quad \text{Eq. (1)}$$

$$DL\% \left( \frac{w}{w} \right) = \frac{\text{amount of CP in the formulation} - \text{amount of free drug}}{\text{weight of the lipids}} \times 100 \quad \text{Eq. (2)}$$

$$DR\% \left( \frac{w}{w} \right) = \frac{\text{amount of CP in the formulation}}{\text{Amount of CP added}} \times 100 \quad \text{Eq. (3)}$$

### *In vitro* skin penetration studies of free and nanoencapsulated clobetasol

CP penetration studies were carried out in a modified

Franz-type diffusion cell (Microette; Hanson Research, Chatsworth, USA) (Figure 1). The receptor media was a 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution. The solubility of CP in the receptor medium was determined by addition of an excess of CP to the medium, followed by 24 h agitation at 37 °C, filtration and quantitation of the dissolved drug. The solubility of CP was 0.47 mg/mL. Solutions of CP in 1% (w/v) SDS aqueous solution or CP-loaded NLCs were added to the donor media. In both cases, 80 µg of CP was used in the donor cell to ensure sink conditions. The diffusion area available between the receptor and donor compartments was 1.86 cm<sup>2</sup>. The stirring rate and temperature were maintained at 300 rpm and 37 °C, respectively. Experiments were performed for 3 h and 6 h (n=6). After each experiment, the skin surface was washed with deionized water to remove excess CP on the skin surface. Washed skin was subjected to drug extraction and quantitation as described below.

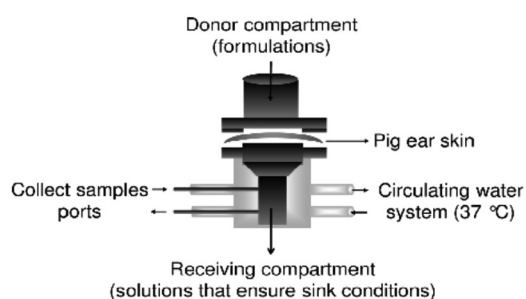


FIGURE 1 - Schematic representation of a Franz-cell apparatus.

### Skin sample preparation

Pig ears were used as a model of human skin. The material was collected immediately after the slaughter of the animals. Ears were then dermatomed in the laboratory. Then, the dermatomized skin (700 µm thick) was kept at -80 °C for a maximum of 30 days. After the *in vitro* penetration study, the stratum corneum (SC) was separated from the remaining skin (RS) using the tape-stripping technique. Skin was tape-stripped 15 times with adhesive tapes (Durex Original 500, 3M, Sumaré, Brazil). Tape strips with SC and RS were placed in individual test tubes for the extraction procedure.

### CP extraction from SC and RS

For the CP recovery studies, blank samples of adhesive tapes with SC were spiked with a solution of CP in acetonitrile and allowed to dry. Dried tapes were then immersed in 5 mL of acetonitrile for 20 min to obtain a CP final con-

centration of 50 µg/mL. The mixture was vortexed for 2 min and analyzed by HPLC-UV. The RS was also spiked with a solution of CP in acetonitrile. Following the evaporation of the solvent, 5 mL of acetonitrile was added to the tube. The RS was then processed with a tissue homogenizer (T25 ULTRA-TURRAX®, IKA, Staufen, Germany) for 2 min at 6,000 rpm, followed by bath sonication (USC 1400, Unique, Indaiatuba, Brazil) for 20 min. The skin homogenate was then filtered and analyzed by HPLC-UV. CP extraction from samples collected during the *in vitro* penetration studies was performed as described above (n=3).

### Apparatus and chromatographic conditions

Samples were analyzed with an HPLC system consisting of an isocratic pump (ProStar 210, Varian, Palo Alto, USA), autosampler (ProStar 400, Varian, Palo Alto, USA), and UV detector (ProStar 325, Varian, Palo Alto, USA). Separation was achieved using an Agilent ZORBAX Eclipse XDB-C18 column (250 x 4.6 mm, 5 µm). The mobile phase was a 30:70 (v/v) mixture of water and acetonitrile. The flow rate was 1.0 mL/min, the injection volume was 50 µL, and UV detection was carried out at 240 nm. Data acquisition was performed using Galaxie Chromatography Data System software.

### Method validation

The method was developed and validated for linearity, accuracy, precision, and quantitation limit in accordance with the Food and Drug Administration Guidelines for the validation of analytical methods (FDA, 2000).

The selectivity was investigated against formulation ingredients, skin endogenous components, and adhesive tapes. Blanks of remaining skin and stratum corneum tape-stripped from six sources of skin were analyzed for the absence of interfering compounds at CP retention time. Precision (repeatability and intermediate precision) was expressed as the relative standard deviation (RSD%) of replicate measurements. Accuracy was calculated as the percentage difference between the measured and true values. Accuracy was expressed as the relative error of measurement (E%). Recovery was determined by computing the ratio of the amount of CP extracted from spiked skin to the amount of CP added.

## RESULTS AND DISCUSSION

### Method development and validation

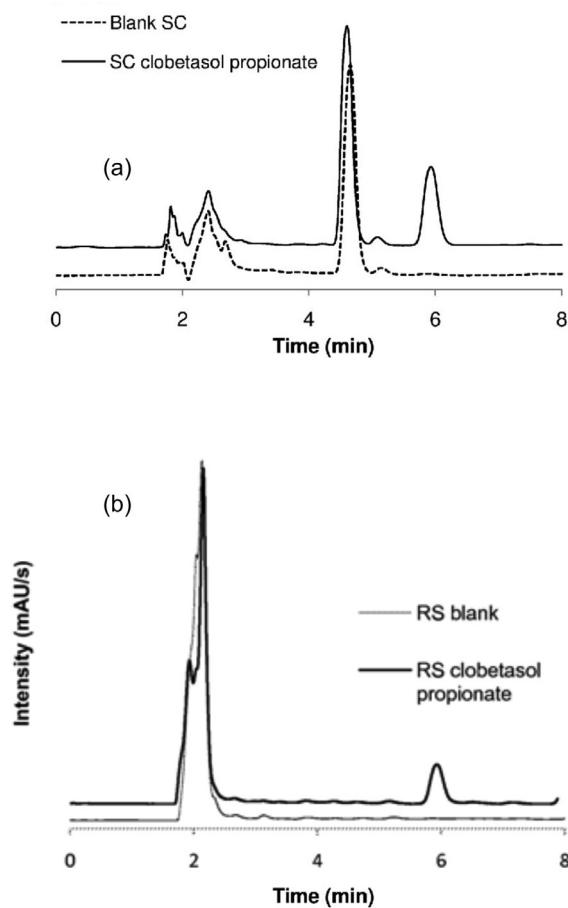
CP is the most important corticosteroid in derma-

tological therapy; however, there are few reports on the quantitation of CP in skin matrices in the literature (Senyigit *et al.*, 2010; Fontana *et al.*, 2011; Rao, Murthy, 2005; Kalariya *et al.*, 2005; Weigmann *et al.*, 1999; Mueller, Anissimov, Roberts, 2003), and there are no published data on the development and validation of an analytical procedure capable of quantifying CP in both the stratum corneum and remaining skin (viable epidermis plus dermis). Literature reports on the quantitation of CP in skin are based on isocratic HPLC-RP methods, and analytical separations have been performed with water and acetonitrile as the mobile phase. However, most of the reports, although focused in the application of the method, do not present details on method development or analytical validation data.

One of the challenges for quantifying drugs in biological matrices is the development of an efficient extraction procedure. This issue is particularly true for skin matrices, as the interactions among the analyte, skin lipids, and proteins may result in low drug recovery. High drug recovery values (above 90% from epidermis and dermis) have been reported in the literature (Senyigit *et al.*, 2010). Weigmann *et al.* (1999) developed an exhaustive procedure for CP extraction from human stratum corneum. In the present work, the mean recovery rates of CP from porcine ear skin were 101.48% ( $\pm 5.01$ ) and 86.83% ( $\pm 5.20$ ) for the stratum corneum and remaining skin, respectively, denoting the high efficiency of the extraction procedure employed. It is important to note that the procedure presented here for CP extraction from the stratum corneum is simpler and less time consuming compared to some previously reported methods (Weigmann *et al.*, 1999).

A simple method for the separation and quantitation of CP in the stratum corneum and remaining skin was also developed and validated. Chromatographic conditions resulted in a short run time, with a CP retention time of 6.10 min. The short run time is suitable for the routine analysis of a large number of samples from *in vitro* penetration studies. Typical chromatograms of stratum corneum and remaining skin, both spiked and not spiked with CP, are presented in Figure 2. As seen, the proposed method was able to quantify CP without interference from endogenous skin compounds and formulation ingredients, demonstrating the specificity of the method.

Validation of the analytical method was performed and linearity was found in the concentration range of 0.5-15  $\mu\text{g/mL}$  ( $r=0.999$ ). The detection limit (DL) was 25 ng/mL, and the quantification limit (QL) was 100 ng/mL, which may be considered suitable for CP quantification during *in vitro* penetration studies. Weigmann *et al.* (1999) reported a detection limit of 160 ng/mL, which is far superior to the one reported here.



**FIGURE 2** - Chromatograms obtained from the analysis of clobetasol-spiked or blank stratum corneum (2a) and from clobetasol-spiked or blank remaining skin (2b).

Accuracy and precision are presented in Table I. All values of accuracy and precision were within the acceptable limits, with E% and RSD% values no greater than 5% (FDA, 2000).

### NLC characterization

NLCs were prepared with and without CP using the microemulsion technique; the characterization data are shown in Table II.

CP-loaded nanostructured lipid carriers (NLCs) presented low average diameters and low polydispersity index (PdI) values, denoting a uniform size distribution. No significant differences in mean diameter, PdI, or zeta potential were observed in NLCs with or without CP, suggesting that the addition of the drug did not alter the original characteristics of the nanoparticle dispersion.

The CP-NLCs presented low drug recovery (Table II) due to formulation adherence to the experimental apparatus. Therefore, the use of thermostatic devices is ad-

**TABLE I** - Accuracy, repeatability, and intermediate precision of the HPLC method for determining the concentration of CP in organic solutions (n=3)

Concentration ( $\mu\text{g/mL}$ )	Relative Standard Deviation (RSD%)			Relative Error of Measurement (E%)		
	Days			Days		
	1	2	3	1	2	3
1	4.18	3.00	0.61	3.01	3.71	-2.27
5	1.96	2.96	0.68	-0.22	0.52	-2.71
10	3.05	1.65	0.38	4.89	3.98	-2.24

**TABLE II** - Mean diameter, polydispersity index (PdI), zeta potential, entrapment efficiency, drug loading, and drug recovery of NLCs obtained with and without CP

	Mean diameter (nm)	PdI	Zeta potential (mV)	EE% (w/w)	DL% (w/w)	DR% (w/w)
NLCs	195.5 $\pm$ 17.9	0.22 $\pm$ 0.05	-49.8 $\pm$ 2.5	---	---	---
CP-NLCs	157.9 $\pm$ 19.7	0.19 $\pm$ 0.01	-53.9 $\pm$ 1.1	98.6 $\pm$ 0.4	2.34 $\pm$ 0.1	62.2 $\pm$ 3

visible for the transfer procedure to avoid process losses during industrial production of lipid nanoparticles.

Several papers have reported that zeta potential values greater than 30 mV (*in modulus*) denote good electrostatic repulsion between the nanoparticles, improving the physical stability of the system (Freitas, Mueller, 1998). Accordingly, the zeta potential values of the NLCs with and without CP (Table II) suggest that these nanoparticle dispersions have great physical stability. The entrapment efficiency was very high (Table II), which indicates that the lipids and surfactants employed were very suitable for CP nanoencapsulation.

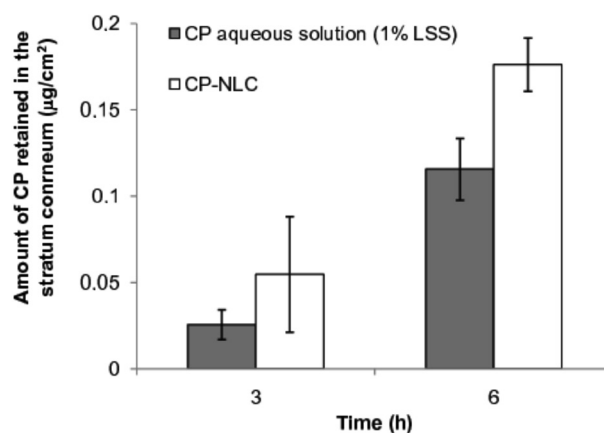
The application of lipid nanoparticles to the skin has been related to several therapeutic benefits, such as the improvement of drug penetration, controlled release, and excellent tolerability (Pardeike, Homoss, Muller, 2009). Small nanoparticles, such as those reported here, ensure close contact of the drug formulation with the stratum corneum and exert occlusive effects, improving skin hydration and the penetration of the drug into the skin (Pardeike, Homoss, Muller, 2009).

### In vitro skin penetration of free and CP-loaded lipid nanoparticles

The nanoparticle formulation was added to the donor compartment of a Franz-type cell apparatus (Figure 1), and CP skin penetration was determined.

In accordance with theoretical expectations, we observed a higher CP accumulation in the stratum corneum

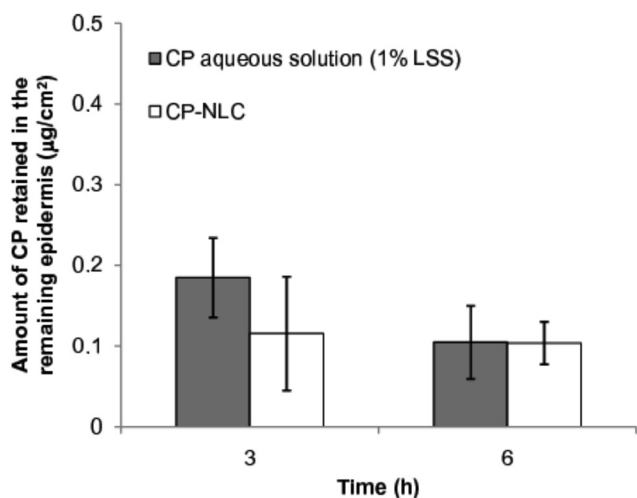
from lipid nanoparticles compared to accumulation from aqueous CP solution (sodium dodecyl sulfate, 1%, w/v) after 6 h of experiment ( $p < 0.01$ ) (Figure 3). In contrast, no significant differences were observed in SC samples after 3 hours during the experiment (Figure 3).

**FIGURE 3** - *In vitro* clobetasol penetration in porcine ear skin stratum corneum after 3 and 6 hours of experimentation.

Several studies have demonstrated that NLCs can increase drug penetration and retention in the skin, likely due to the occlusive effect caused by the formation of an intact film on the skin surface (Marquell-Oliveira *et al.*, 2010). In addition, NLCs may interact with lipids in the outermost skin layers, resulting in stratum corneum fluidization and increased drug penetration. Nanoparticles may

also accumulate in skin appendages, such as hair follicles, which can act as a long-term reservoir for drug release (Knorr *et al.*, 2009). Based on the results presented here, it appears that CP-NLCs accumulate on the skin surface, promoting a controlled release for periods longer than 3 hours. It is important to note that no CP was detected in the receptor media for all formulations tested after 3 or 6 h of the *in vitro* penetration experiment.

There was no significant difference in CP accumulation in the remaining skin 3 or 6 hours after the application of the drug-loaded nanoparticles or aqueous dispersions (Figure 4). Despite this observation, CP nanoencapsulation demonstrated a high potential to improve topical therapy, as the stratum corneum may act as a drug reservoir, and CP higher accumulation in this layer may lead to high CP concentrations in the remaining skin during chronic administration schedules. Indeed, nanoencapsulation may significantly increase the concentration of drug available for skin penetration, particularly for drugs with poor aqueous solubility, which dissolve only a small amount in conventional topical vehicles, such as gels and creams. The preparation of higher drug-loading CP lipid nanoparticles is currently under investigation in our laboratory.



**FIGURE 4** - *In vitro* clobetasol penetration in the viable epidermis plus dermis of the porcine ear skin after 3 and 6 hours of experimentation.

## CONCLUSIONS

The NLCs obtained in this work by the microemulsion technique demonstrated high potential for targeting CP to the skin, as they provide drug accumulation in the stratum corneum. We also demonstrated the suitability of the analytical method developed here for determining

CP concentration in two different skin layers, the stratum corneum and remaining skin. The method demonstrated high sensitivity, specificity, and drug recovery.

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## REFERENCES

- ECHEVARRÍA, L.; BLANCO-PRÍETO, M.J.; CAMPANERO, M.A.; SANTOYO, S.; YGARTUA, P. Development and validation of a liquid chromatographic method for *in vitro* mupirocin quantification in both skin layers and percutaneous penetration studies. *J. Chromatogr. B.*, v.796, n.2, p.233-241, 2003.
- FOOD AND DRUG ADMINISTRATION. Guidance for Industry. Analytical procedures and methods validation: *chemistry, manufacturing, and controls documentation*. Rockville: US Food and Drug Administration, 2000. 33 p.
- FONTANA, M.C.; REZER, J.F.P.; CORADINI, K.; LEAL, D.B.R. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. *Eur. J. Pharm. Biopharm.*, v.79, n.2, p.241-249, 2011.
- FREITAS, C.; MULLER, R.H. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. *Int. J. Pharm.*, v.168, n.2, p.221-229, 1998.
- HORN, E.J.; DOMM, S.; KATZ, H.I.; LEBWOHL, M.; MSOWIETZ, V. KRAGBALLE, K. Topical corticosteroids in psoriasis: strategies for improving safety. *J. Eur. Acad. Dermatol. Venereol.*, v.24, n.2, p.119-124, 2010.
- HU, F.Q.; JIANG, S.P.; DU, Y.Z.; YUAN, H.; YE, Y. Q.; ZENG, S. Preparation and characteristics of monostearin nanostructured lipid carriers. *Int. J. Pharm.*, v.314, n.1, p.83-89, 2006.
- KALARIYA, M.; PADHI, B.K.; CHOUGULE, M.; MISRA, A. Clobetasol propionate solid lipid nanoparticles cream for effective treatment of eczema: formulation and clinical implications. *Indian J. Exp. Biol.*, v.43, n.3, p.233-240, 2005.

- KNORR, F.; LADEMANN, J.; PATZELT, A.; STERRY, W.; BLUME-PEYTAVI, U.; VOGT, A. Follicular transport route – research progress and future perspectives. *Eur. J. Pharm. Biopharm.*, v.71, n.2, p.173-180, 2009.
- MARQUELE-OLIVEIRA, F.; SANTANA, D.C.A.; TAVEIRA, S.F.; VERMEULEN, D.M.; OLIVEIRA, A.R.M.; SILVA, R.S.; LOPEZ, R.F.V. Development of nitrosyl ruthenium complex-loaded lipid carriers for topical administration: Improvement in skin stability and in nitric oxide release by visible light irradiation. *J. Pharm. Biol. Anal.*, v.53, n.4, p.843-851, 2010.
- MUELLER, B.; ANISSIMOV, Y.G.; ROBERTS, M.S. Unexpected clobetasol propionate profile in human stratum corneum after topical application *in vitro*. *Pharm. Res.*, v.20, n.11, p.1835-1837, 2003.
- PARDEIKE, J.; HOMMOSS, A.; MULLER, R.H. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *Int. J. Pharm.*, v.366, n.1-2, p.170-184, 2009.
- RAO, G.; MURTHY, R.S.R. Evaluation of liposomal clobetasol propionate topical formulation for intra-dermal delivery. *Indian J. Pharm. Sci.*, v.62, n.6, p.459-462, 2000.
- SENYIGIT, T.; SONVICO, F.; BARBIERI, S.; OZER, O.; SANTI, P.; COLOMBO, P. Lecithin/chitosan nanoparticles of clobetasol-17-propionate capable of accumulation in pig skin. *J. Control. Release*, v.142, n.3, p.368-373, 2010.
- SOUZA, L.G.; SILVA, E.J.; MARTINS, A.L.L.; MOTA, M.F.; BRAGA, R.C.; LIMA, E.M.; VALADARAES, M.C.; TAVEIRA, S.F.; MARRETO, R.N. Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release. *Eur. J. Pharm. Biopharm.*, v.79, n.1, p.189-196, 2011.
- WEIGMANN, H.J.; LADEMANN, J.; PELCHRZIM, R.V.; STERRY, W.; HAGEMEISTER, T.; MOLZAHN, R.; SCHAEFER, M.; LINDSCHEID, M.; SCHAEFER, H.; SHAH, V.P. Bioavailability of clobetasol propionate: quantification of drug concentrations in the stratum corneum by dermatopharmacokinetics using tape stripping. *Skin Pharmacol. Appl. Skin Physiol.*, v.12, n.12, p.46-53, 1999.
- ZHANG, J.; SMITH, E. Percutaneous permeation of betamethasone 17-valerate incorporated in lipid nanoparticles *J. Pharm. Sci.*, v.100, n.3, p.896-903, 2011.

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