International Journal of Pharmaceutics 477 (2014) 519–526

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

A new topical formulation for psoriasis: Development of methotrexate-loaded nanostructured lipid carriers

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ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 29 October 2014
Accepted 30 October 2014
Available online 1 November 2014

Keywords:
Methotrexate
Lipid nanoparticles
Skin permeation
Psoriasis
Transdermal drug delivery

ABSTRACT

The aim of the present work was to develop and assess the potential of nanostructured lipid carriers (NLCs) loaded with methotrexate as a new approach for topical therapy of psoriasis. Methotrexate-loaded NLCs were prepared via a modified hot homogenization combined with ultrasonication techniques using either polysorbate 60 (P60) or 80 (P80) as surfactants. The produced NLCs were within the nanosized range (274–298 nm) with relatively low polydispersity index (<0.25) and zeta potential values around −40 mV. NLCs demonstrated storage stability at 25 °C up to 28 days. The entrapment efficiency of methotrexate in NLC-P60 and -P80 was ~65%. Cryo-SEM images showed the spherical shape of the empty and methotrexate-loaded NLCs. FT-IR confirmed methotrexate presence within the NLCs. The *in vitro* release of methotrexate from the NLCs followed a fast release pattern reaching ~70% in 2 h. *In vitro* skin penetration study demonstrated that methotrexate-loaded NLCs-P60 had higher skin penetration when compared to free methotrexate, suggesting a significant role of drug-nanocarriers on topical administration. Methotrexate-loaded NLC-P60 provided drug fluxes of 0.88 μg/cm^2/h, higher ($P < 0.001$) than with the free drug (control, 0.59 μg/cm^2/h). The results indicate the potential of NLCs for the delivery of methotrexate to topical therapy of psoriasis.

1. Introduction

Psoriasis is a chronic inflammatory skin disease that affects 1 to 3% of the world population, with equal gender distribution (O'Daly, 2011; Raho et al., 2012). Incidence rates vary from 50 to 140 new cases per 100,000 people per year (Raho et al., 2012). It is associated with high levels of distress and morbidity, as well as a general decrease in the quality of life of the patient, even though it is not usually life-threatening. Nonetheless, severe psoriasis increases the risk of mortality, in comparison to the general population (Chandran and Raychaudhuri, 2010).

Psoriasis is a life-long disease and the management and treatment of psoriasis are different depending on the severity of the disease. The first line of active treatments for psoriasis is the use of topical agents (Chong et al., 2013). This type of therapy is typically sufficient in the management of mild to moderate psoriasis (Murphy and Reich, 2011), namely when this disease affects less than 10% of the body surface area (Mitra and Wu, 2010). Topical agents comprise coal tar (Bhatia et al., 2011) and dithranol (Rahman et al., 2012), corticosteroids (Horn et al., 2010), vitamin D analogs (Kamangar et al., 2013) and retinoids (Murphy and Reich, 2011), as well as keratolytic agents such as salicylic acid (Paul et al., 2012).

When the effects arising from topical therapies strategies are suboptimal or when the extent of the disease makes it unfeasible for the use of topical therapy, phototherapy and systemic therapy may need to be considered (Laws and Young, 2012). Currently available systemic therapies for psoriasis include non-biological and biological therapies. As previously stated, these are commonly used as monotherapies or in combination with other modalities of treatment in patients with moderate to severe psoriasis (body surface area higher than 10%) (Chong et al., 2013). The most prominently used non-biological systemic agents are methotrexate (MTX), cyclosporine and orally administrated retinoids (such as acitretin) (Chong et al., 2013; Laws and Young, 2012). Current therapeutic strategies for the treatment of psoriasis generally employ oral and parenteral administration routes for MTX as it inhibits epidermal cell proliferation (Shen et al., 2012) and has anti-inflammatory action at low doses (Micha et al., 2011; Shen et al., 2012). It should be noted that there is a large number of adverse effects (such as liver toxicity, gastrointestinal side-effects, including nausea, vomiting, diarrhea and stomatitis) associated to

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http://dx.doi.org/10.1016/j.ijpharm.2014.10.067
0378-5173/

systemic administration of MTX (Micha et al., 2011). Nevertheless, some side-effects can be minimized by the concomitant supplementation of folate to the patient (Micha et al., 2011; Montaudié et al., 2011).

In the scope of the management and treatment of psoriasis, nano-dermatology and the development of nanoparticles for dermatological applications is without a doubt an area of increasing magnitude and interest (Saraceno et al., 2013). Drug carriers can provide a sustained drug release over a prolonged period of time, (Papakostas et al., 2011) and shields it from degradation. Hence, therapeutic effect can be maximized and toxicological concerns related to drug overdose and clearance can be minimized (Gupta et al., 2012). Additionally, patient compliance is higher, as these therapeutical strategies enable a reduction in the frequency of drug administration (Papakostas et al., 2011).

Lipid nanoparticles, as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are frequently used to incorporate and deliver lipophilic drugs (Kumar and Randhawa, 2013). Lipid nanoparticles are produced with generally regarded as safe (GRAS) lipids and surfactants. Other advantages include low production cost, easy to scale up and additionally low toxicity, as organic solvents are not required for the synthesis of these nanoparticles. NLCs are composed of solid and liquid lipids which allows the formation of an overall amorphous nanostructure with many imperfections within its matrix, providing NLCs with higher drug capacity and a lesser degree of drug expulsion during storage then SLNs (Wang et al., 2013). In the scope of topical administration, formulations of NLCs are characterized by their occlusive ability of creating a mono-layered lipid film onto the skin, thereby avoiding water evaporation and increasing skin moisture and hydration and, consequently, drug permeation (Gupta et al., 2012; Kumar and Randhawa, 2013). Indeed, literature concerning the topical administration of drug-carrying nano-systems as therapeutic strategies for psoriasis presents interesting examples of the application of lipid nanoparticles (Agrawal et al., 2013, 2010; Lin et al., 2010; Pradhan et al., 2013; Raza et al., 2013). Recently novel strategies for preparing SLNs *in situ* using electrospraying have been described, to overcome resolving problems associated with the formulation of poorly water-soluble drugs (Yu et al., 2011a,b).

Overall, the present work intends to present NLCs formulations that would be pertinent and efficacious for the dermal treatment of psoriasis. The success associated with the use of MTX by dermal application would also be mirrored in increased patient compliance, as topical administration of therapeutic substances constitutes a much less invasive and more comfortable and convenient route of administration.

2. Materials and methods

2.1. Materials

Witepsol® S51 was kindly provided by Cremer Oleo (Hamburg, Germany), oleic acid was purchased from May & Baker Ltd. (Dagenham, England) and polysorbates 60 (Tween 60) and 80 (Tween 80) were obtained from Merck (Darmstadt, Germany). All chemicals and solvents were of analytical grade. Aqueous solutions were prepared with double-deionized water (Arium Pro Sartorius AG, Göttingen, Germany, conductivity less than 0.1 $\mu S\,cm^{-1}$). MTX was kindly provided by Excella (Feucht, Germany) as a gift.

2.2. Preparation of NLCs

MTX-loaded NLCs were prepared by high-shear homogenization followed by the ultrasound method. Briefly, the lipid phase was prepared by melting Witepsol® S51 at 50 °C, then adding oleic acid, the surfactant (polysorbate 60, P60 or polysorbate 80, P80), and finally 1 mg of MTX was added to the hot solution. The melted lipid phase was dispersed in pre-warmed 50 °C double-deionized water to obtain a microemulsion by high-shear homogenizer (YSTRAL GMBH X10/20-E3, Ballrechten-Dottingen, Germany) at 12,000 rpm for 2 min. This microemulsion was then homogenized with a probe-type sonicator (VCX130, Sonics & Materials, Newtown, CT, USA) for 15 min with frequency amplitude of 70% in order to obtain a nanoemulsion. It was then cooled at room temperature, allowing the inner oil phase to solidify forming NLCs dispersed in the aqueous phase. As a control, drug-free NLCs were prepared in a similar manner without the addition of MTX to the lipid phase. The compositions of the NLCs are given in Table 1. The produced NLCs were transferred into glass vials and then freeze-dried using a Modulyo 4K freeze-dryer from Edwards (Crawley, West Sussex, U. K.) at 0.09 mbar for 72 h, with a condenser surface temperature of -60 ± 5 °C.

2.3. Determination of entrapment efficiency

Entrapment efficiency (%EE) of MTX in NLCs was determined by UV spectrophotometry. A 1:50 dilution of NLC formulations in double-deionized water was subsequently centrifuged (Heraeus™ Multifuge™ ×1R Centrifuge, USA) through centrifugal filter units (Amicon® Ultra Centrifugal Filters, Ultracel – 50 KDa, Darmstadt, Germany) at $2260 \times g$, 20 °C during 30 min or until complete separation between the NLCs retained in the filter unit and the aqueous phase corresponding to the filtrate. The filtrate was used to quantify the amount of non-incorporated MTX by UV–vis spectrophotometry (Jasco V-660 Spectrophotometer, USA) at λ_{max} 303 nm, which is the maximum absorption of MTX in aqueous solution (Lin et al., 2010). A standard curve of MTX in water was used to determine the concentration of MTX and the results are expressed as mean ± standard deviation ($n = 3$).

The results were compared to drug-free NLCs used as control. Taking into account the drug initially added to the NLCs formulation and subtracting the free MTX remaining in the filtrate, it was possible to determine the amount of drug incorporated in the NLCs and thus the entrapment efficiency by the following equation:

$$\%EE = \frac{\text{Total amount of MTX} - \text{free MTX in the filtrate}}{\text{Total amount of MTX}} \times 100$$

Table 1
Composition of the developed NLCs formulations.

Formulation code	Witepsol S51	Oleic acid	Polysorbate 60	Polysorbate 80	MTX
NLC-P60	700	300	200	–	–
MTX_NLC-P60	694	300	200	–	6
NLC-P80	700	300	–	200	–
MTX_NLC-P80	694	300	–	200	6

Amounts expressed in mg and all formulations were prepared in 8.8 mL of double-deionized water.

2.4. Determination of particle size, surface charge and physical stability

The particle size and polydispersity index (PDI) of each batch produced were determined by dynamic light scattering (DLS), while the zeta potential (surface charge) was evaluated by electrophoretic light scattering (ELS); both determinations were done using a ZetaPALS zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). A 1:400 dilution of each formulation was made using double-deionized water followed by determination of both particle size and PDI at 25 °C. For each sample, the corresponding mean diameter ± standard deviation values were obtained from six determinations and calculated by the multimodal analysis. Concerning surface charge analysis, the zeta potential Smoluchowski mathematical model was used to obtain the corresponding measurements. For each sample, the corresponding mean ± standard deviation values were obtained from six runs of ten cycles. For each final formulation, at least three batches were analysed for mean particle size, polydispersity and zeta potential as described.

The stability of the prepared NLC formulations was determined by measuring the particle size, the surface charge using ZetaPALS zeta potential analyzer and the amount of MTX by spectrophotometry after storage at 25 °C for 28 days. Each reported value was the average of six measurements, of four independent batches.

2.5. Fourier transform infrared spectroscopy (FTIR)

The freeze-dried formulations with and without MTX, and also pure MTX, were evaluated using an FT-IR Spectrophotometer (Frontier™, PerkinElmer; Santa Clara, CA, USA) equipped with a diamond crystal. All samples were run in triplicate, several controls were run in parallel. A background run (to remove the background noise of the instrument) was carried out as a negative control, while the free MTX analysis was carried out as positive control; the NLCs with and without MTX were also analysed. Spectra was recorded between 4,000 and 600 cm^{-1} with a spectral resolution of 4 cm^{-1}.

2.6. Scanning electron microscopy

The morphology (shape, size and surface structure) of the NLCs was evaluated by scanning electron microscopy (SEM) using high resolution scanning electron microscope with X-Ray microanalysis and cryo-SEM experimental facilities at the Materials Centre of the University of Porto, Portugal (CEMUP). A 1:400 dilution of NLCs in double-deionized water was dropped on a support and rapidly frozen in liquid nitrogen. Cryofactures were then performed using an ALTO 2500 (Gatan Alto 2500 (Pleasanton, CA, USA)), with subsequent sublimation and coating with Au/Pd by sputtering for 35 s. The samples were then observed at −150 °C using a JSM 6301F microscope (JEOL, Tokyo, Japan).

2.7. Determination of in vitro drug release

The release of MTX from different NLCs was assessed by a dialysis bag diffusion technique. A defined amount of drug-loaded NLCs dispersion containing 0.25 mg MTX was transferred into cellulose dialysis bags with a 6,000–8,000 Daltons, (Cellu•Sep® T2; Membrane Filtration Products Inc., Frilabo, Maia, Portugal). The dialysis bags were immersed in a receptor compartment containing 80 mL of phosphate buffer saline (PBS) pH 7.4 and placed over a heating and magnetic stirring plate (IKAMAG®, Staufen, Germany) with an agitation of approximately 360 rpm and at a temperature of 37 °C. At predetermined time intervals (0.5, 1, 2, 3, 4, 6, 7, 8 and 24 h), aliquots of 1 mL were withdrawn and the drug content determined spectrophotometrically (Jasco V-660 spectrophotometer, USA), as described previously, using a standard curve of MTX in PBS pH 7.4. Results are the mean values of three runs. Sink conditions were maintained, as the maximum solubility of MTX in aqueous mediums is of <2 mg/mL, and the concentration of MTX in the receptor medium was always at least 30 times lower than this value.

2.8. In vitro skin permeation

The skin permeation of MTX, MTX-loaded NLCs and void NLCs were evaluated using a Franz cell assembly (9 mm unjacketed Franz Diffusion Cell with 5 mL receptor volume, o-ring joint, clear glass, clamp and stir-bar; PermeGear, Inc., USA) (Zhang et al., 2013). Pig ear skin was used as a barrier.

The donor medium consisted of 0.5 mL of vehicle containing drugs free or loaded on NLCs systems. The actual amount of methotrexate in donor was 250 μg. The receptor medium (5.5 mL) was buffer containing 10% DMSO to maintain sink conditions. The available diffusion area between cells was 0.785 cm^2. The stirring rate and temperature of receptor were respectively kept at 600 rpm and 35 °C. The temperature of skin surface could be maintained at about 32 °C after this setting, which was near the *in vivo* status. At appropriate intervals, 500 μL aliquots of the receptor medium were withdrawn and immediately replaced with equal volumes of fresh buffer. The cumulative amount of MTX was determined by HPLC. The quantification of MTX was conducted by HPLC rather than UV–vis spectrophotometry to overcome interferences from skin components released during the experiment.

The HPLC system included MD-2015 multi-wavelength detector (Jasco, Easton, MD, USA) programmed for peak detection at 303 nm, a high-pressure pump (PU-2089), an autosampler (AS-2057) and a controller (LC-Net II/ADC) mastered by ChromNAV software. A Chromolith 4.6-mm reversed-phase monolithic column (Merck, Darmstadt, Germany) connected to a guard column of the same material was used as a stationary phase. The MTX quantification was performed according to method previously described (Moura et al., 2014). In particular, standard MTX solutions were prepared at 0.75, 1.5, 3, 6, 12.5 and 25 μg/mL in mobile phase and in permeation buffer enriched upon pig skin contact for defined times (0, 1, 3, 5 and 8 h).

2.9. Statistical analysis

All mean values are presented as means ± SD. The Student's *t*-test (two-tailed) was used to evaluate the statistical significance of any differences in mean values in the experimental groups. The one-way analysis of variance-test was used to assess the differences in means between formulations in the *in vitro* skin permeation.

3. Results and discussion

3.1. Methotrexate-loaded NLCs characterization: particle size, PDI, surface charge and storage stability

NLCs formulation were prepared using Witepsol S51 as solid lipid and oleic acid as liquid lipid (oil). These ingredients were chosen taking into account that the successful entrapment of a hydrophobic drug, such as MTX, into a lipid nanoparticle depends on its adequate solubility or miscibility with the lipid. After heating all the NLCs ingredients at 50 °C, the solution was clear and no crystals were detected, indicating complete solubilization of the MTX in the lipid matrix. MTX was successfully incorporated into NLCs by coupling high shear homogenization with ultrasound. This method has many advantages compared to preparations

Table 2
Characterization of NLCs.

Formulation code	Particle size (nm)	PDI	Zeta-potential (mV)	% EE
NLC-P60	298 ± 3	0.19 ± 0.01	−40 ± 1	–
MTX_NLC-P60	292 ± 2	0.18 ± 0.02	−39 ± 3	64 ± 2
NLC-P80	274 ± 7	0.14 ± 0.02	−36 ± 3	–
MTX_NLC-P80	292 ± 9*	0.12 ± 0.02	−37 ± 3	64 ± 4

*$P < 0.05$, Student's *t*-test showed mean particle size to be statistically different between empty and MTX-loaded NLC-P80 ($P = 0.0179$).

performed using only one of these techniques, such as high homogeneity and reduced dimensions of nanoparticle dispersions (Puglia et al., 2013).

The particle sizes of the NLCs as the mean hydrodynamic diameter of the particles assessed by DLS are presented in Table 2. At the time of production, both void and MTX- NLCs-P60 or NLCs-P80 showed a mean diameter in the range of 274–298 nm and a polydispersity index below 0.20. Statistical analysis indicated differences between void and MTX-loaded NLCs-P80 ($P < 0.05$) while no statistically significant differences were observed when polysorbate P60 was used as surfactant (Table 2). Part of the MTX contained in the produced NLCs-P80 could be adsorbed to their surface, thus, resulting in an increase in particle size. The polydispersity parameter, gave important indications concerning sample homogeneity; as values below 0.25 reflect relatively homogeneous nanoparticles, with minimum predisposition to aggregation (Mitri et al., 2011). All formulations reported particle size below 300 nm, aiming the topical administration. For this route, a desirable value of particle size for NLCs is established as ≤500 nm, in order to penetrate the skin epithelium (Kohli and Alpar, 2004).

The zeta potential is an important parameter that allows predictions on the physical stability of colloidal dispersions. In theory, higher values of zeta potential, either positive or negative, tend to stabilize the suspension and aggregation phenomena are less likely to occur for charged particles with pronounced zeta potential| (>|30|), due to the electrostatic repulsion between particles with the same electrical charge (Gonzalez-Mira et al., 2010). In this study, all formulations of void and MTX-loaded NLCs-60 and–NLCs-P80 presented zeta-potential values between −36 and −40 mV, which predicts a good skin permeation and long-term stability. Indeed, upon 28 days of storage at room temperature, the same narrow interval of values was maintained for the zeta-potential (Fig. 1C). It has been described that only negatively charged particles are able to permeate the skin (Kohli and Alpar, 2004). Altogether, the storage stability study indicates good physical stability of the lipid nanoparticles, most probably due to the surfactant used in their preparation (Saupe et al., 2005).

The physical stability of the NLCs was also evaluated by examining changes of particle size and polydispersity index during storage conditions for 28 days at 25 °C. Both lipid nanoparticles (NLCs-P60 and NLCs-P80) with and without MTX did not show statistically significant changes in their mean particle size (Fig. 1 A) and PDI values (Fig. 1B, $P > 0.05$) when stored as aqueous suspensions at room temperature for at least 28 days. Also, the amount of MTX present in the NLCs after this storage period was identical to the values determined with fresh produced formulations.

3.2. MTX entrapment efficiency

For the preparation of MTX-loaded NLCs, two types of polysorbates, P60 and P80, were used as surfactants. A 0.5% (w/w) of MTX relative to total excipients content was selected based on preliminary solubility studies, corresponding to the solubility of MTX in the molten lipid. Both formulations were able to incorporate MTX with an EE of 63.8 ± 1.9% and 63.5 ± 4.2%, for

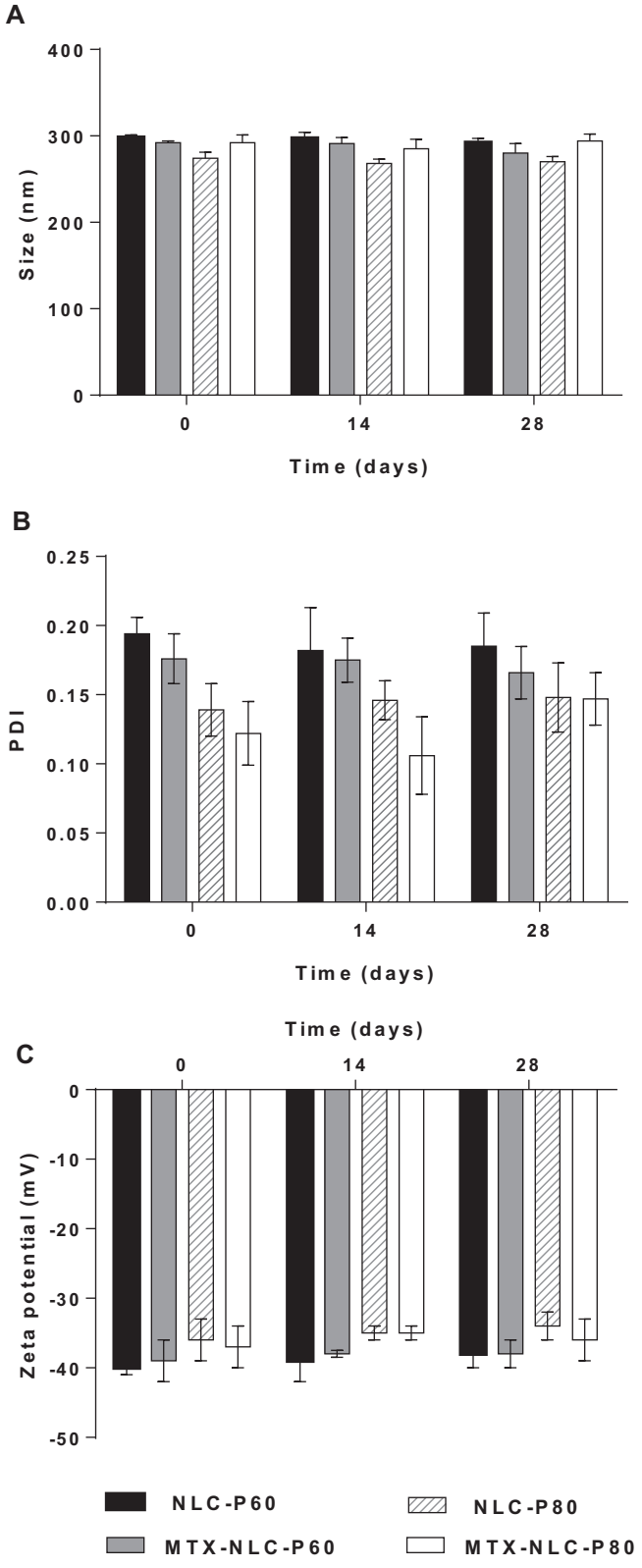

Fig. 1. Storage stability. Effect of time of storage at 25 °C on particle size (A), PDI (B) and zeta potential (C) of void and MTX-loaded NLCs. All data represent the mean ± standard deviation ($n = 4$). No statistically significant differences were observed over the time for any formulation ($P > 0.05$).

NLC-P60 and NLC-P80, respectively and no statistical differences were observed ($P > 0.05$), thus, both types of surfactants could be considered suitable for MTX incorporation in lipid nanoparticles.

3.3. Morphology

To gain more information on the particle size, shape and surface morphology, cryogenic scanning electron microscopy (cryo-SEM) was performed in all the dispersions, as samples in frozen condition are investigated close to their natural state. All formulations exhibited a spherical shape and a smooth surface, regardless of their composition (Fig. 2). The sizes of the NLCs observed by cryo-SEM were in the range of 230–280 nm, with no observable difference being noted between void and MTX-loaded NLCs. Analysis of particles by cryo-SEM involve solvent removal that can explain the decrease found in particle size, when compared with the data measured by DLS (Das and Chaudhury, 2011).

3.4. Fourier transform infrared (FTIR) spectroscopy

To demonstrate that it was possible to incorporate MTX in both types of NLCs, infrared spectra of free MTX, MTX-loaded NLCs-P60 and–P80 were obtained, and are presented on Fig. 3. The band at 1648 cm^{-1} is due to the vibration of the functional groups —COOH present in the structure of MTX (Kohler et al., 2005), and can be seen in the graphs for FTIR spectra of free MTX and MTX-loaded NLCs-P60 and–P80. The spectra from void NLCs did not present this peak, indicating successful incorporation of MTX in the NLCs formulations.

3.5. In vitro *MTX release assay*

In lipid nanoparticles systems, the drug is incorporated in the lipid matrix either in dissolved or in dispersed form (Muller et al., 2002). Therefore, the solubility of the drug in the lipid matrix becomes a very important controlling factor for the release of drug from NLCs. To evaluate the release of MTX from the NLCs-P60 and–P80 formulations, *in vitro* release assays were performed using a dialysis diffusion technique. All the presented *in vitro* release assays were performed for the simulated physiological conditions of the plasma, employing a receptor medium composed of PBS pH 7.4 buffer at a temperature of 37 °C. Comparative studies were carried out for free MTX solution (data not shown), MTX-loaded NLCs-P60 and -P80 and results are shown in Fig. 4. Both

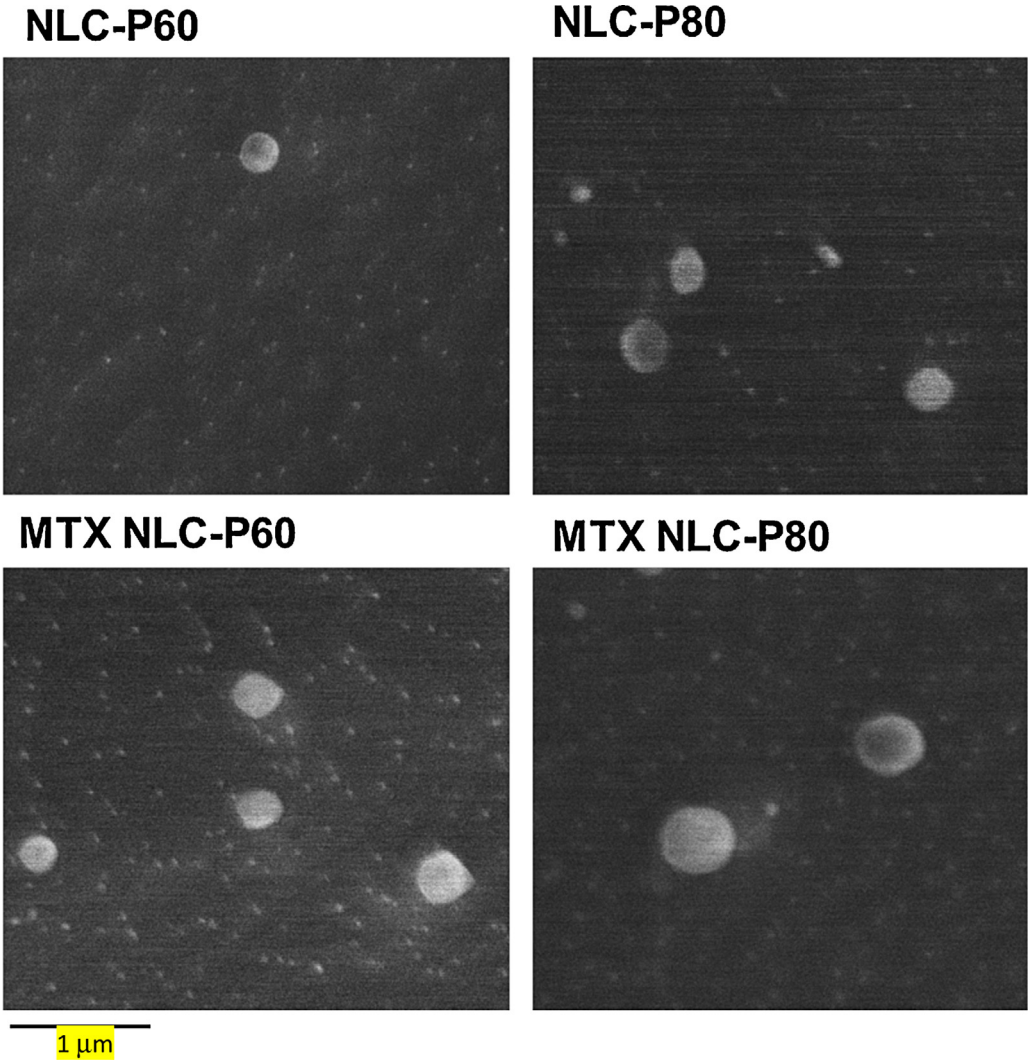

Fig. 2. NLCs images. Cryo-scanning electron microscopy (cryo-SEM) images of NLC-P60, NLC-P80, MTX-loaded NLC-P60 and MTX-loaded NLC-P80. The scale indicated below the pictures is 1 μm. Amplification: ×20,000.

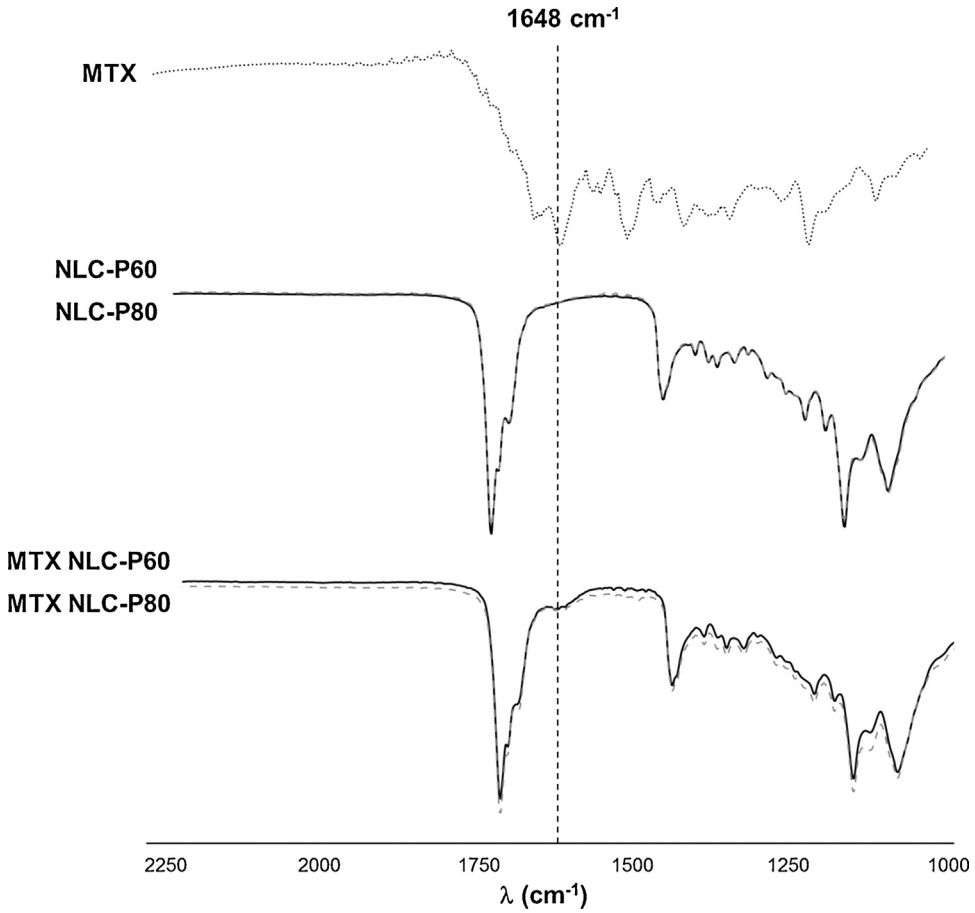

Fig. 3. MTX presence within NLCs. FT-IR spectra obtained for free MTX, void NLC-P60 and NLC-P80 and MTX-loaded NLC-P60 and -P80. The vertical dotted-line indicate peaks characteristic of the presence of MTX (1648 cm^{-1} for free and MTX-loaded NLC-P60 and NLC-P80).

MTX-loaded NLCs systems showed a fast initial phase of drug release of 64 ± 16% and 64 ± 10%, for NLC-P60 and NLC-P80, respectively in the first 2 h, followed by sustained release until 8 h. In this study, the initial drug loading is below the MTX solubility limit, and its release is achieved by simple diffusion through the lipid matrix (Muller et al., 2002). During preparation of NLCs, cooling from high temperature to room temperature favors the enrichment of drug in the outer layers of the particles resulting in superficial entrapment causing initial burst release (zur Muhlen and Mehnert, 1998). While the acquisition of slower and more prolonged drug release patterns is of most interest in the application of nanoparticulate drug-delivery systems, it can be concluded that this drug release profile is nonetheless pertinent with regard to the intended topical application. Its rapid but still gradual release of MTX would avoid the loss of therapeutic agent (due to daily actions like changing clothes and physiological reactions such as sweating) and allow its enhanced administration through the skin (in regard to the hydrating/occlusive properties of NLCs), followed by a more gradual and prolonged release of this drug (further reinforcing over time the effect of the main drug dose administrated to the patient).

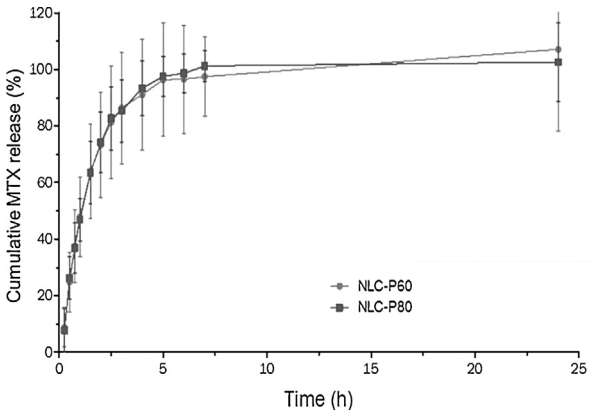

Fig. 4. *In vitro* release of MTX from NLCs. MTX release from NLC-P60 and NLC-P80 through a dialysis bag diffusion technique to a receptor medium containing PBS pH 7.4 buffer at 37 °C. Data points correspond to the mean ± standard deviation for $n = 3$.

3.6. In vitro *drug skin penetration studies*

The aim of present research was to deliver the drug in the skin for psoriasis topical therapy. Drug penetration into certain layers of the skin can be achieved using NLCs (Muller et al., 2002). In this context, to assess the *in vitro* MTX skin penetration, the Franz diffusion cells were used and as a model barrier, pig ear skin was chosen, since, pigs soft tissue is very similar to its human counterpart when it comes to morphology and function. Results presented in Fig. 5 revealed MTX penetration enhancement upon entrapment into NLCs of Witepsol and oleic acid. Statistical significant difference was observed for MTX-loaded NLC-P60 ($P < 0.01$) when compared with free MTX. After 8 h the amount of MTX permeated through skin was 5.8 ± 0.2 and 4.2 ± 0.1% from NLCs-P60 and -P80, respectively while for free MTX was 3.6 ± 0.2%. From this, it can be concluded that NLCs may play important role in

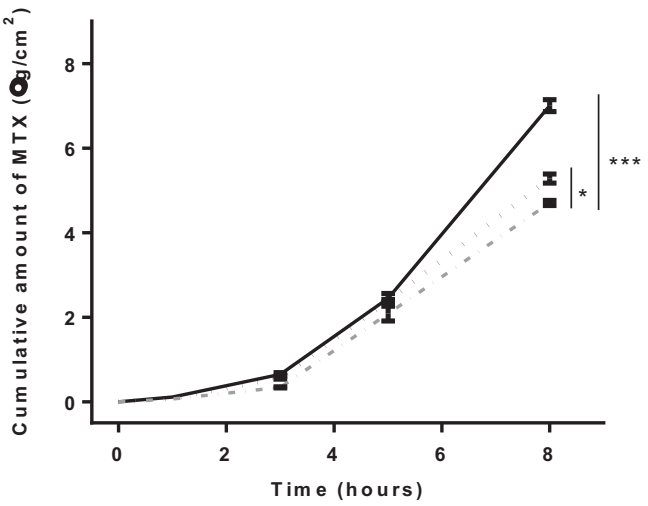

Fig. 5. MTX skin permeation. *In vitro* cumulative amount of MTX permeated over time in pig ear skin as free drug (dotted grey line), within NLC-P60 (black line) or NLC-P80 (dotted black line). Each value represents the mean ± SD ($n = 3$).

Table 3
MTX flux between its free form and loaded-NLCs formulations after 8 h.

Formulation code	Flux (μg/cm²/h)
Free MTX	0.59 ± 0.01
MTX_NLC-P60	0.88 ± 0.02***
MTX_NLC-P80	0.66 ± 0.01

controlling the release of MTX from NCLs as well as targeting of drug to the skin.

Diffusion of drugs and associated drug-carriers through the epidermis can also be affected by the interaction between the formulation and the myriad of membrane components, enzymes and transporters comprised in this layer (Gupta et al., 2012). Diffusion rates can similarly be affected by surface charge, properties of the nanomaterial, drug-loading efficiency, mode of application and hydrogen bonding ability (Desai et al., 2010). In this study, the choice of surfactant affected the flux rate of MTX, as NLC-P60 exhibited the highest diffusion rate through the skin when compared with NLC-P80 ($P < 0.05$) and even free MTX ($P < 0.01$) (Table 3). Similar flux rates were described to NLCs of Precirol ATO 5 and squalene loaded with lipophilic calcipotriol and methotrexate when assessed in normal skin (Lin et al., 2010). On the other hand, when the skin barrier is compromised, due to pathological processes or artificially, nanoparticle penetration might be considerably enhanced. Indeed, the alteration of the stratum corneum associated with inflammation and keratinocyte hyperproliferation could aid in the penetration of nanoparticles in psoriatic lesions, thereby favoring the use of these drug carriers for topical administration of drugs (Desai et al., 2010; Lin et al., 2010). Nonetheless, it should be noted that the effects of psoriasis on skin barrier integrity, specifically concerning nanoparticle penetration, is yet to be studied (Prow et al., 2011).

4. Conclusions

Psoriasis is one of the major chronic inflammatory diseases that plague modern society, being associated with high morbidity factors. While it still remains incurable, successful psoriasis management can be reached by an extensive number of therapeutic agents. Even so, multiple and wide-ranging adverse consequences can arise from every treatment strategy available. MTX is considered the gold standard for treatment of severe psoriasis and its use is associated with an extensive list of side effects to the patient. Thus, as a way of bypassing these troubling limitations, the role of nanomedicine can be emphasized, as its adequate application holds the potential of targeted, more efficient and less adverse administration of pertinent therapeutic substances for the treatment of psoriasis.

Through this work, it was possible to successfully produce NLCs loaded with MTX, which possess characteristics that would render them a promising alternative for the psoriasis topical treatment and even as a substitute for current intravenous MTX administration. The produced NLCs had acceptable dimensions (<300 nm) accompanied by elevated absolute zeta potential levels (>30 mV), low values of PDI (<0.25) and reasonably high values of encapsulation efficiency (>60%), with a spherical shape and smooth surface in terms of morphology. The elevated absolute zeta potential values and low PDI values suggested high physical stability of the formulations, which was confirmed for storage at room temperature for at least 28 days.

In vitro evaluation of drug release from the produced NLCs in a simulated physiological environment revealed a biphasic drug release profile, characterized by a quick initial phase of drug release followed by a phase with prolonged release until 8 h. Evaluation of the *in vitro* skin permeation of MTX showed its capability to go through the skin barrier when loaded within NLCs formulation. Indeed, it was expected that these drug-carrying nano-systems would enhance the permeation of this drug, due to their multiple beneficial properties in the context of topical application. Overall, the results of the present work confirm the high potential of NLCs as carriers for MTX and feasibility for topical delivery. Patient compliance should be increased, as topical applications are much more comfortable and friendlier to patients.

Acknowledgments

This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project Pest-C/EQB/LA0006/2013. This work was also funded by ON.2 QREN – Quadro de Referência Estratégico Nacional – QREN, by FEDER funds through the Programa Operacional Factores de Competitividade – COMPETE and national funds through Fundação para a Ciência e a Tecnologia (FCT, Portugal) through project NORTE-07-0124-FEDER-000067. CN thanks FCT (Fundação para a Ciência e Tecnologia) for the Post-Doc Grant (SFRH/BPD/81963/2011). The authors would like to acknowledge Excella for kindly providing the MTX.

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