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Design and Evaluation of Miconazole Nitrate loaded Nanostructured Lipid Carriers (NLC) for improving the Antifungal therapy

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ABSTRACT

The aim of this study was to prepare and evaluate gels incorporating nanostructured lipid carriers (NLC) of Miconazole nitrate (MN) for systemic delivery of the active after topical application. MN has been used as model drugs to be incorporated into nanostructured lipid carriers, once they are very well established as anti-mycotics for the treatment of topical fungal infections. NLC designed for topical administration of MN, were prepared by the hot high pressure homogenization technique. This MN-NLC was characterized for particle size, entrapment efficiency, and SEM. The lipid nanoparticles were incorporated in gels for convenient topical application and were evaluated forfor particle size, Rheological analysis Texture analysis , *In vitro* drug release studies and *Ex Vitro* skin permeation Studies. The preparation of aqueous NLC dispersions with a mean particle size lower than 300 nm has been obtained with uniform size distribution (PI < 0.350). The prepared semi-solid systems showed mean particle size remained lower than 250 nm and PI remained lower than 0.500 after 3 months of storage. An initial rapid release was observed in the case of Marketed gel, whereas MN- NLC Gel depicted a slow initial release with a lag time of 0.5 h and 1 h, respectively. High amount of MN release was facilitated through abdominal skin of rats from marketed gel than MN-NLC Gel. Research work could be concluded as successful development of MN-loaded NLC-bearing hydrogel to increase the encapsulation efficiency of colloidal lipid carriers with advantage of improved performance in terms of stability and provides a sustaining MN topical effect as well as faster relief from fungal infection.

INTRODUCTION

Miconazole nitrate (MN) is a broad-spectrum antifungal agent of the imidazole group (Bennett *et al.,* 2001). It acts by means of a combination of two mechanisms: ergosterol biosynthesis inhibition, which causes lysis of fungal cell membranes because of the changes in both membrane integrity and fluidity and direct membrane damage of the fungal cells. The drug is primarily used as a topical treatment for cutaneous mycoses; poor dissolution and lack of absorption make it a poor candidate for oral administration. However, MN can be used as a systemic antifungal agent when amphotericin B or ketoconazole is either ineffective or contraindicated. MN poor skin-penetration capability presents a problem in the treatment of cutaneous diseases by topical application. For effective treatment, the drug must be delivered in sufficient concentration to the site of infection (Gossel *et al.,* 1985). Various approaches have been used to enhance the access of such poorly skin-partitioned drug molecules. For example, the use of complexation with cyclodextrins has been reported to improve oral and topical delivery of MN (Pedersen *et al.,* 1993 and Tenjarla *et al.,* 1998). Biodegradable nanoparticles, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) (Joshi *et al.,* 2006) are stable colloidal systems with notable advantages as drug delivery systems, i.e. physicochemical stability, versatility, biocompatibility, biodegradability and controlled drug release. SLN and NLC are colloidal carrier systems providing controlled release profiles for many substances (Teeranachaideekul *et al.,* 2008). Aqueous dispersions of lipid nanoparticles are being investigated as drug delivery systems for different therapeutic purposes. One of their interesting features is the possibility of topical use, for which the systems have to be incorporated into commonly used dermal carriers, such as creams or hydrogels, in order to have a proper semisolid consistency (Muller *et al.,* 2002).

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Compared with traditional carriers, SLN are well tolerated, have high bioavailability, a nice targeting effect and are amenable to large scale production (Muller *et al.,* 1996 and Maaben *et al.,* 1993 and Yang *et al.,* 1999) However, due to the high crystallization of the solid lipids or blends of solid lipids, drugs tend to be released from the nanoparticles, thus leading to drug expulsion and low loading capacity (Muller *et al.,* 2002a). To overcome the limitations of SLN, a new generation of lipid nanoparticles, nanostructured lipid carriers (NLC) have been developed in recent years (Muller *et al.,* 2002a).

NLC are prepared by mixing solid lipids with liquid lipids (oils). Hu et al (2005) prepared stearic acid (SA) NLC with varying oleic acid content and Saupe et al(2006) obtained NLC based on a mixture of cetyl palmitate and Miglyol 812 (caprylic/capric triglycerides). It is supposed that the oil incorporation impacted the crystalline state of the solid lipid. Jenning et al. (2000) found the formation of oily nanocompartments within the solid matrix whereas Jores et al. showed that high oil loads may lead to phase separation. The aim of this study was to develop topical gels containing NLC dispersions loaded with MN. The NLC were prepared by high-pressure homogenization method. Nanoparticles were characterized in terms of particle size, morphology, encapsulation efficiency and crystalline structure. The influence of the NLC on ex-vivo drug skin permeation was evaluated and compared with a conventional gel.

MATERIALS AND METHODS

Materials

Miconazole nitrate was gifted by Glenmark Pharma, Ltd., Mumbai, India. Dynasan 116 (glyceryl tripalmitate) and Miglyol 812 were obtained from Guangdong, China. Poloxamer 188, Methyl Paraben and Propylene glycol were purchased from SD Fine Chemicals, Mumbai, India. Carbopol 934P was obtained as a gift sample from Colorcon Asia Pvt. Ltd., Mumbai, India. All the other chemicals were of the analytical grade. Water was used in double-distilled quality.

Methods

Screening of components

One of the most important factors that determine the loading capacity of the drug in the lipid is the solubility of drug in melted lipid. 10 mg of MN was dispersed in a mixture of melted lipid (1g) and 1 ml of hot distilled water and shaken for 30 min in a hot water bath.

Aqueous phase was separated after cooling by ultracentrifugation and analyzed for drug content by spectrophotometric method at 272 nm (Bhalekar *et al.,* 2009). Solubility of drug in the lipid phase is one of the most important factors that determine the loading capacity of the drug in the lipid carrier. The solubility of MN was determined in different liquid lipids and surfactants. An excess of drug was added individually to liquid lipids and surfactants (5 ml each) in screw capped tubes. After 24 h, each sample was centrifuged and 0.5 ml of the clear supernatant layer was diluted suitably with methanol, and analyzed by spectrophotometric method at 272 nm.

Preparation of NLC dispersions

The NLC dispersions were prepared using hot high-pressure homogenization method (HPH). Table 1 reports the composition of the prepared NLC dispersions. Blank and drug loaded NLC were prepared using the elsewhere reported HPH technique, slightly modified (Muller *et al.,* 2002b). In order to prepare NLC, the lipid phase has been melted at 5-10ºC above the melting point of the solid lipid.

At the same time, an aqueous surfactant solution has been prepared and heated at the same temperature. The hot lipid phase was then dispersed in the hot surfactant solution using an Ultra-Turrax T25 Stirrer (IKA-Werke, Staufen, Germany) at 8000 rpm for 4 min. The obtained pre-emulsion was homogenized at a temperature 5ºC to 10ºC higher than the melting point of the bulk lipid, using an homogenizer (APV Micron Lab 40 Italy) and applying a pressure of 500 bar and 5 homogenization cycles. The obtained dispersion was cooled in an ice bath in order to solidify the lipid matrix and to form NLC.

Characterization of NLC Dispersion

Particle size and zeta potential determination

Particle size and size distribution measurements of the NLC suspended in the original dispersions were performed using photon correlation spectroscopy (PCS). The average particle size (z-average size) and polydispersity index (PI) were measured by photon correlation spectroscopy (PCS, Malvern Mastersizer Hydro 2000G U.K.) at 25 ^{0}C under a fixed angle of 90^{o} in disposable polystyrene cuvettes. The count rate was kept at around 200 kcps with varying duration greater than 50s.

The dispersant used was water and its RI (1.33), viscosity (0.8872 cP) and Dielectric constant (78.5) were kept constant for all determinations. Zeta potential was measured in folded capillary cells using the Nano ZS90 zetasizer. 1 ml sample was taken from each formulated nanosuspension and dispersed with 10ml of double distilled water.

The samples were ultrasonicated for 5 min prior to size determination to measure the primary particle size. Then the sample was taken in disposable sizing cuvette and placed in the instrument for size and zeta potential measurements. In the case of NLC-based semi-solid formulations, prior to particle size analysis by PCS, the formulations have been diluted with double-distilled water to weak opalescence.

Scanning electron microscopy

The morphology (shape and surface characteristics) of NLC was studied by scanning electron microscopy (SEM) (model JSM 840A, JEOL, Japan). The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current of about 80MA.

Table. 1: Composition of NLC formulations (%, m/m), Particle size, Polydispersity Index (PI), Zeta potential and % Drug entrapment of different NLC formulations obtained immediately after production.

Formulation code	Composition					Parameters (Immediately after production)			
	Miconazole nitrate	Dynasan 116	Miglyol 812	Poloxamer 188	Water ad	Particle size (nm)	Polydispersity Index (PI)	ZP (mV)	% Drug Entrapment
NLC	-	13.5	5.5	5.0	100	238.4±7.51	0.350±0.003	-19.6±0.9	-
MNNLC-1	1.0	13.5	6.5	5.0	100	206.3±1.48	0.311±0.002	-13.2±0.4	97.08±2.12
MNNLC-2	1.0	13.5	6.5	2.5	100	218.2±2.08	0.334±0.001	-13.4±0.2	92.13±4.31
MNNLC-3	1.0	12.5	5.5	2.5	100	233.9±2.52	0.352±0.001	-13.9±0.1	88.32±1.66
MNNLC-4	1.0	12.5	5.5	5.0	100	229.7±2.51	0.344±0.002	-13.5±0.8	86.31±2.81

Dug entrapment efficiency

The amount of encapsulated MN was calculated by subtracting the free amount of the drug from MN-NLC dispersion by ultracentrifugation at 55,000 rpm for 1 hr. The solution was filtered and diluted with methanol and MN content was determined spectrophotometrically. Entrapment efficiency (EE %) was calculated from the following equation

$$EE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug laded expected}} \times 100$$

DSC Analysis

DSC analyses were performed on pure Miconazole nitrate, Dynsyan 116 and Miglyol 812 by a Mettler Toledo DSC 8220 instrument (Perkin-Elmer DSC-7). 1-2 mg of solid lipid has been accurately weighted in 40 µl aluminium pans. DSC scans have been recorded at a heating rate of 10 ^{0}C /min and was run over the range 25-300 ^{0}C, using an empty pan as reference. For the analysis of pure model drugs (8-10 mg) were carefully transferred and heated in crimped to the aluminum pans for accurate results.

Preparation and Characterization of NLC-Based Hydrogel

For the preparation of hydrogel, the gel-forming polymer Carbopol 934P was dispersed in double distilled water containing glycerol, stirred for 10 min at 1500 rpm and neutralized by triethanolamine under gentle stirring and immediately neutralized with triethanolamine until pH 6.0. Hydrogel were further allowed to equilibrate for 24 hours at room temperature and then used to disperse a freshly prepared NLC suspension. Aqueous NLC dispersion and hydrogel were mixed in a high speed stirrer (Remi, Mumbai, India) at 1000 rpm for the next 5 min. The gel was allowed to stand overnight to remove entrapped air. The formulative composition of the gels is documented in Table2.

Table. 2: Composition of the Carbapol based Hydrogel and NLC-based semi-solid formulations.

Composition	Carbapol based Hydrogel formulation	NLC-based semi-solid formulation
Miconazole nitrate	1.00%	1.00%
Dynasan 116	-	9.00%
Miglyol 812	-	2.50%
Polxamer 188	2.50%	2.50%
Carbapol 934 P	0.50%	0.50%
Methyl Paraben	0.05%	0.05%
Propylene glycol	3.50%	3.50%
Glycerine	2.50%	2.50%
Triethanolamine	0.25%	0.25%
Water ad	100%	100%

Rheological measurement

In the present work, the rheological analysis of NLC based gel and Blank gel was performed using a stress control rheometer (Viscotech Rheometer, Rheologica Instruments AB, Lund, Sweden), equipped with Stress Rheologic Basic Software, version 5, using cone-plate geometry with the diameter of the cone being 25 mm and a cone angle of 1°, operating in the oscillation and static mode. Continuous shear investigations have been applied to characterize of the developed semi-solid formulations, evaluating the shear stress as a function of shear rate. In order to determine if the systems are thixotropic, this study started applying 0 s^{-1} up to a maximum shear rate of 100 s^{-1} and back to 0 s^{-1}, and the resulting shear stress and viscosity were measured. The average of three readings was used to calculate the viscosity. (Tamburic *et al.,* 1995).

Texture Analysis

For the characterization of the developed semi-solid formulation three different parameters have been evaluated i.e. adhesiveness, consistency and gel strength. These mechanical properties have been assessed using the texture analyzer TA-XT Plus (Stable Micro Systems, Goldalming, UK). Data acquisition and mathematical analysis have been performed using a computer equipped with the Texture Expert softwsare.

In Vitro Drug Release

The in vitro drug release profile of MN-loaded NLC-bearing hydrogel and marketed formulation were studied using a dialysis bag. Formulations were taken into a dialysis bag (molecular weight cut-off, 12 KDa, Himedia, India) and placed in a beaker containing 20 ml of mixture of methanol: PBS (pH 6.4) (30:70). Then, the beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 1°C throughout the study. Samples (1 ml) were withdrawn at definite time intervals and replaced with equal amounts of fresh buffer. The samples were analyzed for drug concentration by UV-VIS spectrophotometer at 272 nm.

Ex Vitro skin permeation Studies

In vitro permeation of MN from NLC based gel and marketed formulation (Flucos Gel, Cosme Pharma ltd, India) were performed using excised full thickness hairless abdominal skin of rats (Male albino rats, Sprague Dawley; 100–150 g). The skin samples were mounted on modified Franz diffusion cells (Crown Glass Co., NJ) with a surface of 3.14 cm^2 and a receptor volume of

10 ml such that the dermal side of the skin was exposed to the receptor fluid [methanol:PBS (pH 6.4), i.e. 30:70] ratio and the stratum corneum remained in contact with the content of donor compartment. Formulations were placed in the donor compartment enabling one to cover the entire skin surface evenly. The temperature was maintained at 37 ± 1°C. Serial sampling (0.5 ml) was performed at specified time intervals (1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24 h) by removing the contents of the receptor compartment and replacing it with the fresh medium. The samples were analyzed using UV-VIS spectrophotometer at 272 nm and mean cumulative amount diffused Q (mg/cm^2) at each sampling time a point was calculated. At the end of 24 h, the skin was cut, homogenized, and extracted, first with methanol and then filtered; them ethanolic extract was evaporated and the residue was again extracted with DMF, filtered, diluted with 0.1 N HCl, and analyzed spectrophotometrically at 272 nm.

RESULTS AND DISCUSSION

Preparation and Characterization of NLC

For the current study, NLC were successfully prepared and the composition of the formulations prepared is shown in Table 1. Calibration curve (y=0.0215x+0.0134, R^2=0.9993) of MN was used to calculate the concentration of MN in the aqueous phase. Partition coefficients (ratio of the amount of MN in lipid to the amount of miconazole nitrate in aqueous phase) obtained by analyzing drug content in aqueous phase were 39.10±3.34, 56.67±6.13, and 78.81±2.56 for Stearic acid, compritol 888 ATO and Dynasan116. Dynasan116 has been selected as the solid lipid for NLC because MN exhibited higher partition coefficient and after usual inspection of drug crystals in different melted lipids, based on the light they scatter using a black and white background light box. Among the selected liquid lipid oils that were screened, maximum solubility of MN was found in Miglyol 812 (79.52 ± 4.9 mg/g) followed by Migloyl 808 (61.33 ± 8.3 mg/g). The selection of Miglyol 812 as liquid lipid for NLC preparation was based on solubility studies.

For the production of NLC formulations the optimized ratio between Dynasan 116 and Miglyol 812 has been determined after screening different proportions of both lipids by DSC studies to evaluate the absence of free oil in the melted mixtures. No free oil has been detected after running the mixtures of solid and liquid lipids until -50ºC (Sato *et al.,* 2001). Mixtures of Dynasan116 and Miglyol 812 at different ratios have been melted at 85ºC and further analysed by DSC (Fig.1). In the present investigation, five different NLC formulations were produced by hot high pressure homogenization. Various parameters were optimized by varying one parameter while keeping others constant. The MN-NLC dispersion was white in color and odorless and did not show sedimentation even after centrifugation at 2,000 rpm for 30 min. Yields of production obtained were always relatively high and was in the range 80–98%. Lipids show positive influence on entrapment efficiency, this result can probably be attributed to the high affinity of the lipophilic drug for the lipidic material. MNNLC-1 and MNNLC-2 show of about 90% while samples MNNLC-3 and MNNLC-4 show less % entrapment efficiency.

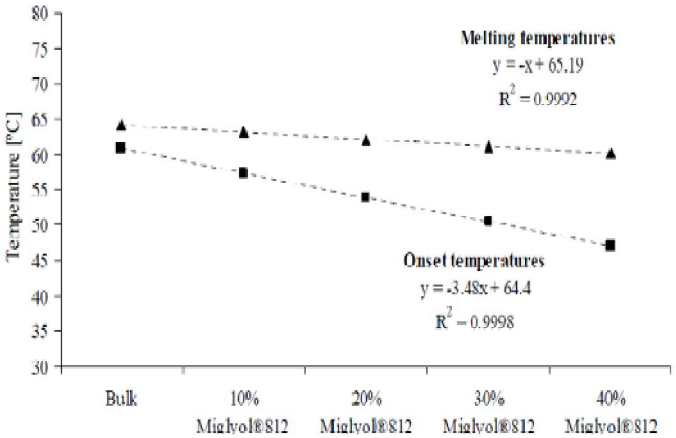

Fig. 1: Melting temperature (= peak maximum) and onset temperature values of bulk lipid (Dynasan 116) with increasing amounts of liquid lipid (Miglyol 812) from 10% to 40% (m/m).

It is known that the particle size distribution is one of the most important characteristics for the evaluation of the stability of colloidal systems and also influences the penetration mechanism of drugs into the skin (Souto *et al.,* 2004). Therefore, the particle size parameters and the surface electrical charge (ZP) have been evaluated immediately after production of the systems, and during one month of storage at three different temperatures 4ºC, 25ºC and 40ºC. Under optimized production conditions (500 bar and 5 homogenization cycles) very small lipid nanoparticles with a negatively charged surface could be obtained. The preparation of aqueous NLC dispersions with a mean particle size lower than 250 nm has been obtained in previous studies using only 5% of surfactant (Poloxamer 188) stabilizing 20% of lipid mass. In this work, a relatively uniform size distribution has been obtained (PI < 0.350). The incorporation of MN decreased the electrical charge at the surface of NLC and lower ZP values. The developed formulations have been stored at three different temperatures to challenge the systems under stress conditions. In all storage temperatures, the systems remained in their colloidal particle size range (< 1 µm). The mean size was maintained lower than 300 nm, with a PI in the same magnitude as the values obtained immediately after production (PI < 0.350). After one month of storage, all lipid nanoparticles showed a negative charge at their surface. Also the pH values did not vary notably between the variables investigated. Particle size and polydispersity index of formulation are shown in Fig.2. The differences between the evaluated parameters were not significant, neither under different storage temperatures nor with the presence of drug molecules, meaning that the systems NLC for topical delivery of antifungals physicochemically stable under stress conditions. No gel formation has been observed after one month of shelf life at three different temperatures. Poloxamer 188 could stabilize the developed formulations even under stress conditions. Zeta potential of NLC based formulation is shown in Fig.3.

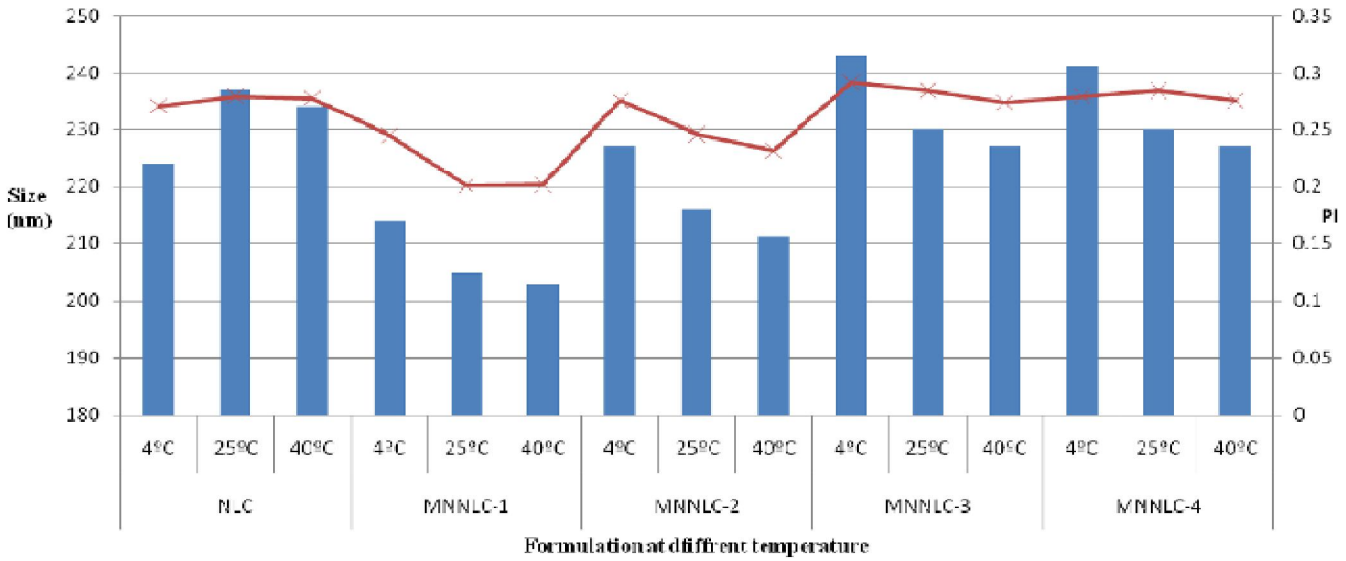

Fig. 2: Particle size parameters and Polydispersity Index (PI) of NLC formulations stored at different temperatures and obtained one month after production.

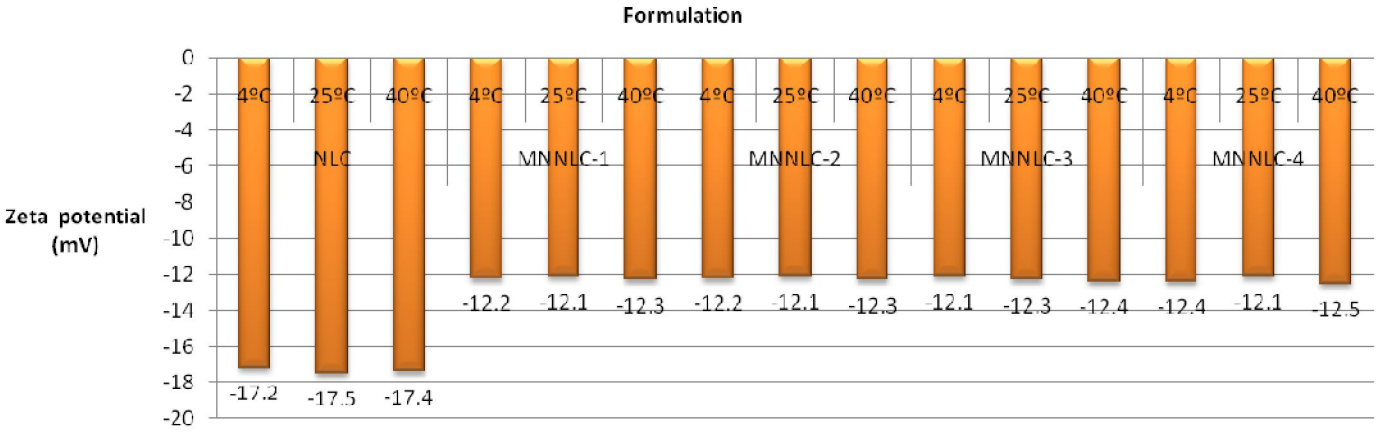

Fig. 3: Zeta potential of NLC formulations stored at different temperatures and obtained one month after production.

The polymorphic modifications of Dynasan 116 and MN have been investigated by DSC. The observed results do not support a crystalline character of MN, on the contrary reveal that the drug is dissolved in the melted lipid. Based on the production process, the physical mixtures were heated from 25ºC to 85ºC to give MN possibility to dissolve to its maximum solubility, and then the mixtures were cooled in order to recrystallize. This procedure imitates the production process of the lipid nanoparticles. Then the mixtures were heated a second time. Before tempering the mixture of Dynasan 116, Miglyol 812 and MN, the presence of the stable polymorph β of the lipid was hardly detected, while after tempering the presence of β form was recorded at approximately 63ºC. Before tempering, the heating curve revealed a less pronounced shoulder, which corresponds to the β' modification of tripalmitin. After tempering no more shoulder was visible being substituted by a well defined small peak at approximately 61.5ºC. The main peak in both curves corresponds to the stable β modification. The influence of Miglyol 812 was also observed during the cooling process in both curves.

Concerning the cooling curves, the peak recorded between 40ºC and 25ºC both before and after tempering shows the presence of Miglyol 812. The difference of shape between them is due to the presence of well defined polymorphic modifications. The calculated melting enthalpy of the lipid fraction in the mixtures shows little difference in comparison to the melting enthalpy of the bulk Dynasan 116. Based on this observation, it can be stated that all mixtures might be preferentially in the β' modification with an onset temperature higher than 40ºC. This is the main pre-requisite for preparation of lipid nanoparticles for topical drug delivery. Taking into account that the lipid particle matrix should be in the solid state at skin temperature the selected mixtures seem to be appropriated for the preparation of MN-loaded NLC. The endotherms recorded in Fig. 4 Show the crystals of Miglyol 812 (small shoulder) of the main peak of the cooling scan. Shape and surface morphology of the NLC prepared with optimized parameters was observed by scanning electron microscopy. The study revealed that most of the NLC were fairly spherical in shape, the surface of the particle showed a characteristic smooth surface.

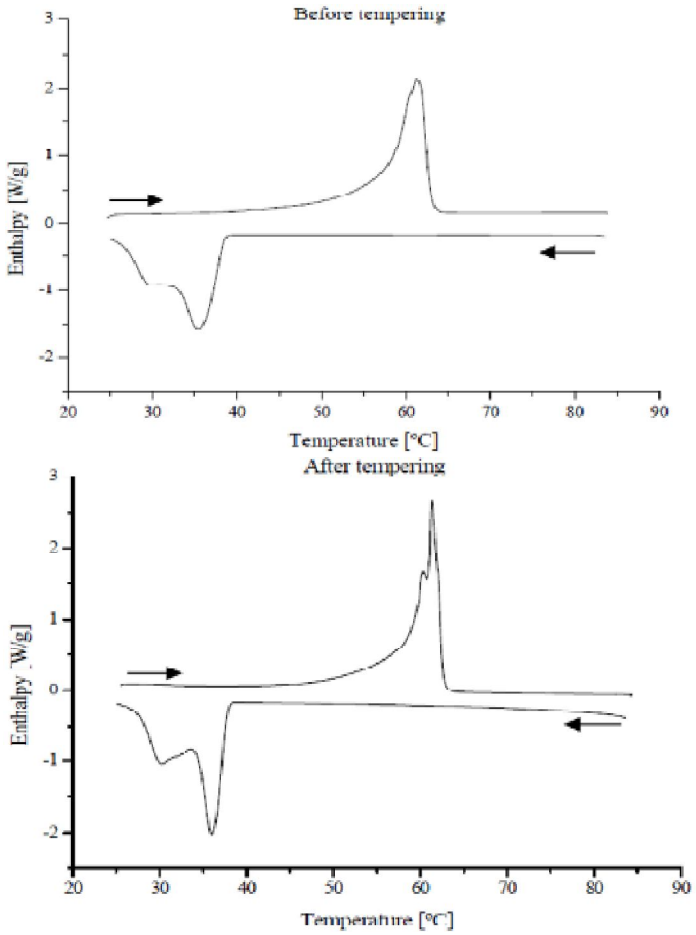

Fig. 4: DSC patterns of physical mixtures of Dynasan 116, Miglyol 812 and miconazole nitrate (69.3+29.7+1) recorded before (upper) and after (lower) tempering the mixture under heat exposure (90ºC) for 1 hr.

Characterization of NLC-Based Hydrogel

The prepared semi-solid systems showed a white appearance after dispersing the lipid nanoparticles in the hydrogels, which was maintained during the storage time at three different temperatures. A gel loaded with MN-NLC dispersion was white in color and odorless with smooth appearance. The pH of the MN-loaded NLC bearing hydrogel was determined using a Digital pH meter, standardized using pH 4.0 and 7.0 standard buffers before use. The pH was found to be 5.6± 0.4 for NLC-based semi-solid formulation which is acceptable for topical applications. The prepared semi-solid systems showed a white appearance after dispersing the lipid nanoparticles in the hydrogels, which was maintained during the storage time at three different temperatures. The data depicted in Table II revealed that the values of the ZP of MN-loaded NLC increased significantly. Particles remained negatively charged after their entrapment in the gel network a least for three months of storage at different temperatures. The decrease of the ZP values during storage time was not significantly relevant for affecting the physical stability of lipid nanoparticles. In fact, no particle aggregation has been obtained, as observed by the particle size results. The mean particle size obtained by PCS remained lower than 250 nm. The PI remained lower than 0.500 after 3 months of storage. These results are in agreement with the theory, which says that increased ZP provides increased stability by electrostatic repulsion. Thus, no size increase should occur. The increase of ZP values can be explained by adsorption of negatively charged Carbopol molecules onto the surface of the lipid nanoparticles (Fig.5).

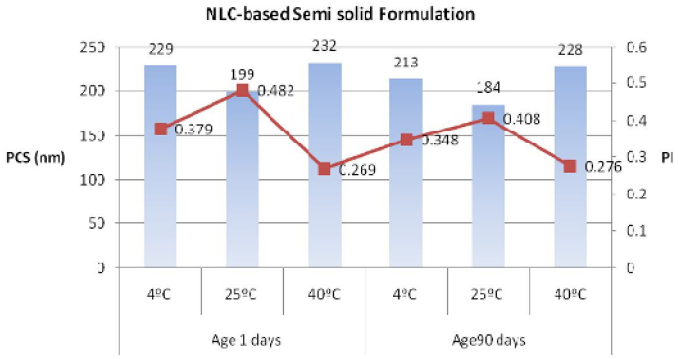

Fig. 5: Particle size and PI parameters of NLC-based semi-solids after one day and three months (90 days) of storage at 4ºC, 25ºC and 40ºC

The rheological properties of carbomer gels have been characterized in several studies (Liu *et al.*, 2007). The focus of the present investigation was the rheological behavior of such gels when lipid nanoparticles are entrapped into their network. According to this, analysis has been performed for NLC-based formulations and Fig. 6 shows, respectively, the obtained results recorded after one week of storage at 4ºC, 25ºC and at 40ºC.

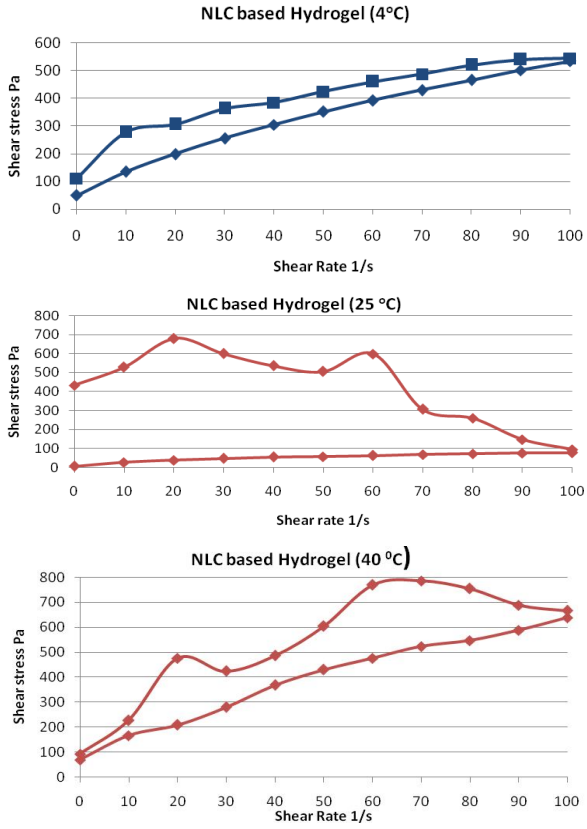

Fig. 6: Shear rate [1/s] versus shear stress [Pa] of NLC-based semi-solid formulations obtained after one week of storage at three different temperatures. *values represent mean ±SD (n=3)

In the range of shear rates studied in this work, the shear stress was not proportional to the shear rates in NLC systems. The characteristic concavity of the rheogram toward the shear rate axis indicates that all developed formulations exhibited pseudoplastic flow. This pseudoplasticity results from a colloidal network structure that aligns itself in the direction of shear, thereby decreasing the viscosity as the shear rate increases (Martin A. *et al.*, 1993). During all experiments, the temperature has been accurately maintained at 20±0.1ºC using a thermostated water bath. It is important that the temperature does not change during the rheological determination to avoid obtaining false positive results in the test for thixotropy. From Fig.6 it can be stated that all systems show thixotropy, which may be defined as an isothermal and comparatively slow recovery, on standing of a material, of a consistency lost through shearing. In complex systems such as NLC-loaded hydrogels in which a loose network connects together the sample, thixotropy proceeds from structural breakdown and re-aggregation. The incorporation of aqueous NLC dispersions into Carbopol hydrogels affects the texture of the developed formulations in terms of adhesiveness, consistency and gel strength. The adhesiveness of freshly prepared pure Carbapol 934P hydrogels has been compared to NLC-based semi-solid formulations (Fig.7).

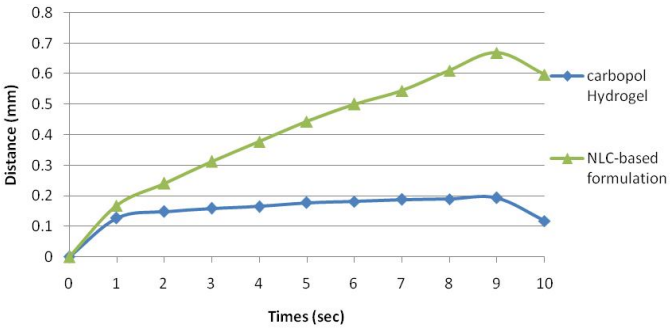

Fig. 7: Adhesiveness patterns of Carbapol 934P gel obtained on day 0, in comparison to freshly prepared carbopol hydrogel and NLC-based semi-solid formulations. *values represent mean ±SD (n=3)

The analysis of the hydrogels texture shows the decrease of adhesive properties of polyacrylate hydrogels with the presence of lipid nanoparticles. NLC-based formulations have shown to be more adhesive. This observation is in good agreement with the assumption that swelling is not interrupted by the water insoluble lipid nanoparticles dispersed in the gel network. The adhesiveness was also evaluated according to the storage temperatures (4ºC, 25ºC and 40ºC) of NLC-based semi-solid formulations. Fig. 8 show the obtained results after one week of storage. NLC-based semi-solid formulations stored at 40ºC were more adhesive than those stored at 4ºC. As reported previously NLC-based semi-solid formulations stored at 25ºC showed the highest hysteresis loop, i.e. area of thixotropy, in the flow curves. This means that under these conditions the systems are more sensitive to shear deformation, which might be related to the lowest adhesive properties in comparison to the ones stored at higher (40ºC) and lower (4ºC) temperatures.

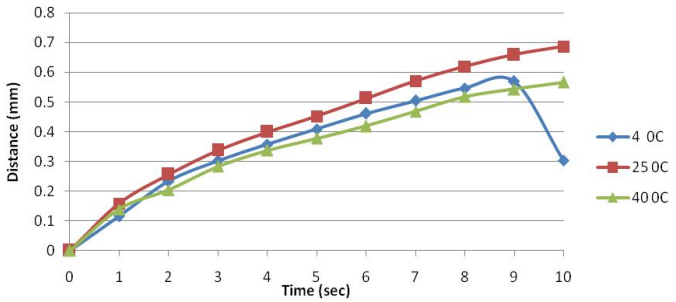

Fig. 8: Adhesiveness patterns of NLC-based semi-solid formulations obtained after one week of storage at three different temperatures. *values represent mean ±SD (n=3)

A test for measuring the consistency of NLC-based semi-solid formulations has been developed by adapting a texture-profile analyzer to pull the test sample placed on the base of the instrument upwards from 0 mm to 2 mm, and downwards from 2 mm to 0 mm. The force (N) needed to lift the sample probe to the pre-set distance has been recorded. At a distance of 2 mm, NLC-based formulations stored at 25ºC recorded a force of 0.1558 N, emphasizes the higher consistency of NLC-based formulations. Comparing those values with the consistency of pure Carbopol hydrogels, i.e. without lipid nanoparticles (0.3702 N); it confirms that both consistency and adhesiveness of hydrogel decreases with the incorporation of lipid nanoparticles. The penetration force (N) needed to break the sample placed in the base of the instrument to a pre-set depth of 2 mm has been recorded and translated as the gel strength. The higher value recorded for the penetration force was obtained for the Carbapol 934P gels stored at 4ºC, followed by those stored at 40ºC and the lowest was observed for gels stored at room temperature. This means that the lowest gel strength was measured in the samples stored at 25ºC, those which showed lower adhesiveness after incorporation of aqueous NLC dispersions. Table III shows the areas (N.sec) of the curves obtained after one week of storage applying the same test. Comparing the obtained areas at 25ºC, NLC-based formulations showed higher gel strength values than Carbapol 934P gels. At 4ºC and at 40ºC the values were not significantly different. These results emphasize the fact that gel strength is not directly related to adhesiveness and consistency of hydrogels containing lipid nanoparticles.

Table. 3: Zeta potential and particle size parameters of NLC-based semi-solids formulation after one day and three months (90 days) of storage at 4ºC, 25ºC and 40ºC.

Size parameters	Age (Days)	Temperature ºC	NLC-based semi-solid formulations
ZP (mV)	01	4	-32.1±0.2
		25	-31.5±0.6
		40	-26.8±0.4
	90	4	-27.3±0.1
		25	-25.9±0.9
		40	-21.2±0.3
PCS (nm)	01	4	225.6±1.4
		25	190.4±3.8
		40	239.8±6.2
	90	4	213.4±4.8
		25	184.2±9.3

		40	228.1±1.7
		4	0.319±0.03
	01	25	0.388±0.01
		40	0.264±0.04
PI		4	0.348±0.06
	90	25	0.408±0.08
		40	0.276±0.02

To evaluate the drug release pattern, the dialysis bag method was used where a mixture of methanol: PBS (pH6.4) (30:70) was used as the diffusion medium. Different release patterns were observed from the MN-NLC Gel and Marketed gel. An initial rapid release was observed in the case of Marketed gel, whereas MN-NLC Gel depicted a slow initial release with a lag time of 0.5 h and 1 h, respectively (Fig.9).

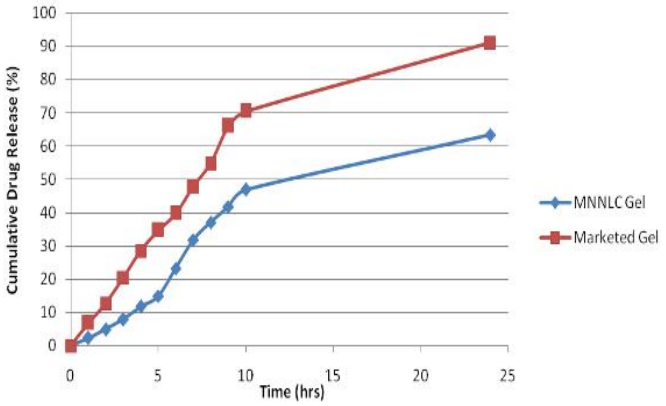

Fig. 9: In vitro release studies of MNNLC Gel and Marketed gel. *values represent mean ±SD (n=3).

MN-loaded NLC-bearing hydrogel formulations depicted better controlled drug release profile for a prolonged period, suggesting their applicability in topical drug delivery. Both burst releases as well as sustained release are of interest for dermal application. Burst release is useful to improve the penetration of drug and for faster onset of action, while a sustained release supplies the drug over a prolonged period of time. MN-loaded NLC bearing hydrogel formulations depicted better controlled drug release profile for a prolonged period, suggesting their applicability in topical drug delivery. The release data were fitted to various kinetic models in order to calculate the release constant and regression coefficients (R^2). Among the models tested, the drug release profiles for the MN-NLC Gel were best fitted with Hixon crowell cube root model based on the regression coefficients (R^2 of 0.95 and 0.97 respectively). The diffusion exponent (n) values for both batches were within 0.4 which indicated that drug release mechanism followed pure Fickian diffusion. The goal of a permeation study is to compile a kinetic profile that reflects how the concentration of an active ingredient changes in time as it diffuses through the skin. The ex vivo permeation of MN from MN-NLC Gel and Marketed gel was evaluated using Franz diffusion cell. The mean cumulative amount diffused Q (mg/cm^2) at each sampling time point was calculated (Fig.10);

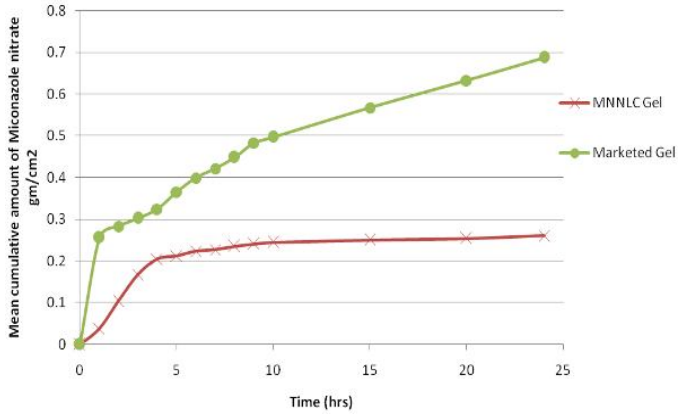

Fig. 10: Ex Vitro release of Miconazole nitrate from MNNLC Gel and Marketed gel. *values represent mean ±SD (n=3).

high amount of MN release was facilitated through abdominal skin of rats from marketed gel (0.687 mg/ cm^2) of MN than MN-NLC Gel (0.260 mg/cm^2). The deposition potential of the gels was assessed at the end of 24 h after application. MN 'percentage permeated', 'percentage deposited' and 'percentage remained on the skin' were calculated. Highest permeation was obtained for marketed gel, which can be attributed to its high flux value. MN-NLC gel produced significantly higher deposition of MN in skin (67±0.13%) than marketed gel (36±0.34%) as shown in Table IV. NLC gel indicates that approximately 21% of the drug is still remained on the skin at the end of 24 h, suggesting that the permeation and deposition values might present an altogether different picture at a time period beyond 24 h. However, studies need to be carried out beyond 24 h to validate this hypothesis. These observations confirmed the earlier findings about nanoparticles being deposited in the skin, thus acting as a depot to give sustained release. Nanoparticulate gel shows higher localization of MN in skin as compared to conventional gel. Thus, drug-localizing effect in the skin seems possible with novel colloidal particulate drug carriers such as NLC. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin (Fang *et al.*, 2008).

Table. 4: Recorded force (N) during the consistency test and Areas (N.sec) of NLC-based semi-solid formulations stored at three different temperatures for one week.

Formulations		Recorded force (N) at different distances (mm)			Areas (N.sec) obtained after one week of storage
Sample ID	Storage temp.	0 mm	2 mm	0 mm	
Carbapol 934P	4ºC	0.0243	0.1492	-0.0464	0.073
	25ºC	0.0235	0.3702	-0.0538	0.873
	40ºC	0.0238	0.1324	-0.0258	0.264
NLC-based semi-solid formulations	4ºC	0.0236	0.0783	-0.0139	0.219
	25ºC	0.0249	0.1558	-0.0144	0.279
	40ºC	0.0235	0.0931	-0.0142	0.265

CONCLUSION

The MN-loaded NLC could be fabricated with the help of a modified HPH method and successfully incorporated into hydrogel for topical application. The *In vitro* and *Ex vitro* skin permeation data indicate that MN-loaded NLC bearing hydrogel provides sustained release of MN. The obtained results reflect the potential of NLC as a carrier for topical administration of MN which is demonstrating greater drug deposition into skin. In conclusion, the developed systems are promising alternative drug carriers for topical pharmaceutics.

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