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SOLID LIPID NANOPARTICLES (SLN) –BASED HYDROGEL FORMULATION FOR TOPICAL DELIVERY OF MICONAZOLE NITRATE

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ABSTRACT

The aim of this study was to prepare and evaluate gels incorporating solid lipid nanoparticles (SLNs) of Miconazole nitrate (MN) for systemic delivery of the active after topical application. Solid lipid nanoparticles designed for topical administration of MN, were prepared by the hot high pressure homogenization technique. MN-SLN was characterized for particle size, entrapment efficiency and SEM. The lipid nanoparticles were incorporated in gels for convenient topical application and were evaluated for particle size, Rheological analysis Texture analysis , *In vitro* drug release studies and *Ex Vitro* skin permeation Studies. The preparation of aqueous SLN dispersions with a mean particle size lower than 250 nm has been obtained with uniform size distribution (PI < 0.400). 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- SLN Gel depicted a slow initial release with a lag time of 0.5 h and 1 h, respectively. Ex-vivo tests showed that SLN were able to control the drug release through the stratum corneum; the release rate depended upon the lipid content on the nanoparticles. High amount of MN release was facilitated through abdominal skin of rats from marketed gel (0.687 mg/cm^2) than MN-SLN Gel (0.260 mg/cm^2). It was observed that MN-loaded SLN - bearing hydrogel was more efficient in the treatment of candidiasis. Results indicate that MN-loaded SLN-bearing hydrogel provides a sustaining MN topical effect as well as faster relief from fungal infection.

Keywords: **Miconazole nitrate; Solid lipid nanoparticles (SLN); Topical gels; Topical delivery**

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INTRODUCTION

During the past several years, solid lipid nanoparticles (SLN) began to act as a topical carrier not only for pharmaceutical molecules, but also for cosmetic products. Solid lipid nanoparticles have emerged as an alternative to liposomes due to various advantages such as improved physical stability, low cost compared to phospholipids and ease of scale-up and manufacturing[1,2]. Compared with conventional carriers such as cream, tincture and emulsion, SLN combine their advantages such as controlled release, in vivo good toleration and protection of active compounds[3]. Especially, SLN can favor drug penetration into the skins[4, 5], maintain a sustained release to avoid systemic absorption[6], act as a UV sunscreen system[7] and reduce irritation[8, 9].Miconazole nitrate (MN) is a broad-spectrum antifungal agent of the imidazole group[10]. 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[11]; 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's poor skin-penetration capability presents a problem in the treatment of cutaneous diseases by topical application.The stratum corneum is the target organ of anti-mycotic treatment, and the improvement of local bioavailability leads to enhanced efficacy of the applied formulation. For effective treatment, the drug must be delivered in sufficient concentration to the site of infection. 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[12, 13] . Several reports have described the potential use of liposomes to topically deliver drugs into the deep layers of the skin.The aim of this study was to develop topical gels containing SLN dispersions loaded with Miconazole nitrate. The SLN were prepared by hot high-pressure homogenization method. Nanoparticles were characterized in terms of particle size, morphology, encapsulation efficiency and crystalline structure. The influence of the SLN 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) was obtained from Lipoid GmbH, Germany. 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.

Partitioning Behavior of Miconazole Nitrate in Various Solid Lipids

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[14]. 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.

Preparation of SLN Dispersion

The SLN dispersions were prepared using hot high pressure homogenization method. Table1 reports the composition of the prepared SLN dispersions. The lipid was heated up to 90 ºC and MN was added to the melted Dynasan 116. 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 (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 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 SLN. Each preparation was carried out in triplicate.

Table I

Composition of various SLN formulations (%, m/m). Particle size, Polydispersity Index (PI), Zeta potential and % Drug entrapment parameters of different SLN formulations obtained immediately after production

Formulation code	Composition				Parameters			
	MN	Dynasan 116	Poloxamer 188	Water	Particle size (nm)	Polydispersity Index (PI)	ZP (mV)	% Drug Entrapment
SLN	-	19.0	5.0	100	243.8±325	0.392±0.012	-26.3±0.2	-
MNSLN-1	1.0	19.0	5.0	100	215.1±2.18	0.325±0.002	-15.1±0.4	94.62±5.08
MNSLN-2	1.0	19.0	2.5	100	228.2±2.08	0.342±0.002	-13.7±0.2	91.82±4.53
MNSLN-3	1.0	18.0	5.0	100	235.6±2.53	0.351±0.003	-14.2±0.3	84.77±1.38
MNSLN-4	1.0	18.0	2.5	100	237.1±2.51	0.353±0.001	-15.5±0.8	87.03±7.15

Characterization of SLN Dispersion

Particle size and zeta potential determination

Particle size and size distribution measurements of the SLN 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 SLN-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 (SEM)

The morphology (shape and surface characteristics) of SLN 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.The condenser lens position was maintained between 4.4 to 5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD=39mm.

Dug entrapment efficiency

The amount of encapsulated MN was calculated by subtracting the free amount of the drug from MN-SLN 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 fficiency (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 and Dynsyan 116 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 SLN-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 SLN suspension. Aqueous SLN 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 SLN-based semi-solid formulations

Composition	Carbapol based Hydrogel formulation	SLN-based semi-solid formulation
Miconazole nitrate	1.00%	1.00%
Dynasan 116	-	9.00%
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%

The physical stability of SLN in the original dispersions and after their incorporation into hydrogel was monitored by particle size examination to investigate the possible aggregation of particles. The stability was determined immediately after the preparation of SLN gels. The SLN based gels dispersions were characterized for their physicochemical properties such as color, odor, pH and stability.

Rheological measurement

In the present work, the rheological analysis of SLN 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.

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 SLN-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

Ex vitro permeation of MN from SLN 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 Jasco UV-VIS spectrophotometer at 272 nm and mean cumulative amount diffused Q (mg/cm^2) at each sampling time points 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 SLN

For the current study, SLNs 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 miconazole nitrate 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 SLN 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. In the present investigation, five different SLN formulations were produced by hot high pressure homogenization. Various parameters were optimized by varying one parameter while keeping others constant.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[15,16]. Therefore, the particle size parameters and the surface electrical charge (zeta potential, ZP) have been evaluated immediately after production of the systems (Table 1), and during one month of storage at three different temperatures (4ºC, 25ºC and 40ºC). The preparation of aqueous SLN 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. As reported in Table 1 in formulation, a relatively uniform size distribution has been obtained (PI < 0.400). The incorporation of MN decreased the electrical charge at the surface of SLN 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. 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 SLN for topical delivery of antifungals was 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. Yields of production obtained were always relatively high and were 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. MNSLN-1 and MNSLN-2 show of about 90% while samples MNSLN-3 and MNSLN-4 show less % entrapment efficiency. SLN are prepared from solid lipids or blends of solid lipids. After hot high pressure homogenization the lipid recrystallizes in higher energy modifications, i.e. α and β-forms[17]. During storage, these polymorphic forms can transform to a more ordered modification (β), which is characterized by a lower energy modification and a higher degree of crystallinity. These parameters are strongly correlated with drug incorporation[18]. Drug expulsion in SLN can occur when the lipid matrix transforms from high energy modifications, characterized by the presence of many imperfections, to the β-modification forming a perfect crystal with no room for guest molecules. A high amount of drug could be incorporated in nanoparticle dispersion. Such high incorporation was possible because of lipid solubility of MN.Shape and surface morphology of the SLN prepared with optimized parameters was observed by scanning electron microscopy. The study revealed that most of the SLN were fairly spherical in shape, the surface of the particle showed a characteristic smooth surface.Figure 1 shows the results of DSC analyses of EN (raw material), bulk material of Lipid (Dynasan 116) and drug-loaded SLN batch (SLN 1). For the bulk material of Dynasan 116, the melting process took place with maximum peak at 63.47°C and MN shows peak at 186°C shown in Fig. 1 (A, B). DSC thermogram of MNSLN-1 showed an endotherm at 62°C, which can be attributed to melting of Dynasan 116 in SLN Fig.1 (C).

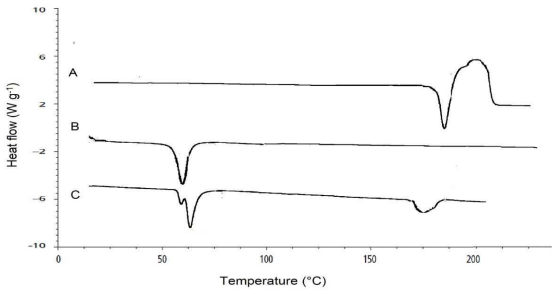

Figure 1
Differential scanning thermograms of bulk material of miconazole nitrate (A), Dynasan 116 (B), and MNSLN-1 (C)

DSC measurements offer a close look at the melting and crystallization behaviour of crystalline material like lipid nanoparticles[19]. In all cases, the presence of Dynasan 116 endothermic peak and the disappearance of the EN melting endotherm were observed.

However, the curve C exhibite a weak broad exotherm between 175°C and 190°C, probably due to dissolution of drug crystals into the molten lipid. Therefore, the differences in the shape of the calorimetric curves of drug-loaded SLN could be attributed to either the drug amorphization or its dissolution in the molten carrier during DSC scan.

Characterization of SLN-Based Hydrogel

The lipid nanoparticles were incorporated in gels by using Carbapol. 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-SLN dispersion was white in color and odorless with a smooth appearance. The pH of the MN-loaded SLN 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 SLN -based semi-solid formulation which is acceptable for topical applications[25].The data depicted in Table 3 revealed that the values of the ZP of MN-loaded SLN increased significantly. Particles remained negatively charged after their entrapment in the gel network atleast 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. Advantages of the use of nanoparticles in comparison to microparticles is the increase of surface area which allows therefore, the increase in contact area between the skin and the system and the increase of drug partition between both phases and bioavailability. For long term stability studies the particle size distribution of SLN entrapped in hydrogels needs to be monitored and notify any changes on storage. The particle size analysis may often detect a potentially unstable formulation long before any other parameter changes markedly. Lipid nanoparticles may grow or suffer aggregation as a gel network breaks down on storage, thus allowing Brownian motion to bring the particles into contact so that they aggregate.

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

Size parameters	Age (Days)	Temperature (ºC)	SLN-based semi-solid formulations
ZP (mV)	01	4	-25.3±0.1
		25	-31.4±0.4
		40	-29.9±0.8
	90	4	-20.8±0.2
		25	-26.5±0.7
		40	-23.3±0.1
PCS (nm)	01	4	238.5±2.5
		25	207.3±9.7
		40	245.7±1.7
	90	4	229.2±4.7
		25	199.1±0.9
		40	232.9±2.3
PI	01	4	0.352±0.04
		25	0.429±0.01
		40	0.258±0.02
	90	4	0.379±0.06
		25	0.482±0.03
		40	0.269±0.09

The rheological properties of carbomer gels have been characterized in several studies [20,21]. 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 SLN-based formulations and Fig.2 shows, respectively, the obtained results recorded after one week of storage at 4ºC, 25ºC and at 40ºC.

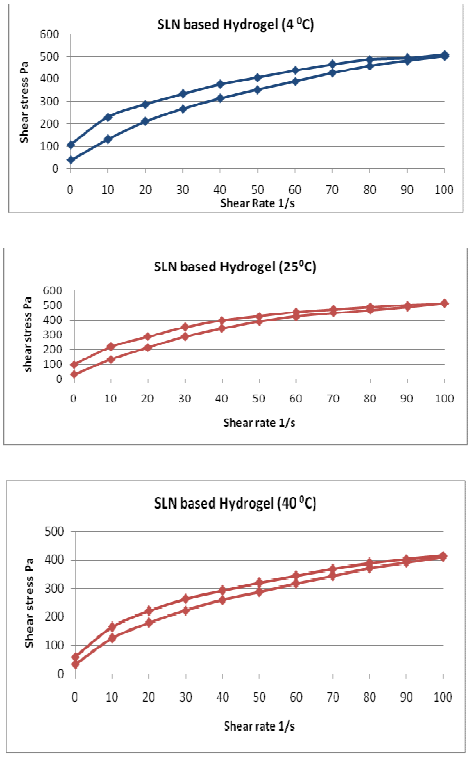

Figure 2

Shear rate [1/s] versus shear stress [Pa] of SLN-based semi-solid formulations obtained after one week of storage at 3 different temperatures. Data are means ± s.d., n = 3

In the range of shear rates studied in this work, the shear stress was not proportional to the shear rates in systems (SLN). 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. 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 Figs.2 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 SLN-loaded hydrogel in which a loose network connects together the sample, thixotropy proceeds from structural breakdown and re-aggregation. The behaviour of SLN-based semi-solid systems was more dependent on the storage temperature. The incorporation of aqueous SLN dispersions into Carbopol hydrogel affects the texture of the developed formulations in terms of adhesiveness, consistency and gel strength. The adhesiveness of freshly prepared pure Carbapol 934P hydrogel has been compared to SLN-based semi-solid formulations (Figure 3). The analysis of the hydrogel texture shows the decrease of adhesive properties of polyacrylate hydrogel with the presence of

lipid nanoparticles. SLN-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 SLN-based semi-solid formulations. Figure 4 shows the obtained results after one week of storage. SLN-based semi-solid formulations stored at 40ºC were more adhesive than those stored at 4ºC. As reported previously SLN-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.

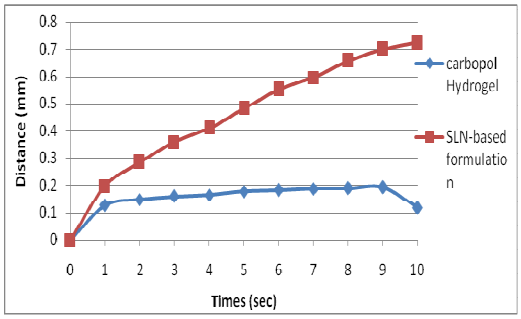

Figure 3

Adhesiveness patterns of Carbapol 934P gel obtained on day 0, in comparison to freshly prepared SLN- based semi-solid formulations. Data are means ± s.d., n = 3

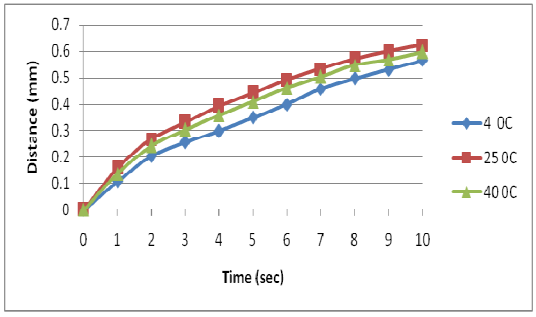

Figure 4

Adhesiveness patterns of SLN-based semi-solid formulations obtained after one week of storage at three different temperatures. Data are means ± s.d., n = 3

A test for measuring the consistency of SLN-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, SLN-based formulations stored at 25ºC recorded a force of 0.0688 N, emphasizes the higher consistency. Comparing those values with the consistency of pure Carbopol hydrogel, i.e. without lipid nanoparticles (0.3702 N), it confirms that both consistency and adhesiveness of hydrogel decreases with the incorporation of lipid nanoparticles.Gel strength analysis has been performed in the Carbapol 934P gels without lipid nanoparticles once stored at three different temperatures. 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 SLN dispersions. The areas (N.sec) of the curves obtained after one week of storage applying the same test. Comparing the obtained areas at 25ºC, SLN - 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 hydrogel containing lipid nanoparticles (Table 4).

Table 4

Recorded force (N) during the consistency test of the SLN -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
SLN-based semi-solid formulations	4ºC	0.0233	0.0598	-0.0063	0.086
	25ºC	0.0247	0.0688	-0.0298	1.739
	40ºC	0.0241	0.0563	-0.0049	0.265

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 MNSLN Gel and Marketed gel. An initial rapid release was observed in the case of Marketed gel, whereas MNSLN Gel depicted a slow initial release with a lag time of 0.5 h and 1 h, respectively (Figure 5). MN-loaded SLN-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 MNSLN 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. MN-loaded SLN-bearing hydrogel formulations depicted better controlled drug release profile for a prolonged period, suggesting their applicability in topical drug delivery [22].

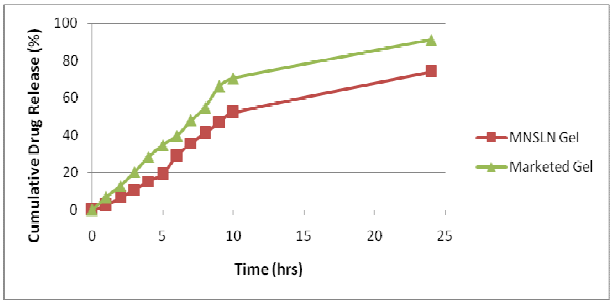

Figure 5

In vitro release studies of MNSLN Gel and Marketed gel. Data are means ± s.d., n = 3

The ex vivo permeation of MN from MNSLN 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; high amount of Miconazole nitrate release was facilitated through abdominal skin of rats from marketed gel (0.687 mg/ cm^2) of Miconazole nitrate than MNSLN Gel (0.260 mg/cm^2) (Figure 4). MN-SLN gel produced significantly higher deposition of Miconazole nitrate in skin ((55%±0.09) than marketed gel (36%±0.34) (Table 5). 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 SLN. 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[23]. The release of active substances from the lipid matrix is influenced by the crystal structure of the lipid molecules. Drug penetration into certain layers of the skin can be achieved using SLN as a consequence of the creation of a supersaturated system[24].

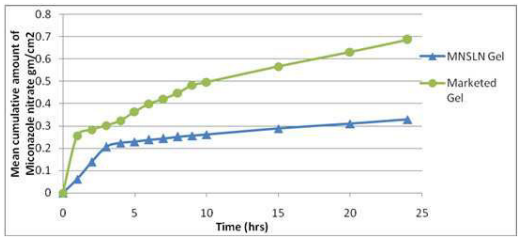

Figure 6
Ex Vitro release of Miconazole nitrate from MNSLN Gel and Marketed gel

Table 5
Mean Amount of Miconazole Nitrate Deposited into abdominal skin of rats

Test formulation	Amount of MN Deposited skin (%) mean (±SD)	Receptor compartment (%) mean (±SD)	Remained on the skin (%) mean (±SD)
Marketed Gel	36±0.34	24±0.17	50±0.09
sMNSLN Gel	55±0.09	18±0.31	27±0.24

CONCLUSION

The MN-loaded SLN could be fabricated with the help of a hot high pressure homogenization method and successfully incorporated into hydrogel for topical application. The in vitro and ex vitro data indicate that MN-loaded SLN bearing hydrogel provides an excellent adhering and sustained release of MN. The obtained results reflect the potential of SLN as a carrier for topical administration of MN which is demonstrating greater drug deposition into the skin. In conclusion, the developed systems are promising alternative drug carriers for topical pharmaceutics.

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