Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 407 - 416

**Research Article** **Pharmaceutics**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# PREPARATION AND EVALUATION OF CLOTRIMAZOLE NANOSTRUCTURED LIPID CARRIER FOR TOPICAL DELIVERY

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## ABSTRACT

The purpose of this study was to prepare Clotrimazole loaded nanostructured lipid carriers (CM-NLC) effective for topical delivery of clotrimazole (CM) by hot homogenization method. Stearic acid as solid lipid, Oliec acid as Liquid lipid, combination of four types of surfactants: Tween 80, Poloxamer-188, Sodium lauryl sulphate (SLS) and Lecithin were used to stabilize NLC dispersion. CM-NLC were characterized by laser diffraction and all the NLC dispersions exhibited average size between 200 and 300 nm. Transmission electron microscopy studies showed that the CM-NLC formulation had a spherical shape. All the formulations had high entrapment efficiency. DSC analysis showed that CM was dispersed in NLC in an amorphous state. The penetration of Clotrimazole from the CM-NLC formulations into cadaver skins was evaluated *in vitro* using Franz diffusion cell. The CM-NLC formulations could significantly increase the accumulative uptake of CM in skin over the marketed gel and showed a significantly enhanced skin targeting effect. These results indicate that the studied CM-NLC formulation with skin targeting may be a promising carrier for topical delivery of clotrimazole (CM).

**KEYWORDS:** **Clotrimazole; Nanostructured lipid carriers ; Skin targeting; Topical delivery**

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# 1. INTRODUCTION

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been looked upon as promising carriers for presenting several attractive features for transdermal drug delivery. SLN are identical to an oil-in water emulsion, except that the liquid lipid (oil) portion of the emulsion is replaced by a solid lipid having a mean photon correlation diameter (PCS) ranging between 80 and 1000 nm[1].The major advantage of SLN is the possibility of production on large industrial scale[2]. However, depending on the drug some potential problems can occur, such as drug leakage during storage and insufficient total drug load. To overcome the limitations of SLN, nanostructured lipid carriers have been developed. The later consist of a solid lipid matrix with a high content of liquid lipid[3]. As a vehicle for controlled release of active substances and targeting to skin layers, nanodisperse systems such as liposomes, nanoemulsions, and lipid nanoparticles are gaining more and more importance. Solid lipid nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) are the new generation of nanoparticulate active substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use. Compared with polymeric nanoparticles, lipid nanoparticle has lower toxicity because of the absence of solvents in the production process and also relatively low cost of the excipients. NLC combine their advantages such as controlled release, biodegradable and protection of active compounds. Especially, NLC can favor drug penetration into the skins, maintain a sustained release to avoid systemic absorption, act as a UV sunscreen system, reduces irritation[4,5]. SLN represents a particulate system, which can be produced by one of the following techniques, namely, high pressure homogenization[6], microemulsion template technique[7], solvent emulsification evaporation technique[8], solvent displacement technique[9], solvent emulsification diffusion method[10], phase inversion[11] and a very recently introduced membrane contractor technique[12]. Stratum corneum is the main barrier in the percutaneous absorption of topically applied drugs. Small size and relatively narrow size distribution of SLN permit site-specific delivery to the skin[3]. SLN have high affinity to the stratum corneum, and therefore an enhanced bioavailability of the encapsulated material to the skin is achieved. SLN enhance the penetration and transport active substances particularly lipophilic agents and thus intensify the concentration of these agents in the skin[13]. Recently, the research activities on SLN and NLC has gradually focused on the cosmetic and topical product, NLC as a topical carrier were used for topical delivery of several drugs including clotrimazole, prednicarbate and betamethasone 17-valerate and SLN was reported to have a skin targeting potential[14,15]. SLN was found to have a skin targeting which can result in a high accumulation of podophyllotoxin in skin[16]. The skin targeting of NLC for topical delivery aroused our interest. In this work, NLC was used for topical delivery of clotrimazole and the long-term aim is to explore a novel formulation with skin targeting effect for the treatment of fungal infections. Clotrimazole is an imidazole anti-fungal agent, which is clinically administered both in orally and topical formulations. Clotrimazole is used as a highly lipophilic model drug in the present study. This study investigates the formulation and characterization of clotrimazole NLC (CM-NLC). The lipid nanoparticles were incorporated in gels for convenient topical application and were evaluated for *in vitro* skin penetration and *in vivo* skin hydration.

# 2. MATERIALS AND METHOD

### *2.1. Materials*

Stearic acid, Oleic acid, Tween 80, Sodium lauryl sulphate and Carbopol 940 were provided by LOBA CHEMIE.Poloxamer 188 was provided by BASF and Lecithin was kindly gifted by Sigma. Clotrimazole was obtained from Sun Pharma, Mumbai. All

other chemicals were of reagent grade and used without further purification.

### *2.2. Preparation of SLN*

The lipid phase (stearic acid and Oleic acid in ratio 7:3) was melted with lecithin (with formulation A) and CM was dispersed in the lipid melt. The dispersion medium (ie, distilled water with surfactant) was heated to the temperature of 80°C and the hot lipid phase was emulsified in the dispersion medium by high speed stirring using Ultra-turrax T 25 (IKA-
Werke, Staufen, Germany) at 9,000 rpm for 5min. This dispersion was then subjected to high-pressure homogenization (HPH) using (Niro soavi, Italy) homogenizer at 500 bars and 5 cycles. The dispersion thus obtained was allowed to cool to room temperature, forming lipid nanoparticles by recrystallization of the dispersed lipid[17]. On the basis of physical stability the dispersion was optimized with different percent of surfactants as listed in table 1.

**Table 1**
***NLC dispersions of Clotrimazole with different % of lipid and surfactants***

| Sample | CM % | Stearic acid % | Oleic acid% | Lecithin % | Tween 80 % | Poloxamer 188 % | SLS % |
|---|---|---|---|---|---|---|---|
| A | 1 | 7 | 3 | 4 | | | |
| B | 1 | 7 | 3 | | | | 4 |
| C | 1 | 7 | 3 | | 4 | | |
| D | 1 | 7 | 3 | 1 | 1.5 | 1.5 | |
| E | 1 | 7 | 3 | 0.5 | 1.5 | 1.5 | 0.5 |

### *2.3. Characterization of CM-NLC*

The prepared NLC were characterized with respect to the particle size, shape, entrapment efficiency, crystallinity and stability study.

### *2.3.1. Particle size*

NLC dispersions were characterized for average particle size (z-average size) using Laser diffraction (Malvern Mastersizer 2000 SM, Malvern Instruments Corp) ) with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration..Polydispersity index was also determined, which is the measurement of width of the particle size distribution is given by $d_{0.9}$ , $d_{0.1}$ and $d_{0.5}$ are the particle size diameters determined at $90^{th}$,$50^{th}$ and $10^{th}$ percentile of particle undersized. All Analysis were carried out in triplicate.

### *2.3.2 Determination of CM entrapment in NLCs*

CM-NLC dispersion was separated by ultracentrifugation at 35,000 rpm (Beckmann instrument, Italy). The collected samples were added in chloroform and warmed to dissolve completely, and then it was extracted with dicloromethane (DCM) which dissolved only clotrimazole. The solution was filtered diluted with methanol and clotrimazole content was determined spectrophotometrically. EE was calculated by following formula

$$EE\% = \frac{\text{The amount of entrapped drug in SLN}}{\text{The amount of entrapped drug in SLN and free drug in dispersion}} \times 100$$

### *2.3.3 Determination of unentrapped clotrimazole*

Free drug expressed as percentage of the added drug remained untrapped in supernatant liquid which was obtained after ultra-centrifugation. Each estimation was repeated three times.

### *2.3.4. Transmission electron microscopy (TEM)*

Transmission electron microscopy was performed using JEOL 1010 (JEOL Ltd, Tokyo, Japan). One drop of nanoparticulate dispersion was placed on the grid, dried for 3 to 5 minutes, and drained on the filter paper. The grid was further dried by keeping it in the petri plate; then it was loaded in the transmission electron microscope, and areas were scanned for observation of nanoparticles. The picture was taken under the electron microscope.

### *2.3.5. DSC measurements*

Differential Scanning Calorimetry (DSC) on CM, CM-NLC and stearic acid was performed by Mettler-Toledo DSC $821^e$ (Columbus, OH) instrument, and an empty standard aluminum pan was used as reference. DSC scans were recorded at heating rate of 10°C/min in temperature range 30°-250°C.

### *2.3.6. Stability of the CM-NLC*

The chemical and physical stabilities of CM-NLC were evaluated at 2–8°C for 1 month via clarity, particle size, and drug content.

### *2.3.7. Preparation and evaluation of Clotrimazole NLC gel*

Carbopol 934 was used as a gelling agent for NLC dispersion. Different concentrations of Carbopol 934 ranging from 0.5 % to 1 % were used and concentration giving optimum rheological properties was used for further studies. Carbopol 934 (0.6%) was added to the nanoparticle dispersion under overhead stirring at 800 rpm. Stirring was continued till the carbopol got dispersed. The carbopol dispersion was neutralized using 0.05% (w/w) triethanolamine and the pH was adjusted to 6.0. The gel was allowed to stand overnight to remove entrapped air. CM-NLC gel was assayed by dissolving gel in chloroform and sample was analyzed by suitably diluting with methanol.

### *2.3.8. In Vitro Skin Penetration Studies*

Human cadaver skin from the abdominal region was used, after removing hair and subcutaneous fat tissue, was mounted on the Franz diffusion cell. Phosphate buffer pH 6.8 served as receptor fluid. A small quantity (0.1 g) of the gel was applied to the skin surface. At the end of 24 h, the skin was cut homogenized and extracted with methanol and suitably diluted and analyzed spectrophotometrically.

## RESULT AND DISCUSSION

### *3.1. Preparation of CM loaded Nanostructured Lipid Carrier Dispersion*

NLC dispersion of CM was prepared using different concentration and combination of surfactant as shown in Table 1.It indicates that Batch A was prepared using only Lecithin and was found to convert into gel soon at 4 $^0$C therefore its particle size could not be determined. Batch B and batch C were prepared with only ionic surfactant SLS and non ionic surfactant Tween 80 respectively which gives opalescent appearing NLC dispersion having stability of few days. Then, Combination of Non ionic surfactants along with lecithin was tried in batch D which gives Translucent NLC dispersion having fine stability but phase separation occurred in few weeks. If ionic and non-ionic surfactant were used in combination it gives transparent dispersion having good stability.

Hot Homogenization method was used to prepare CM-NLC dispersion which includes raising the temperature above melting point of lipid and then lowering it which coverts nano-emulsion into solid particles. New solid surface has immediately formed which requires extra emulsifiers to cover this surface. If this new naked surface is not immediately covered by surfactant it leads to flocculation under the effect of intermolecular wander wall forces. When lecithin was used alone (Batch A), it gets entrapped in lipid vesicle so unable to cover the naked solid surface leads to formation of gel. But, in subsequent batches it was seen that no gelation occurs and by using combination of ionic and non ionic surfactants gives stable CM- NLC dispersion. It can be explained that ionic surfactant (SLS) provide the electrostatic stabilization while non-ionic surfactant (Poloxamer-188 and Tween 80) gives steric stabilization to the NLC dispersion.

### *3.2. Particle Size Analysis and Entrapment Efficiency*

The particle size analysis of the nanoparticulate dispersion by laser diffraction using Malvern Mastersizer showed a mean particle size of 210 nm of optimized Batch E , as sample E showed maximum stability. Table 2 indicates particle size of all the batches in nanometer range. When single surfactant was used as in Batch B and C particle size was on higher side with less stability. After Blending four kind of surfactant (Batch E) particle size was decreased. Combinations of ionic and non ionic surfactant not only provide electrostatic and static stabilization to formulation but also decrease the particle size because of its synergism

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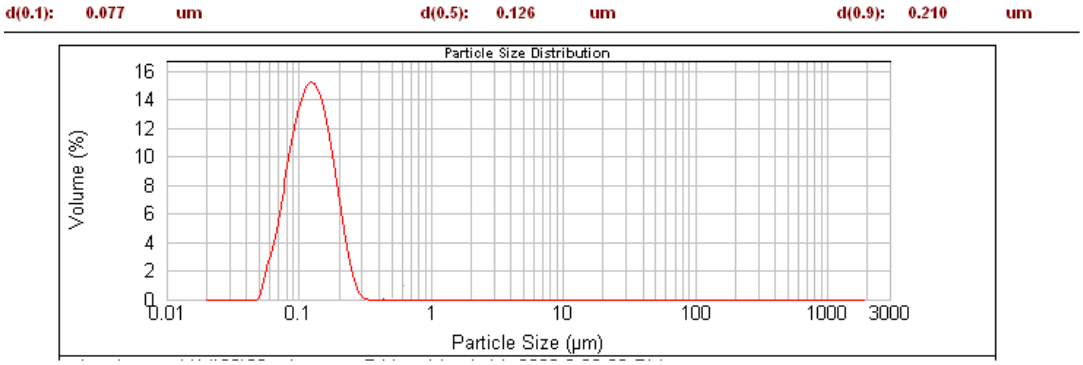


**Figure 1**
***Particle size distribution curve of Batch E***

A high amount of drug could be incorporated in nanoparticle dispersion. Such high incorporation was possible because of lipid solubility of CM. The average particle size and %EE of different formulations prepared is shown in Table 2. From the % EE values was clear that, it remains nearly same for all the batches as composition of lipid was kept constant.

**Table 2**
***Mean Percent Clotrimazole entrapped in NLCs and particle size***

| Sample | Entrapment efficiency (%) ± SEM | Particle size (nm) ± SEM |
|---|---|---|
| A | -- | -- |
| B | 93.43±510 | 730±4.86 |
| C | 94.38±0.645 | 310±2.67 |
| D | 94±3.54 | 228±2.51 |
| E | 94±3.60 | 210±2.67 |

### *3.3. Transmission Electron Microscopy*

Transmission electron micrograph (TEM) of CM-NLC dispersion sample E illustrates the spherical shape of nanoparticles entrapping the drug. The homogeneous monolayer coating of surfactant at the periphery of the nanoparticles surrounding the lipid core can be clearly seen (fig 2). The particle size observed is 200 nm, which was in confirmation with particle size obtained with laser diffraction.

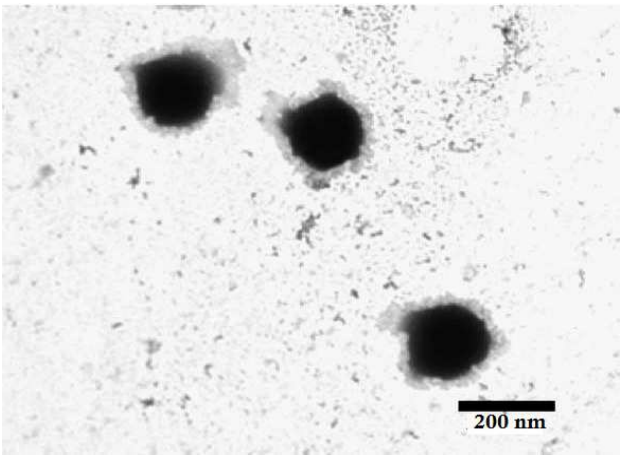


**Figure 2**
***Transmission electron micrograph of CM-NLCs of Sample E***

### *3.4. Differential Scanning Calorimetry*

DSC is a highly useful means of detecting drug-excipient incompatibility in the formulation. Stearic acid alone and in formulation was studied using DSC. For the bulk material of Stearic acid, the melting process took place with maximum peak at 71.97°C and CM show peak at 168°C shown in Fig.3. DSC thermogram of CM-NLC showed an endotherm at 70°C, which can be attributed to melting of Stearic acid in NLC. The peak of Stearic acid in formulation shows a shift to the lower temperature side. This could be due to reduction in particle size and increase in surface area leading to decrease in melting enthalpy as compared with heat flow through larger crystals, which require more time. The higher melting enthalpy value suggests higher ordered lattice arrangement. For the less-ordered crystal/ amorphous state the melting of the substance requires less energy than the perfect crystalline substance, which needs to overcome lattice force. Decrease in melting point and transformation of a sharp peak to a peak with shoulder associated with the numerous lattice defects and the formation of amorphous regions in which the drug is located. Also shows a new peak at 200°C due to presence of clotrimazole.

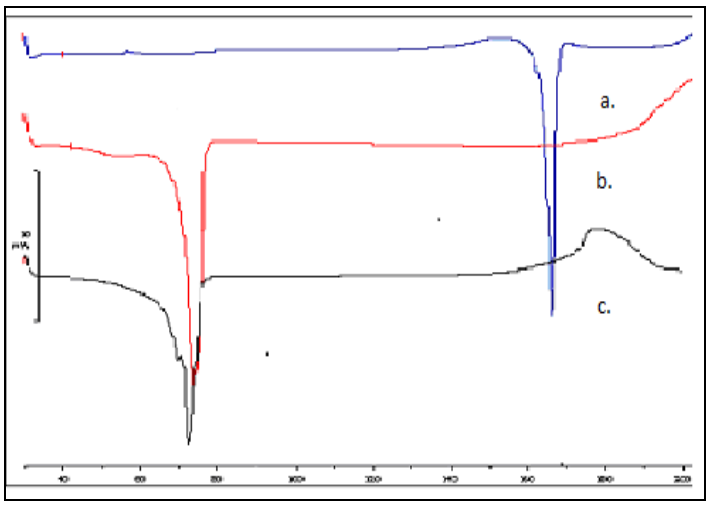


**Figure 3**
***Differential scanning thermograms of bulk material of a. clotrimazole, b. stearic acid and c. CM-NLCs***

### *3.5. Stability study*

After one month refrigerated storage the NLC dispersion showed little difference in particle size and entrapment efficiency. No obvious change of clarity and degradation was observed. The particle size and entrapment efficiency of sample E stored for 1 month was 220 nm ± 3.18 and 92% ± 2.69 content. Centrifugation at 3000 rpm for 30 min also showed that the CM-NLC had a good physical stability.

### *3.6. Preparation and evaluation CM-NLC gel*

Gel of CM-NLC was prepared by using different concentrations of carbopol 934 (0.5%- 1%) out of that 0.6% concentration showed good rheological properties. Drug content in gel was 98% ± 0.85 (n=3).

### *3.7 Rheological Characterization*

In order to get comprehensive information on the rheological behavior of the NLC-CM gel, oscillatory measurements yielding information about viscous and elastic properties of the investigated carrier were performed. Frequency sweep measurements give good information on microstructures of the gel system. In this the degree of dependency of G' and G'' is examined over the applied frequency range. It revealed the storage modulus G' (elastic response) which is a measure of energy stored and the loss modulus G'' (viscous response) which reflects the energy lost. If performed within the linear viscoelastic region (LVR), a frequency sweep provides a fingerprint of a viscoelastic system under non-destructive conditions. Thus the systems are examined in their rheological ground state without disrupting the structure like continuous shear techniques[19]. The frequency stress sweep response of 0.6 % carbopol NLC gel is shown in figure 4.From figure it was concluded that 0.6% carbopol NLC gel system had shown independent behavior of G' and G'' over applied frequency range. It is possible that monotonic increase in G' at higher frequencies means the partial breakage of the interconnected network, inferred from the existence of the plateau region at lower frequencies, which represents a true cross-linked polymer gel network. It is, therefore, concluded that the gel network is retained at low frequencies and, on the other hand, destroyed by the more frequent changes of the displacement at higher frequencies due to the formation of too rigid and brittle structures[20]. The Elastic modulus is higher than the viscous modulus over the whole frequency range, indicating the presence of a gel-like structure. The higher values of the elastic modulus show that the investigated system is more elastic than viscous in the investigated frequency range. Thus, taking into rheological properties, 0.6% carbopol NLC gel was found to be stable and appropriate for topical application.

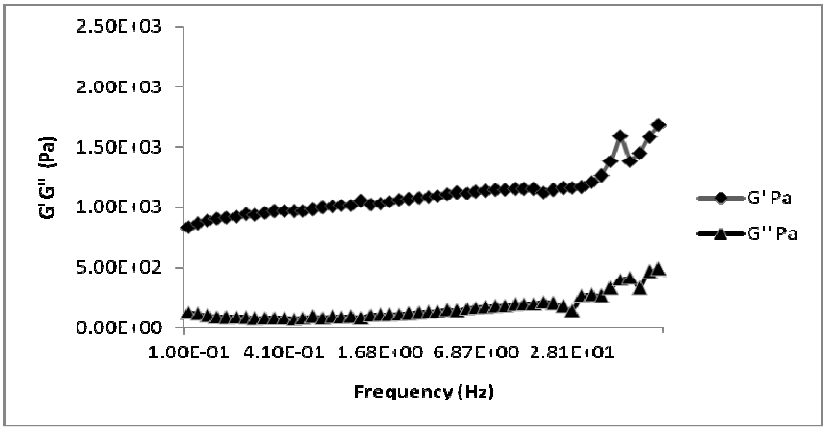


**Figure 5**
***Effect of Frequency (Hz) on elastic (G') and viscous (G") modulus***

### *3.8. Ex-vivo study using HCS*

The ex-*vivo* permeation of CM through human cadaver skin from CM-NLC gels was evaluated using Franz diffusion cell. The mean cumulative amount diffused Q (mg/cm$^2$) at each sampling time point was calculated.

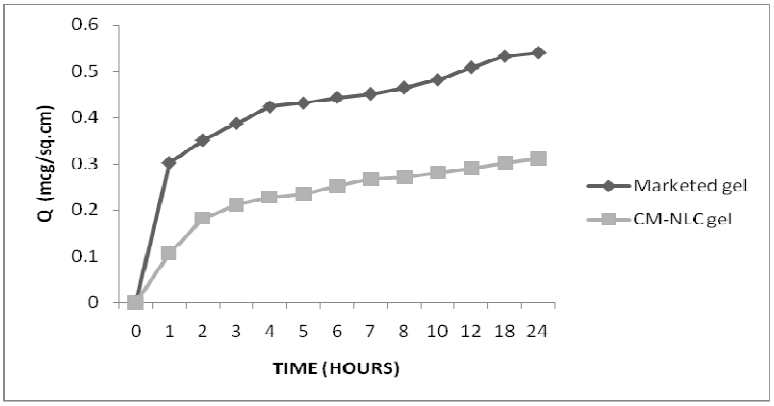


**Figure 5.**
***Ex-vivo release of Clotrimazole from marketed and 1 % CM-NLC gel.***

In present investigation CM-NLC gel produced significantly higher deposition of CM in skin (62 %) than Marketed Gel (30 %). 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[18].

**Table 3**
***Mean amount of Clotrimazole deposited into Human Cadaver skin***

| Test formulation | Human cadaver skin (%) mean (±SEM) | Gel (%) Mean (±SEM) |
|---|---|---|
| CM marketed gel | 30 ± 2.67 | 45 ± 3.21 |
| CM-NLC gel | 62 ± 1.96 | 28 ± 2 .14 |

***Mean of three determinations (n=3)***

## CONCLUSION

In conclusion, stearic acid based NLC dispersions containing clotrimazole having low particle size and long-term physical stability are prepared successfully using high-pressure homogenization (HPH) technique. Lipid content and surfactant play important role in drug entrapment and particle size. With gel containing nanoparticulate dispersion, a greater quantity of drug remained localized in the skin with lesser amount penetrating into the receptor compartment *exvivo* as compared with conventional gel thus enabling drug targeting to skin.

## ACKNOWLEDGMENT

The author would like to thank Dr. S.P Pande and Dr. K.K Tapar for their assistance. Further, we are thankful to BASF, Mumbai for kindly supplying the surfactant used in this study.

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