

**FORMULATION AND DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIER  
LOADED EMULGEL OF CICLOPIROX OLAMINE**

**R. B. Saudagar\*<sup>1</sup> and A. S. Bornare<sup>2</sup>**

<sup>1</sup>Department of Pharmaceutical Chemistry, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

<sup>2</sup>Department of Quality Assurance Techniques, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

\*Corresponding Author: R. B. Saudagar

Department of Pharmaceutical Chemistry, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

Article Received on 28/12/2017

Article Revised on 17/01/2018

Article Accepted on 07/02/2018

**ABSTRACT**

The aim of this study was to prepare and evaluate emulgels incorporating nanostructured lipid carriers (NLC) of Ciclopirox olamine (CPO) for topical application. Ciclopirox olamine has been used as model drug to be incorporated into nanostructured lipid carriers, once they are very well established as antifungal for the treatment of topical fungal infections. NLC designed for topical administration of Ciclopirox olamine, were prepared by the hot high pressure homogenization technique. This Ciclopirox olamine nanostructured lipid carrier was characterized for particle size, zeta potential, entrapment efficiency, and SEM. The lipid nanoparticles were incorporated in emulgels for convenient topical application and were evaluated for pH, Rheological analysis, Drug content, Spreadability, *In vitro* drug release studies, Antifungal studies and Stability studies. The preparation of aqueous NLC dispersions with a mean particle size lower than 300 nm has been obtained with uniform size distribution (PI < 0.518). An initial rapid release was observed in the case of Marketed gel, whereas optimized formulation depicted a slow initial release, the drug release of Marketed gel up to 12 hours (96.32%) and optimized formulation drug release up to 24 hours (98.13%). the antifungal activity of optimised formulation was better than Marketed gel. Research work could be concluded as successful development of nanostructured lipid carrier loaded emulgel of Ciclopirox olamine to increase the entrapment efficiency of colloidal lipid carriers with advantage of improved performance in terms of stability and provides a controlled Ciclopirox olamine topical effect as well as faster relief from fungal infection.

**KEYWORDS:** Ciclopirox olamine, Nanostructured lipid carrier, Topical emulgel, Topical drug delivery.

**INTRODUCTION**

Ciclopirox olamine (CPO) is a broad-spectrum antifungal agent. In contrast to the azoles and other antimycotic drugs, Mechanism of action of Ciclopirox is poorly understood. However, loss of function of certain catalase and peroxidase enzymes has been implicated as the mechanism of action, as well as various other components of cellular metabolism. In a study conducted to further elucidate ciclopirox's mechanism, several *Saccharomyces* mutants were screened and tested. Results from interpretation of the effects of both the drug treatment and mutation suggested that ciclopirox may exert its effect by disrupting DNA repair, cell division signals and structures (mitotic spindles) as well as some elements of intracellular transport. It acts by inhibiting the membrane transfer system.<sup>[1]</sup>

The drug is BCS class II (low solubility, high permeability) therefore there is need for enhance the solubility of drug is incorporated in lipid matrix.

Biodegradable nanoparticles, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) 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. 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 compared with traditional carriers, SLN are well tolerated, have high bioavailability, a nice targeting effect and are amenable to large scale production. 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. To overcome the limitations of SLN, a new generation of lipid nanoparticles, nanostructured lipid carriers (NLC) has been developed in recent years.<sup>[2,3,5]</sup>

NLC are prepared by mixing solid lipids with liquid lipids (oils), prepared with varying oleic acid content and Sodium lauryl sulphate obtained NLC. It is supposed that the oil incorporation impacted the crystalline state of the solid lipid found the formation of oily nanocompartments within the solid matrix whereas showed that high oil loads may lead to phase separation. The aim of this study was to develop topical emulgels containing NLC dispersions loaded with Ciclopirox olamine. The NLC were prepared by high pressure homogenization method. Nanoparticles were characterized in terms of particle size, zeta potential, surface morphology and entrapment efficiency. The influence of the NLC on in vitro drug release and antifungal activity was evaluated and compared with a Marketed gel.<sup>[6,7,8,9]</sup>

## MATERIALS AND METHODS

### Materials

Ciclopirox olamine was gifted by Glenmark Pharma, Ltd., Nashik, India. Stearic acid was purchased from

Research-Lab Fine Chem Industry, Mumbai and Oleic acid obtained from Loba Chemie Pvt Ltd, Mumbai. Sodium lauryl sulphate (SLS) and Carbopol 934 were purchased from Molychem, Mumbai, India. All the other chemicals were of the analytical grade. Water was used in double-distilled quality.

### Methods

#### 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. In order to prepare NLC, the lipid phase has been melted at 80-90°C 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 (IKA-Werke, Staufen, Germany) at 25000 rpm for 10 min. The obtained pre-emulsion was homogenized at a temperature 5°C to 10°C higher than the melting point of the bulk lipid, and applying a pressure of 500 bar and 5 homogenization cycles. The obtained dispersion was cooled at room temperature and to form Nanostructured lipid carriers.<sup>[10,11,12,13,14]</sup>

**Table 1: Composition of NLC formulation.**<sup>[36,37]</sup>

Formulation code	F1	F2	F3	,F5	F6	F7	F8	F9
<b>Ingredient</b>								
Ciclopirox olamine (w/v)	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
Stearic acid (w/v)	60	60	60	50	50	35	35	35
Oleic acid (v/v)	1.85	1.85	1.85	1.75	1.75	1.6	1.6	1.6
SLS (w/v)	1.3	1.3	1.3	1.2	1.2	1.1	1.1	1.1
Water (v/v)	100	100	100	100	100	100	100	100

## CHARACTERIZATION OF NLC

### Determination of Particle size, Zeta potential, and Polydispersity Index

The particle size and Zeta potential analysis was determined by zetasizer instrument. The yielded the mean diameter of the particle and polydispersity Index (PDI) as a measure for the width of the particle size distribution. For measurement, all the samples were diluted with double distilled water to make the suitable concentration for measurement by Malvern Zetasizer (Zs, Malvern Instrument, UK) Zeta potential is key indicator of the stability of formulation. The magnitude of zeta potential indicates the degree of electronic repulsion between adjusts, similarly charge particle in dispersion. Zeta potential was measured folded capillary cells using the zetasizer. 1ml sample was taken from formulated nanosuspension and dispersed with 10 ml double distilled water. The sample (optimized formulation F1) was ultrasonicated for 5 min prior size determination to measure the primary particle size. Then the sample was taken in disposable cuvette and placed in the instrument for size and zeta potential measurement.<sup>[9,10]</sup>

### Dug entrapment efficiency<sup>[10,11,12,13]</sup>

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

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

### Scanning electron microscopy

The morphological characteristic of Nanostructured lipid carrier (optimized formulation F1) is determined by a scanning electron Microscope. One drop of sample is placed on a slide and excess water is left to dry at room temperature. The slide is attached to the specimen holder using double coated adhesive tape and gold coating under vacuum using a sputter coater (for 10 minutes) to obtain a uniform coating on the sample to enable good quality images for SEM, and then investigated at 20kV (Model : Nova nano SEM NPE P3O3). The SEM was operated at low accelerating voltage of about 15KV with load current of about 80MA.<sup>[7,9,13,14]</sup>

## EVALUATION OF EMULGEL

### Determination of pH

The formulation was intended for skin application; therefore, pH measurement was essential to ensure the non-irritating nature of the formulations was determined at room temperature with a glass electrode pH meter.<sup>[15,16,17,18]</sup>

### Viscosity

The viscosity of different emulgel formulation was determined at room temperature using a Brookfield viscometer type DV-II + PRO at 10, 20, 30, 40, 50 rpm using spindle (LPV) no. 64. All test carried out on 40 ml samples at  $25 \pm 1^{\circ}\text{C}$ .<sup>[19,20,21,22,23]</sup>

### Spreadability

One of the criteria for an emulgel to meet the ideal quantities is that it should possess good Spreadability. It is term expressed to denote the extent of area to which gel readily spread on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. It is performed by 'slip and drag method' by modified spreading apparatus. It consists of glass slide having dimensions 10×5 cm fixed on tripod stand. An excess of 2 g of emulgel is placed on the fixed slide over which another slide is placed to which a weight is attached by thread. A weight of 500 gm is placed over both the slides to expel air for 5 minutes. Then weight is removed over the two slides. Weight of 80gm is attached and time required for slide to travel per marked distance i.e. 7.5 cm was noted. Lesser the time taken for separation of two slides, better the Spreadability.<sup>[24,25,26,27]</sup>

It is calculated by using the formula,

$$S = M \times L / T$$

Where, M = weight tied to upper slid, L = Length of glass slide, T =time taken to separate the slides

### Drug content

Quantity of emulgel was taken containing 10 mg drug in volumetric flask and sufficient quantity of phosphate buffer pH 6.8 was added to dissolve the formulation completely and volume was made up to 10 mL with phosphate buffer pH 6.8 to get a concentration of 1000µg/mL. The absorbance of prepared solution was measured at  $\lambda$  max by using UV visible spectrophotometer and % drug content was calculated.<sup>[28,29]</sup>

### In Vitro Drug Release

Laboratory-assembled apparatus resembling a Franz diffusion cell was used to determine the release profile of drug emulgel. The cell consisted of two chambers, the donor and the receptor compartment between which a diffusion membrane (egg membrane) was mounted. The donor compartment, with inner diameter 24 mm, was open i.e. exposed to the atmosphere at one end and the receptor compartment was such that it permitted sampling. The diffusion medium used was phosphate

buffer solution pH 6.8 (PBS). 1 mL of the drug containing emulgel was placed in the donor compartment separated from the receptor compartment by the egg membrane. The egg membrane was previously soaked for 24 hr. in PBS. The donor and receptor compartments were held together using a clamp. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was fixed on a magnetic stirrer. The receptor compartment with 100 mL PBS was placed on a thermostatically controlled magnetic stirrer. It was maintained at  $37 \pm 0.5^{\circ}\text{C}$  and stirred constantly at 50 rpm. Samples of 1 mL were collected at predetermined time intervals, diluted sufficiently with phosphate buffer 6.8 to get 10 ml solution, analysed for drug content by UV Spectrophotometer at  $\lambda$ max against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal.<sup>[30,31,32]</sup>

### Antifungal activity

An agar diffusion method was used for determination of antifungal activity of formulation. Standard 9 cm diameter; 0.5 cm depth petri dish containing medium were used. Inoculum were prepared by suspending 1-2 colonies of *candida albicans* (NCIM NO.3102) from 24 hr. Cultures in Potato dextrose agar medium in to tube contain 10 ml of sterile saline. The tubes were diluted with saline. The inoculum spread over the surface of agar medium. The plates were dried at  $35^{\circ}\text{C}$  for 15 min prior to placing the formulation. The boars of 0.5 cm diameter were prepared and 20µl sample of formulation (0.77% w/v) were added in the bores. After incubation at  $35^{\circ}\text{C}$  for 24 hr. the zone of inhibition around the boars were measured. Comparison study was done with the marketed formulation, Drug suspension, formulated cream and Optimized formulation.

### Stability

To investigate the long- term stability as a function of storage condition, the selected drug loaded nanostructured lipid carrier formulations was stored at different temperatures accelerated and room temperature ( $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 75% RH  $\pm$  5% RH and  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 60% RH  $\pm$  5% RH) for 3 month and physical appearance in terms of clarity, pH, viscosity, drug content were evaluated.<sup>[38]</sup>

## RESULT AND DISCUSSION

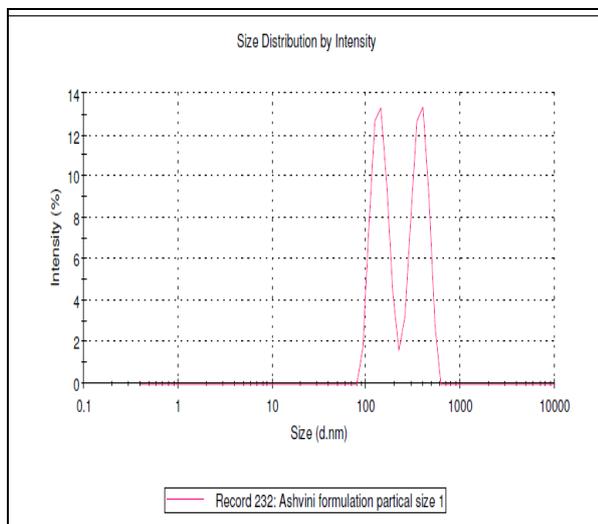
### Particle size and Zeta potential

Amount of lipid in NLC is an important factor for determination of the physicochemical characteristics. As the amount of solid lipid was increased, the particle size was decreased up optimum level after that particle size was again increased. But when increase in the pressure particle size was decrease at certain level, after word shows inverse effects. The amount and type of lipid was affects the particle size and stability of the formulation. The amount of emulsifier should be optimum to cover

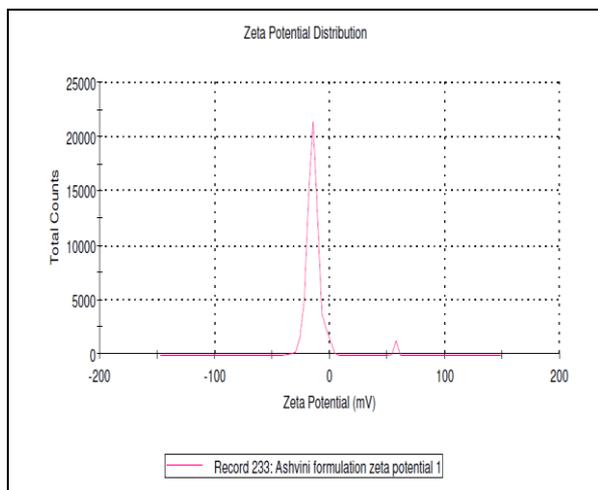
the surface of the nanoparticle in the formulation to improve the stability of the formulation.

**Table 1: Particle size, PDI, and Zeta potential of optimized formulation.**

Formulation	Particle size (nm)	PDI	Zeta potential(mV)	Zeta deviation (mV)
Optimized Batch (F1)	Peak 1: 370.6 Peak 2: 140.7	0.789	-14.4	11.7



**Figure 1: Graph of Particle size of optimized formulation (F1).**



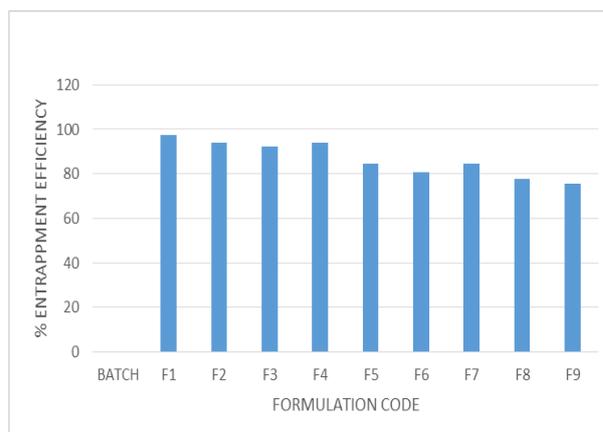
**Figure 2: Graph of Zeta potential of Optimized formulation (F1).**

#### Entrapment Efficiency

The maximum Entrapment efficiency was found to be 97.40% and the minimum Entrapment efficiency was found to be 75.32% (figure 3). The effect of Stearic acid on drug entrapment efficiency in NLC was investigated. It has been observed that the drug entrapment efficiency of optimised batch (F1) containing 60:40 (solid lipid: liquid lipid) and 2% surfactant at 25000 pressure. It might be due to the incorporation of liquid lipids into solid lipids which have led to massive crystals order disturbance. Greater imperfection in the crystal lattice leaves enough space to accommodate drug molecules, which ultimately improved drug entrapment efficiency.

**Table 2: Entrapment efficiency of all formulation.**

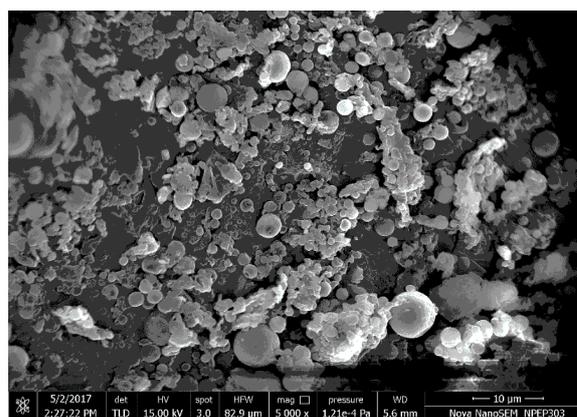
Sr.no.	Formulation code	% Entrapment Efficiency ( $\pm$ SD)
1.	F1	97.40 $\pm$ 0.1
2.	F2	94.15 $\pm$ 0.07
3.	F3	92.20 $\pm$ 0.1
4.	F4	94.15 $\pm$ 0.09
5.	F5	84.41 $\pm$ 0.2
6.	F6	80.51 $\pm$ 0.2
7.	F7	84.44 $\pm$ 0.1
8.	F8	77.92 $\pm$ 0.2
9.	F9	75.32 $\pm$ 0.2



**Figure 3: Entrapment efficiency of F1 to F9.**

#### Scanning electron Microscopy

Scanning electron microscopy of NLC is shown in figure 4. The shape of the NLC was spherical and the size of the NLC was found to be near smooth and regular outer surface. These characterizations were expected because of the increased oleic acid content of the formulation.



**Figure 4: Scanning Electron Microscopy for Optimized formulation (F1).**

### pH of Emulgel

pH of various emulgel was found to be in range of 6.26 to 6.34. pH values indicate the suitability of emulgel for topical application.

### Viscosity

Viscosity v/s rpm plots for all formulations shown decrease in viscosity as shear rate (rpm) was increased. Concentration of Stearic acid and Speed of homogenizer was a major factor affecting viscosity of formulations. Formulations exhibited considerable increase in viscosity when concentration of Stearic acid increased over the range 7% w/v to 12% w/v.

### Spreadability

For topical preparation Spreadability is one of the important for ease of application and better patient compliance. Prepared nanostructured lipid carrier loaded emulgel was easily spreadable.

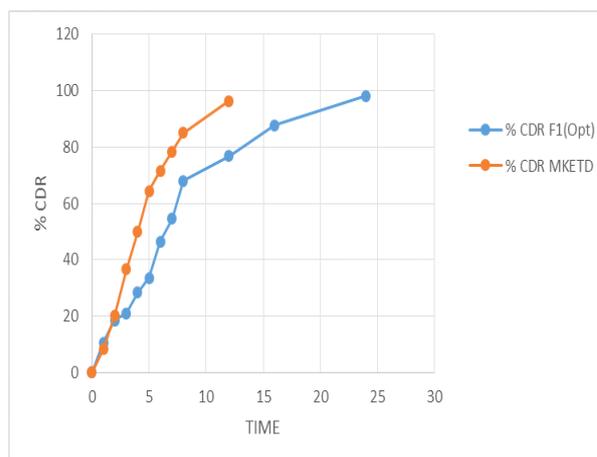
### Drug content

The percentage drug content of all prepared emulgel formulations was found to be in the range of 95-98 %. Therefore uniformity of content was maintained in all formulations.

### In Vitro Drug Release

The in-vitro release of Ciclopirox olamine from its various emulgel formulae are represented in the figure and table. It was observed that all formulae had become liquefied and diluted at the end of the experiment, indicating water diffusion through the membrane. In general, it can be observed that from figure 6 that the release of the drug from optimized (F1) emulgel

formulation was higher than the commercial cream. (Loprox 0.77% cream). The drug release of optimized formulation shown the controlled release up to 24 hours (98.13%) and the marketed formulation was obtained drug release up to 12 hours (96.32%).



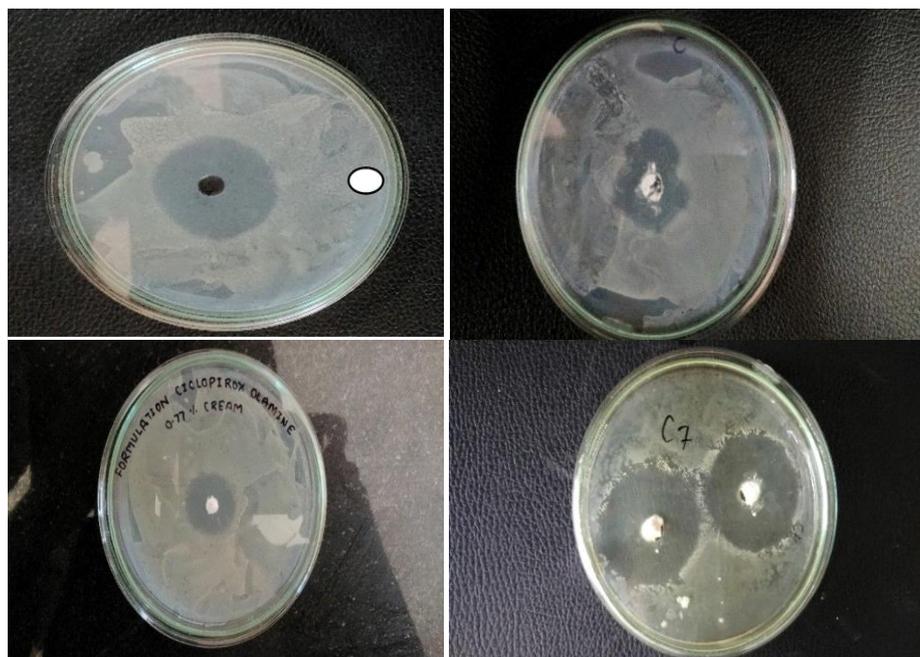
**Figure 6: Comparison of optimized batch with marketed formulation.**

### Antifungal activity

The antifungal activity of optimized formulation was compared with that of marketed cream. The results are represented in table 3 and shown in figure 7, percentage inhibition was taken as a measure of the drug antifungal activity. The results showed that all of the formulation had a greater activity against *candida albicans* than marketed cream, drug suspension and plain formulated cream.

**Table 3: Zone of Inhibition of formulation against *candida albicans*.**

Sr. no	Formulation code	Zone of Inhibition (mm)	% Efficacy
1.	F1	29 mm	100
2.	F2	28 mm	96.55
3.	F3	26 mm	89.65
4.	F4	28 mm	96.55
5.	F5	27 mm	93.10
6.	F6	25 mm	86.20
7.	F7	24 mm	82.75
8.	F8	23 mm	79.31
9.	F9	21 mm	72.41
10.	Drug suspension	29 mm	100
11.	Marketed formulation	23 mm	79.31
12.	Formulated cream	22 mm	75.86



**Figure 7: Zone of Inhibition of optimized formulation against *candida albicans* and comparison with Drug suspension, Formulated cream and marketed formulation.**

#### Stability<sup>[38]</sup>

The optimized formulation was evaluated after storage at accelerated condition and Room temperature. The results of stability studies show that the formulation was stable at accelerated temperature condition ( $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 75% RH  $\pm$  5% RH) and also at room temperature conditions ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 60% RH  $\pm$  5% RH). There is not significant so as to affect the quality and safety of the formulation after storage.

#### CONCLUSION

Stearic acid based nanostructured lipid carrier dispersion containing Ciclopirox olamine having low particle size and long- term physical stability are prepared successfully using high pressure homogenization technique. Lipid content and surfactant play important role in drug entrapment and particle size. Gel containing nanoparticulate dispersion, a greater quantity of drug remained localized in the skin with lesser amount penetrating into the receptor compartment. The optimized formulation was compared with marketed preparation which concludes that optimized formulation showed better results than the marketed preparation, it's revealing that these nanoparticles are promising delivery system.

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