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Formulation and Evaluation of Topical Gel Containing Nanostructured Lipid Carriers Dispersion of an Antifungal Drug

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ABSTRACT

Fungal infection are the common dermatological diseases. Drug delivery systems for topical use have shown significant advantages in targeting the drug to the action site in the body and also reduces the systemic side effects.

In the present study an attempt was made to prepare econazole nitrate loaded nanostructured lipid carrier (NLC). Different formulations were prepared by hot homogenization technique using solid lipid and liquid lipid (GMS, GMO) and surfactants (Poloxamer 188, Poloxamer 407). Formulations were characterized for entrapment efficiency, viscosity, spreadability, pH and *in vitro* drug release.

Entrapment efficiency of formulation F1-F8 was found to be 65.81-74.63%. The drug release of NLC gel followed zero order kinetics. NLC gel were stable at 40 ± 2°C and 75 ± 5% RH. Thus, the prepared NLC gel proved to be a potential candidate as a topical nanoparticulate sustained drug delivery system for econazole nitrate.

Keywords: Nanostructured lipid carrier, Econazole nitrate, Glyceryl monostearate, Glyceryl monooleate, hot homogenization.

INTRODUCTION

Fungal infections are the common dermatological diseases and more than 150 million people are affected with fungal infections, which have impact on human lives. Drug delivery systems for topical use have shown significant advantages in targeting the drug to the action site in the body and also reduces the systemic side effects. With the help of carrier's antifungal drug administration into skin can be improved including vesicular carriers and lipid nanoparticles. [1,2,3]

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NLC are colloidal nanoparticles produced by mixing liquid lipid (oils) with the solid lipid in which the liquid lipid is either embedded into the solid matrix or localized at the surface of solid particles. These nanoparticles vary in the submicron size range of 10-1000nm[4]. Lipid based drug delivery systems are more prominent because of site specific action[5,6]. As it has the potential to increase the solubility and improve the bioavailability of lipophilic drugs**.** Econazole nitrate (ECN) is an imidazole derivative compound, used primarily in the treatment of superficial infections. Some have reported that econazole nitrate is used for topical administration to treat fungal infections[7]. ECN has low aqueous solubility due to its hydrophobic nature, this can be a drawback on antifungal efficacy, it can be overcome by preparation of nanostructures lipid carriers by using lipid mixture and addition of surfactants and co surfactants for the formulation.

In the present study, an attempt was made to improve the permeability of drug, sustained drug delivery and side effects of the antifungal drug loaded nanostructured lipid carrier containing gel.

METHODOLOGY

Materials

Econazole nitrate was obtained as gift sample from Gufic Biosciences Ltd. Glyceryl monostearate was purchased from Central Drug House(P), Ltd. New Delhi. Glyceryl monooleate was purchased from Yarrow Chem Products, Mumbai. Poloxamer 188 and poloxamer 407 was purchased from Yarrow chem Ltd. All the other chemicals used were of analytical grade.

Methods

Selection of lipid phase

5 mg of Econazole nitrate was dispersed in mixture of melted lipid (5 g) and hot distilled water (5 ml) and Stirred continuously for 30 minutes under magnetic stirring and temperature maintained above 10° C melting point of lipid. Then aqueous phase was separated by ultracentrifugation at 5500 rpm and drug content was analyzed[7,8]

Melting point determination

Melting point of econazole nitrate was determined by using Thiele's tube method. Melting point of a drug sample has been the first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range.

Compatibility studies

FTIR analysis

FTIR spectral studies were carried out for pure drug econazole nitrate, freshly prepared and six months old 1:1 SDs and individual substances to check the compatibility between drug and polymers using Bucker Tensor-27 (Bucker, Germany)

Differential Scanning Calorimeter (DSC)

DSC studies were carried out on DSC Q60, Shimadzu, Japan. Sealed and perforated aluminum pans were used in the experiments for all samples. Temperature calibrations were performed using indium as standard. An empty pan sealed in the same way as for the sample was used as a reference. The entire samples were run in nitrogen atmosphere at a scanning rate of 10° C/ min from 50-300°C. By comparing the DSC curves of a pure drug sample with that of formulation, the presence of an impurity can be detected in a formulation[9].

Preparation of econazole nitrate loaded nanostructured lipid carriers

Econazole nitrate loaded nanostructured lipid carriers were prepared by using hot homogenization method. The solid lipid and liquid lipid were melted to approximately 10° C above its melting point; Econazole nitrate was dispersed in this molten lipid mixture. An aqueous phase was prepared by dissolving poloxamer 188 and poloxamer 407 as cosolvent in distilled water. The hot aqueous phase was added to the molten lipid mixture under magnetic stirring with same temperature maintained homogenized using Ultra Turax Homomgenizer Then obtained solution was kept in lyophilizer to get the final product (econazole nitrate nanostructured lipid carriers)[10-15].

Table 1. Formulation chart of econazole nitrate nanostructured lipid carriers

Formulation code	Econazole nitrate (g)	Glyceryl mono stearate (g)	Glyceryl monooleate (ml)	Poloxamer 188 (g)	Poloxamer 407 (g)	Distilled water (ml)
F1	0.1	1	1	0.5	2	100
F2		1	2	1.0	1.5	100
F3		1	3	1.5	1.0	100
F4		1	4	2	0.5	100
F5		1	5	0.5	0.5	100
F6		1	6	1.0	1	100
F7		1	7	1.5	1.5	100
F8		1	8	2	2	100

Preparation of nanostructured lipid carrier gel of econazole nitrate

2 g of carbopol was weighed and transferred slowly into 100 ml distilled water taken in a beaker. This solution was stirred at 200 rpm for 3 h under magnetic stirring. To this 10 g base gel, calculated amount of lyophilized nanostructured lipid carrier of econazole nitrate was added and stirred at 100 rpm for 2 h; followed by the addition of methyl paraben and propyl paraben. Triethanolamine about 1-3 drops was added to get the proper gel consistency[16-18].

Table 2. Formulation of Econazole nitrate-NLC gel

Sl.No.	Ingredients	Quantity taken
1	Carbopol	2 g
2	Methyl Paraben	0.2 g
3	Propyl Paraben	0.3 g
4	Optimized econazole nitrate-nanostructured lipid carrier (F3)	192mg
5	Triethanolamine	q.s.
6	Distilled water	Upto 100 ml

Evaluation of econazole nitrate NLC

Particle size analysis

The particle size of nanostructured lipid carrier (NLC), were measured by using Malvern Zeta sizer Nano ZS-90. Before analysis, nanosuspension was further diluted with HPLC graded water followed by sonication for 30 min. The mean particle size was decisived from the particle size distribution data.

Zeta potential

Zeta potential is defined as a measure of the magnitude of the electrostatic or charge repulsion or to allure between particles in liquid suspension. Its measurement will give detailed insight into the causes of dispersion, or flocculation, aggregation and can be applied to enhance the composition of dispersions, emulsions and suspensions. The unit of zeta potential is usually milli volt (mV). Before analysis, nanosuspension was further diluted with HPLC graded water followed by sonication for 30 min[19,20].

Scanning electron microscopy (SEM)

SEM photographs were taken for the prepared nanoparticles using a scanning electron microscope (Carl Zeisus FESEM model number: Ultra 55 USA.**)** at different required magnifications at room temperature. The photographs were analyzed for morphological characteristics.

Drug entrapment efficiency

The prepared NLC dispersion was centrifuged at 6000 rpm for 30 min at 4°C using REMI cooling centrifuge. Then the free drug content was evaluated by using supernatant liquid. The entrapment efficiency (%) of drug was calculated by the following equation[21].

$$\% \text{ Entrapment Effiency} = \frac{\text{Total drug loaded-Free drug content}}{\text{Total drug loaded}} \text{ x } 100$$

Loading Capacity

Loading capacity is the amount of drug loaded per unit weight of the nanoparticles, indicating the percentage of mass of the nanoparticles that is due to the encapsulated drug.

Loading capacity can be calculated by the amount of total entrapped drug divided by volume of water that is required to re-suspend the nanoparticles[22].

$$\text{Loading capacity} = \frac{\text{Total drug loaded-Free drug content}}{\text{Volume of water required to resuspend Nanostructured lipid carrier}}$$

In vitro drug release for econazole nitrate nanostructured lipid carrier

The *in vitro* drug release profile of econazole nitrate loaded NLC were studied using vertical diffusion cell. The dialysis membrane was soaked overnight in the pH 5.5 phosphate buffer. Then calculated amount of product was kept in the donor compartment above the dialysis membrane. 250 ml of pH 5.5 phosphate buffer was taken in 250 ml beaker. Then the beaker was placed over a magnetic stirrer, the temperature and rpm was maintained at 34±0.5°C and 100 rpm throughout the study. Samples (5 ml) were withdrawn at predetermined intervals of time (1, 2, 3, 4, 5, 6, 7, and 8 h) and replaced with equal amounts of fresh buffer. After suitable dilution the samples were analyzed for drug concentration by UV spectrophotometer at 271 nm[23,24].

In vitro drug release for econazole nitrate nanostructured lipid carrier gel

The *in vitro* drug release profile of nanostructured lipid carrier gel of econazole nitrate and the standard econazole nitrate gel were studied using Franz diffusion cell. About 0.1 g of gel was kept on the dialysis membrane was mounted over the donor compartment and fixed on it. The dialysis membrane was soaked overnight in pH 5.5 phosphate buffer. The receptor compartment was filled with the buffer. Then the beaker was placed over a magnetic stirrer, the temperature and rpm was maintained at 37±0.5°C and 100 rpm throughout the study. Samples (5 ml) were withdrawn at predetermined intervals of time (1, 2, 3, 4, 5, 6, 7 and 8 h) and replaced with equal volume of fresh buffer. After suitable dilution the samples were analyzed for drug concentration by UV spectrophotometer at 271 nm[25,26,27].

Kinetic analysis of drug release

To reveal the kinetics of drug release from the NLC gel, the results obtained from *in vitro* release studies was fitted to various kinetic models such as Zero-order, First-order, Higuchi and Krosmeyer Peppas model. The precedent for selecting the most convenient model was based on a goodness-of-fit test[28].

Evaluation of econazole nitrate loaded nanostructured lipid carrier gel

Spreadability

The spreadability of formulations was determined by using horizontal glass plate method. A standard weight (5 g) was tied to the upper glass plate and about 1 g of econazole nitrate nanostructured lipid carrier gel was placed between two horizontal glass plates. The whole set was hold in the vertical position. The time required for the plate to slide off from the other plate was noted. The spreadability was calculated from the formula[21].

Spreadability = M ∗ L/ T

M = Weight tied to upper slide (g)

L = Length of glass slide (cm)

T = Time taken (sec)

pH determination

Before the analysis pH meter is calibrated using pH 4.0, 7.0 and 9.2 standard solutions. After the calibration the glass electrode was immersed in the gel (50 g) and the pH was noted[21].

Viscosity

Viscosity of the nanostructured lipid carrier gel was determined by using Brookfield viscometer. The temperature of the gel was maintained at 25°C. Helipath T-bar Spindle no. 96F was fixed to viscometer and immersed in the beaker containing 50 g of NLC gel. The viscometer was operated at different rpm and reading was noted in centipoises (cps)[21].

Drug content estimation

Accurately weighed 1 g of the gel transferred to the 100 ml of volumetric flask containing 20 ml of phosphate buffer pH 5.5. The volumetric flask was shaken for 30 min and the volume was made up to 100 ml with phosphate buffer pH 5.5 solution. After suitable dilution, the sample was analyzed using Agilent technologies carry 60 UV- visible spectrophotometer at 271 nm.

Skin irritation study

The experimental protocol for the skin irritation study was approved by Institutional Animal Ethics Committee. Skin irritation study was carried out using 6 rats of either sex weighing between 200-250 g. The animals were kept in

polypropylene cages with free access to a standard laboratory diet and water. Animals were divided into 2 groups of 3 animals each. Hairs were depleted from the abdominal region using scissors and blade. After hair depletion, gel was applied and covered with cotton bandage. The reaction at the site of application was studied and scored[29,30].

Stability studies

Stability studies were carried out on most satisfactory formulation as per ICH guidelines at 40±2°C and 75 ±5% RH. The most satisfactory formulation stored in a sealed in aluminum foil. These were stored at room temperature. After 3 months, particle size, zeta potential, entrapment efficiency, *in vitro* drug release of most satisfactory formulation was determined[31,32].

RESULTS AND DISCUSSION

Preformulation study

Melting point determination

The melting point of econazole nitrate was determined by Thiele's tube method and the melting point range was found to be 161-162°C.

Compatibility study - FTIR study

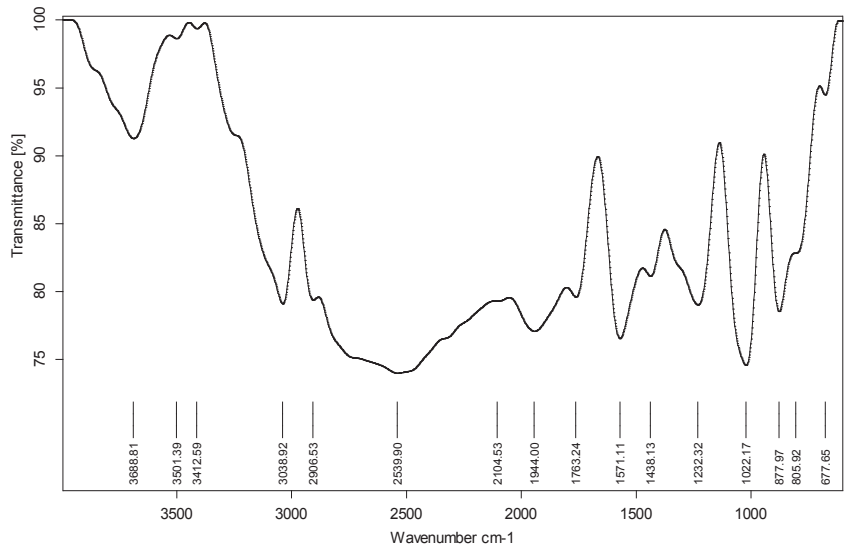

Figure 1. FTIR spectrum of econazole nitrate

Figure 2. FTIR spectrum of econazole nitrate + GMS

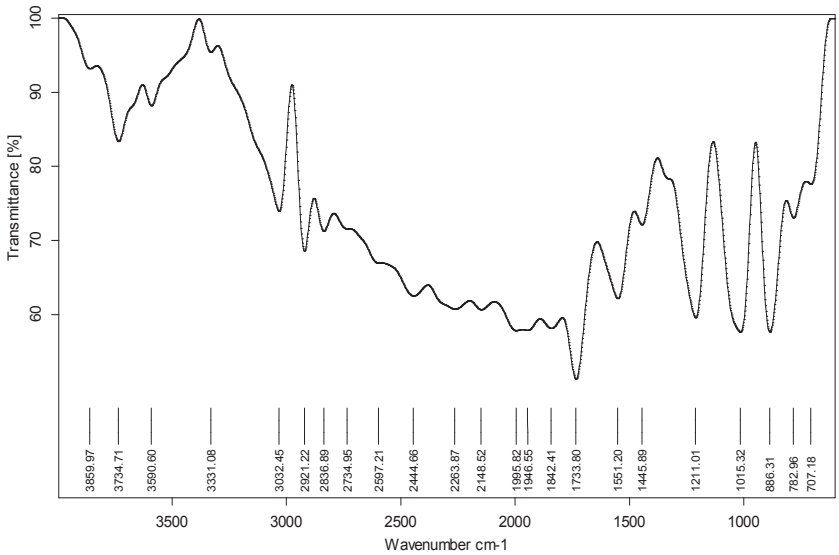

Figure 3. FTIR spectrum of econazole nitrate+ GMO

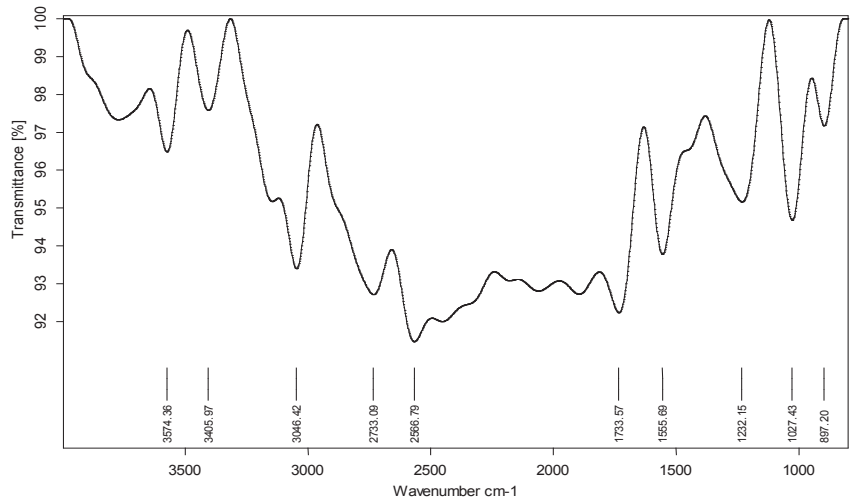

Figure 4. FTIR spectrum of econazole nitrate+ carbopol

FTIR spectra of econazole nitrate and its combination with glyceryl monostearate and also with glyceryl monooleate were shown in Fig:1,2,3,4. From the obtained spectra it was observed that the characteristics peaks of econazole nitrate (1763cm-1, 1022cm-1, 877cm-1) were present in the combination spectra thus indicating the compatibility of the drug with the lipids used. It shows that there was no significant change in the chemical integrity of the drug and spectral data and it complies with IP.

3.1.2 DSC study

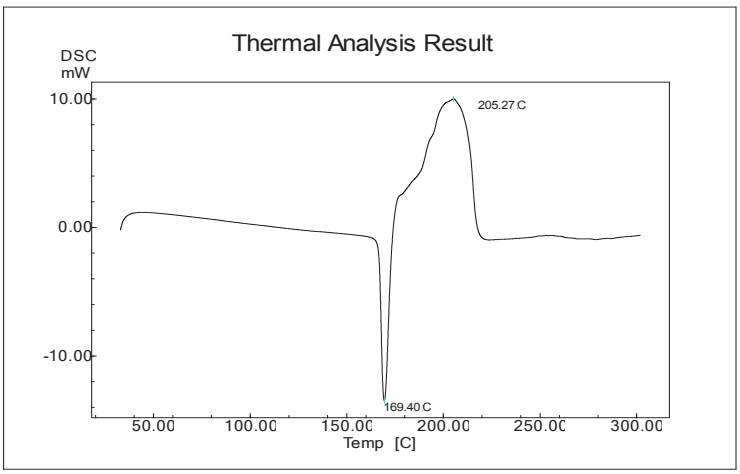

Figure 5. DSC thermogram of econazole nitrate

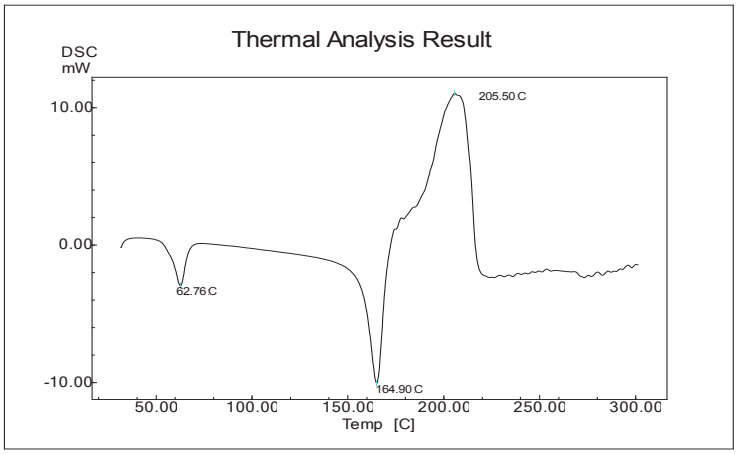

Figure 6. DSC thermogram of econazole nitrate + GMS

DSC spectrum of pure drug econazole nitrate exhibit a sharp endothermic peak at 169.40°C (Fig:5,6) and the mixture of glyceryl monostearate and drug shown a blunt endothermic peak at 164.90°C. These results infer that the compatibility between the lipids and drug.

Evaluation parameters of Nanostructured lipid carrier - Particle size, polydispersity index and zeta potential

Table 3. Physicochemical characteristics of NLC F1-F8

Formulation code	Particle size* (nm) Mean ± SD	Polydispersity index* Mean ± SD	Zeta potential* (mV) Mean ± SD
F1	142.1±0.92	0.27±0.01	-22.16±0.32
F2	162.8±0.28	0.32±0.002	-41.8±0.35
F3	153.3±0.66	0.34±0.002	-47.4±0.32
F4	160.24.5±5	0.38±0.002	-26.36±0.30
F5	182.73±0.45	0.32±0.0025	-31.46±0.25
F6	173.4±0.88	0.39±0.005	-30.53±0.35
F7	142.83±0.60	0.354±0.003	-25.43±0.35
F8	134.8±0.65	0.36±0.003	-27.4±0.3

*n=3

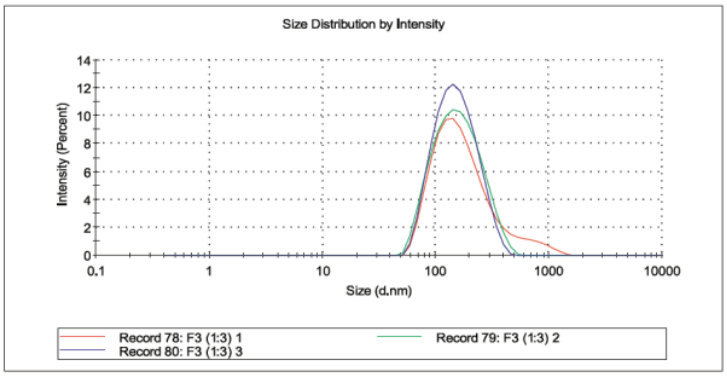

Figure 7. Particle size distribution of F3 formulation

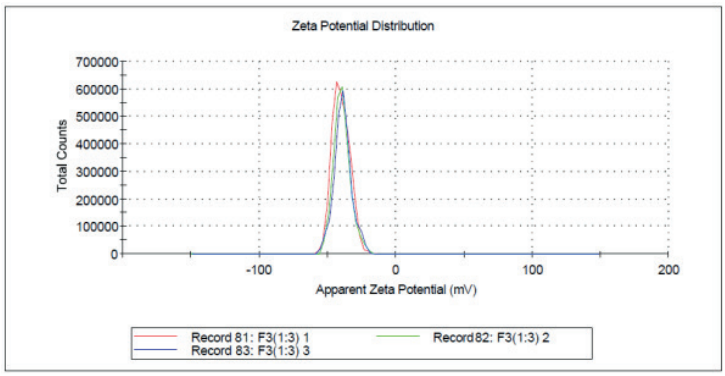

Figure 8. Particle size distribution of F3 formulation

Average particle sizes of econazole nitrate NLC were found in the range of 134.83 nm to 182.8 nm. It showed that the particles were in nanometre range. The polydispersity index (PDI) was found in the range of 0.27 to 0.39 for glyceryl monostearate and glyceryl monooleate as mentioned in (Table 3). This showed the polydispersity of particle was below 1 which infers the more homogeneity of the particles. The stability of the econazole nitrate nanostructured lipid carrier was evaluated by measuring the zeta potential of the NLC. The zeta potential of the formulations ranges from -22.16 to -47.4 mV. The zeta potential of best formulation F3 was found to be -47.4 mV which indicates that the formulation was stable has been given in (Table 3 and Fig:7,8). From the observations it was found that the nanostructured lipid carrier has been good homogeneity because polydispersity index was found to be less than one. The zeta potential is negative due to presence of negative surface charge of the drug.

Surface morphology

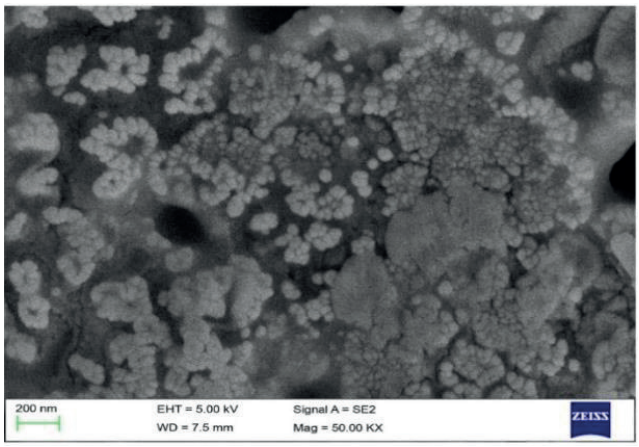

Figure 9. SEM micrograph of F3 at 50KX magnification

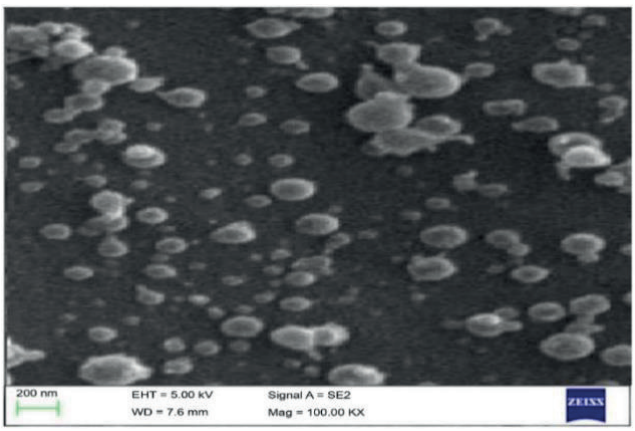

Figure 10. SEM of F3 at 100KX magnification

The morphological observation of sample under the SEM revealed that nano-structured lipid carrier of econazole nitrate are spherical in shape with different sizes ranging from 50.87 nm to 200.54 nm as shown in fig: 9,10.

Drug entrapment efficiency % and loading capacity

Table 4. Drug entrapment efficiency of nanostructured lipid carrier (F1-F8)

Formulation code	Drug entrapment efficiency* (%) Mean±SD	Loading capacity* (mg/ml) Mean±SD
F1	70.96±0.042	1.65±0.0009
F2	65.81±1.02	1.8±0.02
F3	67.29±0.05	1.6±0.01
F4	68.29±0.39	1.70±0.0009
F5	67.54±0.024	1.50±0.0005
F6	68.42±0.045	1.62±0.001
F7	72.89±0.056	1.61±0.0012
F8	74.63±0.04	1.58±0.0010

Highest entrapment efficiency of econazole nitrate NLC was found to be 74.63±0.04%. As the lipid concentration was increased the entrapment efficiency also increased, Table 4.

In vitro diffusion study of nanostructured lipid carrier of econazole nitrate

Table 5. *In vitro* drug release data of formulations F1-F4

Time (h)	Percentage cumulative drug released* (%) Mean±SD			
	F1	F2	F3	F4
1	12.49±0.47	12.07±0.46	10.82±0.2	7.56±0.39
2	18.75±0.38	23.17±0.38	12.21±0.47	8.76±0.31
3	29.56±0.75	28.08±0.67	15.36±0.49	12.12±0.39
4	36.31±0.77	35.81±0.75	17.96±0.27	16.79±0.52
5	42.52±0.65	39.5±0.92	35.02±0.87	27.4±0.62
6	51.74±1.15	47.38±1.01	46.46±0.96	33.4±0.69
7	65.78±1.24	53.9±1.09	52.92±1.07	56.38±0.85
8	74.43±1.38	69.45±1.27	66.8±1.03	66.75±1.05

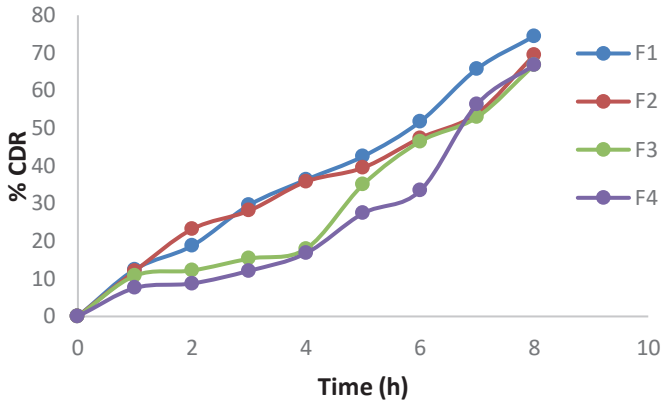

Figure 11. Percentage cumulative drug released profiles of formulations F1-F4

Table 6. *In vitro* drug release data of formulations F5-F8

Time (h)	Percentage cumulative drug released (%) Mean±SD			
	F5	F6	F7	F8
1	6.4±0.28	5.7±0.29	5.35±0.17	5.42±0.15
2	8.07±0.34	7.12±0.24	6.96±0.23	6.3±0.17
3	10.58±0.33	9.4±0.24	9.4±3.0.37	8.57±0.2
4	14.54±0.46	12.42±0.26	12.19±0.5	12±0.47
5	24.69±0.18	22.35±0.35	19.96±0.5	19.91±0.48
6	31.24±0.28	28.82±0.14	27.52±0.62	28.49±0.75
7	43.24±0.48	41.51±0.71	40.47±0.39	39.5±0.83
8	55.23±0.66	52.19±1.04	50.33±0.54	48.81±0.99

Figure 12. Percentage cumulative drug released profiles of formulations F5-F8

In vitro drug released study was carried out for 8 h for formulation F1-F8. The cumulative percent drug release after 8 h was found. At low lipid concentration in F1=43.47, % drug release was found to be 74.43%, whereas at high lipid concentration in F8=68.70, % drug release was found to be 48.81%. % Decrease in drug release was found as the liquid lipid is increased, this may be due to high viscous and thickness of lipid layer, Table no: 5,6 and Fig: 11,12.

Evaluation of nanostructured lipid carrier gel of econazole nitrate, spreadability, pH, drug content and viscosity

Table 7. Profile of ECN-NLC gel in terms of spreadability, pH, drug content and viscosity data's

Formulation code	Spreadability coefficient (g.cm/sec) Mean±SD	pH Mean±SD	Drug content (%) Mean±SD	Viscosity (cps)
NLC gel of econazole nitrate	0.467±0.0374	6.84±0.045	74.08±0.51	15372±35.72

In vitro drug diffusion study of nanostructured lipid carrier gel of econazole nitrate

Table 8. ECN-NLC gel in terms of *in vitro* diffusion study

Time (h)	Percentage drug released (%) Mean±SD
	NLC gel of ECN
1	9.2940±0.61
2	11.4372 ±0.33
3	16.813±0.57
4	26.488±1.29
5	31.364±1.28
6	33.6301±0.36
7	42.1698±2.10
8	50.557±2.64

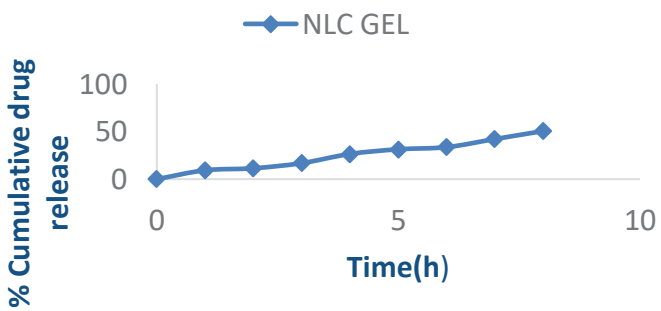

Figure 13. % Cumulative drug released profile of ECN-NLC gel

The cumulative percent drug release from the nanostructured lipid carrier gel showed 50.55±2.64 after 8 h of diffusion study given in (Table 8 and Fig:13. This may be because of the NLC gel containing the drug loaded lipid particles. The penetration of the drug, concentrates on the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin.

Skin irritation study

The primary skin irritation index was found to 0.00 for erythema, Eschar and edema formation in the rats of control group, test group I and test group II. From the results of primary skin irritation index, it was concluded that there is no skin irritation in rats.

Stability study

Table 9. Stability studies of nanostructured lipid carrier loaded econazole nitrate

Days	Spreadability* (g.cm/sec) Mean±SD	Viscosity* (cps) Mean±SD	pH* Mean±SD	Percentage drug released* (%) Mean±SD
30	0.335 ± 0.010	15351 ± 17.00	6.7 ± 0.07	50.33±0.12
60	0.338±0.011	15324 ± 22.12	6.7±0.10	49.67±0.17
90	0.315±0.01	15283 ± 10.44	6.7±0.1	49.32±0.20

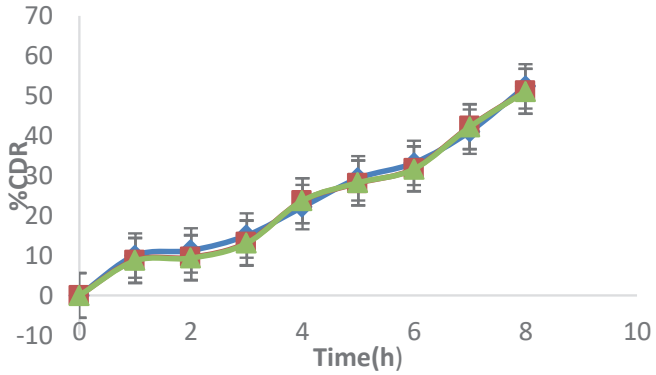

Figure 14. Drug release profile of ECN-NLC

Nanostructured lipid carrier gel of Econazole nitrate These studies revealed that NLC gel of Econazole nitrate was stable in terms of spreadability, pH, viscosity and cumulative percent drug release after storage for three months at 40±2°C, 75±5% RH. There was no significant difference after 3 months of stability study as given in Table 9 and Fig:14.

By Hot homogenization method ECN-NLC gel reduces particle size, improves stability due to Glyceryl monostearate, Glyceryl monooleate, Poloxamer108 and 407 and Carbopol 934P and therefore it is favourable for topical delivery system. The developed formulation overcomes and alleviates the drawbacks (drug loading, skin irritation) and limitations of econazole nitrate formulations.

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