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DESIGNING AND CHARACTERIZATION OF ECONAZOLE NITRATE NANOSTRUCTURED LIPID CARRIERS GEL FOR TOPICAL DELIVERY

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ABSTRACT

The focus of present work was to prepare and evaluate Econazole nitrate (ECN) loaded Nanostructured lipid carriers (NLCs) to elevate the topical efficacy of the drug for cure of fungal infections. ECN loaded NLCs were formulated by high speed homogenization followed by sonication technique using oleic and stearic acid as a liquid lipid and solid lipid, respectively. Tween 80 and sodium lauryl sulfate (SLS) were used as surfactant. DSC and XRD studies revealed transformation of crystalline nature of ECN to amorphous form. The selected NLCs formulation showed 18.26 ± 5.62 µm mean diameter with polydispersity index 0.612 and zeta potential -51.2 ± 7.23 mV that impart good stability. SEM analysis displayed smooth discrete somewhat spherical particles. The selected NLCs dispersion was gelled in carbopol and assessed for physical parameters like appearance, viscosity and spreadibility. *In-vitro* release of selected ECN loaded NLCs gel formulation showed 73.8% drug release over 9 hours of study. Therefore, it can be deduced that ECN loaded NLCs gel prolongs the duration of drug release and can be used as effective drug delivery method for cure of fungal infections.

KEYWORDS: Econazole nitrate; Nanostructured lipid carriers; Drug delivery; Scanning electron microscopy.

INTRODUCTION

Nanocarriers have been used since long as a carrier to deliver drug. Several polymeric nanoparticles were designed for variety of drugs. However, despite extensive research the polymeric nanoparticle based products do not exert in markets due to lack of pilot plant scale up techniques, to overcome this dilemma solid lipid nanoparticles (SLN) were established.[1] The advantages offered by SLN over polymeric nanoparticles were its biodegradability, biocompatibility, and availability of large scale production. However, they exhibited certain flaws like limited drug loading and drug expulsion, these limitations payed the path for the researchers to design a new lipid carrier, the nanostructured lipid carrier (NLC) with controlled nanostructure.[2,3] NLC is prepared by blending solid and liquid lipids exhibiting difference in structure. The structural difference results in formation of imperfect amorphous cluster in which drug gets entrapped. The formation of the imperfect alignment and creation of amorphous nature helps in overcoming the constraints associated with SLN.[4,5]Econazole nitrate (ECN) is an imidazole compound with a broad spectrum antifungal activity mainly for the cure of fungal infections caused by the fungus of genus *Candida albicans*.[6] The Econazole nitrate interferes in the synthesis of ergosterol by obstructing the enzyme Cytochrome P-450, which increased the permeability of cell results in leakage of cellular content, causes cell death.[7-9] The plasma protein binding is about 98% and the absorption is very poor when administered topically.[10] In the present work, Nanostructured lipid carrier for Econazole nitrate were developed in which the drug molecule were entrapped in the amorphous cavity constituted by the 3D arrangement of lipid blends with an objective to overcome its side effects and to escalate its follicular uptake and minimize its oral use, so that it could be effectively and Safely used, and improves patient compliance. The aim of the present work was to formulate ECN loaded NLCs formulation using high homogenization followed by sonication technique. Characterization of NLCs was done by Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies. For knowing the morphology, diluted NLCs were subjected to SEM study. And, then optimized NLCs was entrapped into gel using carbopol and to performed *in-vitro* studies.

MATERIALS AND METHODS

Materials

Econazole nitrate (ECN) was purchased from Yarrow chemical products, (Mumbai, India). Oleic acid and stearic acid were obtained from SDFCL, (Mumbai, India). Tween 80, sodium lauryl sulfate (SLS), stearylamine and methanol were procured from S.D. Fine Chemicals, Ltd. (Mumbai, India). All other chemicals and reagents used in research work were analytical reagent grade.

Methods

Selection of binary lipid phase ratio

The liquid and solid lipid with the best solubilizing probability for ECN in various ratios were mixed i.e. 1:9, 2:8, 3:7, 4:6 and vice versa in order to determine the miscibility of two different lipids. At 200 rpm for 60 min. at 80 °C lipid mixture were agitated using a magnetic stirrer. To examine the miscibility of two components the cooled solid mixture was spread on to a filter paper, followed by observation visually to ascertain the droplets of oil on the filter paper. The melting point of the mixture showing above 40 °C which didn't affirm the existence of droplets of oil on the filter paper was preferred for the formulation of ECN loaded NLCs.

Preparation of ECN loaded NLCs

NLCs loaded with ECN were formulated by using high speed homogenization followed by sonication technique.[11, 12] The lipids phases were heated at temperature 10-15 °C above from its melting point and then ECN were added to the melted lipids. Simultaneously, surfactants were heated at the same temperature. Then the hot lipids phase was dispersed into surfactants mixture using continuous stirring at 4000 rpm for 1 hour to obtain the primary emulsion. The pre-emulsion was then homogenized well and then sonicated for 45 minutes. The prepared NLCs formulations were stored at 4 °C in a Refrigerator. The compositions of different NLCs formulations are shown in Table 1 and schematic representation of method shown in Fig. 1.

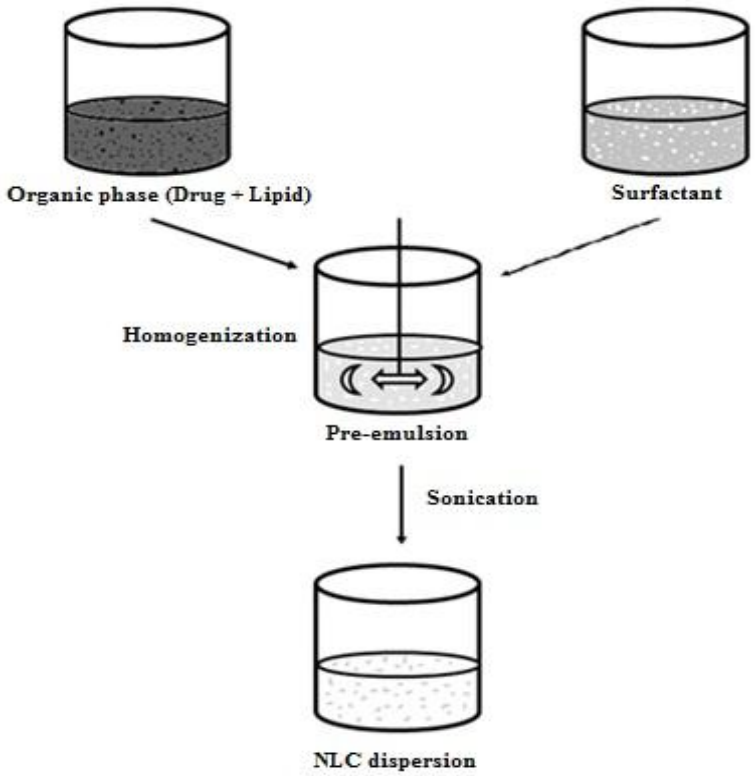

Fig. 1: Method of preparation of NLCs by high speed homogenization method.

Table 1: Formulation chart of ECN loaded NLCs.

Code	Drug (w/w)	LL: SL	Surfactants	
			Tween 80 (ml)	SLS (g)
NLC 1	1	7:3	4	0
NLC 2	1	7:3	0	4
NLC 3	1	7:3	2	2
NLC 4	1	3:7	4	0
NLC 5	1	3:7	0	4
NLC 6	1	3:7	2	2

Evaluation of ECN loaded NLCs

Fourier transform infrared spectroscopy study

FTIR study of drug (ECN) and physical mixture of drug and excipients were recorded by FTIR Spectrophotometer (IR Affinity-1, Shimadzu, Japan). The samples were prepared by press pellet techniques, using KBr pellets and spectra were scanned in range of 400- 4000 cm^{-1}.

Differential scanning calorimetry analysis

Differential scanning calorimetry studies were used to ascertain the amorphous nature of drug dispersed in the

lipid. DSC analysis of ECN, physical mixture of drug and excipients and formulated ECN loaded NLCs were conducted by Mettler Toledo DSC 8220 instrument. Samples were weighed in aluminum pan and regulated at temperature ranges between 0- 800 °C at a scanning range of 10 °C/min., to maintain the inert atmosphere nitrogen was purged.[13]

X-ray diffraction analysis

XRD analysis of samples were performed by PanlyticalXpert X-ray Diffractometer and Cu was used as a source of radiation. The scanning range was 10°-89° at angle 2θ diffraction. XRD pattern were measured with current of 40 mA and voltage of 45 kV.

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Surface morphology analysis

The morphology (surface and shape characteristics) of NLCs was observed by scanning electron microscopy (SEM) using gold sputtering technique (ZEISS E-40, Carl Zeiss, NTS, North America). The analysis was regulated at accelerated voltage of 18 kV and pressure of 0.8 mmHg.[14]

Zeta potential determination

The zeta potential of formulated NLCs were determined by using Malvern Zetasizer (Malvern Instruments, Worcestershire, UK). The analysis was performed in triplicates after suitable dilutions with deionized water.

Drug entrapment efficiency

For determination of entrapment efficiency, the ECN loaded NLCs dispersion was centrifuged at 15000 rpm for 45 min. at 25 °C. Supernatant were separated and determined by spectrophotometrically at 213 nm.[15, 16] Entrapment efficiency were determined by using following equation.

$$\%\ Entrapment\ efficiency = \frac{[ECN]_{total} - [ECN]_{supernatant}}{[ECN]_{total}} \times 100$$

Where, $[ECN]_{total}$ is the total weight of the drug incorporated and $[ECN]_{supernatant}$ is the weight of the drug analyzed in supernatant

Preparation of ECN loaded NLC gels

The ECN loaded NLCs gels were formulated by dispersing optimized NLCs in 1%w/w and 2% w/w carbopol using a mechanical stirrer (Remi, Mumbai, India). The dispersion was neutralized using stearylamine. The gel was allowed to stand overnight to remove entrapped air. The drug loaded NLCs gel samples were coded as EG1 and EG2 respectively.

Evaluation of ECN loaded NLCs gels

Physical parameters of ECN loaded NLCs gels

The formulated ECN loaded NLCs gels were assessed for viscosity by using Brookfield viscometer (Brookfield engineering laboratories, Inc., MA, USA) using spindle no. 6 at 10 rpm at 37 ± 1 °C temperature.

The spreadability was assessed, by measuring the spreading diameter of 1 g of sample gel between two horizontal glass plates after 1 minute. The standard weight 125 g placed on upper glass plate. The spreadability was calculated by using following formula.

S = m × l/t

Where, "S" is spreadability, "m" is weight applied on upper plate, "l" is the length of glass plate, "t" is time taken.

***In-vitro* drug release studies**

In-vitro drug release of plain drug, ECN loaded NLCs and ECN loaded NLCs gel were executed using Franz diffusion cells. The activated dialysis membrane (HiMedia, Dialysis membrane, Mol. cutoff between 12-14 kDa) were placed between the compartments of diffusion cell. An accurately weighed amount of samples were placed on the donor compartment. The receptor medium contains phosphate buffer (pH 7.4). The medium of receptor compartment were maintained at 37 °C ± 1°C with continuous stirring at 500 rpm. At fixed time interval aliquots of 2 ml were withdrawn and recovered with equal volume of fresh phosphate buffer (pH 7.4). The withdrawn samples were evaluated by using UV-spectrophotometer at a wavelength of 213 nm.

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

RESULTS AND DISCUSSION

Evaluation of ECN loaded NLCs

FTIR study

FTIR spectra of pure drug (ECN) and physical mixture of excipients with drug were obtained and presented in Fig. 2. Characteristic peaks of various groups of Econazole nitrate are C-H stretching for aromatic at 3086 cm^{-1}, C=C stretching for aromatic at 1596 cm^{-1}, $-NO_2$ stretching at 1538 cm^{-1}, C-O stretching for ether at 1089 cm-1 and C-Cl stretching at 674 cm-1 were observed in the physical mixture of pure drug with excipients. This declared that there was no remarkable chemical interaction between excipients and drug or confirms that the drug is in stable nature during the formulation process.

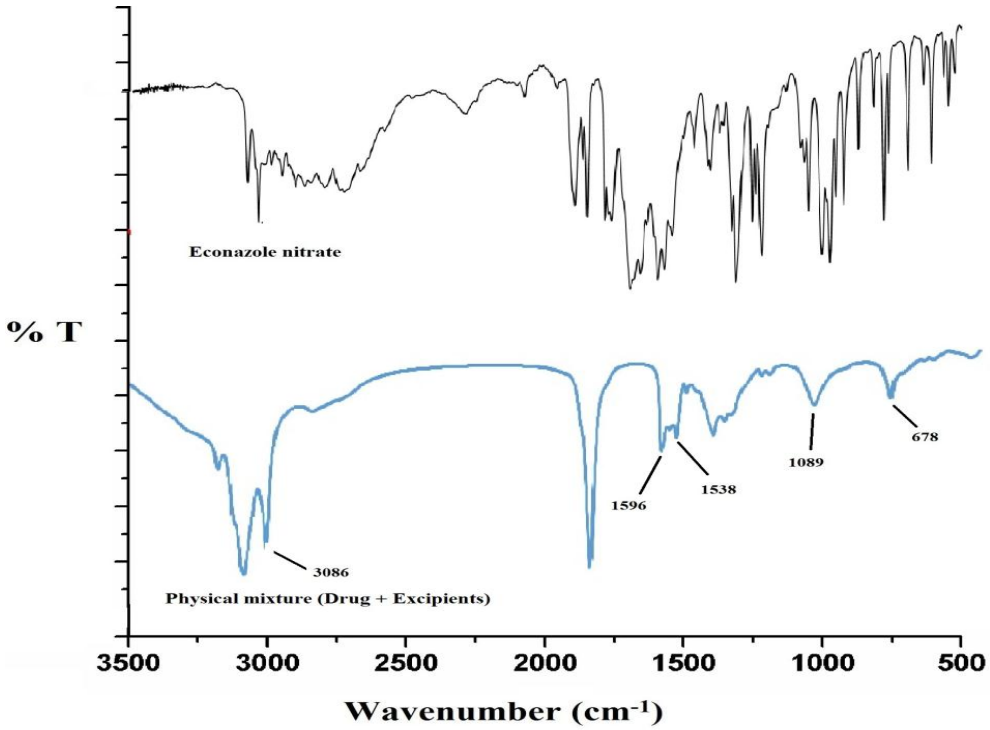

Fig. 2. FTIR spectra of Econazole nitrate and Physical mixture (Drug + Excipients).

DSC analysis

DSC thermogram of ECN, physical mixture of ECN and excipients and formulated NLCs were obtained. The DSC curve of pure ECN (Fig. 3) showed endothermic peak at 164 °C which resemble with their melting point, whereas DSC curve of physical mixture (Fig. 4) and formulated ECN loaded NLCs (Fig. 5) didn't showed any endothermic peak within the range of melting point of ECN, indicates conversion of ECN from crystalline nature to amorphous nature when it is incorporated in solid and lipid matrix.

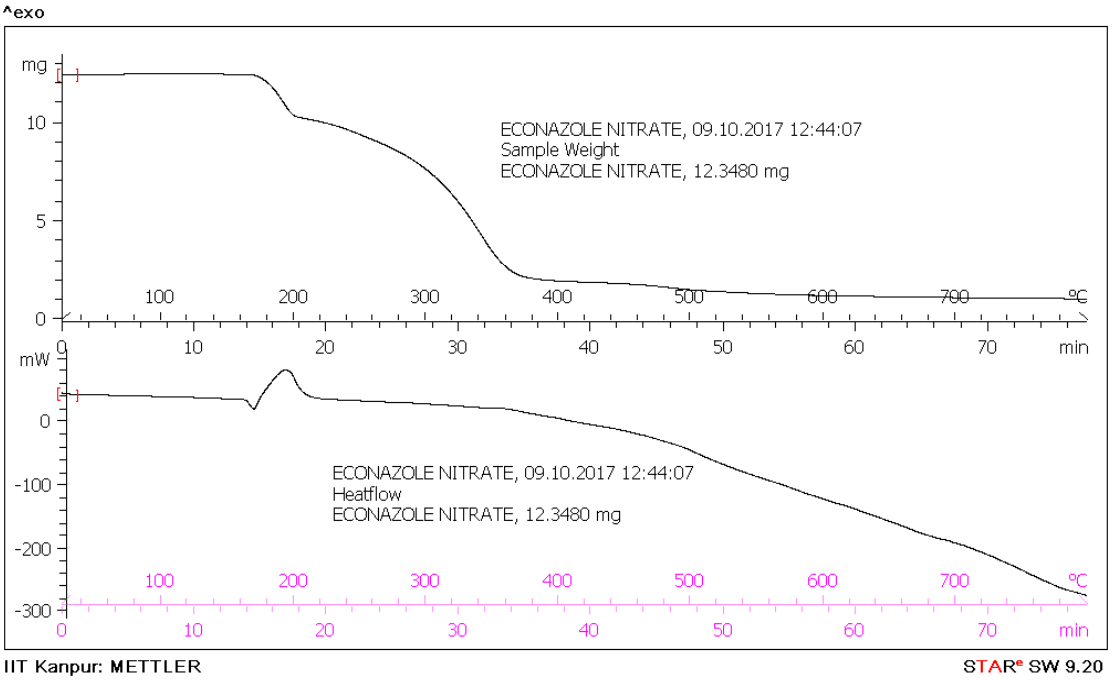

Fig. 3. DSC thermogram of Econazole nitrate

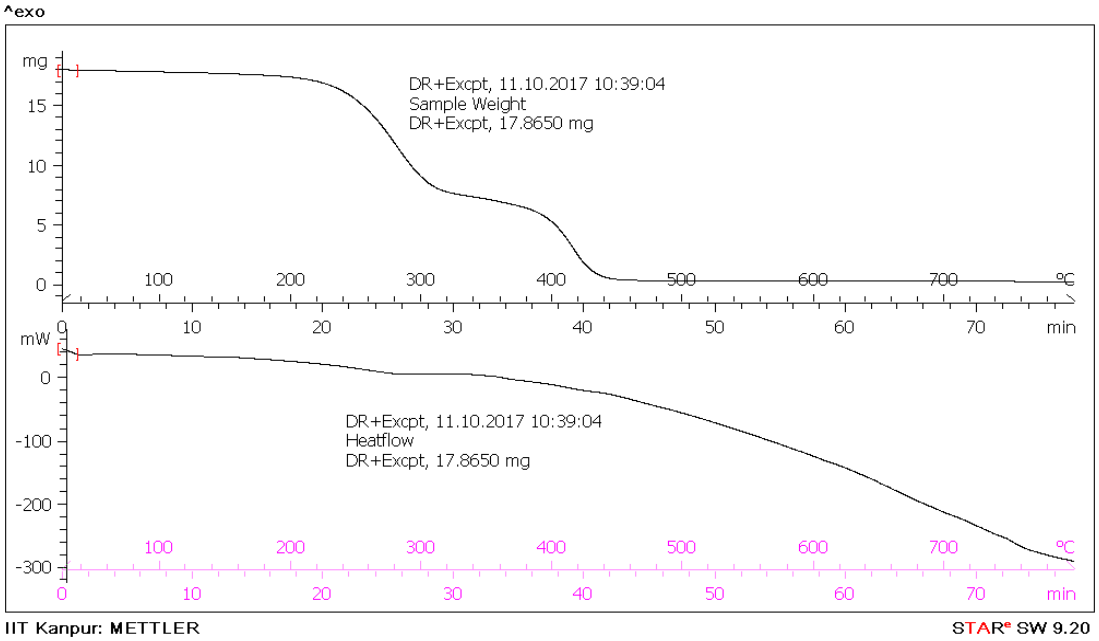

Fig. 4. DSC thermogram of drug and excipients.

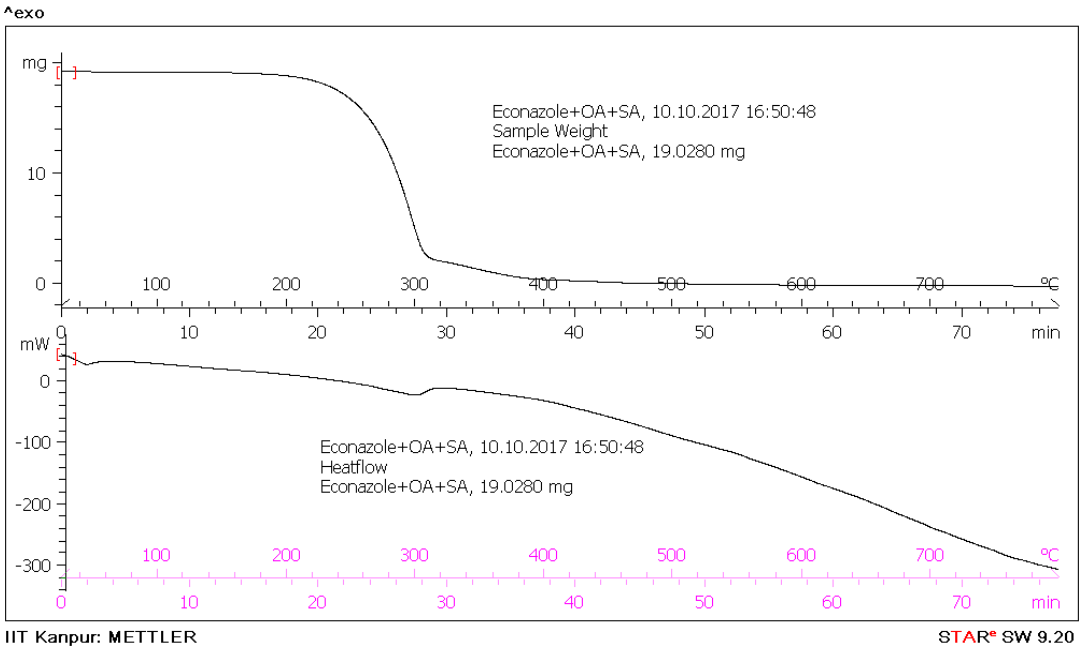

Fig. 5. DSC thermogram of NLC 3 formulation.

XRD (X-ray diffraction) studies

The XRD pattern of ECN (Fig. 6) showed prominent sharp peak at 2θ scale indicates crystalline nature. The intensity of peaks were declined in the XRD pattern of ECN loaded NLCs. Therefore, it confirmed that ECN drug lost their crystalline nature when integrated with liquid and solid lipid. The obtained results were presented in Fig. 6.

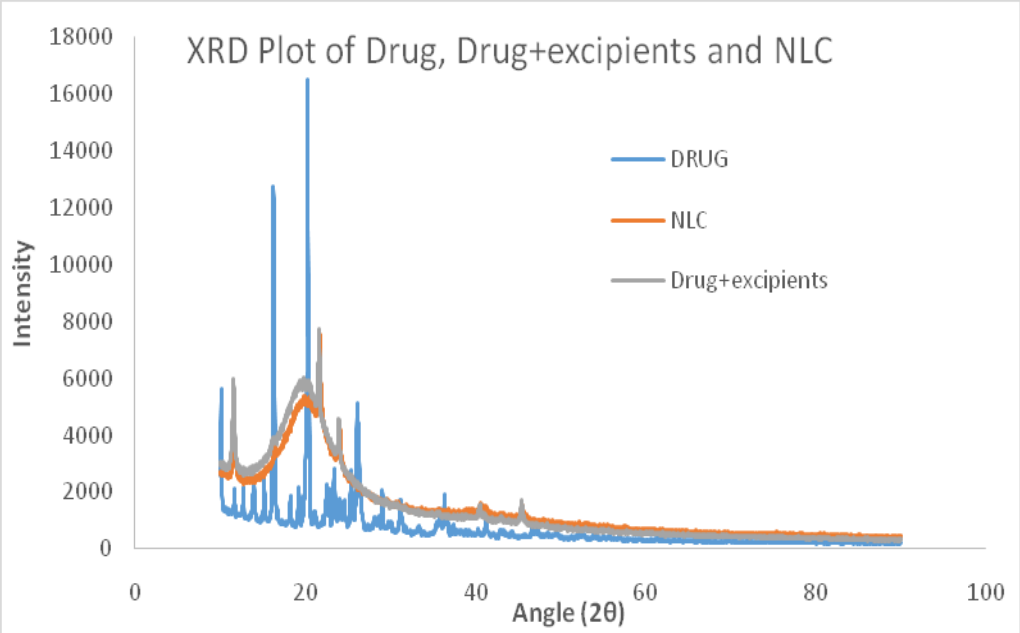

Fig 6: XRD of Drug, excipients and NLC 3 formulation.

Scanning electron microscopy (SEM)

The SEM study showed NLCs were successfully formulated, having smooth surface, distinct integrity and somewhat spherical in shape (Fig. 7). The irregularity in shape may be due to the existence of lipid.

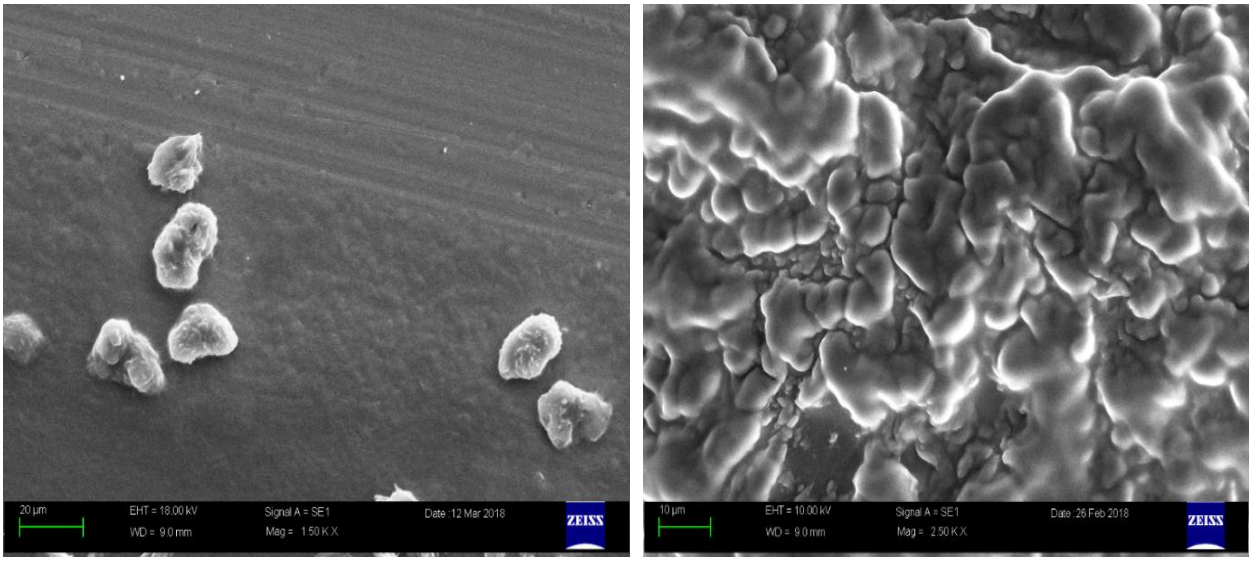

Fig 7: SEM image of Econazole nitrate loaded NLCs (a) 1.50 KX and (b) 2.50 KX.

Zeta potential determination

Zeta potential is the important parameter to analyze the physical stability of NLCs dispersion. The zeta potential of selected ECN loaded NLCs formulation were found to be -51.2 ± 7.23 mV from which it revealed that formulation is stable because combination of electrostatic and stearic effect prevents aggregation of particles with aging. The obtained results of NLC formulations presented in Table 2 and Fig. 8.

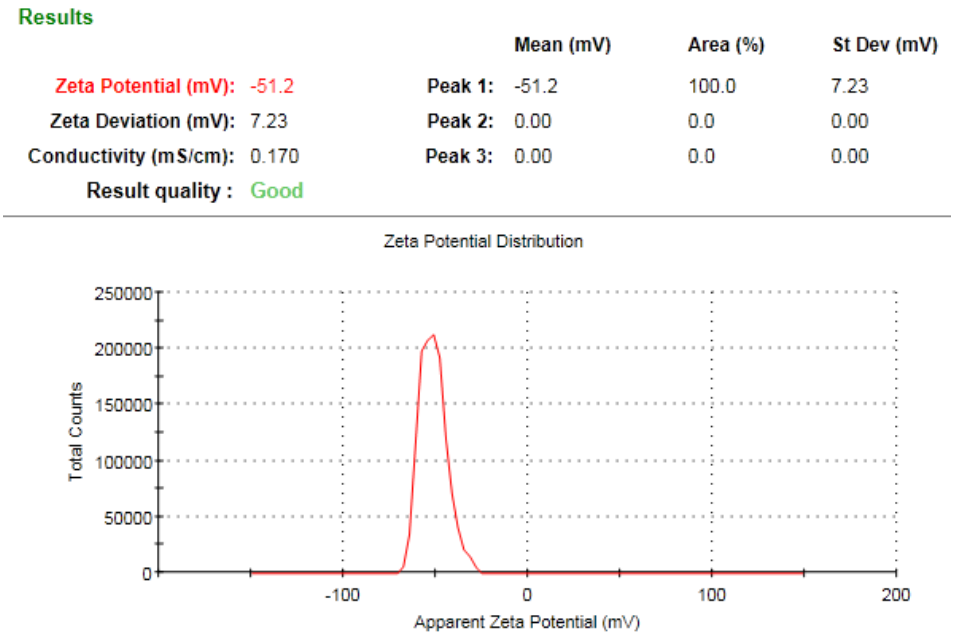

Fig. 8: Zeta potential of NLC 3 Formulation.

Table 2: % EE and Zeta potential of formulated ECN loaded NLCs.

Code	% EE	Zeta potential (mv)
NLC 1	63	-20.6 ± 1.03
NLC 2	25	-
NLC 3	73	-51.2 ± 7.23
NLC 4	54	-48.8 ± 7.37
NLC 5	22	-
NLC 6	69	-50.7 ± 8.35

Entrapment efficiency

The entrapment efficiency of ECN loaded NLCs are presented in Table 2. The result indicates that entrapment efficiency increase with increasing the concentration of lipid. The entrapment efficiency were found within the ranges of 22- 73%. The entrapment efficiency of selected ECN loaded NLCs were found to be 73% with 7% w/v lipid concentration. The higher concentration of lipid affects the amount of drug (ECN) adsorbed to the outer milieu of NLCs and the increase in the concentration of Any individual surfactant decreases % entrapment efficiency due to decrease in accommodation space for drug (ECN).

Evaluation of ECN loaded NLCs gels

In comparison to EG2 gel, EG1 gel having 1% gelling agent was found to be suitable for gelling the ECN loaded NLCs due to desirable viscosity and good spreadability. The results of formulated ECN loaded gels presented in Table 3.

Table 3: Physical parameters of Formulated gels

Code	Carbopol	Appearance	Spreadability (g.cm/sec)	Viscosity (cp)
EG1	1%	Translucent	41.02	32083
EG2	2%	Translucent	36.19	30641

***In-vitro* release studies**

The *In-vitro* release profiles of pure drug, ENC loaded NLCs and ECN loaded NLCs gel were obtained by using Franz diffusion cells. From the results it was observed that 19.47% of drug released in 2 hours from free drug. The selected ECN loaded NLCs formulation showed 53.96% drug released in 7 hours of study whereas ECN loaded NLCs gel showed 73.8% of drug released in 9 hours which revealed that formulation is suitable for extended release of drug and which is effective for topical delivery. The results are presented in Table 4 and Fig. 9.

The *in-vitro* drug release data from optimized NLC gel (EG 1) were then fitted to various models. The results are presented in Table 5. From the values of correlation coefficient (r^2) it was found that NLC gel follows first order kinetic model (r^2 = 0.962) which describes a process in which the release of any drug with respect to time is directly proportional to the concentration of drug embedded in the carrier system.

Table 4: Drug release profiles of Plain drug (ECN), Optimized formulation (NLC 3) and Gel formulation (EG 1)

Time (min.)	% Drug release		
	Plain drug (ECN)	NLCs Formulation (NLC 3)	Gel Formulation (EG1)
0	0.41	0	0
30	3.68	1.21	1.06
60	7.26	3.18	2.18
120	19.47	10.27	6.52
180	-	21.48	18.20
240	-	39.61	34.67
300	-	42.17	41.73
360	-	48.73	48.26
420	-	53.96	58.41
480	-	-	67.6
540	-	-	73.8

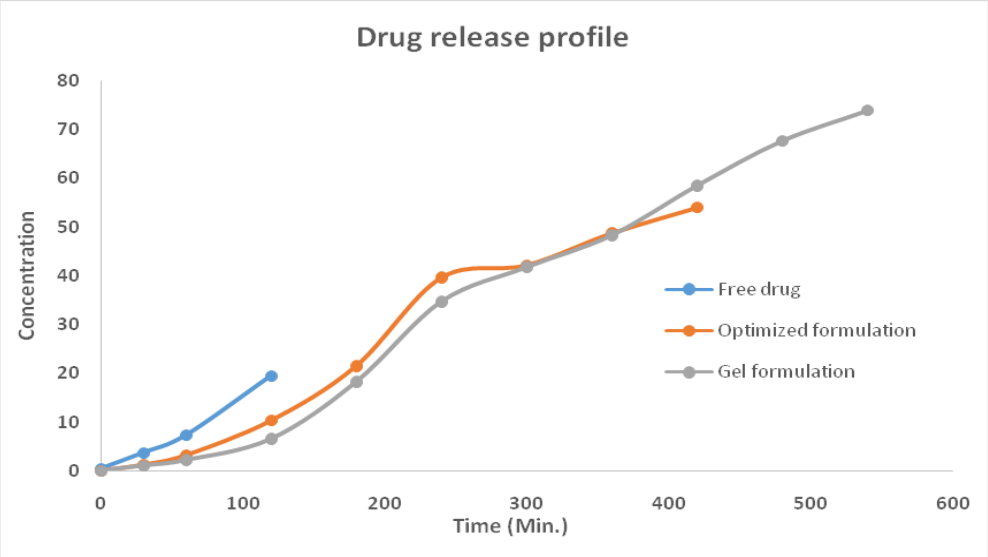

Fig. 9. *In-vitro* release profile of free drug (ECN), selected NLCs (NLC 3) and gel formulation (EG1).

Table 5: Mathematical models of Gel formulation (EG 1).

S. No.	MODELS	NLC GEL (EG 1)
		Correlation coefficient (r^2)
1	Zero order	0.8591
2	First order	0.9626
3	Higuchi	0.6096
4	Krosmeyer Peppes	0.4035

CONCLUSION

In the present study, ECN loaded NLCs were formulated using combination of oleic acid as lipid phase and stearic acid as solid phase were formulated by high speed homogenization method followed by sonication. Tween 80 and SLS used as a stabilizer. The factors affecting the NLCs formulation were optimized. The FTIR study of ECN and excipients confirmed that there was no remarkable chemical interaction between them. XRD and DSC studies confirm the transformation of crystalline nature of drug into amorphous nature. The SEM analysis showed the NLCs were formulated and having irregular shape, due to presence of lipids. The results of zeta potential predicted good particle stability because of the repulsive forces prevent aggregation with aging. The *in-vitro* release studies showed that when Econazole nitrate NLCs incorporated in cabopol gel, 73.8% of the drug is released in 9 hours, which is more than the % release of pure drug as well as drug NLCs. The NLC gel (EG 1) follows first-order kinetic model. Therefore, it can be concluded that the Econazole nitrate NLCs in gel form can be used to extend the duration of drug release and as an efficient topical drug delivery carrier for treatment of fungal infections.

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Conflict of Interest

The authors declare no conflict of interest.

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