

# Luliconazole Loaded Niosomal Topical Gel: Factorial Design, *in vitro* Characterization and Antifungal Study

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## ABSTRACT

**Aim/Background:** This research work intended to make use of prospective advantages of niosomes for the improvement in topical delivery of poorly soluble luliconazole. **Materials and Methods:** Luliconazole, an anti-fungal agent used in the treatment of fungal infection, was successfully formulated as niosomal topical gel by using a polymer carbopol 934. The  $3^2$  full factorial statistical design was employed as a means for the optimization of niosomal formulation to check the impact of two formulation factors namely, concentration of cholesterol and span 60 on two dependent variables % drug release and % drug entrapment efficiency in formulated niosomal formulations. **Results:** The mean vesicular size, Zeta potential, drug release and entrapment efficiency of optimized formulation was found  $150.6 \pm 11.1$  nm,  $-23.5 \pm 5.08$ ,  $92.41 \pm 2.44\%$  and  $92.36 \pm 2.85\%$ , respectively. The scanning electron microscopy study reveals that the optimized formulation was spherical, porous and rough in surface. The thermogram of differential scanning calorimetry study states that the drug may be solubilise or converted to amorphous form in niosomal vesicles. The viscosity and spreadability of niosomal gel were observed satisfactorily for the easy topical application. The *ex vivo* study of optimized niosomal gel showed significant higher drug release ( $97.31 \pm 3.18\%$ ) in comparison with marketed product ( $82.20 \pm 1.95\%$ ). The antifungal study showed considerable antifungal effectiveness of niosomal gel against the conventional marketed preparation. **Conclusion:** Henceforth, it can be concluded that the niosomes loaded topical gel of luliconazole may be useful for reducing the application frequency of conventional topical product and thereby improves the patient compliance.

**Keywords:** Poorly soluble drug, Nanocarrier, Topical application, Antifungal study.

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## INTRODUCTION

Although the oral drug delivery is the utmost favourable route for administration of medication, it has restricted specifically in the therapy of skin fungal diseases. The drug delivery though topical way have become better options compare to oral delivery as it exhibits no presystemic metabolism with decline in a biological toxicity and a larger amount of client satisfaction. The skin protects the superficial layer of human body against the atmospheric condition. Among the topical drug delivery systems, the niosomal gels have become greater prominent carrier because of the easiness in the application and improved topical permeation than the other semisolid formulation.<sup>1-3</sup> The niosomes are formulated using non-ionic surfactants those are safe to human body and have a capability of entangling both polar and non-polar molecules. The niosomal formulation have emerged at industrial scale due to failure of liposomal carries in

providing good stability, cheapness and inappropriate quality concerns of phospholipids.<sup>3,4</sup> The niosomal vesicles have a high capacity for skin permeation compared to free drug.<sup>5,6</sup> An antifungal drug, luliconazole is available currently in market in the form of parental dosage form, topical creams and oral forms that have significant aggressive effects on taste and gastrointestinal disturbances.<sup>7</sup> The use of topical formulations of Luliconazole (lecithin-based organogel, gel and hydrogel) is suggested, but such product may washed out in short duration that may result in loss of drug content. The scientists have come up with lipidic formulation that may significantly improve the topical permeation and localized accumulation of drug in the skin.<sup>8,9</sup> The purpose of present research study was to develop Luliconazole (LCZ) loaded niosomal gel for topical delivery to study efficacy of formulation against the fungal infection.

## MATERIALS AND METHODS

### Materials

Luliconazole was gifted from Glenmark Pharmaceuticals Ltd., Mumbai, India. The Span 60, Tween 80 and Cholesterol were procured from Loba Chemie, Mumbai, India. The Sabouraud



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**Table 1: Factors and levels for Response surface method tool.**

Independent variables	Levels		
	Low (-1)	Middle (0)	High (1)
X <sub>1</sub>	20	30	40
X <sub>2</sub>	100	120	140

X<sub>1</sub>: Amount of cholesterol (mg), X<sub>2</sub>: Amount of Span 60 (mg).

Dextrose Agar and Sabouraud Dextrose HiVeg™ Broth were procured from HiMedia laboratories, Mumbai, India.

## Methods

### Statistical Design for the Formulation of LCZ contained Niosomes

A full 3<sup>2</sup> factorial design was utilized to check the impact of two formulation parameters namely concentration of cholesterol (X<sub>1</sub>) and span 60 (X<sub>2</sub>) on two dependent variables % drug release (Y<sub>1</sub>) and % entrapment efficiency (Y<sub>2</sub>) of drug in formulated niosomal formulations using Design – Expert® Software Version 10.0.1. The various parameters and levels specified for optimization of LCZ contained niosomes are shown in Table 1.<sup>10,11</sup> During the optimization of niosomal formulation, Stirring speed (400 rpm) and quantity of drug (30 mg) were kept persistent.

### Preparation of drug loaded niosomes

Accurately weighed quantity of non-ionic surfactant and cholesterol were dissolved in diethyl ether (6mL) to make solvent system A. The solvent system B containing mixture of methanol (2 mL) and luliconazole, was prepared and added to solvent system A. The resulted organic solvent system was injected slowly to phosphate buffer saline of pH 7.4 (10 mL) using 26G needle with the addition rate of 1 mL/min. The temperature of resultant mixture was maintained at 56-58°C with the continuous stirring using magnetic stirrer. This results in the solvent vaporization followed by the formation of niosomes. The formulation was lyophilized with a cryoprotectant sucrose using the freeze dryer.<sup>12,13</sup>

### Optimization of drug loaded niosomes

In ordered to optimize the formulation, different ratios of ingredients were tried to check the effect on response variables. A total of 13 sets of run were prepared by varying the factor levels and evaluated for the responses as shown in Table 2.

### Statistical analysis

Mathematical modelling was performed to describe the relationship of dependent and independent factors.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1 X_2 + b_{12}X_{11} + b_{22}X_{22}$$

Where, b<sub>0</sub> is the intercept, b<sub>i</sub> (b<sub>1</sub> and b<sub>2</sub>) and b<sub>ij</sub> (b<sub>12</sub>). A full and reduce model for response Y was established in polynomial equation by keeping the values of regression coefficients. The Analysis of Variation (ANOVA) statistics was carried out to provide reliability of the polynomial equation. The interaction between the factors and dependent responses was nicely explained by drawing 2D and 3D contour plots.<sup>10,11,14</sup>

### Validation of statistical model

The formulation and dependent variables were utilized to check the reliability of applied statistical model. The % bias was calculated to validate the software output. The lowest value of error indicates the suitability of the model.<sup>14</sup>

### Characterization of LCZ loaded Niosomes

#### Fourier Transform Infrared (FTIR) spectroscopy

To study the compatibleness between all excipients and active ingredient, the spectra were taken by the use of spectrophotometer. The potassium bromide in a suitable mixture ratio was selected to record the spectra in the range of 4000–400 cm<sup>-1</sup>.<sup>15</sup>

### Determination of Entrapment Efficiency (EE)

The EE was estimated by centrifugation of niosomal dispersion for 45 min at 6000 rpm and -10°C. The suitable dilution of supernatant layer with phosphate buffer saline (pH 7.4) was made to estimate the concentration of untrapped drug by the use of UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) at 299 nm. The EE was calculated as follows.<sup>10,13</sup>

$$\% EE = \frac{\text{The theoretical quantity of drug} - \text{The untrapped quantity of drug}}{\text{The theoretical quantity of drug}} \times 100$$

### Determination of globule size and zeta potential

These parameters of luliconazole loaded niosomes was measured using a particle size analyser (Malvern Nono ZS 90, Malvern software ver. 2.2) at room temperature with samples properly diluted with distilled water.<sup>11,16,17</sup>

### Scanning Electron Microscopy (SEM)

The SEM study of optimized formulation was obtained with the help of scanning electron microscope. The two sided tape was utilized to mount the metal stubs containing sample with

**Table 2: Statistical design for LCZ contained niosomes.**

Formulation code	Independent factors		Dependent factors	
	X <sub>1</sub>	X <sub>2</sub>	Y <sub>1</sub> (Mean ± SD)	Y <sub>2</sub> (Mean ± SD)
LCZ 1	-1	-1	80.78 ± 2.05	76.00 ± 2.85
LCZ 2	-1	0	77.19 ± 1.95	79.11 ± 3.95
LCZ 3	-1	1	73.54 ± 3.38	82.44 ± 1.99
LCZ 4	0	-1	87.49 ± 2.78	86.59 ± 3.56
LCZ 5	0	0	92.41 ± 2.44	92.36 ± 2.85
LCZ 6	0	1	90.03 ± 3.09	92.28 ± 1.49
LCZ 7	1	-1	85.77 ± 1.59	88.22 ± 2.28
LCZ 8	1	0	83.29 ± 2.29	91.66 ± 0.86
LCZ 9	1	1	81.33 ± 4.09	92.15 ± 1.84
LCZ 10	0	0	91.22 ± 3.48	89.51 ± 2.84
LCZ 11	0	0	92.39 ± 2.25	90.21 ± 2.08
LCZ 12	0	0	91.96 ± 1.76	89.77 ± 1.64
LCZ 13	0	0	92.68 ± 2.36	89.92 ± 1.83

$n = 3$ , Y<sub>1</sub>: % Drug release, Y<sub>2</sub>: % Entrapment efficiency.

the aid of vacuum. The imageries were recorded by different enlargements. The working distance and voltage were maintained as per the protocol, 20 µm and 15 kV, respectively.<sup>18</sup>

### Differential Scanning Calorimetry (DSC)

The scanning of weighed amount of sample in an aluminium pan, was carried out at different temperature array between 50-300°C with the atmospheric dry nitrogen. The rate heating was maintained at 10°C per min. The graphs were recorded to study any possibility of interaction.<sup>11,19</sup>

### % drug release from niosomal suspension

The % drug release of Luliconazole loaded niosomes was studied by the dialysis sac method.<sup>11,20</sup> Briefly, 2 mL of Luliconazole loaded niosomal suspension was kept into a dialysis sac (Himedia-Dialysis membrane 135, Mol. cut off 12,000–14,000 Da, Mumbai, India) and place in receptor media consisting of phosphate buffer saline pH 7.4 (100 mL). This assembly was maintained at 37±0.5°C with continuous stirring speed of 100 rpm using magnetic stirrer. The aliquots at specified intervals were withdrawn, filtered by 0.45 µm filter and after proper dilution with buffer media, were determined using UV-visible spectrophotometer at 299 nm.<sup>11,20</sup>

### Formulation and optimization of niosomal gel

The optimized composition of LCZ loaded niosomes were formulated to gel made up of various concentrations of Carbopol 934 (1%, 1.5% 2% and 2.5%) with plasticizer Propylene Glycol (PG) (5% and 10%). The pH of the gel was adjusted with Triethanolamine and finally volume was made with distilled water marked up to 100 g.<sup>16,21</sup>

### Evaluation of niosomal topical gel

#### Physical appearance, drug content and pH

The physical appearance of optimized gel was measured visually for colour, uniformity and consistency. The drug content of luliconazole loaded niosomal topical gel was estimated by spectrophotometrically at 299 nm after suitable dilution with solvent. The pH of the gel was determined using a digital pH meter.<sup>22</sup>

#### Viscosity

A 100 g of accurately weighed drug loaded niosomal gel was used to measure a viscosity using a rotational Viscometer with spindle no. 96 (LVDV- III U, Brookfield, WI) at room temperature and estimate by CD-PROGA, DVA-80software.<sup>23-25</sup>

#### Spreadability

The spreadability study of gel was analyzed using wooden block and glass slide apparatus method.<sup>26</sup> The mobile top portion of apparatus was kept on the gel (5g), which was placed on the bottom portion of apparatus. The Spreadability was measured by noting the time period for the top portion to move 5cm from the initial point.

#### % Drug release study

The % drug release of optimized gel was studied by the dialysis sac method as discussed earlier.<sup>11,20,27</sup> The study was performed using Franz diffusion cell utilizing a dialysis sac. The dialysis bag was tightly placed between donor and receptor compartments. An accurately weighed drug loaded niosomal gel (1g) was kept

in donor compartment. The other experimental conditions are as same as discussed earlier.

### Ex vivo drug diffusion study

The diffusion study was carried out using a goat ear skin as a tissue membrane. The previously equilibrated animal membrane was placed in between parts of Franz diffusion cell. The equivalent of 10mg of drug in optimized gel and commercially available cream (LuliFresh-1%w/w) were utilized to perform the study. The study was performed by maintaining specified physiological condition (temperature  $37 \pm 0.5^\circ\text{C}$ ). The aliquots at specified intervals were withdrawn, filtered by  $0.45 \mu\text{m}$  filter and after proper dilution with buffer media, were determined using UV-Visible spectrophotometer at 299 nm.<sup>28</sup>

### Antifungal activity

The antifungal susceptibility study of optimized gel was carried out by agar well diffusion method in comparison with marketed product (LuliFresh-1%w/w). The Petri dishes inoculated with fungal strain (*Candida albicans*) in sabouraud dextrose agar, were bored using 9 mm sterile steel bore. Samples kept in the respective bores were undergone incubation period as per the standard specifications ( $48 \text{ hr}$  at  $37^\circ\text{C} \pm 0.5^\circ\text{C}$ ) followed by measurement of zone of inhibition.<sup>29-31</sup>

### Stability study

The stated ICH guidelines were followed to check the stability of optimized gel for the period of 30 days. The samples were put in sealed glass vials in stability chamber and maintained at identified storage conditions  $25 \pm 1^\circ\text{C}/60\% \pm 5\% \text{ RH}$  and  $40 \pm 1^\circ\text{C}/75\% \pm 5\% \text{ RH}$ . The stored gels were analysed for drug content, physical appearance, clarity, pH and viscosity for the specified time period.<sup>10</sup>

### Statistical analysis

The ANOVA was utilized to check the data statistically using a software Graph Pad Instat program version 3.01. The  $p < 0.05$  was considered as statistical significance. The all parameters were checked in triplicate.<sup>10,11</sup>

## RESULTS AND DISCUSSION

### Relations between the variables

A suitable statistical model was applied to study the relationship between formulation variables (concentration of cholesterol -  $X_1$  and concentration of span 60 -  $X_2$ ) on dependent variables (% drug release -  $Y_1$  and % entrapment efficiency -  $Y_2$ ). The highest % entrapment ( $92.36 \pm 2.85$ ) and drug release ( $92.41 \pm 2.44$ ) was obtained at minimum level (0) of  $X_1$  and  $X_2$  as depicted in Table 2. The integrity of model was examined by compiling the data in various statistical models and calculating the residual errors. The PRESS value was utilized for the determination

of dependent variables using different statistical models. The quadratic model was suggested by the statistical software due to the lowest PRESS value. The full model was applied to check the consequence between  $X_1$  -  $X_2$  and  $Y_1$  -  $Y_2$ .

$$Y_1 = 92.03 + 3.15X_1 - 2.52X_2 + 0.70X_1X_2 - 11.64X_1^2 - 0.12X_2^2$$

$$Y_2 = 89.95 + 5.88X_1 - 2.81X_2 - 0.33X_1X_2 - 4.51X_1^2 - 0.46X_2^2$$

The factors which are having non-significant effect on the result were omitted from the study to provide revised reduced model.

$$Y_1 = 92.00 + 3.15X_1 - 2.52X_2 - 11.68X_1^2$$

$$Y_2 = 89.81 + 5.88X_1 - 2.81X_2 - 4.68X_1^2$$

As per the calculation of  $p$  value given by software, the formulation factors such as  $X_1$ ,  $X_2$  and  $X_1^2$  were having positive impact on the dependent factors. For the successful prediction of model,  $F$  value is calculated. This value was very lesser than the value provided in the table ( $\alpha = 0.05$ , 2) that indicates model does not have any impact by removing such non significant terms.

### The 2D and 3D plots

The plots depicted in Figure 1 that showed relationship of formulation parameters on the predicted values.

### Check point analysis

The formulation was optimizing after setting the appropriate targets for each parameters. Based on set target of maximum drug release and entrapment efficiency, the model has provided a range of composition of formulation for achieving utmost aspiring value. The predicted formula with selected specified amounts of cholesterol and span 60 was formulated and studied for the predicted responses. The overlay plot is depicted in Figure 2.

The responses obtained for observed value were having equivalent data with that of predicted value as depicted in Table 3.

### Mean vesicular diameter, Zeta potential, Drug content, Drug release and Entrapment efficiency

The diameter was checked with the validated and precised tool. The optimized niosomal formation showed  $150.6 \pm 11.1 \text{ nm}$  vesicles diameter with PDI value of  $0.145 \pm 0.11$ . The optimized formulation was showing  $-23.5 \pm 5.08$  zeta potential that shows good stability of optimized formulation. Drug content was found  $27.108 \pm 0.31 \text{ mg}$ . The optimized formulation showed  $92.41 \pm 2.44\%$  drug release and  $92.36 \pm 2.85\%$  entrapment efficiency.

### FT-IR study

The FT-IR study depicted in Figure S1 reveals that the important all distinguished peaks  $\{1464.03 \text{ cm}^{-1}$  (C=C Chlorobenzene),  $1676.21 \text{ cm}^{-1}$  (C=C stretching),  $2150.72 \text{ cm}^{-1}$  (C≡N stretching),



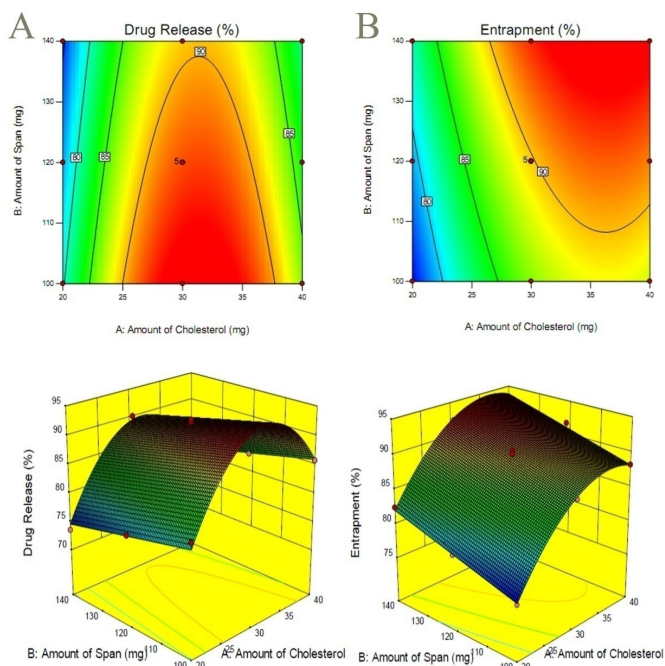
**Table 3: Check point analysis.**

Formulation Code	Independent variables		Response (%)	Predicted Value	Observed value	% Error
	X <sub>1</sub> (mg)	X <sub>2</sub> (mg)				
LCKP 1	31.00	126.00	Y <sub>1</sub>	91.44	90.86	1.53
			Y <sub>2</sub>	91.20	92.14	-2.31
LCKP 2	30.45	135.94	Y <sub>1</sub>	90.11	91.44	-1.89
			Y <sub>2</sub>	92.31	91.05	-1.38
LCKP 3	32.02	122.84	Y <sub>1</sub>	91.80	92.76	2.09
			Y <sub>2</sub>	91.20	92.74	2.71

**Table 4: Stability study for the optimized niosomal formulation.**

Time (days)	0		30	
	25°C ± 1°C/60% ± 5% RH		40°C ± 1°C/75% ± 5% RH	
Appearance	White, homogenous and smooth texture		White, homogenous and smooth texture	
Clarity	Translucent		Translucent	
pH	5.54 ± 0.01		5.51 ± 0.01	
Drug content (%)	99.2 ± 0.88		99.1 ± 0.35	

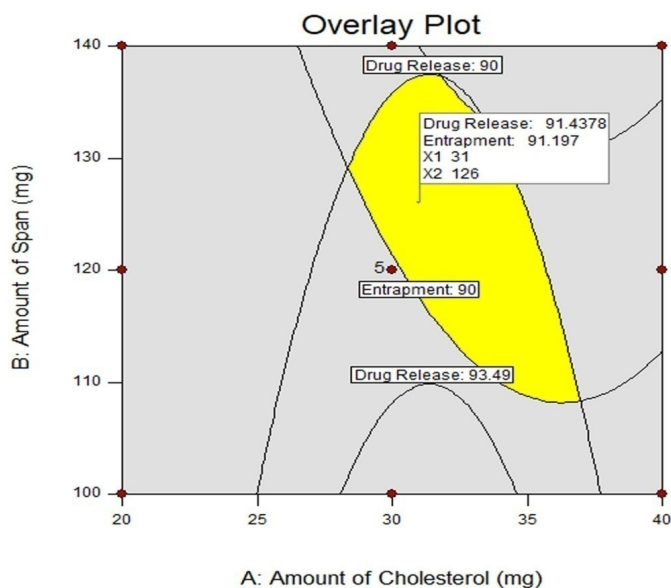
Value are expressed as mean ± SD; n=3.

**Figure 1:** Graphical presentation of 2D plots and 3D plots for (A) % drug release and (B) % Entrapment efficiency.

2571.22 cm<sup>-1</sup> (S-H stretching) and 3032.23 cm<sup>-1</sup> (C-H stretching)} found in pure chemical compound were present in the optimized niosomal formulation. This result proved that there are no any possible redundant reactions in niosomal formulation.

### SEM study

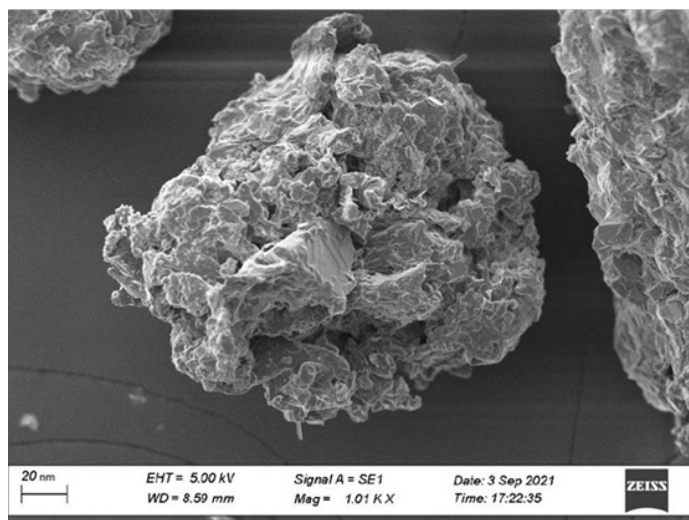
The SEM study reveals that the optimized formulation was spherical, porous and rough in surface as depicted in Figure 3.

**Figure 2:** Overlay plot for check point analysis.

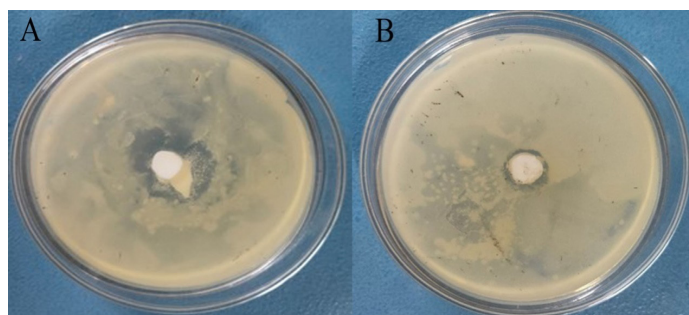
The study also confirmed the nano structure of the formulation that supports the data obtained with Zeta sizer for vesicular size.

### DSC Study

The DSC diagram (Figure S2(A)) of pure drug showed sharp melting point peak at 151.60°C which was found absent in optimized niosomal formulation. This result confirmed the fact



**Figure 3:** SEM image of optimized lyophilized niosomes.



**Figure 4:** Antifungal comparison study between (A) Optimized LCZ niosomal formulation and (B) Marketed LCZ product.

that the drug may solubilise or converted to amorphous form in niosomal vesicles as shown in Figure S2(D).

### Characterization of niosomal gel

The 1% w/w Luliconazole loaded niosomes was included into the gel base comprising of gelling agent (Carbopol 934-2%), plasticizer (PG-10%) and pH modifier (Triethanolamine) and sufficient vehicle (distilled water).

### pH, Viscosity, appearance and Spreadability

The Luliconazole loaded niosomal gel was is white, homogenous, smooth and translucent in nature. The pH was observed  $5.54 \pm 0.01$  which is suitable for topical application. The viscosity and spreadability of gel were observed 12587 cp and  $20.83 \pm 3.89$ g. cm/sec, respectively that are convenient for the easy application.

### % Drug release study

The effect of gel composition on % drug release from Luliconazole loaded niosomal gel and its comparison with marketed product (LuliFresh-1%w/w) was carried out for 8hr. The optimized formulation showed  $92.96 \pm 2.69\%$  significant higher drug release compared with  $85.95 \pm 2.05\%$  of marketed product. This confirmed that the novel formulation such as niosomes has

significant contribution in improving dissolution rate of poorly soluble drug.

### Ex vivo drug release study

This study was performed to correlate experimental condition with the physiological circumstance of application site. The optimized formulation showed  $97.31 \pm 3.18\%$  significant higher drug release compared with  $82.20 \pm 1.95\%$  of marketed product. This study contributes to the same result obtained with *in vitro* release study discussed earlier.

### Antifungal study

*In vitro* antifungal activity for luliconazole loaded niosomal gel was carried out to check the efficacy of nano formulation against fungal strain (*Candida albicans*) in comparison to marketed luliconazole preparation. The mean inhibition zone of niosomal gel and marketed product were  $25 \pm 6$  mm and  $10 \pm 3$  mm, respectively as shown in Figure 4. This data showed significant antifungal effectiveness of niosomal gel against the conventional marketed preparation.

### Stability study

The outcomes of this ageing study are shown in Table 4. The results after one month study showed no significant difference with the initial data of physical appearance of niosomal gel. The pH of the formulation was showing satisfactory results compared to the data before the period of stability study. The drug content at accelerated condition showing little degradation of drug that holds the statement that this storage condition is not favourable for niosomal gel. Therefore, it can be concluded that the  $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$  is a more favourable state for niosomal gel for a long duration of time.

## CONCLUSION

Luliconazole, an anti-fungal agent used in the treatment of fungal infection, was successfully formulated as niosomal topical gel by using carbopol 934. The rheological parameters and % drug content of optimized topical gel was having satisfactory results. The % drug release study (*in vitro* and *ex vivo*) have shown significant drug release compared to marketed product. The antifungal activity also showed significant effect that with marketed topical product. Henceforth, it can be said that the niosomes loaded topical gel of luliconazole may be useful for reducing the application frequency of conventional topical product and thereby improves the patient compliance.

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to Glenmark Pharmaceutical Ltd., Mumbai, for supplying the Luliconazole.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**LCZ:** Luliconazole; **RPM:** Revolutions per minute; **26G:** 26 gauge; **°C:** Degree Celsius; **mL:** Milliliter; **mg:** Milligram; **SD:** Standard deviation; **n:** Total numbers; **3D:** Three dimensional; **2D:** Two dimensional; **%:** Percentage; **FTIR:** Fourier transform infrared spectroscopy; **EE:** Entrapment efficiency; **UV:** Ultra Violet; **nm:** Nanometre; **SEM:** Scanning electron microscopy; **DSC:** Differential scanning calorimetry; **Da:** Dalton; **Mol.:** Molecular weight; **PG:** Propylene glycol; **g:** Gram; **µm:** Micrometre; **w/w:** Weight/Weight; **hr:** Hours; **min:** Minutes; **ICH:** International Council for Harmonisation; **ANOVA:** Analysis of variance; **RH:** Relative humidity; **cm:** Centimetre; **cp:** Centipoise.

## SUMMARY

The challenge was made to solve the problems allied with the use of marketed products which have significant aggressive effects on taste and gastrointestinal disturbances. The lipidic formulation such as niosomes may significantly improve the topical permeation and localized accumulation of drug in the skin. The luliconazole loaded niosomes were optimized by a full  $3^2$  factorial design to check the impact of two formulation parameters namely concentration of cholesterol ( $X_1$ ) and span 60 ( $X_2$ ) on two dependent variables % drug release ( $Y_1$ ) and % entrapment efficiency ( $Y_2$ ) of drug using statistical tool. The mean vesicular size, Zeta potential, drug release and entrapment efficiency of optimized formulation was found  $150.6 \pm 11.1$  nm,  $-23.5 \pm 5.08$ ,  $92.41 \pm 2.44\%$  and  $92.36 \pm 2.85\%$ , respectively. The scanning electron microscopy study reveals that the optimized formulation was spherical, porous and rough in surface. The *ex vivo* study of optimized niosomal gel showed significant higher drug release ( $97.31 \pm 3.18\%$ ) in comparison with marketed product ( $82.20 \pm 1.95\%$ ). The antifungal study showed considerable antifungal effectiveness of niosomal gel against the conventional marketed preparation. The stability study revealed that the  $25 \pm 2^\circ\text{C}/60 \pm 5\%$  RH is a more favourable state for niosomal gel for a long duration of time.

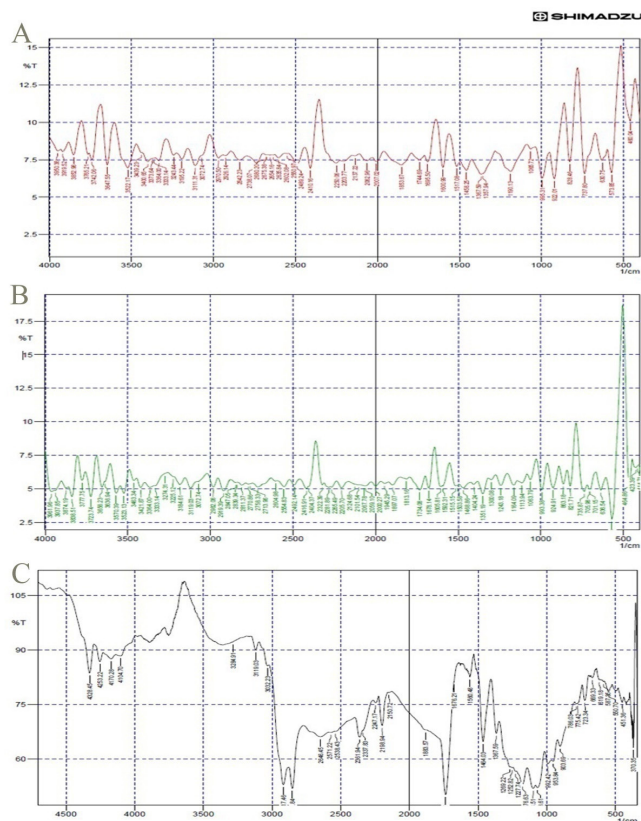
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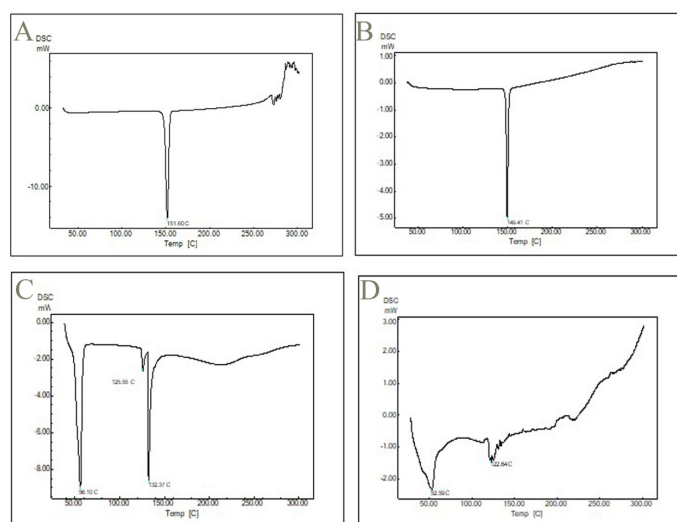


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**Figure S1:** FTIR spectra of (A) LCZ, (B) Mixture of LCZ, Cholesterol and Span 60 and (C) Optimized niosomal formulations.



**Figure S2:** DSC Thermogram of (A) LCZ, (B) Cholesterol, (C) Span 60 and (D) Optimized niosomal formulation.