RESEARCH ARTICLE

Formulation and Assessment of Lipid-based Nanoformulation of Luliconazole

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ABSTRACT

The luliconazole preformulation study aimed to determine the best way to administer medicine using SNEDDS. The plain white powder, luciconazole, showed a number of different crystal structures and was very soluble in isopropyl myristate and oleic acid, as confirmed through partition coefficients in n-octanol. With a robust linear standard curve at 295 nm, UV spectroscopy verified its dependability for analysis. Luliconazole and the necessary excipients for SNEDDS formulation were found to be compatible according to fourier-transform infrared spectroscopy (FTIR) spectra. Using a 3^2-factorial design enhanced the efficacy and stability of SN-7 batches, especially when diluted with water. Thermal studies revealed formulation dynamics-critical thermal behaviors and crystalline structures (DSC and XRD). The end SNEDDS showed a strong suppression of *Candida albicans* growth and a higher viscosity, allowing for longer surface retention. Ethical approval facilitated comparative evaluations against a commercial formulation. High-performance liquid chromatography (HPLC) analysis validated SN-7's purity, enabling a benchmark for monitoring luliconazole plasma concentrations post-application and *ex-vivo* permeation studies, vital for potential fungal infection treatments. A rigorous three-month stability study affirmed SN-7's unwavering properties across diverse environmental conditions, ensuring sustained effectiveness. An IEAC-approved skin irritation study on albino wistar rats endorsed SN-7's minimal irritancy, underscoring its safety and comfort, which is pivotal for pharmaceutical acceptance in topical drug delivery.

Keywords: Luliconazole, SNEDDS, DSC, XRD, TEM, Skin irritation test.

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INTRODUCTION

Invasions of fungi, like *Candida* or dermatophyte, can throw body out of whack and create unsightly symptoms like redness and itching. The topical antifungal medicine luliconazole's physical, chemical, and biological properties are thoroughly examined in preformulation experiments. Solubility tests are important to find the right vehicles for topical application, and stability studies ensure it works in different environments. When developing a formulation, it is crucial to consider excipient compatibility to guarantee drug integration without degradation. These findings help address a widespread health concern by facilitating the development of topical antifungal therapies that are both safe and effective.[1,2]

Improved drug solubility and bioavailability are the goals of luliconazole-containing SNEDDS. Stable nanoemulsions containing luliconazole are produced by painstakingly choosing surfactants, oils, and co-surfactants, and then adjusting their ratios. Among the many advantages of this novel system is its enhanced drug absorption and controlled release, which bodes well for the future of sustained delivery systems and the amelioration of problems associated with medications that are not highly water-soluble.

A battery of *in-vitro* experiments is conducted as part of the formulation process for SNEDDS drug distribution based on lucinazole. Important for determining the formulations' continuous drug carriage capabilities, *in-vitro* drug release studies measure the efficacy of medication's release over time, in this case lucinazole or itraconazole. To better understand release patterns and optimize formulations, researchers conduct release kinetics investigation, which investigates drug release processes. For SNEDDS to be reliable in real-world settings, it must undergo dilution studies that assess its stability and performance. Emulsification by oneself factors affecting patient compliance and the ease of application include the rate of stable

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nanoemulsion formation under skin-like circumstances and the passage of time. Patient comfort and overall efficacy are impacted by adequate handling, application, and spreadability of SNEDDS, which can be achieved through viscosity tests. Together, these investigations prove that the formulations successfully treat localized skin diseases through continuous drug administration.[3-5]

MATERIALS AND METHOD

A complimentary sample of luliconazole was obtained as a gift sample from an Indian industry located in Aurangabad called Glenmark. All chemicals and solvents utilized were of analytical reagent (AR) grade and were procured from LOBA Chemie Pvt. Ltd., Mumbai.

Preformulation Study

Organoleptic properties of luliconazole

In ensuring sensory satisfaction and compliance with topical antifungal treatments containing luliconazole, the parameters evaluated included its color, odor, and flavor. Additionally, a method was established to characterize luliconazole's polymorphic behavior through crystallization studies, focusing on stability, solubility, and bioavailability in formulations. Through these steps, formulations were optimized to enhance patient acceptance and adherence.

Melting point

It was determined using the capillary method. Luliconazole was loaded into a sealed capillary one end, and the thials tube was heated in which the capillary is immersed until the substance melted. The temperature at which the melting occurred was recorded as the melting point of luliconazole.

Solubility studies

To determine the solubility of a solute such as luliconazole, prepare solutions of various solvents at a predetermined concentration (e.g., 0.1 mol/L) using analytical grade chemicals, ensuring proper labeling and accuracy. Maintain a constant temperature (typically 25°C) throughout the experiment. Add a dry, contaminant-free sample of the solute to each solvent solution, vigorously stirring or shaking to facilitate dissolution. Allow the mixtures to equilibrate, monitoring visually for signs of solubility such as clarity or turbidity. Record observations on whether the solute completely, partially, or does not dissolve in each solvent. Repeat the process for reliability and compile results into a tabular format, noting any relevant parameters. Conclusions drawn from the solubility data can inform the formulation and development of pharmaceutical products or other applications, with thorough documentation of experimental procedures and results for future reference.

Investigation of partition coefficient

Prepare aqueous and organic solutions containing the solute, allowing them to equilibrate before separating the phases. Measure the concentration of the solute in each phase using analytical techniques, then calculate the partition coefficient using a formula.

Investigation of UV-visible spectra

First it was determined in phosphate-buffered saline solution were determined as follows: A standardized contents of saline phosphate buffer (SPB) with a pH of 7.4, was prepared. Subsequently, the UV absorption spectra of the solution were recorded using a spectrophotometer, and the maximum absorbance wavelength (λmax) was identified at 295 nm. To establish the calibration curve, solutions of luliconazole ranging in strength from 1 to 10 µg/mL were prepared using the SPB as the solvent. Light absorbed was investigated at 295 nm using UV spectroscopy, and the data were documented. A calibration curve was then constructed.. This procedure was repeated using a phosphate buffer with a pH of 6.8 and methanol (60:40 Volume/Volume) as the solvent. Luliconazole solutions within the 1 to 10 µg/mL concentration were formulated, and their optical density was gauged at 261 nm. A calibration curve was generated accordingly. The correlation coefficient (R2) was calculated to ensure the accuracy and reliability of the calibration curves.

Preparation of Luliconazole based SNEDDS

Phase solubility experiments were used to choose surfactants and lipids for the self-nano emulsifying drug delivery systems (SNEDDS) based on liconazole. The purpose of these investigations was to find out how soluble luconazole was in different types of oils, co-surfactants, and surfactants. In order to conduct phase solubility studies, luliconazole was separately combined with a variety of oils, including olive oil, castor oil, soy oil, cotton seed oil, isopropyl palmitate, isopropyl myristate, and propylene glycol, as well as with surfactants and co-surfactants PEG 200, PEG 400, tween 80, and span 80. The solubility of luliconazole in each surfactant, co-surfactant, and oil was ascertained using appropriate analytical techniques. With special emphasis paid to oleic acid and isopropyl myristate, which demonstrated maximum solubility and were recognized as viable candidates for the formation of microemulsion carriers, the solvents were selected based on their capacity to create microemulsions.

Lipids and surfactants selection

In order to find viable options for microemulsion formulation, the phase solubility experiments evaluated luliconazole's solubility in a range of oils, surfactants, and co-surfactants. The highest solubility of some oils, such as isopropyl myristate (IPM) and oleic acid, suggests they are suitable carriers. After luliconazole was gradually added and stirred until equilibrium was attained, clear solutions of oils, surfactants, and co-surfactants were created. Analytical techniques were used to assess solubility, with oleic acid and IPM exhibiting the greatest solubility among the oils examined. These results led to the development and optimization of formulations for stable microemulsions, and their promise for improved luliconazole drug delivery was further confirmed by characterization and assessment.

Drug-lipid compatibility

In the material and methods section of the research paper, drug-lipid compatibility was assessed using fourier-transform infrared spectroscopy (FTIR). The absorption wave numbers observed in the FTIR spectrum of luliconazole and the physical mixture of luliconazole-loaded SNEDDS were analyzed. FTIR spectra were obtained using appropriate instrumentation, with specific attention to absorption peaks associated with functional groups of interest. Additionally, selected formulations for SNEDDS were prepared in small batches with varying compositions of surfactants. These formulations were then subjected to FTIR analysis to assess drug-lipid compatibility. Spectral data were interpreted to evaluate any shifts or changes in absorption peaks indicative of interactions between luliconazole and the lipid excipients. This procedure aimed to elucidate the compatibility of luliconazole with lipid components for the development of effective SNEDDS formulations.

Luliconazole loaded SNEDDS optimization using factorial design

A 9-run design layout was employed, including a center point replicate to estimate experimental error and enhance robustness of the analysis. The design layout, detailed in Table 1, involved three factors at two levels each, resulting in 8 runs with an additional center point. Factors were coded as -1 (lower level), 0 (mid-level), and +1 (higher level) for independent variables, as shown in Table 2. Luliconazole-based SNEDDS formulations were prepared according to the designed experimental matrix using DOE with variations in the amounts of tween 80 and PEG 400. The response variables, including particle size and zeta potential, were measured for each formulation to evaluate their performance. This procedure aimed to optimize the formulation parameters to enhance the characteristics and efficacy of luliconazole-loaded SNEDDS.

With three factors at two levels each ($2^3 = 8$ runs), a 9-run design allows a center point replicate to be included. The center point can aid in estimating experimental error, enhancing the robustness of the analysis.

Table 1: Design layout of 3^2 factorial design

Code	*Variable (X1)*	*Variable (X2)*
Variable (X1)	-1	-1
SN-1	-1	-1
SN-2	-1	0
SN-3	-1	+1
SN-4	0	-1
SN-5	0	0
SN-6	0	+1
SN-7	+1	-1
SN-8	+1	0
SN-9	+1	+1

Table 2: Various variables with numeric values

Value	*Factors*	
	X1 (Ratio)	*X2 (Amount of surfactant)*
-1	1:3.5	3.5
0	1:4	4
+1	1:45	4.5

Characterization of Luliconazole-loaded SNEDDS

Multiple techniques were employed to characterize the SNEDDS loaded with liconazole. Differential scanning calorimetry (DSC) analysis was conducted to explore the heat flow during temperature variations for liconazole, surfactants (Tween 80), and lipid constituents (isopropyl myristate, oleic acid, PEG 400). X-ray diffraction (XRD) investigation was utilized to examine the crystalline structure of luliconazole and its components (Tween 80, isopropyl myristate, oleic acid, PEG 400). Viscosity measurements were taken to assess the thickness of SNEDDS formulations, aiding in understanding their potential for prolonged precorneal surface retention. Finally, transmission electron microscopy (TEM) analysis was performed on luliconazole-loaded SNEDDS batch SN-7 to observe the nanostructures and confirm stable drug encapsulation. Each characterization technique provided valuable insights into the physical and chemical properties of the SNEDDS formulations, contributing to their comprehensive evaluation and optimization.

Evaluation of Luliconazole-loaded SNEDDS

Drug release examination

A pH 7.4 Franz diffusion cell was used to assess the liconazole SNEDDS formulations in the *in-vitro* drug release investigation. To keep sink conditions stable, samples were taken on a regular basis and quantities were supplied appropriately. Luliconazole's concentration was measured at 295 nm using ultraviolet-visible spectrophotometry.

Analysis of release kinetics

Several mathematical models were used to examine the release kinetics in the kinetic monitoring of the *in-vitro* drug release for formulations of luliconazole SNEDDS. Zero-order, first-order, Higuchi, Hixon Crowell, and Kores Meyer Peppas models were used to assess the formulations. To evaluate the quality of fit, the coefficient of determination (R2) values were computed for every model. It would help in comprehending the drug release characteristics and formulation optimisation by offering insights into the release mechanics and kinetics of luliconazole from the SNEDDS preparations.

The dilution study

The dilution study aimed to assess how dilution affects the qualities of luliconazole SNEDDS formulations, focusing on factors like homogeneity, formation speed, and transparency of the emulsion. The procedure involved diluting each formulation with varying volumes of distilled water and

simulated tear fluid (pH 7.2) and observing the resulting emulsion qualities. Grading was then assigned according to the observed characteristics. This study provides insights into the stability and performance of the SNEDDS formulations under dilution conditions, aiding in their optimization for effective drug delivery applications.

Self-emulsification time, drug entrapment efficiency, and viscosity

The following procedure was followed to assess self-emulsification time, drug entrapment efficiency, and viscosity of luliconazole SNEDDS formulations. Firstly, self-emulsification time was determined by gently mixing each formulation with 37°C distilled water in a beaker and measuring the time taken for standardized nanoemulsions to form. Then, drug entrapment efficiency was evaluated through centrifugation and UV-visible spectrophotometry to measure the free drug content. Finally, viscosity was assessed using a Brookfield viscometer, providing crucial information for characterization and stability assurance of the SNEDDS formulations.

Ex- vivo permeation study

With Protocol number: IAEC/SKCP/11/2022-23/09, the investigational protocols for this study were accredited by the IEAC for Animal Use. In order to determine how effective topical luliconazole treatment is, a comparative *in-vivo* bioavailability evaluation was performed, with the *ex-vivo* permeation knowledge playing a crucial role. Batch SN-7, the experimental formulation, was contrasted with a commercially available luliconazole formulation in this investigation. The main goal was to determine how Luliconazole penetrates skin and how its plasma concentration changes when given topically. The HPLC analysis of batch SN-7 revealed the presence of only one peak with a retention duration of 6.59 minutes. This chemical was utilized to make comparisons. Results from this *ex-vivo* permeation investigation of batch SN-7 shed light on the drug's skin penetration and bloodstream entry capabilities.[6,7]

Stability study

Batch SN-7's stability study guarantees the consistency and efficacy of the dosage forms for three months. Conditions: relative humidity of 15, 65, and 75%; temperatures of 4, 25, and 40°C. Light exposure is meticulously managed and tracked throughout various storage environments. Periodically, samples from the SN-7 formulation batch are evaluated for a variety of physical and chemical attributes, such as potency, appearance, purity, and so on. The results demonstrated that batch SN-7 of the luliconazole formulation was stable and safe to use since it did not change or lose its effectiveness throughout the course of the three months of testing.[8-11]

Skin irritation study

In order to determine the safety and tolerability of the transdermal drug delivery optimized Batch SN-7 and the commercial formulation, the albino Wistar rats were used in the skin irritation testing. By using rigorous procedures and sticking to established norms, this study ensured reliability in its assessment of skin irritation. The rats in this investigation had 0.5 grams of the formulation applied to their skin. For three days, we tracked and recorded every instance of skin irritation, including redness (erythema) and swelling (edema). The results were evaluated using a grading system for skin irritancy, which usually uses a scale from 0 to 2, and is probably based on the guidelines set out by Aqil *et al*. The formulation showed very little to no skin irritation with its low irritancy score. This discovery proves that the formulation is safe for transdermal drug delivery since it does not irritate the skin. A key component of patient safety and comfort when employing transdermal administration systems is ensuring the absence of skin irritation, which is crucial for the formulation's acceptance in pharmaceutical use.[12-14]

Antifungal study

The antifungal study involved experiments with *Candida albicans* using the agar diffusion method to assess the inhibitory effects of various formulations. Firstly, SNEDDS batch SN-7, loaded with luliconazole, was compared against a commercial antifungal agent. Agar plates were inoculated with *Candida albicans*, and wells were created in the agar surface. Solutions of SNEDDS without drug, Marketed formulation, and the luliconazole-loaded SNEDDS formulation (SN-7) were added to the respective wells. After an appropriate incubation period, the zones of inhibition were measured to determine the extent of fungal growth inhibition.[15-16]

RESULTS AND DISCUSSION

Results of Preformulation Studies

Results of organoleptic properties of luliconazole

To ensure sensory satisfaction and compliance in topical antifungal treatments, particularly focusing on luliconazole, it's imperative to assess its organoleptic qualities. Begin by confirming its powdered white color and almost odorless aroma, which enhance its visual appeal and patient acceptance. Next, verify its flavorlessness to reassure patients that its topical application won't yield any unpleasant taste. Additionally, given luliconazole's potential for existing in various crystalline forms, a procedure must be established to characterize its polymorphic behavior. This entails conducting thorough crystallization studies and analysis to understand how different crystalline forms may impact luliconazole's stability, solubility, and bioavailability in formulations. By addressing these aspects comprehensively, formulations can be optimized to enhance patient satisfaction and compliance with topical antifungal treatments containing luliconazole.

Results of melting point

The melting point of luliconazole, determined to be 153.6°C using capillary fusion, aligns with previously reported values, confirming its thermal behavior. This characterization is crucial for quality control and formulation considerations, ensuring the stability and efficacy of luliconazole-based products.

Table 3: Results of solubility studies

S. No.	*Solvents*	*Solute*	*Concentration (mol/L)*	*Temperature (°C)*	*Solubility*
1	Water	Luliconazole	0.1	25	Insoluble
2	Ethanol	Luliconazole	0.1	25	Soluble
3	Acetone	Luliconazole	0.1	25	Soluble
4	Isopropyl alcohol	Luliconazole	0.1	25	Soluble
5	Dimethylformamide	Luliconazole	0.1	25	Soluble
6	Methanol	Luliconazole	0.1	25	Soluble
7	Oleic acid	Luliconazole	0.1	25	Partially soluble
8	Water (60%) and methanol (40%)	Dimethylsalfoxide	Luliconazole	0.1	25
9	Pure solvent (not mentioned)	Luliconazole	0.1	25	Soluble
10	0.1 N HCL	Luliconazole	0.1	25	Soluble
11	PBS pH 6.8	Luliconazole	0.1	25	Insoluble
12	PBS pH 7.2	Luliconazole	0.1	25	Insoluble
13	Saline PBS pH 6.8	Luliconazole	0.1	25	Insoluble

Results of solubility studies

The solubility studies data given in Table 3 revealed that luliconazole exhibited varying solubility profiles across different solvents and buffer solutions, with notable solubility observed in ethanol, acetone, isopropyl alcohol, and dimethylformamide. However, it displayed poor solubility or insolubility in aqueous solutions and phosphate buffer solutions, indicating challenges in formulating aqueous-based formulations. These findings underscore the importance of selecting appropriate solvents and formulation strategies to optimize luliconazole's solubility for effective pharmaceutical applications.

Results of partition coefficient

The determined partition coefficient of 2.80 for n-octanol indicates the preferential solubility of luliconazole in the oil phase, highlighting its hydrophobic nature. This finding underscores the potential for effective delivery and retention of luliconazole in lipid-based formulations for enhanced therapeutic efficacy.

Results of UV-visible spectra

The UV spectrum of luliconazole in the prepared phosphate-buffered saline (PBS) solution exhibited a maximum absorption wavelength (λ_{max}) at 295 nm (Figure 1). This value is consistent with previously reported data for luliconazole in other solvent systems, such as methanol. The presence of a single, well-defined peak suggests minimal interference from excipients or degradation products within the nanoformulation.

This result confirms the successful incorporation of luliconazole into the lipid-based formulation and demonstrates the suitability of the chosen solvent system (PBS) for further analysis using UV spectroscopy. The λ_{max} value can be used for future quantitative analysis of luliconazole content within the nanoformulation.

Construction of calibration curve of luliconazole

The calibration curve for luliconazole demonstrated a strong linear relationship between concentration and absorbance

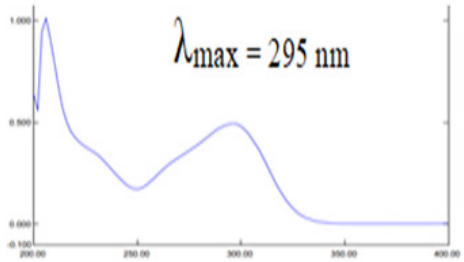


Figure 1: λmax of luliconazole

(Table 4, Figure 2). The correlation coefficient (R^2) value of 0.9988 indicates an excellent fit, signifying a highly reliable method for quantifying luliconazole within the tested concentration range (0–10 µg/mL). This confirms the validity of the chosen analytical technique (likely UV spectrophotometry) for the subsequent analysis of luliconazole encapsulated within the lipid-based nanoformulation. The high R^2 value ensures an accurate determination of drug concentration based on its absorbance measurement, allowing for a precise assessment of drug loading and encapsulation efficiency within the nanoformulation.

Results of luliconazole in combined solvent

Table 5, Figure 3 gives an overview of results of study. The strong linear relationship between concentration and absorbance was evident from the R^2 value of 0.9988, indicating excellent linearity within the tested range (1–10 µg/mL) at a wavelength of 295 nm.

This finding demonstrates the suitability of the chosen analytical technique (likely UV spectrophotometry) for quantifying luliconazole within the lipid-based nanoformulation prepared using the phosphate buffer-methanol mixture. The high R^2 value ensures accurate determination of drug concentration based on its absorbance measurement, allowing for a precise assessment of drug loading and encapsulation efficiency within the nanoformulation.

Table 4: Results of luliconazole in SBP pH (7.4)

Con (µg/mL)	*Absorbance*
0	0 ± 0.00
1	0.123 ± 0.010
2	0.239 ± 0.011
3	0.312 ± 0.015
4	0.425 ± 0.014
5	0.525 ± 0.016
6	0.628 ± 0.023
7	0.716 ± 0.023
8	0.816 ± 0.016
9	0.876 ± 0.014
10	0.996 ± 0.012

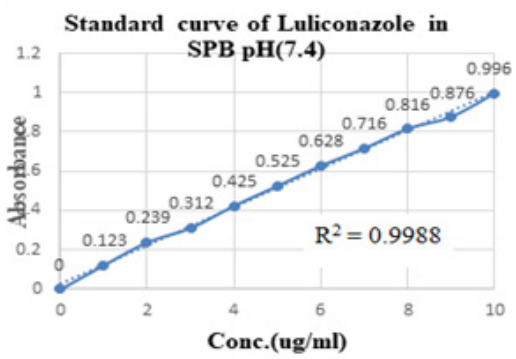


Figure 2: Calibration curve of luliconazole

Table 5: Absorbance data in mixture of solvents

Conc. (µg/mL)	*Absorbance*
1	0.121
2	0.240
3	0.313
4	0.424
5	0.523
6	0.627
7	0.720
8	0.818
9	0.880
10	0.990

Results of Preparation of Luliconazole-based SNEDDS

Lipid and surfactant selection study results

- *Phase solubility studies*

Several oils, surfactants, and co-surfactants were used to investigate the solubility of luliconazole. Tables 6 and 7 reveal that oleic acid and IPM have maximum solubility, which makes them good candidates for a microemulsions carrier.

In Table 6, PEG 400, PEG 200, and propylene glycol displayed considerable solubility of luliconazole, indicating

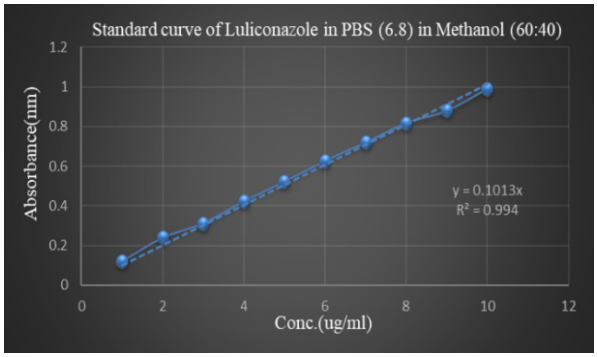


Figure 3: Calibration curve of luliconazole

Table 6: Results solubility of luliconazole in surfactants

Surfactant or co-surfactant	*Amount of dug solubilized (mg/mL)*
PEG 200	30.58 ± 2.04
PEG 400	33.63 ± 2.14
Tween 80	24.14 ± 1.07
Span 80	2.77 ± 0.22
Propylene glycol	25.73 ± 2.23

Table 7: Solubility of drugs in oils

Oils	*Solubility (mg/mL)*	*SD (n = 3)*
Olive oil	13.65	1.07
Isopropyl myristate	14.83	1.19
Cottoon seed oil	12.75	1.02
Oeleic acid	34.69	2.19
Iso-propyl Palmitate	12.05	1.23
Castor oil	5.27	2.91
Soya oil	11.38	1.08
Liquid paraffin	4.11	0.54

their effectiveness as solubilizing agents. Conversely, surfactants such as tween 80 and span 80 showed varying degrees of solubility, with tween 80 demonstrating moderate solubility compared to span 80. These differences can be attributed to the chemical properties and structures of the oils, surfactants, and co-surfactants, as well as their hydrophilic-lipophilic balance (HLB). For instance, oleic acid's high solubility may be attributed to its fatty acid nature, which likely facilitates favorable interactions with luliconazole (Table 7). Overall, these findings provide valuable insights into selecting appropriate carriers and formulations to enhance the solubility and bioavailability of luliconazole for topical delivery, laying the groundwork for further optimization and evaluation of microemulsion formulations in future studies.

Results of drug-lipid compatibility studies

These results outlined in Table 8 and Figure 4 indicate compatibility between Luliconazole and the lipid components studied by FTIR, providing a foundation for further formulation development efforts targeting optimized drug delivery systems.

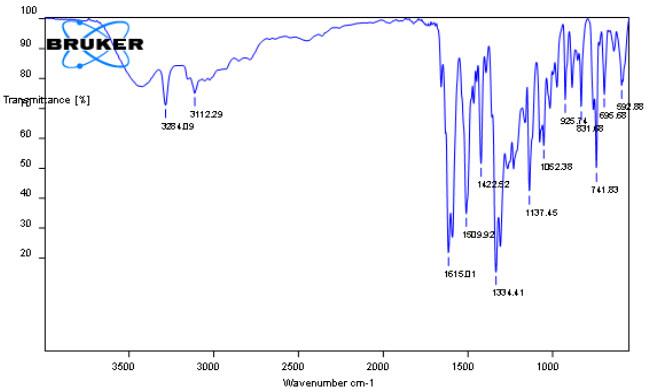


Figure 4: IR spectra of luliconazole

Table 8: Absorption wave numbers observed in the FTIR spectrum of luliconazole

Wave number (cm^{-1})	*Functional group/vibration*
3500–3200	O-H (alcohol, carboxylic acid)
3000–2800	C-H stretching (alkane)
1700–1650	C=O (ketone, ester, carboxylic acid)
1600–1500	C=C (aromatic)

The results of dilutions of ternary mixtures by water, presented in Table 9, show a progressive increase in turbidity with decreasing amounts of oleic acid and isopropyl myristate and increasing water content. This indicates a decrease in the solubilizing capacity of the mixture as the lipid content diminishes, resulting in the formation of turbid solutions. Such observations suggest that oleic acid and isopropyl myristate are crucial for maintaining clarity in the formulations, with higher lipid concentrations yielding transparent or slightly turbid solutions. This underscores the importance of lipid content in stabilizing microemulsions and highlights the need for optimizing lipid concentrations to achieve desired formulation characteristics.

Luliconazole loaded SNEDDS optimization by means of DoE

The factorial design optimization give in Table 10 of luliconazole-based SNEDDS reveals significant impacts of the combination concentration of tween 80 and PEG 400 on particle size and zeta potential. Increasing tween 80 and PEG 400 concentrations generally lead to a reduction in particle size, indicating improved drug dispersion within the formulation. Conversely, zeta potential becomes more negative with higher amounts of both surfactants, indicating enhanced stability due to increased repulsion between particles. Optimal particle size and zeta potential conditions could be achieved with intermediate levels of tween 80 and PEG 400, as observed in runs 7. These findings underscore the importance of carefully balancing surfactant concentrations to achieve desired characteristics in SNEDDS formulations for luliconazole delivery.

Table 9: Results of dilutions of ternary mixtures by water (1:1 Mix)

Mixture	*Tween 80 (mL)*	*Polyethylene glycol 400 (mL)*	*Observation*
1	4.5	4.5	Translucent
2	4	4	Somewhat unclear
3	3.5	3.5	Turbid
4	3	3	Turbid
5	2.5	2.5	Turbid
6	2	2	Turbid
7	1.5	1.5	Turbid
8	1	1	Turbid
9	0.5	0.5	Turbid

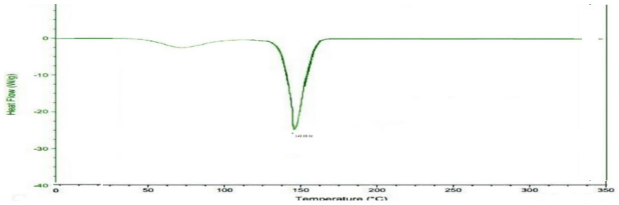


Figure 5: Graph for drug luliconazole

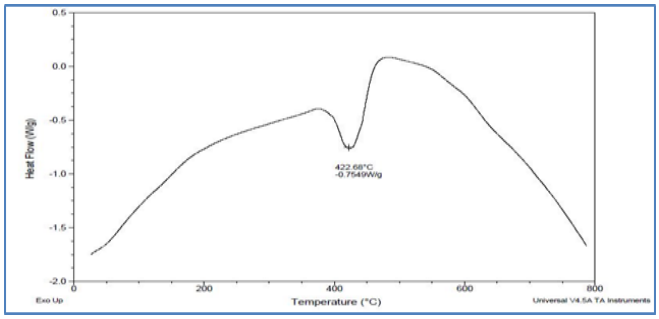


Figure 6: Curve for surfactant tween 80

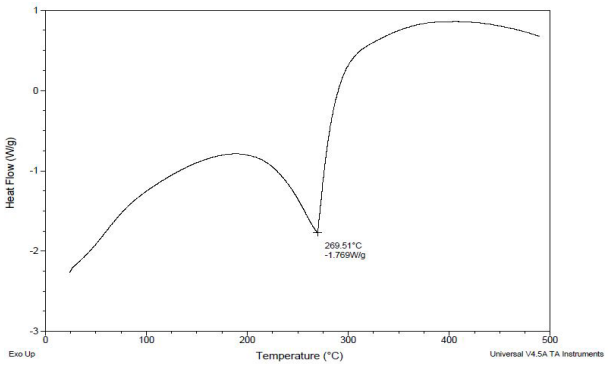


Figure 7: Thermo gram of isopropyl myristate

Characterization of Luliconazole-loaded SNEDDS

DSC studies

Differential scanning calorimetry (DSC) analysis was conducted to explore the heat flow during temperature variations for drug and excipients.

Table 10: Results of optimization investigations

	Factor		*Response*	
	1	*12*	*1*	*2*
Run	*A: Amount of tween 80 (mL)*	*B: Amount of PEG 400 (mL)*	*Particle size*	*Zeta potential*
1	40	44	90.6	-10.2
2	45	44	85.5	-10.5
3	50	44	79.6	-10.9
4	40	45	75.5	-11
5	45	45	60.6	-11.2
6	50	45	50.5	-12.3
7	40	46	55	-15.5
8	45	46	45.5	-16.4
9	50	46	41.2	-18.6

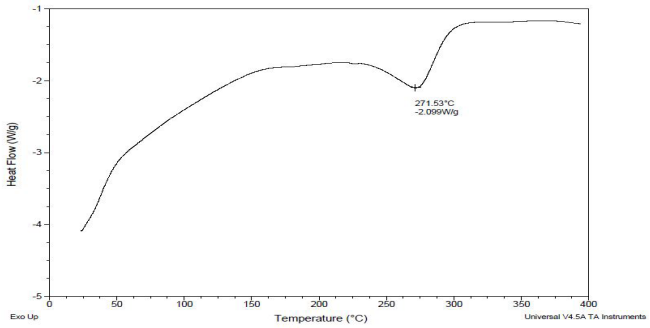


Figure 8: Thermo gram of oleic acid

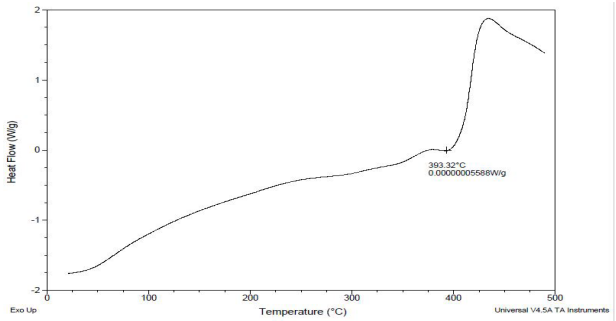


Figure 9: Thermo gram of PEG 400

XRD study

XRD is a crucial tool in materials science, geology, chemistry, and physics because it analyzes X-ray diffraction patterns to determine crystalline structure.

Viscosity

The surfactant in SNEDDS loaded with luliconazole makes them thicker, which means the solution stays on the precorneal surface longer, which could mean better drug penetration. Among 9 luliconazole formulations with varied SNEDDS viscosities, formulation SN-7 demonstrated optimal viscosity (Table 11).

TEM

Nanoprecipitation-prepared SNEDDS formulations of batch SN-7 loaded with luliconazole were studied using TEM.

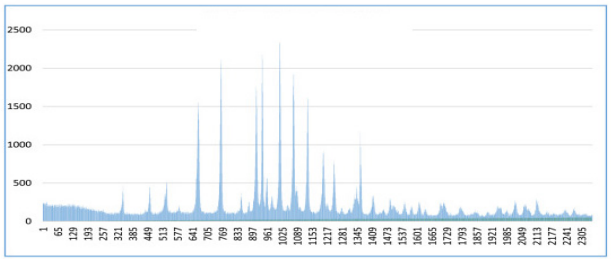


Figure 10: XRD of drug

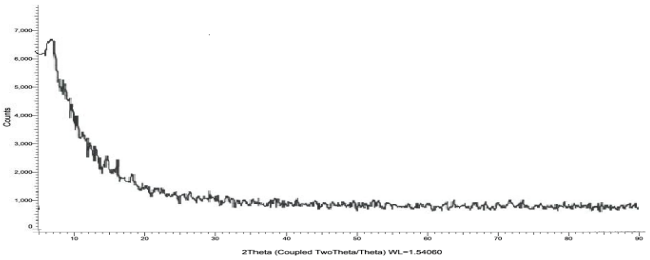


Figure 11: XRD of surfactant tween 80

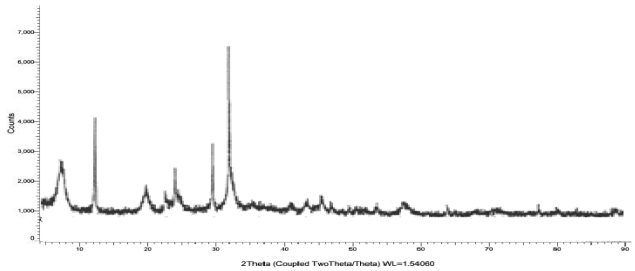


Figure 12: XRD of isopropyl myristate

The comprehensive characterization of luliconazole loaded SNEDDS formulations through various analytical techniques provides valuable insights into their physical and structural properties, crucial for their effectiveness in drug delivery

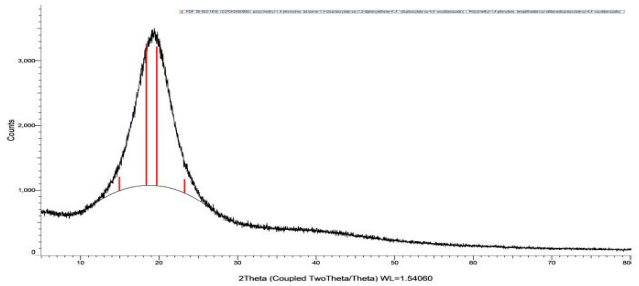


Figure 13: XRD of oleic acid

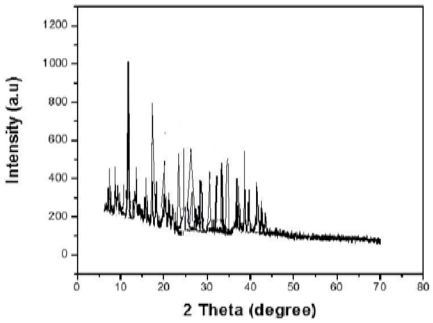


Figure 14: XRD of PEG 400

applications. Differential scanning calorimetry (DSC) analysis depicted the drug's and excipients' heat flow profiles, revealing their thermal behavior and potential interactions. For instance, DSC plots shown in Figures 5 to 9 of luliconazole and its carriers such as tween 80, isopropyl myristate, oleic acid, and PEG 400 elucidated their respective thermal transitions and crystalline structures. Similarly, X-ray diffraction (XRD) studies illustrated in Figures 10 to 14 provided further elucidation of crystalline characteristics, aiding in understanding the physical properties and stability of the formulations. Moreover, viscosity measurements (Figure 15) highlighted the impact of formulation composition on solution thickness, indicating the potential for improved drug penetration and retention on the precorneal surface. SN-7 formulation demonstrated optimal viscosity, suggesting its potential as an effective drug carrier. Furthermore, transmission electron microscopy (TEM) observations confirmed the presence of homogeneous nanostructures as found in Figure 16 in SN-7 formulations, indicating stable drug encapsulation and promising potential for efficient drug delivery.

The combination of DSC, XRD, viscosity analysis, and TEM imaging provided a comprehensive understanding of luliconazole SNEDDS formulations, elucidating their thermal behavior, crystalline structure, viscosity profiles, and nanostructure morphology. These insights are invaluable for optimizing formulation compositions, ensuring stability, and enhancing drug delivery efficiency. SN-7 formulation emerged as a promising candidate with optimal viscosity and stable nanostructures, indicative of its potential for effective drug delivery applications. Further studies and optimization efforts based on these findings are warranted to harness the full therapeutic potential of luliconazole-loaded SNEDDS formulations for enhanced patient outcomes in topical drug delivery.

Table 11: Viscosity of luliconazole loaded in nano delivery system

Coded values	*Viscosity (cps)*
SN:-1	1.4
SN:-2	1.7
SN:-3	2.1
SN:-4	2.6
SN:-5	2.9
SN:-6	3.1
SN:-7	3.6
SN:-8	3.9
SN:-9	4.1

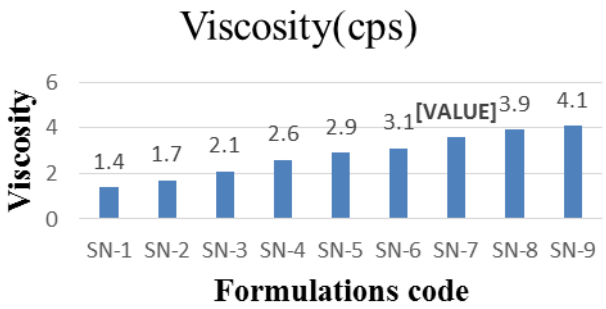


Figure 15: Graphical representation of viscosity of different SNEDDS

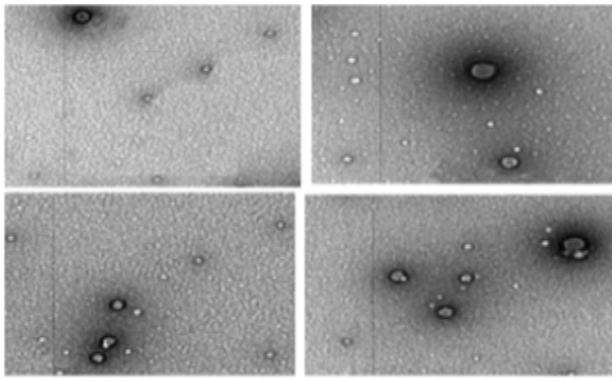


Figure 16: Transmission electron microscopy of luliconazole loaded SNEDDS batch SN-7

Evaluation of Luliconazole-loaded SNEDDS

In-vitro drug release study

In-vitro drug release study was performed and results are mentioned in Table 12.

Release kinetics study

Data about the rate of drug release from its pharmaceutical formulation in batch SN-7 can be found in the kinetic profiles of *in-vitro* drug release for lulicanazole (Table 13).

Dissolution testing is a common method used in this type of investigation to track the controlled release of drugs. The

Table 12: %Drug release of various batches

Time (Hours.)	*SN-1 %*	*SN-2 %*	*SN-3 %*	*SN-4 %*	*SN-5 %*	*SN-6 %*	*SN-7 %*	*SN-8 %*	*SN-9 %*
0.5	39.01408	35.02321	38.02508	32.02508	45.02508	39.02508	50.28169	42.02508	45.02508
1	40.42254	39.52002	39.03252	37.03252	48.03252	42.03252	53.38028	47.03252	48.03252
2	43.23944	40.25121	41.2356	38.2356	52.2356	48.2356	55.07042	48.2356	51.42356
4	48.59155	45.23132	45.25631	42.25631	58.25631	51.25631	57.32394	52.25631	53.25631
6	55.07042	50.26152	52.26333	48.26333	64.26333	54.26333	66.90141	58.26333	58.26333
8	62.85915	55.25147	59.24632	53.24632	69.24632	59.24632	77.32394	63.24632	63.24632
10	68.02817	65.26632	67.00215	62.00215	72.00215	69.00215	84.36623	69.00215	69.50215

Table 13: Luliconazole SNEEDS drug release kinetic patterns *in-vitro*

Code of formulation	R^2					
	Zero Order	*1st Order*	*Higuchi Model*	*Hixon Crowell model*	*Kores Meyer Peppas*	
					R^2	*n (slope)*
SN-1	0.860	0.860	0.933	0.997	0.965	0.655
SN-2	0.945	0.915	0.951	0.914	0.978	0.835
SN-3	0.835	0.835	0.957	0.790	0.951	0.827
SN-4	0.895	0.895	0.947	0.932	0.966	0.900
SN-5	0.940	0.940	0.979	0.978	0.980	0.834
SN-6	0.933	0.897	0.861	0.910	0.762	0.817
SN-7	0.953	0.981	0.961	0.982	0.927	0.917
SN-8	0.932	0.932	0.979	0.974	0.977	0.817
SN-9	0.978	0.978	0.992	0.993	0.988	0.838

Table 14: Results of dilution studies of formulated novel dosage forms

Formulation codes	*Distilled water (mL)*		*Simulated tear fluid (pH 7.2) (mL)*		*Grade found*
	10	*100*	*10*	*100*	
SN-1	less clear		less clear		B
SN-2	less clear		less clear		B
SN-3	less clear		less clear		B
SN-4	less clear		less clear		B
SN-5	less clear		less clear		B
SN-6	Translucent		Translucent		A
SN-7	Translucent		Translucent		A
SN-8	Fine milky emulsion		Fine milky emulsion		C
SN-9	Fine milky emulsion		Fine milky emulsion		C

outcomes usually show the SN-7 batch's release pattern, which can adhere to different kinetic representations.

Dilution study

The investigation focused on how dilution affects emulsion qualities. Grading was done according to the homogeneity, formation speed, and transparency of the emulsion; the grades ranged from A (fast, clear) to E (slow, with visible oil globules) (Table 14).

Results of time required for self-emulsion formation, drug entrapment efficiency and viscosity studies

The characterization studies of luliconazole SNEDDS formulations revealed valuable insights into their performance and stability. Table 15 demonstrates the rapid emulsification time of SN-7, indicating its potential for efficient nanoemulsion formation. The table also illustrates the entrapment efficiency of various batches, with SN-7 exhibiting the highest efficiency,

Table 15: Results of time required for self-emulsion formation, drug entrapment efficiency and viscosity of different batches

Coded batches	*time required for self emulsion formation (sec.)*	*Entrapment efficiency (%)*	*Viscosity (cps)*
SN-1	27.6 ± 1.2	69.4 ± 1.2	1.47 ± 0.008
SN-2	31.3 ± 1.2	60.4 ± 1.2	1.42 ± 0.01
SN-3	36 ± 0.8	66 ± 0.8	1.61 ± 0.01
SN-4	41.6 ± 0.4	60.6 ± 0.4	1.49 ± 0.01
SN-5	25 ± 0.8	52.3 ± 0.9	1.54 ± 0.008
SN-6	30.3 ± 0.9	70.4 ± 1.2	1.65 ± 0.02
SN-7	21.3 ± 1.2	74 ± 0.8	1.66 ± 0.01
SN-8	32.6 ± 1.2	44.7 ± 1.2	1.56 ± 0.01
SN-9	30.3 ± 0.9	40.2 ± 1.2	1.42 ± 0.01

Table 16: *Ex-vivo* permeation study of luliconazole SNEDDS and marketed product

Time (Hr)	*SNEDDS without drug % ± SD*	*Marketed brand % ± SD*	*SNEDDS SN-7 % ± SD*
00	0.00 ± 000	00 ± 00	00 ± 00
01	0.00 ± 000	0.200 ± 0.1	0.302 ± 0.1
03	0.00 ± 000	0.850 ± 0.2	1.023 ± 0.3
06	0.00 ± 000	1.554 ± 0.2	2.530 ± 0.3
09	0.01 ± 000	3.690 ± 0.4	5.033 ± 0.3
12	0.01 ± 000	7.369 ± 0.3	11.365 ± 0.4
15	0.01 ± 000	15.360 ± 0.2	20.303 ± 0.4
18	0.02 ± 000	31.364 ± 0.3	42.036 ± 0.6
21	0.02 ± 000	46.230 ± 0.5	62.236 ± 0.3
24	0.02 ± 000	62.036 ± 0.6	80.210 ± 0.5

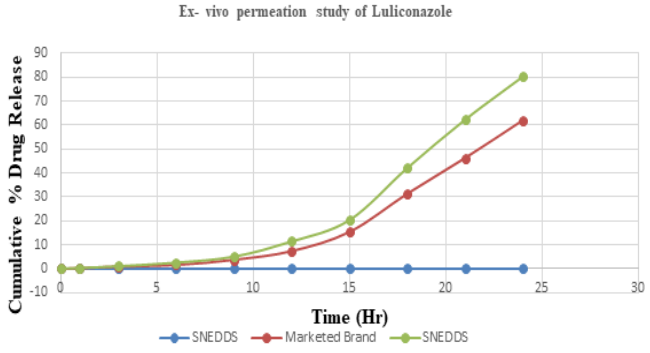


Figure 17: Graph of *ex-vivo* permeation study of luliconazole SNEDDS and marketed formulations

Table 17: Stability study of luliconazole SNEDDS formulation

Temp (°C)/RH (%) condition	*Time period (Days)*	*Observation SN-7*	*Assay*
4.0/15	15	No change	101.0 ± 0.1
4.0/15	30	No change	101.3 ± 0.1
4.0/15	45	No change	101.2 ± 0.1
4.0/15	60	No change	101.1 ± 0.1
4.0/15	75	No change	101.1 ± 0.1
4.0/15	90	No change	101.0 ± 0.1
25/65	15	No change	101.1 ± 0.1
25/65	30	No change	101.1 ± 0.1
25/65	45	No change	101.1 ± 0.1
25/65	60	No change	101.1 ± 0.1
25/65	75	No change	100.1 ± 0.1
25/65	90	No change	100.0 ± 0.1
40/75	15	No change	101.0 ± 0.1
40/75	30	No change	99.90 ± 0.1
40/75	45	No change	98.10 ± 0.1
40/75	60	No change	97.00 ± 0.1
40/75	75	No change	96.00 ± 0.1
40/75	90	No change	0.1

suggesting effective drug encapsulation. Additionally, the table highlights the viscosity of the formulations, influencing their emulsification rate and ultimately affecting drug release kinetics. Overall, SN-7 emerges as a promising formulation with quick emulsification, high entrapment efficiency, and suitable viscosity, demonstrating its potential for enhanced drug delivery and stability.

Ex-vivo permeation study

An *ex-vivo* permeation study was performed, and the results are mentioned in Table 16 and Figure 17.

Results of stability studies

Stability studies were performed, and the results are presented in Table 17.

Skin irritation study

Skin irritation study was performed and the results are mentioned in Table 18.

Antifungal study

The agar diffusion process was employed to conduct microorganism experiments. The findings indicated that, as compared to marketed formulation, luliconazole-loaded SNEDDS batch SN-7 substantially suppresses the growth of *Candida albicans*. Table 19 and Figure 18, which show the mean diameter of the zone of inhibition, provide the data for *C. albicans*.

Table 18: The skin irritation study of the marketed formulation and luliconazole loaded SNEDDS formulation

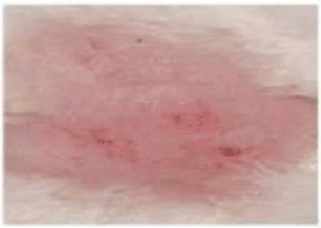
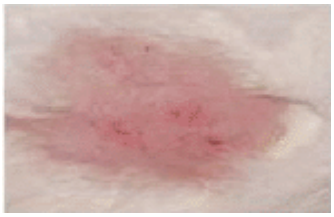
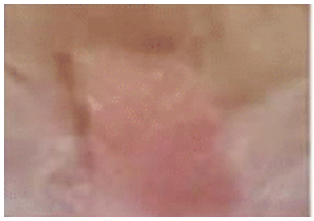
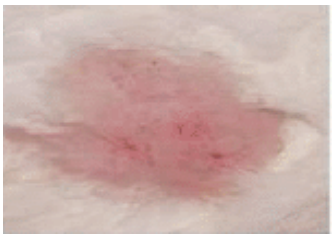
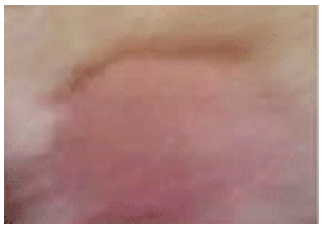
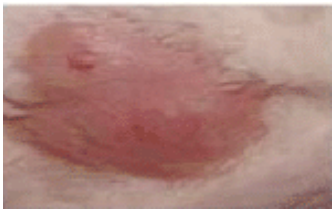
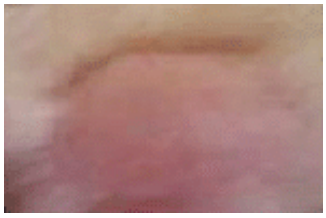
S. No.	*Days*	*Marketed formulation*	*SNEDDS SN-7*	*Observations*
	00			No redness/swelling
	01			No redness/swelling
	02			No redness/swelling
	03			No redness/swelling

Table 19: Results of antifungal studies

Solution	*Amount of growth inhibited (mm)*
Formulation blank	01
Standard drug formulation	09
Formulation with drug inside	12

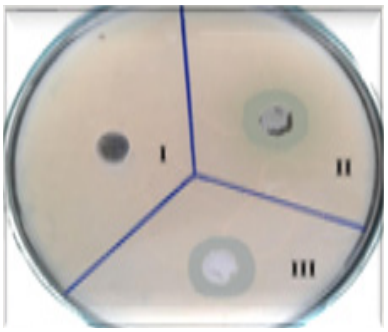


Figure 18: Zone of inhibition

CONCLUSION

The optimization of SN-7 batches through a 3^2-factorial design, revealing enhanced efficacy and stability, particularly upon water dilution, signifies a leap toward developing robust drug delivery systems. Insights from thermal analyses (DSC and XRD) and the observed increased viscosity of SNEDDS emphasize their potential for prolonged therapeutic effects, notably demonstrated by significant inhibition of *C. albicans* growth.

The approval of experimental protocols and successful comparative analyses against commercial formulations substantiate luliconazole's efficacy and potential systemic distribution. *Ex-vivo* permeation studies further enhance our understanding of its bioavailability, which is particularly crucial for designing effective treatments, especially for fungal contagions.

A three-month stability study under diverse environmental conditions and an IEAC-approved skin irritation study on albino wistar rats affirms that SN-7's stability, consistency, and safety are pivotal for patient comfort and wider pharmaceutical acceptance. In conclusion, these findings pave the way for

advancing topical drug delivery systems, ensuring sustained effectiveness, safety, and patient comfort. The insights gained from this study hold promise for developing more efficient and patient-friendly pharmaceutical formulations in the future.

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