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DESIGN AND EVALUATION OF AMPHOTERICIN B AND LULICONAZOLE NANOEMULSIONS FOR TARGETED ANTIFUNGAL DELIVERY

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ABSTRACT
Background: Drugs like Amphotericin B and Luliconazole, which are poorly soluble in water and undergo significant first-pass metabolism, often show low bioavailability. Using nanoemulsion-based delivery systems can enhance absorption and efficacy in the treatment of fungal infections. This study aimed to develop and optimize nanoemulsion formulations of Amphotericin B and Luliconazole to improve their solubility and stability and to demonstrate potential for enhanced bioavailability. **Methods:** Preliminary characterization of Amphotericin B and Luliconazole included solubility analysis in various solvents, melting point determination, particle size, zeta potential, FTIR spectroscopy, DSC, and XRD. Amphotericin B was further evaluated using a validated RP-HPLC method and subjected to forced degradation studies. Pseudo-ternary phase diagrams were constructed to identify suitable Smix ratios for nanoemulsion formation. Formulations were prepared by homogenization and optimized using a central composite design. Key variables included globule size, zeta potential, homogenization speed, and time. **Results and Discussion:** The optimized Amphotericin B nanoemulsion (NE-02-8) exhibited a globule size of 168.2 nm, zeta potential of –28.9 mV, PDI of 0.578, drug content of 99.28%, and 99.48% transmittance. Statistical optimization using a Central Composite Design (CCD) confirmed that homogenization speed and time significantly influenced globule size ($p < 0.05$) and zeta potential ($p < 0.05$). In contrast, the Luliconazole nanoemulsion showed a globule size of 327.5 nm and a zeta potential of –27.9 mV. **Conclusion:** Nanoemulsion formulations of Amphotericin B and Luliconazole demonstrated enhanced solubility, stability, and physicochemical properties, indicating their potential to improve drug solubilization and stability relative to conventional formulations.

INTRODUCTION

Fungal infections remain a significant global health concern, particularly among immunocompromised individuals. The therapeutic management of such infections often relies on antifungal agents like Amphotericin B and Luliconazole, which exhibit broad-spectrum activity against pathogenic fungi. However, both drugs are classified as Biopharmaceutical Classification System (BCS) Class IV compounds, characterized by low aqueous solubility and poor permeability, resulting in suboptimal bioavailability and therapeutic response when administered through conventional dosage forms [1,2].

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Amphotericin B, though potent, is poorly soluble in water and demonstrates considerable toxicity when administered systemically, primarily due to its aggregation and nonspecific interactions with mammalian cell membranes [3]. Similarly, Luliconazole, an imidazole antifungal, is practically insoluble in water, limiting its clinical utility for systemic applications and reducing the efficiency of topical formulations [4].

Two separate nanoemulsions were developed rather than a combined formulation because Amphotericin B and Luliconazole differ significantly in lipophilicity, solubility, therapeutic application, and required dose levels. A combined nanoemulsion would complicate drug–excipient compatibility, co-solubilization, and droplet-size optimization. Therefore, independent nanoemulsions were necessary to optimize each drug's physicochemical parameters [5]. To address these limitations, nanoemulsion (NE)-based drug delivery systems have gained considerable attention. Nanoemulsions are clear, stable mixtures of oil, surfactants, co-surfactants, and water, with droplet sizes typically between 20 and 200 nanometers. These systems offer several advantages, including enhanced solubilization of hydrophobic drugs, improved mucosal permeability, protection from degradation, and prolonged retention time at the site of infection [6,7].

Recent advances from 2022 to 2024 have highlighted the growing interest in nanoemulsion-based delivery of antifungal agents, emphasizing improved permeability, reduced toxicity, and enhanced topical efficacy. These studies underpin the rationale for exploring optimized nanoemulsions for Amphotericin B and Luliconazole [8,9]. Given these advantages, this study was designed to develop and optimize nanoemulsion formulations of Amphotericin B using a Quality by Design (QbD) approach. Preliminary studies, including solubility testing, particle characterization, and structural analysis, were conducted to confirm drug identity and guide formulation development. A pseudo-ternary phase diagram was used to determine the optimal Smix ratio, and the formulations were optimized using a central composite design (CCD) to evaluate the effects of homogenization speed and time on critical quality attributes. The final formulations were assessed for stability, drug content, particle size, zeta potential, and physicochemical characteristics. Castor oil was selected for its high solubilizing capacity for polyene antifungal agents and its established safety in topical and parenteral formulations. Polysorbate 60 and 80 were chosen for their high HLB values (14.5–15), strong emulsification efficiency, and compatibility with highly lipophilic APIs. The QbD-based CCD approach was adopted to systematically evaluate the effects of homogenization parameters on critical quality attributes (CQAs), thereby addressing a gap in the literature, as optimization of Amphotericin B nanoemulsions remains insufficiently explored [10,11].

Several studies have demonstrated that nanoemulsions loaded with Amphotericin B not only improve solubility but also reduce toxicity and enhance antifungal efficacy [12]. Topical nanoemulsions, in particular, offer site-targeted delivery with minimal systemic side effects. Hussain et al. [13] formulated stable, permeable Amphotericin B nanoemulsions with excipients that possess natural antifungal properties, thereby improving skin penetration and enhancing therapeutic efficacy. Studies by Caldeira et al. [14] and dos Santos Matos et al. [15] also supported the potential of Amphotericin B-loaded nanoemulsions for effective antifungal therapy in the treatment of leishmaniasis and other fungal infections, demonstrating high efficacy and favorable safety profiles. Sosa et al. [12] formulated a topical NE for the treatment of candidiasis and aspergillosis, achieving sustained drug release and improved patient compliance.

The current study focuses on the development of nanoemulsions containing amphotericin B and Luliconazole [16-19], composed of the polymer castor oil, Polysorbate 60, and Polysorbate 80. The Homogenization technique was used for formulation, and the resulting Nanoemulsions were assessed to confirm the desired drug release for the management of fungal diseases. Although bioavailability was not experimentally evaluated in this study, the enhanced solubility, reduced droplet size, and improved stability suggest a potential for improved bioavailability, subject to future in vitro permeability or in vivo investigations.

MATERIALS AND METHODS

Chemicals and Reagents

Amphotericin B and Luliconazole were provided as gift samples by Aadhaar Life Sciences Pvt. Ltd. (India). Acetonitrile (HPLC grade) was procured from Qualigens Fine Chemicals (Mumbai, India), and formic acid was obtained from Thomas Baker Chemicals Pvt. Ltd. (Mumbai, India). Surfactants, including Polysorbate 60 and Polysorbate 80, were purchased from Croda Inc. (India), while castor oil was supplied by AOS Products Pvt. Ltd. (India).

All weighing procedures were conducted using NABL-calibrated analytical balances to ensure measurement accuracy. Sample preparation was carried out using Class A borosilicate glassware, and all measurements were performed under standardized laboratory conditions unless otherwise specified.

Methodology

Preliminary analysis of Drugs

The preliminary characterization of Amphotericin B and Luliconazole was conducted to confirm their identities and assess physicochemical properties essential for formulation development. The evaluation included solubility profiling in various solvents, melting-point determination, Fourier transform infrared (FTIR) spectroscopy, particle-size analysis, zeta-potential measurement, differential scanning calorimetry (DSC), and X-ray diffraction (XRD) studies. These analyses were conducted to verify the purity, crystallinity, and thermal behavior of the active pharmaceutical ingredients (APIs). Forced degradation studies of Amphotericin B included acid hydrolysis (0.1N HCl), alkaline hydrolysis (0.1N NaOH), oxidation (3% H_2O_2), photolysis, and thermal stress. Samples were neutralized, filtered, and quantified via validated RP-HPLC. The method was validated for linearity ($R^2 > 0.999$), precision (%RSD < 2), accuracy (98–102%), LOD, LOQ, and robustness as per ICH Q2(R1). Forced degradation studies were selectively performed for Amphotericin B due to its known chemical instability, polyene macrolide structure, and documented susceptibility to hydrolytic, oxidative, and photolytic degradation. These studies were essential for establishing a stability-indicating analytical method and assessing formulation-related protective effects.

In contrast, Luliconazole is a chemically stable imidazole derivative with well-established stability profiles reported in pharmacopeial and regulatory literature. Since the objective of the present study did not include analytical method validation or degradation pathway assessment for Luliconazole, forced degradation studies were not conducted for this drug.

Preformulation studies

Solubility of Amphotericin B in different oils

An excess amount of Amphotericin B was added to different oils (castor, olive, linseed, peanut, Capryol 90, isopropyl myristate, light liquid paraffin, and mineral oil). The mixtures were shaken for 3 hours, stored in a refrigerator overnight, then centrifuged at 2500 rpm for 10 minutes. The supernatants were diluted and analyzed using HPLC.

Solubility of Amphotericin B in different surfactants

Excess drug was mixed with surfactants (Oleth-2, Polysorbate 60/80, Span 80, Transcutol HP/P, Kolliphor RH 40) under the same conditions as the oils, and HPLC was used to determine solubility.

Solubility of Luliconazole in Oils and Surfactants

The solubility of Luliconazole was quantitatively evaluated to inform the selection of excipients for a nanoemulsion formulation. Excess Luliconazole was added to various oils (castor oil, olive oil, isopropyl myristate, mineral oil) and surfactants (Polysorbate 80, Polysorbate 60, Span 80), followed by equilibration, centrifugation, and HPLC analysis. Luliconazole exhibited the highest solubility in castor oil (≈ 412.6 µg/mL), compared to other screened oils. Among surfactants, Polysorbate 80 and Polysorbate 60 demonstrated superior solubilization capacity (≈ 1685.3 µg/mL and 1427.8 µg/mL, respectively). These results justified selecting castor oil as the oil phase and Polysorbates as the Smix components for the development of Luliconazole nanoemulsions.

Construction of pseudo-ternary phase diagram

Phase diagrams were developed by gradually adding water to different Smix ratios (1:1, 2:1, and 1:2). The formation of emulsions was monitored to determine the areas where nanoemulsions were formed. Titration was performed at 25 ± 1°C using incremental water additions (0.1 mL per increment). Clarity was assessed visually and by measuring % transmittance at 650 nm. The criteria for a clear nanoemulsion region were the absence of turbidity, the absence of phase separation, and a transmittance >95%.

Formulation of Prototype Nanoemulsion of Amphotericin B

Formulation of Nanoemulsion without surfactant

From the solubility data, initially 10 mg of Amphotericin B was dissolved in 40 ml of castor oil. Later, 1 ml of this castor oil (containing 250 µg of Amphotericin B) was diluted to 100 ml with water. The prepared solution was homogenized at 10,000 rpm for 30 minutes. This prepared nanoemulsion was evaluated for the drug particle size (Table 1).

Formulation of Nanoemulsion with selected Smix

From the solubility data, the quantity of drug that can be solubilized in the above formulations is given in Table 1. The amount of the drug in the Nanoemulsion formulations ranges from 0.0157% to 0.0456% w/v.

Table 1: Formulation table of Amphotericin B Nanoemulsion

Batches	Amphotericin B (mg)	Castor oil (ml)	Smix (2:1) (ml)		Water (ml)
			Polysorbate 80 (ml)	Polysorbate 60 (ml)	
NE-1	45.63	3.33	20.00	10.00	66.67
NE-2	35.59	5.71	15.24	7.62	71.43
NE-3	28.05	7.50	11.67	5.83	75.00
NE-4	28.52	11.43	11.43	5.71	71.43
NE-5	21.87	12.50	8.33	4.17	75.00
NE-6	18.79	15.00	6.67	3.33	75.00
NE-7	15.70	17.50	5.00	2.50	75.00

Optimization of Amphotericin B Nanoemulsion formulation

The formulation was optimized using Design-Expert software (Version 13) via a Central Composite Randomized Design. The study investigated two independent factors—homogenization speed (X_1) and homogenization time (X_2)—while the outcomes measured were globule size (R_1) and zeta potential (R_2). For clarity, formulations NE-1 to NE-7 correspond to pre-optimization prototype batches, whereas NE-02-1 to NE-02-9 refer to CCD-generated experimental runs. 'Run X' in Table 5 directly corresponds to 'NE-02-X' in Table 6.

Evaluation of Amphotericin B Nanoemulsion

a) Globule Size: Globule size was analyzed using a Horiba SZ-100 (Horiba Scientific) at 25°C via dynamic light scattering (DLS). Samples were appropriately diluted with deionized water and measured using disposable cuvettes under constant refractive index, viscosity, and dielectric constant conditions.

b) Zeta Potential: Assessed using Horiba SZ-100 at 25°C with a disposable cuvette. The sample was diluted in deionized water before measurement.

c) Polydispersity Index (PDI): Determined by DLS using Horiba SZ-100 at 25°C. The diluted sample was analyzed in a disposable cuvette.

d) Drug Content: The drug content was determined using HPLC after diluting the sample with a suitable solvent. The percentage of the drug present was calculated from the peak area.

e) % Transmittance: The absorbance was measured at 650 nm using a UV spectrophotometer. Two milliliters of the formulation were diluted to 100 mL with distilled water, which also served as the blank.

f) Stability Studies: The optimized formulation was stored under accelerated conditions (40 ± 2°C and 75 ± 5% relative humidity) according to ICH guidelines. Samples were evaluated at initial (day 0), 1 month, 2 months, and 3 months intervals for parameters including globule size, zeta potential, polydispersity index (PDI), drug content, and percent transmittance.

The accelerated stability study was limited to three months as an initial predictive assessment, consistent with early-stage formulation development guidelines, to identify potential physical instability trends before extended six-month testing.

Formulation of Nanoemulsion with selected Smix

With reference to Amphotericin B Nanoemulsion, Luliconazole Nanoemulsion will be prepared using castor oil, Smix (2:1), and water by using the same platform technology from the already prepared Amphotericin B Nanoemulsion (Table 2). The prepared solution was homogenized at 7000 rpm for 90 minutes. This prepared Nanoemulsion was evaluated for drug globule size and zeta potential.

Evaluation of Nanoemulsion of Luliconazole

The prepared Nanoemulsion was evaluated for Globule size and Zeta Potential.

Table 2: Formulation table of Luliconazole Nanoemulsion

Batches	Luliconazole (mg)	Castor oil (ml)	Smix (2:1) (ml)		Water (ml)
			Polysorbate 80 (ml)	Polysorbate 60 (ml)	
Luli. Nano emulation	35.59	5.71	15.24	7.62	71.43

RESULTS AND DISCUSSION

Preliminary analysis of Drugs

a) Description

Amphotericin B is a bright yellow powder. Luliconazole is an off-white to pale yellow crystalline powder.

b) Solubility

Amphotericin B is insoluble in water, anhydrous alcohol, ether, benzene, and toluene. It is soluble in DMF, DMSO, and propylene glycol and slightly soluble in methanol. Luliconazole is practically insoluble in water. It is freely soluble in DMF and acetone, and soluble in Acetonitrile and methanol, and sparingly soluble in ethanol.

c) Melting Point

The melting point of Amphotericin B was approximately 170℃. The melting point of Luliconazole was approximately 152℃.

d) FTIR analysis

i. Amphotericin B

The FT-IR spectrum of Amphotericin B is given in Figure 1.

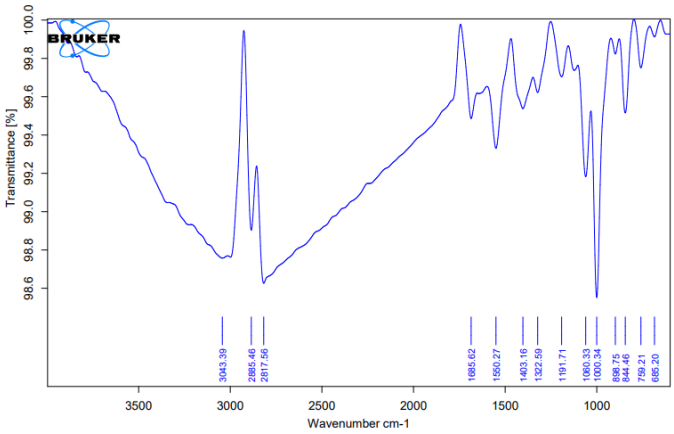

Figure 1: IR spectrum of Amphotericin B

Amphotericin B shows characteristic transmittance. The key peaks in the IR spectrum of Amphotericin B include the stretching vibrations of the following:

2885 cm^{-1} → C–H stretching

1685 cm^{-1} → C=O (carbonyl) stretching

1550 cm^{-1} → Amide II band

1403 cm^{-1} → C–H bending

The spectrum was compared with the standard spectrum of Amphotericin B and was found to agree.

ii. Luliconazole

The FT-IR spectrum of Luliconazole is given in Figure 2. Luliconazole shows characteristic transmittance, and the key peaks in the IR spectrum of Luliconazole include the stretching vibrations of the following:

3011.63 cm^{-1}	C - H stretch
2426.78 cm^{-1}	C ≡ N stretch
1511.31 cm^{-1}	C=C-C Aromatic Ring Stretch
1474.36 cm^{-1}	C ≡ C aromatic Stretch
855.74 cm^{-1}	Para C-H distribution

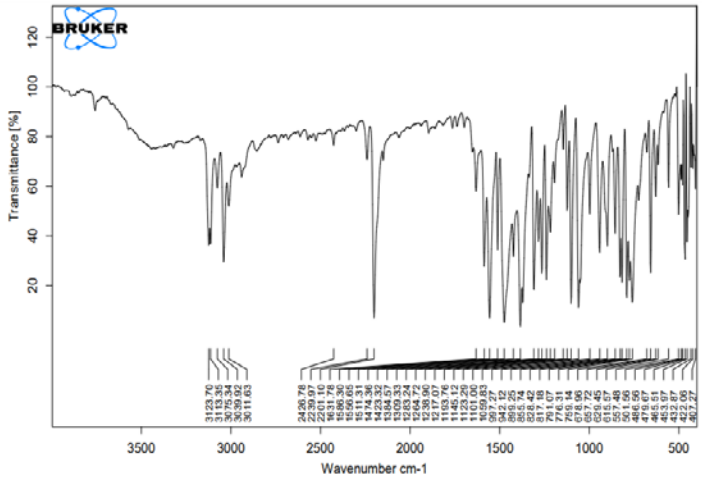

Figure 2: IR spectrum of Luliconazole

The spectrum was compared with the standard spectrum of Luliconazole and was found to comply.

e) Particle Size:

- The measured particle size of Amphotericin B was approximately 908.8 nm.
- The measured particle size of Luliconazole was approximately 254.4 nm.

f) Zeta potential:

- The measured zeta potential of pure Amphotericin B was approximately -29.1 mV, indicating moderate stability due to surface charge.
- Pure Luliconazole exhibited a zeta potential of about -25.1 mV, suggesting a similar level of electrostatic repulsion and dispersion stability.

g) DSC analysis of drugs

The DSC Curve of Amphotericin B is given in Figure 3.

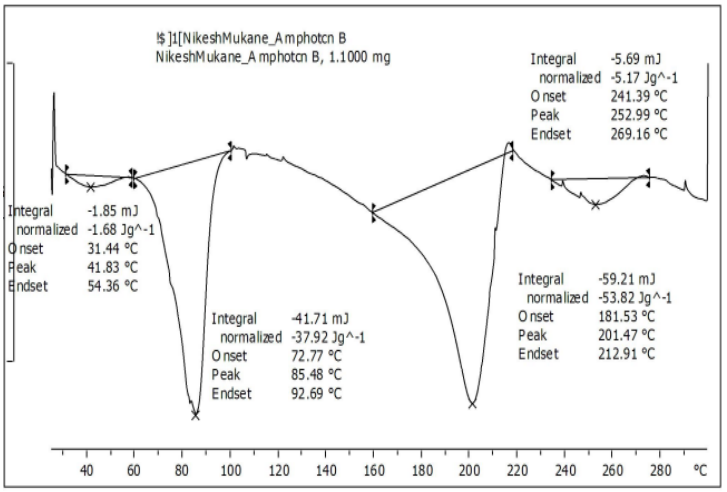

Figure 3: Thermogram of Amphotericin B

The results closely match the literature reference for DSC analysis of amphotericin B.

The DSC Curve of Luliconazole is given in Figure 4

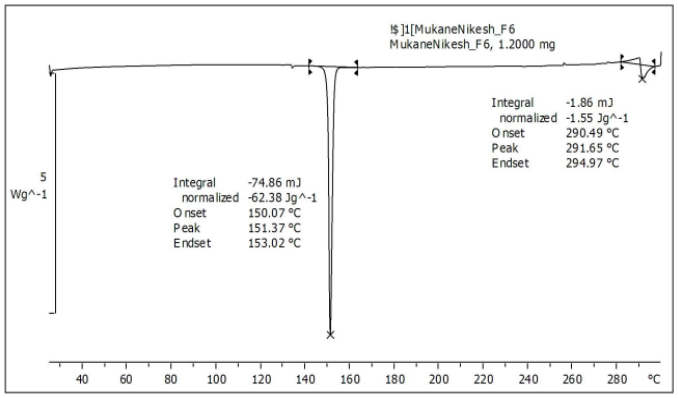

Figure 4: Thermogram of Luliconazole

The results closely match the literature reference for DSC analysis of Luliconazole.

g) XRD of Drugs

The XRD Graph of Amphotericin B is given in Figure 5.

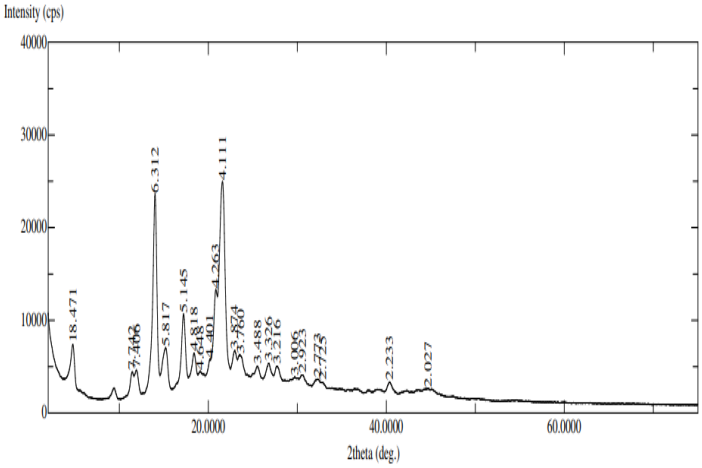

Figure 5: XRD Graph of Amphotericin B

The Amphotericin B API was crystalline, as it exhibited strong diffraction throughout the analysis.

The XRD Graph of Luliconazole is given in Figure 6.

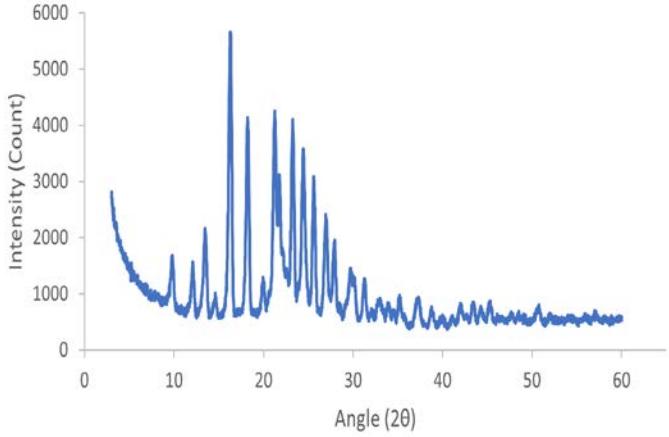

Figure 6: XRD Graph of Luliconazole

The Luliconazole API was crystalline, as it exhibited significant diffraction throughout the analysis.

Preformulation Studies

Solubility of Amphotericin B in different oils

The solubility of Amphotericin B was estimated in different oils. The maximum solubility of AmB was observed in Castor Oil at a concentration of 257.38 µg/ml. Based on the oil-solubility results, Castor Oil was selected for the formulation of a Nanoemulsion. The graphical representation of solubility in different oils is given in Figure 7.

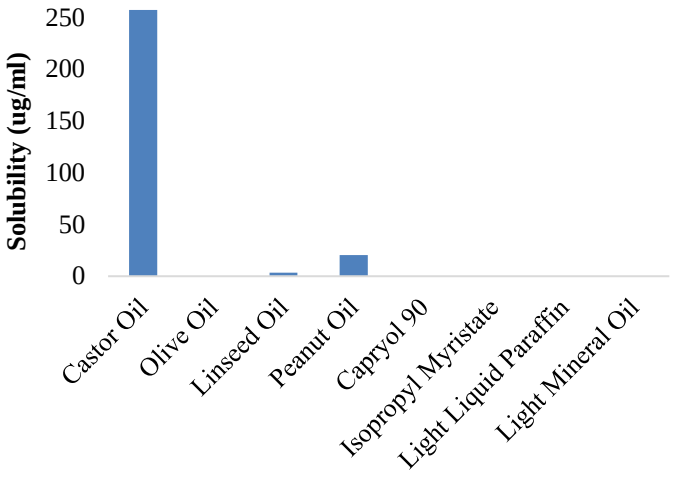

Figure 7: Solubility of Amphotericin B in Oils

Solubility of Amphotericin B in different surfactants

The solubility of Amphotericin B was estimated in different surfactants. From the solubility data, it was observed that Amphotericin B had the highest solubility in polysorbate 80 and 60, at 1583.97 and 1309.54 µg/ml, respectively; therefore, these were selected as the surfactant and co-surfactant for the preparation of Smix (Figure 8).

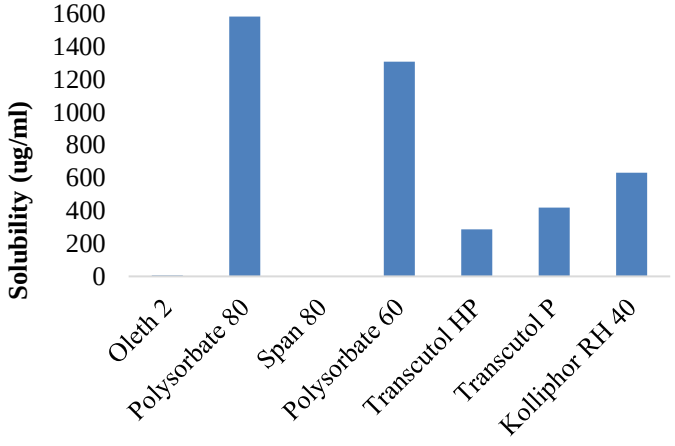

Figure 8: Solubility of amphotericin B in surfactants

Construction of pseudo-ternary phase diagram

A pseudo-ternary phase diagram was constructed by using the water titration method.

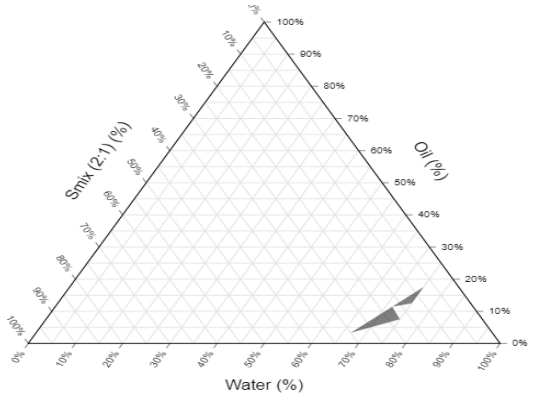

Figure 9: Pseudo ternary phase diagram using Smix 2:1

The grey region in the pseudo-ternary phase diagram represents the nanoemulsion region. The area in all 3 phase diagrams was smaller due to the lower solubility of castor oil in the water and Smix. The Smix with a 2:1 ratio exhibited the largest nanoemulsion region among the two-phase diagrams and was therefore suitable for the formulation of a Nanoemulsion (Figure 9). Quantitative comparison of pseudo-ternary phase diagrams revealed that the Smix ratio of 2:1 exhibited the largest nanoemulsion region, accounting for approximately 38–42% of the total diagram area, compared to 22–25% for Smix 1:1 and 15–18% for Smix 1:2.

Additionally, formulations prepared with Smix 2:1 consistently exhibited lower mean globule size (<200 nm), higher optical clarity (% transmittance >98%), and greater physical stability, as assessed by visual inspection. Based on these quantitative parameters, the Smix 2:1 surfactant-to-co-surfactant ratio was selected as the optimal.

Formulation of Prototype Nanoemulsion

Formulation of Nanoemulsion without surfactant

The nanoemulsion collapsed within an hour (oil globules on the surface), indicating that this type of formulation is unstable and that surfactant must be incorporated to stabilize it. The second limitation is that the drug's solubility in castor oil is low, resulting in a lower drug-loading capacity and incompatibility with the patient.

Formulation of Nanoemulsion with selected Smix

The prepared Nanoemulsion was visually assessed for phase separation (Table 3).

Table 3: Results of Nanoemulsion formulation with Smix

Batches	Observation
NE-1	No Phase separation
NE-2	No Phase separation
NE-3	Phase Separation
NE-4	Phase Separation
NE-5	Phase Separation
NE-6	Phase Separation
NE-7	Phase Separation

Optimization of Amphotericin B Nanoemulsion formulation

Table 4: Actual design for Amphotericin B Nanoemulsion formulations

Run	Factor 1	Factor 2	Response 1	Response 2
	RPM	mins	nm	mV
1	3000	30	382.5	-23.5
3	5000	30	367.4	-23.8
4	7000	30	343.9	-24.7
5	5000	60	286.8	-26.8
6	3000	60	311.2	-26.2
7	7000	60	262.1	-27.1
2	5000	90	195.1	-28.4
8	7000	90	168.2	-28.9
9	3000	90	216.5	-28.2

All values are expressed as mean ± SD (n = 3). Factor 1 A: Homogenization Speed, Factor 2, B: Homogenization Time; Response 1: Globule Size; Response 2: Zeta Potential

ANOVA spectra for Amphotericin B from Design Expert software

Response 1: Globule Size

Figure 10 presents the actual and predicted values. The maximum difference between the actual and predicted Globule size was 2.62 nm. Most of the actual globule sizes were close to the predicted values. Globule size decreases with increasing homogenization time. From the contour plot (Figure 11), it was observed that increasing homogenization time and speed reduced globule size. As the homogenization speed and time increased, the formation of smaller globules produced smaller particles. From the 3D surface Plot (Figure 12), it is evident that as the homogenization time and speed increased, the globule size decreased.

Response 2: Zeta Potential

The maximum deviation between actual and predicted zeta potential values was 0.3500 mV, as shown in Figure 13. A decreasing trend in zeta potential was observed with increasing homogenization speed and time, suggesting that higher

processing intensity may affect the formulation's surface charge and stability. As illustrated in the contour plot (Figure 14), an increase in homogenization time combined with moderate to high homogenization speed increased zeta potential.

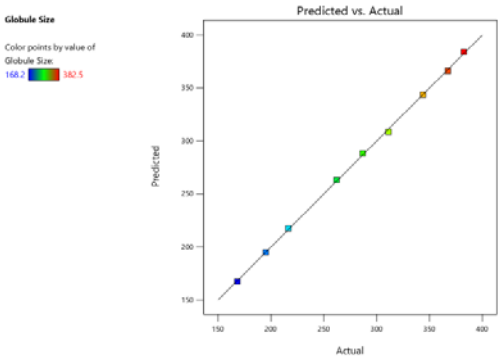

Figure 10: Actual VS Predicted Plot- Globule Size

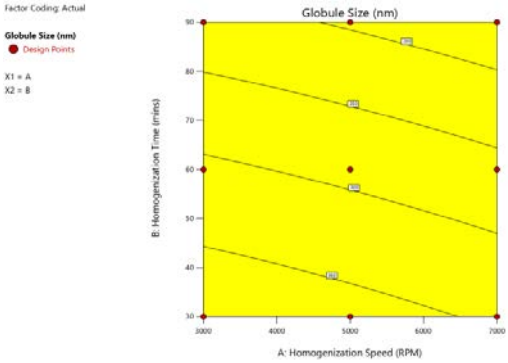

Figure 11: Contour Plot- Globule Size

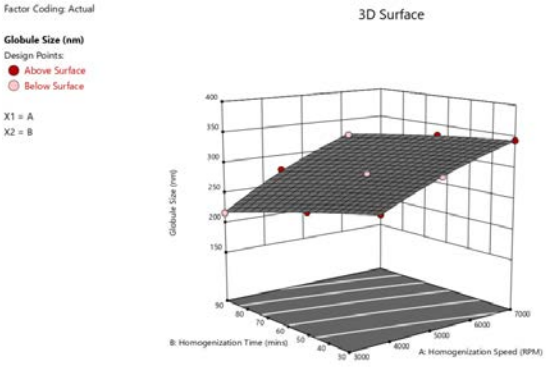

Figure 12: 3D Surface Plot- Globule Size

This suggests that elevated processing conditions increase the surface charge of the nanoemulsion droplets, thereby enhancing electrostatic stabilization. Higher zeta potentials indicate greater formulation stability, confirming that increased homogenization parameters contribute to the formation of a more stable nanoemulsion system. The 3D surface plot (Figure 15) shows that increasing homogenization time, at moderate to high homogenization speeds, results in a corresponding increase in zeta potential. This trend indicates improved electrostatic stabilization of the nanoemulsion at higher processing intensities.

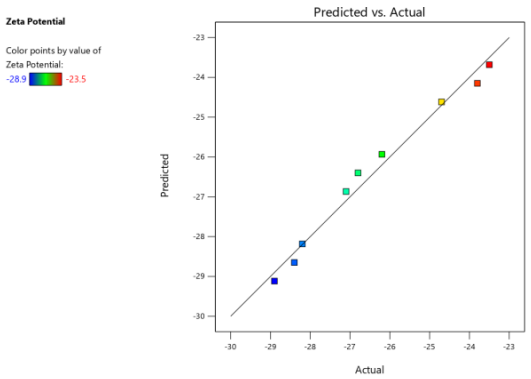

Figure 13: Actual VS Predicted Plot- Zeta Potential

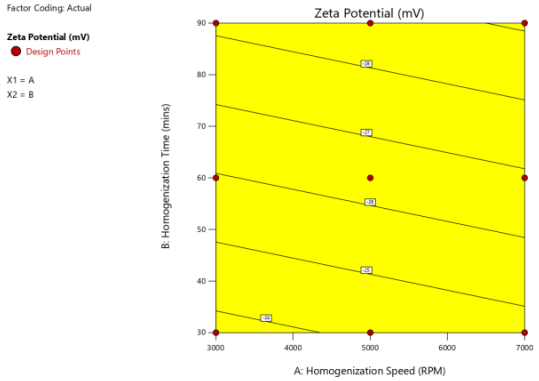

Figure 14: Contour Plot- Zeta Potential

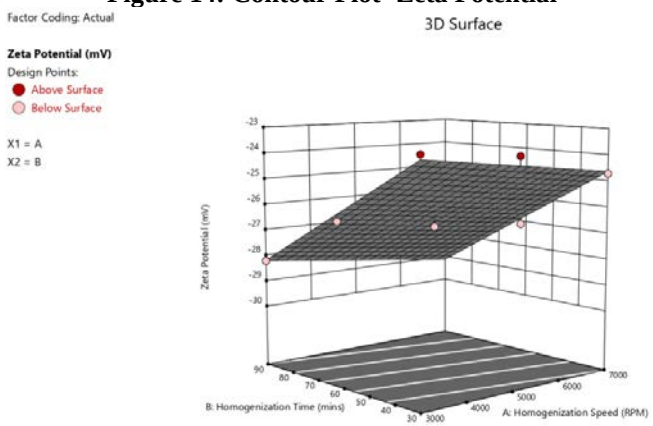

Figure 15: 3D Surface Plot- Zeta Potential

As homogenization speed and time increased, the magnitude of the negative zeta potential also increased (from –23.5 mV to –28.9 mV). Although numerically more negative, this represents a higher absolute surface charge, indicating enhanced electrostatic repulsion between droplets. Consequently, intensified homogenization conditions improved nanoemulsion stability by preventing droplet aggregation.

Evaluation of Amphotericin B Nanoemulsion

Globule Size

Globule size analysis was performed for all AmB Nanoemulsions. The NE-02-8 batch had the smallest particle size, 168.2 nm. The particle size results are presented in Table 5.

Zeta potential

Zeta potential measurements were conducted to evaluate the stability of the nanoemulsion formulations. The pure drug exhibited a zeta potential of –29.1 mV, whereas all formulations showed comparable negative zeta potentials, indicating satisfactory stability. Notably, increased homogenization time and speed further increased the negative zeta potential, thereby improving electrostatic stabilization of the system. The highest zeta potential observed was –28.9 mV for batch NE-02-8. Detailed zeta potential values for all batches are presented in Table 5.

Polydispersity Index

The PDI analysis was performed on all the AmB Nanoemulsions. The NE-02-8 batch exhibited a high PDI of 0.578. The PDI results are presented in Table 5. The relatively higher PDI values observed in the initial optimization runs reflect incomplete droplet disruption and a heterogeneous size distribution at lower homogenization intensities. Progressive optimization of homogenization speed and time significantly reduced droplet heterogeneity by promoting uniform shear-induced breakup. The optimized formulation (NE-02-8) demonstrated improved homogeneity despite a moderate PDI value, which is acceptable for nanoemulsion systems containing highly lipophilic drugs and complex surfactant systems.

Drug Content

Drug content was evaluated to confirm the integrity of the active pharmaceutical ingredient (API) throughout the formulation process and to ensure consistent therapeutic efficacy. All formulations exhibited drug content of 97–100%, indicating no significant loss or degradation during processing. These results also reflect the accuracy of the API incorporation method. Detailed drug content data are presented in Table 5.

% Transmittance

The transmittance (%T) of the selected NE-02 formulations was measured at 650 nm, with distilled water as the blank. The %T values for Amphotericin B-loaded nanoemulsions were close to 100%, indicating that the formulations were clear and transparent. The highest transmittance recorded was $99.48 \pm 0.22\%$ for batch NE-02-8. These results confirm the optical clarity and homogeneity of the nanoemulsions. Detailed %T values are presented in Table 5.

Table 5: Results of Evaluation of AmB Nanoemulsion

Batch	Globule Size (nm)	Zeta potential (mV)	PDI	Drug Content (%)	Transmittance (%)
NE-02-1	382.5	-23.5	0.271	97.31±0.55	97.72±0.56
NE-02-2	195.1	-28.4	0.471	100.44±0.17	99.11±0.11
NE-02-3	367.4	-23.8	0.299	98.37±0.28	97.18±0.25
NE-02-4	343.9	-24.7	0.318	99.49±0.19	97.54±0.61
NE-02-5	286.8	-26.8	0.394	96.82±0.36	98.27±0.37
NE-02-6	311.2	-26.2	0.348	97.54±0.48	97.34±0.15
NE-02-7	262.1	-27.1	0.411	98.72±0.72	98.33±0.16
NE-02-8	168.2	-28.9	0.578	99.28±0.17	99.48±0.22
NE-02-9	216.5	-28.2	0.423	97.21±0.64	98.79±0.06

All values are expressed as mean ± SD (n = 3).

Table 6: Results of the stability study of batch NE-02-8

Time interval	Globule Size (nm)	Zeta potential (mV)	PDI	Drug Content (%)	Transmittance (%)
0 Day	168.2	-28.9	0.578	99.28±0.17	99.48±0.22
1 month	169.3	-28.7	0.526	99.17±0.52	99.19±0.43
2 month	170.1	-28.8	0.511	99.43±0.24	99.56±0.17
3 month	172.5	-27.6	0.528	98.79±0.68	99.33±0.37

All values are expressed as mean ± SD (n = 3).

Stability Studies

Stability testing was conducted for three months under accelerated conditions (40 ± 2°C and 75 ± 5% relative humidity) in accordance with ICH guidelines. The optimized formulation, batch NE-02-8, was subjected to stability assessment to evaluate its performance at elevated temperature, providing insight into its potential long-term stability under ambient storage conditions. The comparative results of these evaluations are

summarized in Table 6. The formulation was stable for more than 90 days at 40 ± 2 °C and 75 ± 5% relative humidity.

Formulation of Luliconazole Nanoemulsion

Luliconazole Nanoemulsion using optimized formulation

Luliconazole nanoemulsion was formulated using a predetermined quantity of castor oil and Smix in a 2:1 ratio, identical to the optimized Amphotericin B nanoemulsion batch. The formulation was prepared under high-homogenization conditions, with 90 minutes of homogenization at 7000 rpm. The resulting nanoemulsion was visually inspected for phase separation; none was observed, indicating good physical stability of the formulation.

Evaluation of Luliconazole Nanoemulsion

Globule Size

Globule size analysis was performed for all Luliconazole Nanoemulsions. The particle size was 327.5 nm**.**

Zeta potential

Zeta potential analysis was conducted to assess the stability of the nanoemulsion formulations. The pure drug exhibited a zeta potential of –25.1 mV, whereas the prepared formulations showed higher negative zeta potential values, indicating improved stability. Increasing homogenization time and speed further increased the negative zeta potential, thereby improving electrostatic stabilization of the system. The zeta potential of the Luliconazole nanoemulsion was recorded at –27.9 mV, reflecting good physical stability of the formulation. The observed difference in globule size between the optimized Amphotericin B nanoemulsion (168.2 nm) and the Luliconazole nanoemulsion (327.5 nm) can be attributed to intrinsic physicochemical differences between the two drugs. Amphotericin B possesses a relatively rigid polyene macrolide structure with amphiphilic characteristics, enabling efficient interfacial alignment and stabilization during high-shear homogenization. Conversely, Luliconazole is a highly lipophilic imidazole derivative with greater molecular flexibility and higher oil affinity, which promotes deeper partitioning into the oil phase. This behavior increases the effective droplet core volume, resulting in comparatively larger globule sizes despite identical formulation composition and processing conditions. These findings highlight that drug-dependent molecular properties, such as lipophilicity, molecular volume, and interfacial behavior, play a decisive role in the formation and size distribution of nanoemulsion droplets. The study is limited by the absence of in vitro release kinetics and antifungal activity testing. These evaluations will be essential in future work to confirm the functional performance of the developed nanoemulsions. Higher surfactant concentrations reduce interfacial tension and increase droplet disruption during homogenization, producing smaller globules. Similarly, increased homogenization speed increases shear forces, promoting more uniform droplet breakup. Reduced droplet size increases surface area, thereby enhancing permeation and retention at infection sites.

CONCLUSION

The present study successfully formulated and evaluated nanoemulsions of Amphotericin B and Luliconazole for the management of fungal infections. Through systematic screening and optimization of formulation components, castor oil and a Smix ratio of 2:1 were identified as suitable for preparing stable nanoemulsions. Homogenization parameters were optimized using a Quality by Design (QbD) approach, and the final formulations were characterized for globule size, zeta potential, polydispersity index (PDI), % drug content, and % transmittance. Stability studies confirmed the physical stability of the optimized formulations under accelerated conditions. The results demonstrated that nanoemulsions effectively improved the solubility and stability of both drugs without compromising formulation clarity or uniformity.

The increased zeta potential values indicated enhanced electrostatic stability, while the high drug content confirmed efficient encapsulation. The study demonstrated significant improvements in solubility, droplet size, and physical stability of Amphotericin B and Luliconazole via optimized nanoemulsion formulations. While these attributes indicate potential for enhanced delivery and therapeutic efficacy, bioavailability was not directly evaluated. Future studies involving in vitro release, permeability, and antifungal testing are required to establish the clinical relevance of these findings.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; PDI: Polydispersity Index; BCS: Biopharmaceutical Classification System; CCD: Central Composite Design; DSC: Differential Scanning; NE: Nanoemulsion; Calorimetry; DLS: Dynamic Light Scattering; FTIR: Fourier-Transform Infrared Spectroscopy; HPLC: High-Performance Liquid Chromatography; QbD: Quality by Design; RH: Relative Humidity; Smix: Surfactant and Co-surfactant Mixture; XRD: X-Ray Diffraction

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Pravin N. Kirdat contributed to conceptualization, experimental design, data collection, and manuscript writing. Meenakshi B. Patel supervised the study, contributed to critical review, data interpretation, and final approval of the manuscript. All authors have read and approved the final manuscript.

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