https://doi.org/10.33472/AFJBS.6.4.2024.5902-5911

African Journal of Biological Sciences

FORMULATION AND CHARACTERIZATION OF ISOCONAZOLE LOADED INVASOMES USING BOX-BEHNKEN DESIGN

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Article History
Volume 6, Issue 5, 2024
Received: 09 May 2024
Accepted: 20 May 2024
doi: 10.33472/AFJBS.6.5.2024.5902-5911

ABSTRACT

The present study focused on the formulation and characterization of Isoconazole-loaded invasomal gel employing a Box-Behnken design. Isoconazole invasomes were synthesized using the conventional thin-layer evaporation method. For optimization, a 2-level 3 factor factorial design was executed using Design of Expert 12 software (DOE 12 trial version). The optimization involved a Response Surface Quadratic model, and 17 experimental runs were designed. The resulting formulations were systematically characterized with a focus on drug entrapment efficiency and particle size. The evaluation of the prepared Invasomes formulations included key parameters such as vesicle size, polydispersity index (PDI), and entrapment efficiency. These parameters are crucial for determining the stability, uniformity, and efficacy of the formulated Isoconazole-loaded Invasomal gel. The obtained results provide insights into the effectiveness of the developed formulations, paving the way for potential applications in dermatological drug delivery systems.

Key words: Formulation, Isoconazole, Characterization, Invasomes, Box-Behnken design

Introduction

Fungal infections, encompassing a wide spectrum of diseases affecting diverse anatomical sites, continue to pose a substantial public health challenge globally. Among the arsenal of antifungal agents, Isoconazole stands out as a versatile drug with proven efficacy against various pathogenic fungi. However, to maximize its therapeutic potential, attention has shifted towards innovative drug delivery systems that can enhance bioavailability, prolong drug release, and improve patient compliance. In this context, invasomes have emerged as a promising carrier due to their unique structure and ability to encapsulate a diverse range of therapeutic agents [1-2].

Invasomes, essentially phospholipid-based vesicular systems, offer advantages such as increased drug penetration, enhanced stability, and controlled release profiles. The phospholipid bilayer structure of invasomes facilitates interaction with the skin barrier, allowing for improved drug permeation. These attributes make invasomes an attractive option for dermatological applications, including the delivery of antifungal drugs like Isoconazole [3, 4].

The Box-Behnken design, a statistical optimization technique, has gained prominence in pharmaceutical research for its ability to systematically explore the effects of multiple factors on a

formulation's outcome [5]. Originally introduced by Box and Behnken in the 1960s, this design enables the identification of optimal conditions by minimizing the number of experimental runs, making it a valuable tool for efficient and cost-effective formulation development [6-7].

This research endeavors to harness the potential of invasomes in dermatological drug delivery, specifically focusing on Isoconazole. The Box-Behnken design will be employed to systematically optimize the formulation process, considering critical variables. By referencing established literature on invasomes and antifungal drug delivery, this study aims to contribute to the advancement of effective and patient-friendly treatment strategies for fungal skin infections.

Material and Methods

Preparation of Isoconazole loaded invasomes

Isoconazole invasomes were produced using a traditional thin-layer evaporation method [8]. Initially, a clean, dry, round-bottom flask was used to dissolve Isoconazole, Phospholipid, and terpene (Limonene) in a solution consisting of chloroform and methanol in a 1:1 (v/v) ratio. The organic solvent was eliminated by rotary evaporation, and any remaining traces were removed under vacuum overnight. The deposited lipid film was hydrated using a mixture of phosphate buffer saline (pH 7.4) and rotated at 60 rpm for 1 h at room temperature. The resulting vesicles were left to swell for 2 h at room temperature to produce large multilamellar vesicles. To create smaller particles, large particles were subjected to probe sonication at 4°C, with an output frequency of 40% at 40 W. The compositions of the various formulations are listed in Table 1.

Table 1: Box-Behnken Design's Optimization of Invasomes

Formulation Code	Std	Run	Factor 1: Phosphotidylcholine (%)	Factor 2: Terpenes (%)	Factor 3: Ethanol (%)
F1	4	1	1.25	0.25	7.5
F2	13	2	0.75	0.175	7.5
F3	11	3	0.75	0.1	10
F4	3	4	0.25	0.25	7.5
F5	16	5	0.75	0.175	7.5
F6	2	6	1.25	0.1	7.5
F7	7	7	0.25	0.175	10
F8	6	8	1.25	0.175	5
F9	14	9	0.75	0.175	7.5
F10	10	10	0.75	0.25	5
F11	12	11	0.75	0.25	10
F12	9	12	0.75	0.1	5
F13	5	13	0.25	0.175	5
F14	8	14	1.25	0.175	10
F15	17	15	0.75	0.1	7.5
F16	15	16	0.75	0.175	5
F17	1	17	0.25	0.1	7.5

Experimental design and data analysis

A regular 2 level 3 factor factorial design was utilized to screen for significant formulation and process variables in the development of Invasomes, with three factors considered. Table 2 outlines the high and low levels of the various variables that were screened for their influence on the development of Isoconazole loaded Invasomes. Optimization of all process and formulation

variables was carried out using a 2 level 3 factor factorial design through Design of Expert 12 software (DOE 12 trial version) in the Invasomes formulations. For optimization purposes, a Response Surface Quadratic model was employed to design 17 runs. The prepared formulations were subsequently characterized for drug Vesicle size, PDI and entrapment efficiency.

Table 2: List of variables employed in 2^3 factorial designs

Factors	Levels	
	Low (−1)	High (+1)
Isoconazole (mg)	50	
Phosphotidylcholine	0.2500	1.25
Terpenes	0.1000	0.2500
Ethanol	5.00	10.00

ANOVA for Quadratic model

Final equation in terms of coded factors

Vesicle size = +208.79 -3.98 A+4.39 B-28.17 C-2.72 AB-8.57AC-12.85 BC+7.73 A^2-3.85 B^2-20.54 B^2- 20.54 C^2

Final equation in terms of actual factors

Vesicle size = -37.41288+9.76520 Phosphotidylcholine+866.16283 Terpenes+55.16730 Ethanol-72.46667 Phosphotidylcholine * Terpenes-6.85400 Phosphotidylcholine * Ethanol-68.52000 Terpenes * Ethanol+30.90765 Phosphotidylcholine^2-683.95722 Terpenes ^{2}-3.28698 Ethanol^2

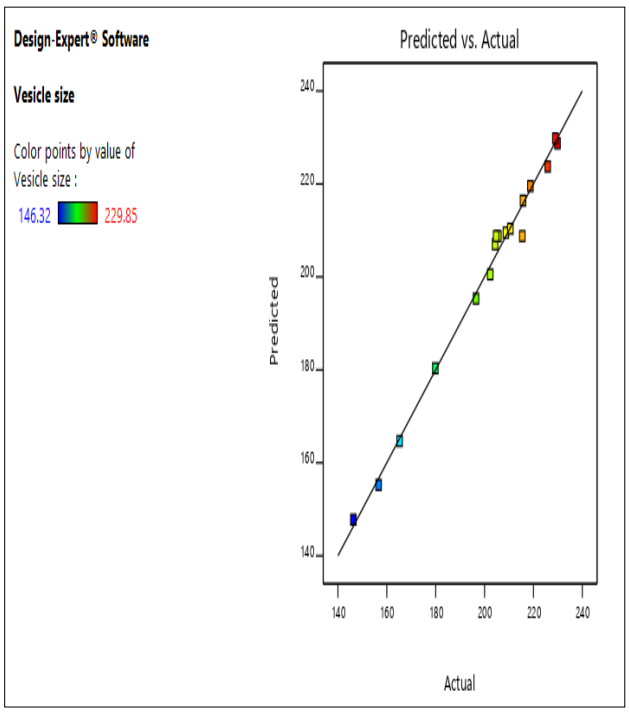

Figure 1: Response surface plots for vesicle size (Predicted vs. Actual)

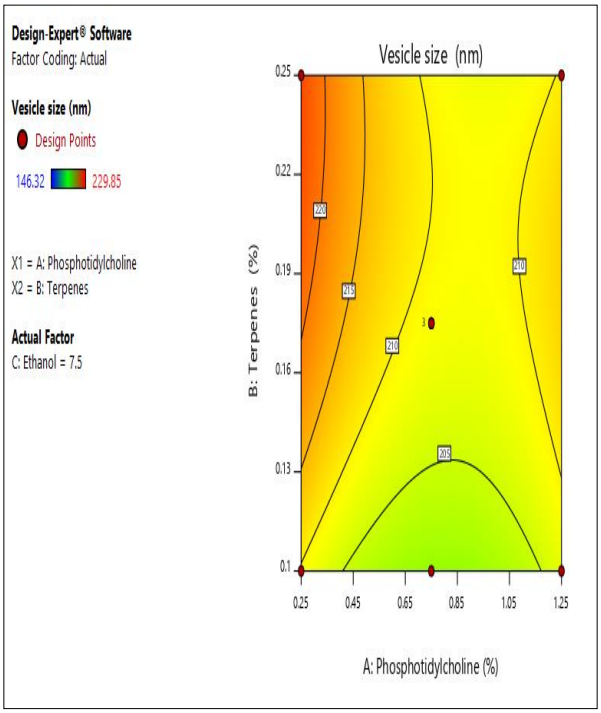

Figure 2: Contour plot for vesicle size between Phosphatidylcholine and Terpenes

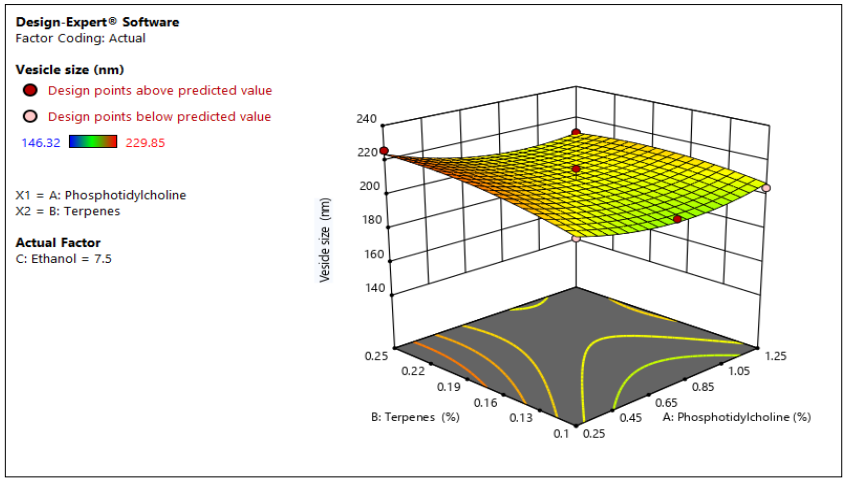

Figure 3: 3D surface plot for vesicle size between

Phosphatidylcholine and Terpenes

ANOVA for Quadratic model

Final equation in terms of coded factors

Polydispersity index = +0.1787-0.0177A-0.0201B-0.0264C-0.0160AB-0.0065AC-0.0125 BC+0.0143 A^2-0.0019 B^2-0.0110 C^2

Final Equation in Terms of Actual Factors

Polydispersity index = +0.081398-0.007532 Phosphotidylcholine + 0.671980 Terpenes +0.031377 Ethanol-0.426667 Phosphotidylcholine * Terpenes-0.005200 Phosphotidylcholine * Ethanol-0.066667 Terpenes * Ethanol+0.057132 Phosphotidylcholine^2+0.057132 Phosphotidylcholine^2-0.344326 Terpenes^2-0.001757 Ethanol^2

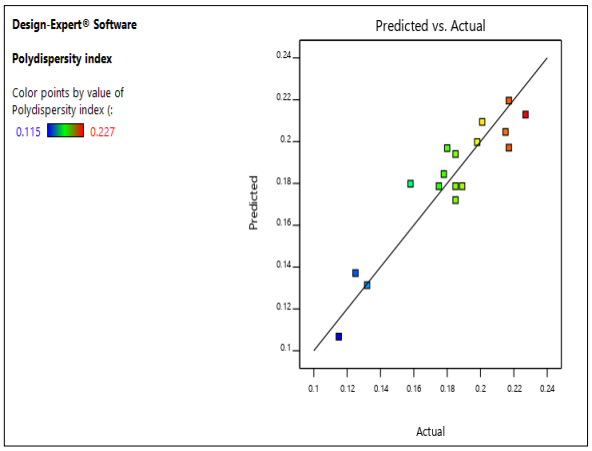

Figure 4: Response surface plots for PDI (Predicted vs. Actual)

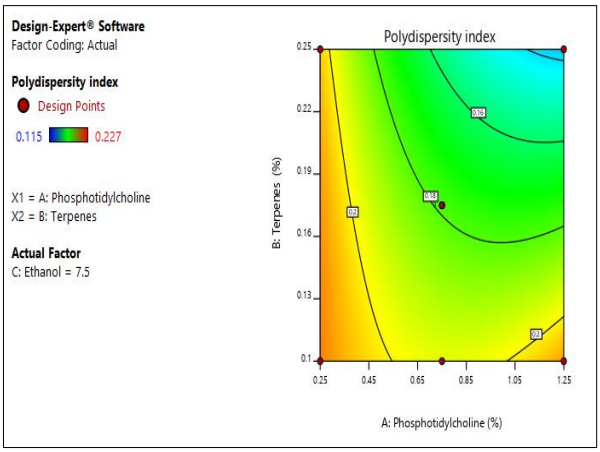

Figure 5: Contour plot for PDI between Phosphotidylcholine and Terpenes

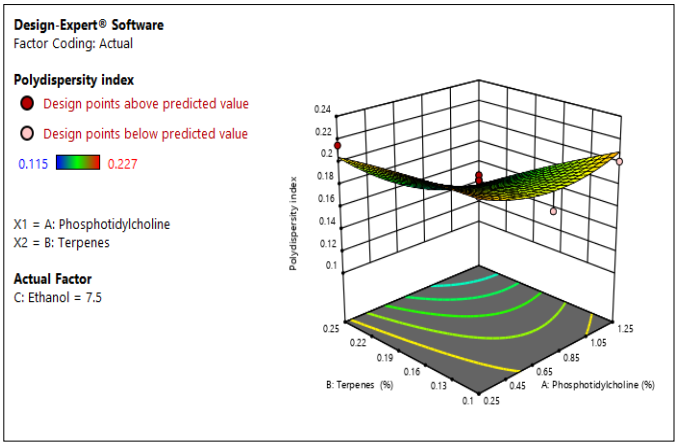

Figure 6: 3D surface plot for PDI between Phosphotidylcholine and Terpenes

ANOVA for Quadratic model

Final equation in terms of actual factors

Entrapment efficiency = +125.67602-28.08031 Phosphotidylcholine -235.87144 Terpenes-8.69350 Ethanol +108.06667 Phosphotidylcholine * Terpenes +0.400000 Phosphotidylcholine * Ethanol +8.40000 Terpenes * Ethanol +4.76743 Phosphotidylcholine^2 +309.37760 Terpenes^2+0.536331 Ethanol^2

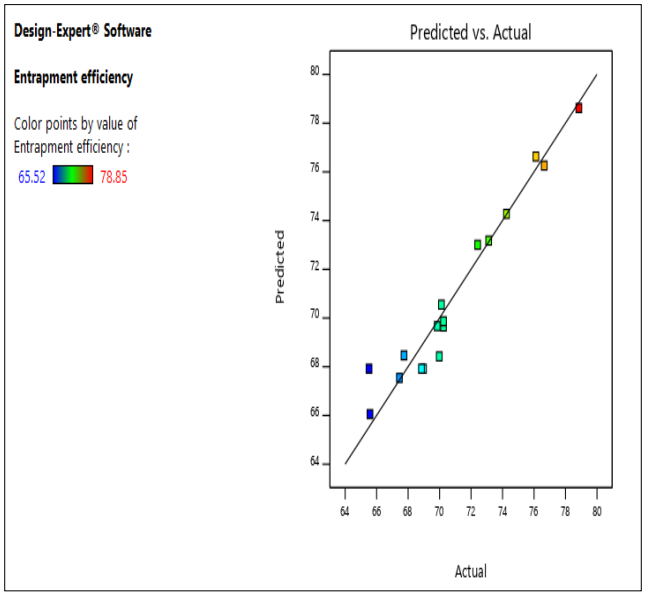

Figure 7: Response surface plots for Entrapment efficiency (Predicted vs. Actual)

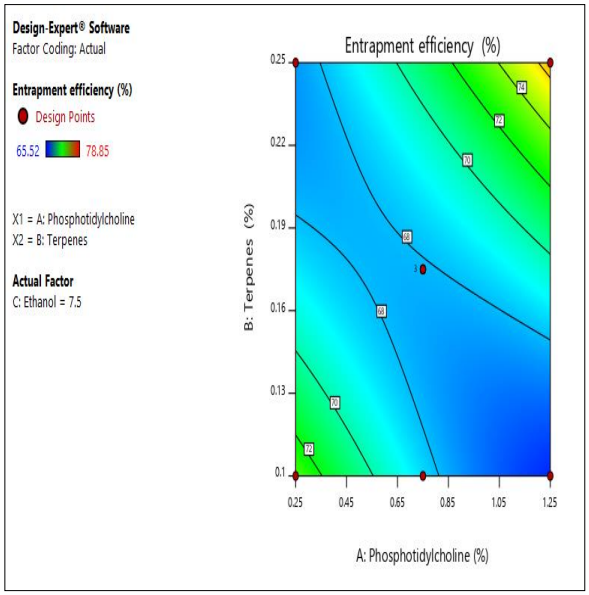

Figure 8: Contour plot for entrapment efficiency between PC and Terpenes

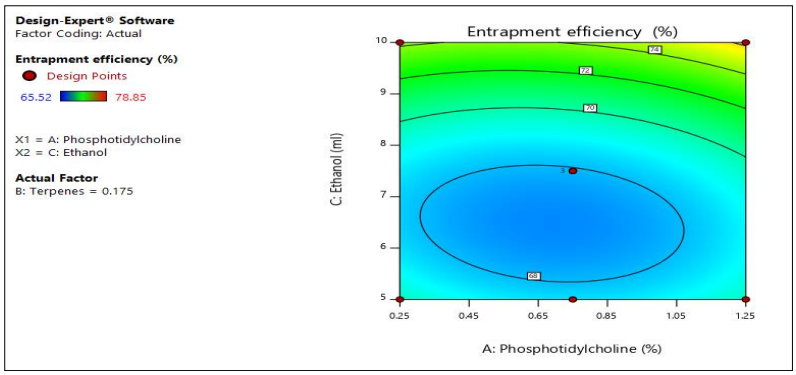

Figure 9: Contour plot for entrapment efficiency between Phosphotidylcholine and Ethanol

Characterization of Isoconazole loaded invasomes

Vesicle size and Polydispersity Index (PDI)

The Vesicle size of Invasomes was measured using dynamic light scattering, Malvern zetasizer (Malvern zetasizer, Worcestershire, UK). Formulation was diluted with double distilled water and vortex for 5 minutes and then placed in the cell of the zeta sizer for analyze particle size of nanosponges [9-10]. Polydispersity Index (PDI) of nanosponges were also determined using with photon correlation spectroscopy by using same instrument.

Entrapment Efficiency

Entrapped Isoconazole in the Isoconazole loaded Invasomes was calculated by estimating the amount of unentrapped drug recovered in the supernatant after centrifugation of the resultant nanosuspension [11]. Briefly, Invasomes suspension was centrifuged by cooling centrifuge at 15000 RPM for 10 min at 10°C and the unentrapped drug was estimated in the supernatant with the help of UV Vis. Spectroscopy. Further, the total amount of the drug and the unentrapped drug in the supernatant was substituted in the following equation to calculate % Entrapment Efficiency.

$$Drug\ entrapment\,(\%) = \frac{Concentration\ of\ total\ drug - Concentration\ of\ unentrapped\ drug}{Concentration\ of\ unentrapped\ drug} \times 100$$

In-vitro drug diffusion study

The in-vitro diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion [12]. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.

Stability Studies

Stability study was carried out for drug loaded invasomes at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28 ± 2°C) for 3 months. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for Entrapment efficiency, Vesicle size and Polydispersity index [13].

Results of Evaluation

The results presented in Table 3 provide valuable insights into the evaluation of prepared invasomes formulations, focusing on vesicle size, polydispersity index (PDI), and entrapment efficiency. Each formulation, denoted by a unique code, exhibits variations in these critical parameters, reflecting the influence of formulation components and preparation methods on the characteristics of the invasomes. The vesicle size, an essential parameter influencing drug delivery and bio distribution, ranges from 146.32 nm (formulation F11) to 229.85 nm (formulation F8). Generally, smaller vesicle sizes are favorable for enhanced permeability and prolonged circulation

in the bloodstream. Therefore, formulations with smaller vesicle sizes, such as F11, F3, and F14, are likely to exhibit improved pharmacokinetic profiles and tissue penetration.

Polydispersity index (PDI) values indicate the uniformity of vesicle size distribution within each formulation. Lower PDI values, such as those observed in formulations F11 (0.115) and F14 (0.132), suggest a narrower size distribution and greater homogeneity among vesicles. This uniformity is crucial for ensuring consistent drug delivery and minimizing variability in therapeutic response.

Entrapment efficiency, reflecting the proportion of drug encapsulated within the vesicles, ranges from 65.52% (formulation F2) to 78.85% (formulation F11). Higher entrapment efficiencies indicate greater drug loading capacity and reduced potential for drug leakage or premature release. Formulations with high entrapment efficiencies, such as F11, hold promise for achieving therapeutic concentrations at lower doses, thereby minimizing side effects and improving patient compliance.

The experimental data with predicted responses, as summarized in Table 4, further support the selection of formulation F11 as the optimized formulation for further evaluation. This selection is based on the comparison between the actual values obtained through experimentation and the predicted values derived from the experimental data.

Formulation F11 exhibits excellent agreement between the actual and predicted values for key parameters, including vesicle size, polydispersity index (PDI), entrapment efficiency, and zeta potential. The vesicle size, a critical determinant of drug delivery efficiency, was predicted to be 147.77 nm, closely matching the actual experimental value of 146.32 nm. This indicates the reliability of the predictive model in estimating the vesicle size of F11.

Similarly, the PDI, which reflects the uniformity of vesicle size distribution, was predicted to be 0.1068, in close proximity to the experimental value of 0.1150. This suggests that formulation F11 maintains a narrow size distribution, as anticipated by the predictive model.

Table 5 provides the results of in-vitro drug release for the optimized formulation F11 over various time intervals. The cumulative drug release percentages indicate the release profile of isoconazole from the invasomal formulation at different time points. This data is crucial for understanding the release kinetics and efficacy of the formulation in delivering the drug to the target site.

The drug release profile of formulation F11 demonstrates a sustained and controlled release pattern over time. Initially, at 0.5 hours, only 8.85% of the drug is released, indicating a slow release rate during the early stages. However, as time progresses, the cumulative drug release increases gradually, reaching 96.65% at 12 hours.

The sustained release behavior observed with formulation F11 suggests that the invasomal delivery system effectively encapsulates and releases the drug in a controlled manner. This sustained release profile is advantageous for prolonged therapeutic effect, minimizing the frequency of administration and optimizing drug bioavailability.

The gradual increase in drug release over time could be attributed to factors such as the diffusion of the drug through the invasomal lipid bilayers or the degradation of the lipid components of the invasomes in the release medium. This sustained release behavior ensures a prolonged presence of the drug at the target site, potentially enhancing its therapeutic efficacy while minimizing potential side effects associated with fluctuating drug concentrations.

The stability study of the optimized formulation F11, as presented in Table 6, provides valuable insights into the effects of storage conditions on the physical and chemical characteristics of the invasomes over time.

Firstly, the study evaluates the impact of temperature variations on the vesicle size of the invasomes. At the initial assessment point (1 month), when stored at 4.0 ± 0.2°C, the average vesicle size is recorded at 146.85 nm. This indicates that under refrigerated conditions, the invasomes maintain their size integrity effectively. However, when subjected to ambient temperature (25-28 ± 2°C) for 1 month, there is a slight increase in vesicle size to 168.85 nm. This elevation suggests some degree of aggregation or fusion of the vesicles, possibly due to increased kinetic energy at higher temperatures. Similarly, at subsequent time points (2 and 3 months), both refrigerated and ambient conditions lead to further increases in vesicle size, albeit within an acceptable range, indicating moderate stability over time.

Secondly, the stability of the encapsulated drug, represented by the percentage of entrapment efficiency (% EE), is assessed under different storage conditions. At the outset (1 month), the % EE is relatively high, indicating efficient drug encapsulation within the invasomes. However, as the storage duration extends to 2 and 3 months, a gradual decrease in % EE is observed under both refrigerated and ambient conditions. This reduction in entrapment efficiency suggests potential drug leakage or degradation over time, which could compromise the formulation's efficacy.

Table 3: Results of Evaluation of prepared Invasomes formulations (Vesicle size, Polydispersity index (PDI), Entrapment efficiency)

Formulation Code	Vesicle size (nm)	Polydispersity index (PDI)	Entrapment efficiency (%)
F1	210.56	0.125	76.12
F2	205.65	0.175	65.52
F3	165.25	0.185	72.42
F4	225.85	0.215	67.45
F5	215.45	0.189	68.85
F6	204.32	0.201	65.58
F7	179.85	0.158	74.25
F8	229.85	0.217	70.25
F9	204.74	0.185	68.98
F10	228.98	0.178	70.25
F11	146.32	0.115	78.85
F12	196.52	0.198	70.12
F13	218.78	0.217	69.85
F14	156.65	0.132	76.65
F15	202.32	0.180	69.98
F16	215.74	0.185	67.74
F17	208.74	0.227	73.12

Experimental data with predicted response

On the basis of experiment data with predicted response formulation F11 select as optimized formulation for further evaluation.

Table 4: Experimental data with predicted response

Run Order	Formulation Code	Parameters	Actual Value	Predicted Value
11	F11	Vesicle size	146.32	147.77
		PDI	0.1150	0.1068

		Entrapment Efficiency (%)	78.85	78.63
		Zeta potential	-38.78	

Table 5: Results of *In-vitro* drug release of optimized formulation F11

S. No.	Time	% Cumulative drug release
1	0.5	8.85
2	1	12.32
3	2	22.32
4	4	35.58
5	6	49.98
6	8	65.85
7	10	88.85
8	12	96.65

Table 6: Stability Study of optimized formulation of invasomes F11

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temperature	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Average Vesicle size (nm)	146.85	168.85	148.98	178.85	152.23	185.45
% EE	78.25	75.65	77.85	73.36	76.65	70.15

Conclusion

The formulation and characterization of isoconazole-loaded invasomes represent a promising approach for enhancing drug delivery efficiency and therapeutic outcomes. The optimized formulation, F11, holds significant potential for further development and eventual clinical translation. However, additional studies, including in vivo efficacy and safety assessments, are warranted to fully elucidate the therapeutic benefits of this innovative drug delivery system. Overall, this work contributes to the advancement of pharmaceutical science and holds promise for improving the treatment of various dermatological conditions.

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