

Original Research Article

Surface optimization, fabrication, *in vitro* investigation, and antifungal activity of Novasome nanovesicles loaded with Itraconazole

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Abstract

Purpose: To develop, optimize, and characterize the antifungal activity of itraconazole-loaded novasomes (ILN) as a new nanovesicular delivery system

Methods: The modified ethanol injection method was used to formulate the ITZ-loaded novasomes. The Box Behnken design was utilized for optimization of formulations and to evaluate the effect of formulation variables on vesicle size, polydispersity (PDI), and entrapment efficiency (EE %). Furthermore, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used for characterization, and antifungal activity was evaluated using Mueller-Hinton agar.

Results: The optimized formula demonstrated a vesicle size of about 299.1 nm, PDI of 0.2815, and a high EE of 99 %. The FTIR spectra confirmed no chemical interaction between itraconazole and the excipients. Also, SEM images showed spherical, smooth surfaces of itraconazole-loaded novasomes without aggregation or visible drug crystals, suggesting good drug encapsulation. Antifungal testing of ILN against *Candida tropicalis* produced an inhibition zone diameter of 10 mm.

Conclusion: Itraconazole-loaded novasomes showed improved physicochemical properties. The recommended delivery system represents a promising approach for enhancing the solubility and therapeutic efficacy of itraconazole.

Keywords: Antifungal activity, Itraconazole, Novasomes, Response surface

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INTRODUCTION

Fungal infections play a significant role in global public health, affecting millions of individuals worldwide. Fungi infections range from superficial conditions like athlete's foot and vaginal candidiasis to life-threatening systemic infections such as aspergillosis. Management of these infections is largely dependent on triazole antifungal agents [1]. Itraconazole ($C_{35}H_{38}N_2O_4$; ITZ) is a commonly administered

triazole antifungal agent with a wide spectrum of activity against multiple fungal pathogens, such as dermatophytes, *Candida*, and *Aspergillus*, with clinical significance in both superficial and systemic fungal infections. Compared to other azoles, it is more effective, safer, and has a broader range [2]. It acts by inhibiting production of ergosterol, a significant cell membrane constituent of fungi [3]. Despite the therapeutic efficacy of ITZ, it faces marked pharmaceutical formulation challenges including solubility. It is an

almost water-insoluble medication (class II in the biopharmaceutical classification system), showing that low aqueous solubility is the most important factor for decreased plasma concentration, low bioavailability (approximately 55 %), irregular and inadequate absorption from the gastrointestinal tract, need for high doses to achieve the required effect, and increased potential for adverse effects [4]. In an effort to overcome these limitations, nano drug delivery systems have become a new approach to enhance the solubility and bioavailability of poorly soluble medications.

Novasomes are new nanosystems composed of surfactants, free fatty acids, and cholesterol, characterized by their ability to encapsulate both hydrophobic and hydrophilic medications. It offers several unique benefits, including improved aqueous solubility, enhanced permeability, good drug release, reduced toxicity, and high stability [5]. Despite previous investigations on different ITZ delivery systems such as nanosuspensions, niosomes, and nanoemulsions, studies on ITZ-loaded novasomes remain largely unexplored, with a lack of comprehensive studies on formulation, optimization, characterization, and antifungal efficacy. This study developed, characterized, and evaluated ITZ novasomal system to address the solubility restrictions and therapeutic improvements.

EXPERIMENTAL

Material

The ITZ powder used in this study was obtained from Baoji Guokang Bio-technology Co., Ltd., China. Span 60 was obtained from Loba Chemie Pvt. Ltd, India. Palmitic acid was obtained from HIMEDIA Laboratories Pvt. Ltd, India. Ethanol was purchased from RCI Lab Scan Limited, Thailand. Methanol was obtained from Chem-Lab NV, Belgium. Chloroform was purchased from Thomas Baker, India. All other materials were analytical grade and applied as received.

Design

Box-Behnken design (version 13, Stat-Ease, USA) was applied to design and optimize the formulation of I-LN. Three independent variables (span 60, free fatty acid, and probe sonication time) were evaluated across 30 experimental runs, among which 6 were repetitions to validate data accuracy [6].

Particle size, polydispersity index (PDI), and entrapment efficiency (EE %) as dependent responses were applied to assess vesicle properties critical for uniformity, stability, and loading capacity (Table 1).

Preparation of ITZ- Novasome nanovesicle formulation

Preliminary studies were conducted to synthesize Novasomes by using a trial-and-error method in order to select suitable factors and levels such as Span 60, free fatty acid, and cholesterol. Novasomes were produced by the modified ethanol injection method according to previously reported procedures. Itraconazole (50 mg; Baoji Guokang Bio-technology Co., Ltd., China), cholesterol, Span 60 (Loba Chemie Pvt. Ltd, India), and selected free fatty acid (palmitic acid; HIMEDIA Laboratories Pvt. Ltd, India) were completely dissolved in an organic solvent mixture of ethanol and chloroform (2:1 v/v) [7]. Thereafter, the organic solution was injected by a 23 G syringe at 1 mL/min into a preheated aqueous solution (20 mL) and maintained at 60 °C under 1000 rpm stirring for 1 h, followed by stirring for 30 min on a cold surface. The resultant dispersion was sonicated using a probe sonicator to reduce vesicle size and achieve uniform distribution. Eventually, the mixture was cooled for further examination.

Particle size (PS) and polydispersity index (PDI)

Particle size and polydispersity (PDI) were measured using dynamic light scattering, and Malvern zeta sizer (UK).

Table 1: The dependent and independent factors in the investigation

Independent factors	Low	High
X1:amount of span 60 (mg)	100	150
X2:amount of FFA (mg)	10	50
X3:probe sonication time (min)	0	8
Dependent factors	Desirability constraints	
Y1=PS (nm)	Minimize	
Y2=PDI	minimize	
Y3=EE %	maximize	

FFA: free fatty acid, PS: particle size, PDI: polydispersity index, EE %: encapsulation efficiency percent

Approximately 0.1 mL of each mixture was diluted with 10 mL of distilled water. Average particle size and polydispersity (PDI) were obtained for each formulation [8,9].

Entrapment efficiency measurement (EE %)

The indirect method was used to determine the encapsulation efficiency (EE) of I-LN. The vesicular dispersions were centrifuged to separate the unentrapped drug from the encapsulated drug. Thereafter, 2 mL of each formulation was centrifuged at 15,000 rpm for 30 min at 4 °C [10]. The supernatant containing the free drug was examined spectrophotometrically at 262 nm using a UV spectrophotometer (Shimadzu, Japan). Entrapment efficiency was calculated using Eq 1.

$$EE (\%) = \{(A-B)/A\} 100 \dots\dots\dots (1)$$

Where: A – total amount of drug loading, B – amount of free drug

Solubility

Solubility of the optimized formulation was determined using the shake flask method. The sample (1.5 mL) was dissolved in 2 mL of the respective media (distilled water with 1 % Brij 35 and 0.1 N HCl with 1 % Brij 35). The mixture was placed in a shaker at 25 °C for 48 hr, centrifuged at 5,000 rpm for 10 min, and a clear supernatant was filtered through a 0.45 filter syringe. The filtrate was quantified using UV visible spectrophotometry at 263 nm in water with Brij and 255 nm in 0.1 N HCl with Brij compared to pure ITZ solubility [11-12].

Scanning electron microscopy (SEM)

The morphological features of the vesicular system for the optimized formulations were examined. To improve conductivity, 0.1 mL of each formulation was diluted with 10 mL of distilled water and spotted on glass slides attached to aluminium stubs using carbon tape. The field emission scanning electron microscope (FESEM, Inspect F50, FEI company, USA) was used to examine the samples to observe shape, size, and distribution [13].

Transmission electron microscopy (TEM)

Shape of the selected novasome formula was determined by a high-resolution transmission electron microscope. A drop of the selected formula was diluted with distilled water and allowed to dry on a carbon-coated copper grid, then evaluated with TEM. The microscope

allowed investigators to observe the structure and shape of the novasome and other properties in the sample [14].

Fourier transform infrared (FTIR) evaluation

The FTIR spectra of ITZ, physical mixture, and optimized ITZ novasome formula were obtained by Shimadzu FTIR spectroscopy. The FTIR spectra were captured with an instrument resolution of 4 cm⁻¹ in the 4000-400 cm⁻¹ spectrum area.

Assessment of biological activity

An agar well diffusion approach was used to evaluate the biological activity [15]. In this study, blinding was used for agar well diffusion. Selected strains for this investigation were *Candida tropicalis*, *Candida albicans*, and *candida para-psilosis*. Choice of different *Candida* species was influenced by strong activity of ITZ against *Candida* compared to other antifungal agents [16]. A fresh fungal culture was aseptically transferred to the center of a Petri dish, after which molten and cooled Mueller–Hinton agar was added and thoroughly mixed with the inoculum. Following solidification, wells of 6 mm diameter were bored into the agar using a sterile cork borer. The plates were then incubated at 37 °C for 24 h, after which the inhibition zone diameter were measured [17].

Statistical analysis

Design Expert (Version 13, Stat-Ease Inc., USA) was used to perform statistical analysis. Data were fitted to a quadratic response surface model and evaluated by analysis of variance (ANOVA). Model fit was confirmed via R², adjusted R², and *p* < 0.05 was considered statistically significant.

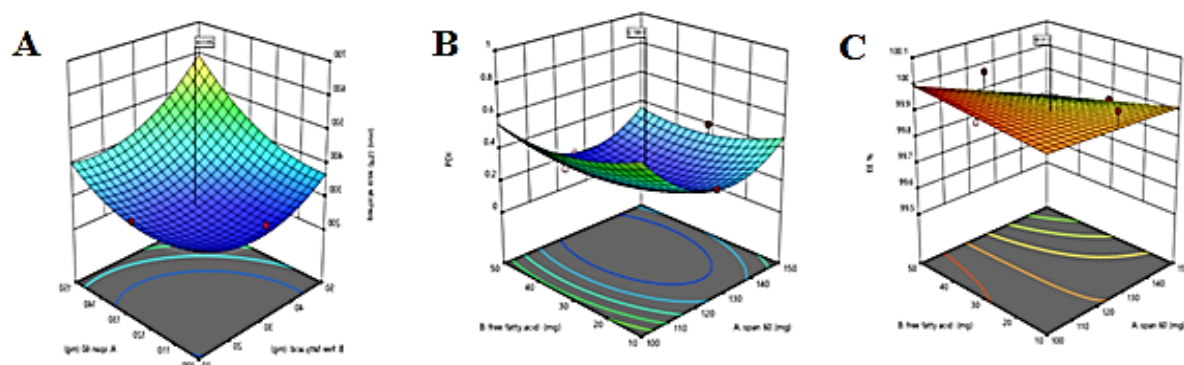
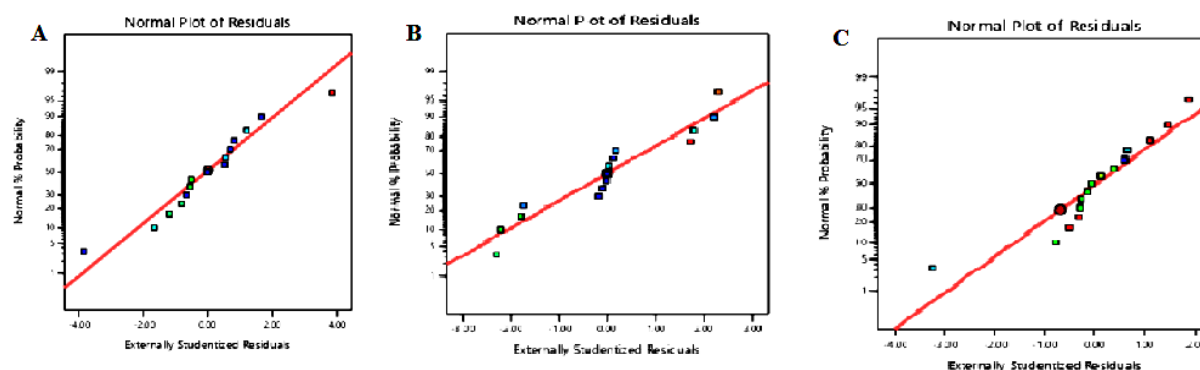
RESULTS

Box-Behnken design of ITZ-loaded Novasomes

Response surface method design (Box-Behnken) was used to prepare and optimize ITZ-loaded novasomes (Table 2). The effect of various independent variables on the measured responses evaluated in the regression equations was explained through comparison of the factor coefficients. The 3-D response diagram in the figure illustrated the influence of span 60, FFA, and probe sonication time on VS, PDI, and EE % of novasomes nanovesicles (Figure 1).

Table 2: Constituents of the formulations and response outcome

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: span 60 (mg)	B: FFA (mg)	C: probe time (min)	PS (nm)	PDI	EE
1	150	30	0	390.4	0.321	99.97
2	150	10	4	370.3	0.4342	99.96
3	125	30	4	333.2	0.2546	99.8
4	100	30	0	377.5	0.5912	99.57
5	125	50	8	410.2	0.2111	99.97
6	150	50	4	655.1	0.3111	99.75
7	125	10	0	290.5	0.3675	99.84
8	125	50	0	510.5	0.2012	99.67
9	125	30	4	290.9	0.2619	99.81
10	150	30	8	409.1	0.4012	99.86
11	100	30	8	309.2	0.4556	99.95
12	100	10	4	300.1	0.8765	99.76
13	125	30	4	310.9	0.2734	99.67
14	100	50	4	425.9	0.7521	99.73
15	125	10	8	320.7	0.3321	99.99

**Figure 1:** Three-dimensional response charts represent the influence of independent factors. (A) PS, (B) PDI, (C) EE (%)**Figure 2:** Plots of predicted versus actual values showing the linear correlation. A: PS, B: PDI, C: EE %

Effect of independent factors on PS

The effect of the selected independent variables, namely Span 60, cholesterol, and palmitic acid, may be associated with the observed particle size of the novasomal formulations. Because higher concentrations of Span 60 made the membrane stiffer and the bilayer more stable, resulting in bigger vesicles. This is due to the high phase transition temperature and low HLB

of span 60. Similarly, increased cholesterol may cause vesicles to enlarge by tightening the membranes, thickening the bilayer, and decreasing fluidity of the membranes. As a saturated fatty acid, palmitic acid may also strengthen hydrophobic interactions in the vesicular bilayer, further stiffening the membrane and promoting the formation of bigger, more stable vesicles (Table 3).

Table 3: Analysis of variance summary for the particle size (PS) response

Model	1.280E+05	9	14225.80	10.81	0.0087	significant
A-span 60	21238.60	1	21238.60	16.14	0.0101	
B-free fatty acid	64818.00	1	64818.00	49.27	0.0009	
C-probe sonication time	1791.01	1	1791.01	1.36	0.2959	
AB	6320.25	1	6320.25	4.80	0.0799	
AC	1892.25	1	1892.25	1.44	0.2841	
BC	4257.56	1	4257.56	3.24	0.1319	
A ²	12156.44	1	12156.44	9.24	0.0288	
B ²	17479.43	1	17479.43	13.29	0.0148	
C ²	23.15	1	23.15	0.0176	0.8996	
Residual	6578.15	5	1315.63			
Lack of Fit	5682.63	3	1894.21	4.23	0.1971	not significant
Pure Error	895.53	2	447.76			
Cor Total	1.346E+05	14				

Effect of independent factors on PDI

The PDI values ranged from 0.2012 to 0.8765, indicating that the selected formulation factors significantly influenced size distribution of the produced novasomal vesicles. This may be due to the influence of Span 60, cholesterol, and palmitic acid on organization of bilayers and assembly of vesicles. Alterations in the quantities of these components might affect the stiffness and interactions among molecules inside the vesicular system. This may alter vesicle formation and thus modify PDI values of the produced formulations.

Effect of independent factors on encapsulation effect

The encapsulation effectiveness of the developed novasomal formulations was

consistently higher, reaching 99 % across all formulations. This outcome may stem from the synergistic actions of Span 60, cholesterol, and palmitic acid on bilayer organization and vesicle stability. The lipophilic nature of Span 60 may facilitate the incorporation of pharmaceuticals into the vesicular bilayer, whereas cholesterol may enhance membrane rigidity, reducing likelihood of drug leakage. Palmitic acid may enhance hydrophobic interactions inside the vesicular membrane, aiding in retention of medicines within the vesicles. The remarkable encapsulation effectiveness may be attributed to the lipophilicity of itraconazole and low water solubility. This increases the likelihood of remaining within the hydrophobic bilayer portion of the novasomal vesicles, hence enhancing drug entrapment.

Table 4: Analysis of variance summary for polydispersity (PDI) response

Model	0.5035	9	0.0559	7.08	0.0221	significant
A-span 60	0.1824	1	0.1824	23.08	0.0049	
B-free fatty acid	0.0358	1	0.0358	4.52	0.0867	
C-probe sonication time	0.0008	1	0.0008	0.1035	0.7606	
AB	4.225E-07	1	4.225E-07	0.0001	0.9944	
AC	0.0116	1	0.0116	1.47	0.2790	
BC	0.0005	1	0.0005	0.0649	0.8090	
A ²	0.2257	1	0.2257	28.56	0.0031	
B ²	0.0254	1	0.0254	3.22	0.1329	
C ²	0.0172	1	0.0172	2.18	0.2000	
Residual	0.0395	5	0.0079			
Lack of Fit	0.0393	3	0.0131	145.93	0.0068	significant
Pure Error	0.0002	2	0.0001			
Cor Total	0.5430	14				

Table 5: Analysis of variance summary for encapsulation efficiency (EE %) response

Model	0.1968	6	0.0328	6.52	0.0093	significant
A-span 60	0.0351	1	0.0351	6.98	0.0296	
B-free fatty acid	0.0231	1	0.0231	4.60	0.0644	
C-probe sonication time	0.0648	1	0.0648	12.89	0.0071	
AB	0.0081	1	0.0081	1.61	0.2400	
AC	0.0600	1	0.0600	11.94	0.0086	
BC	0.0056	1	0.0056	1.12	0.3211	
Residual	0.0402	8	0.0050			
Lack of Fit	0.0280	6	0.0047	0.7657	0.6618	not significant
Pure Error	0.0122	2	0.0061			
Cor Total	0.2370	14				

Optimization process

The optimization process to determine the best formulation variables that produced the expected outcomes was performed using the Box-Behnken design. The response factors examined were the particle size, entrapment efficiency, and polydispersity, while the independent variables were free fatty acid, span 60, and probe sonication time. Based on the desirability value, the optimized formulation was composed of palmitic acid (27.647 mg), span 60 (123.502 mg) and probe time (8 min) yielding a predicted vesicle size of 289.504 nm, PDI of 0.199, and encapsulation efficiency of 99.915 %, which produced 299.1 nm, 0.2815, and 99 % particle size, PDI and EE %, respectively in laboratory work. These results represented good agreement with the predicted values, confirming the validity of the optimization model.

Determination of solubility

The pure medication exhibited very low solubility in distilled water with Brij 35 (0.00365 mg/mL), while in 0.1 N HCl with Brij 35, the solubility was

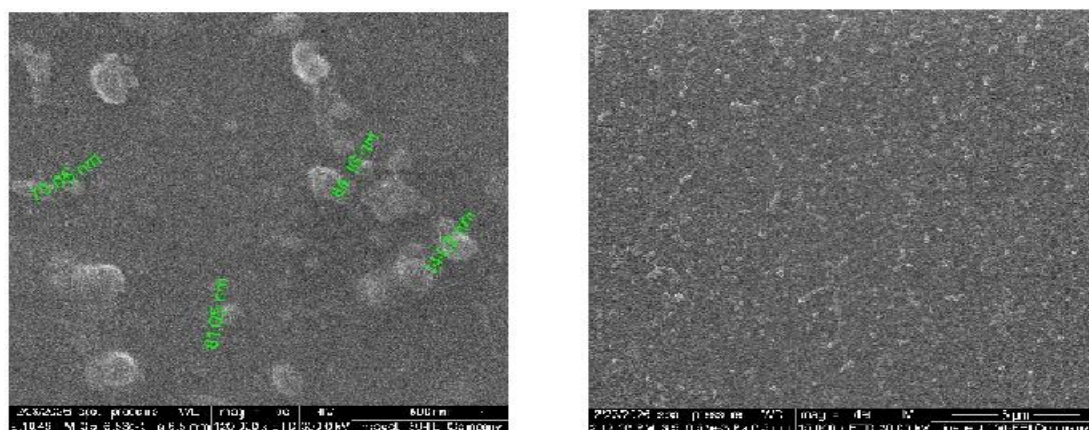
0.07605 mg/mL. The optimized formula (S1) showed higher solubility compared to the pure drug across both media, reaching 0.0897 mg/mL in distilled water with Brij 35, and 1.04 mg/mL in 0.1 N HCl with Brij 35, respectively.

Morphology of novasome

The morphological characteristics of the selected novasome formula were assessed using both SEM and TEM. Optical microscopic examination of the optimized formulations showed that the produced vesicles were aspherical in shape, had a uniform size distribution, and clearly marked sealed structure (Figure 3 and Figure 4).

FTIR spectral analysis of ITZ-loaded novasomes

The FTIR analysis illustrated that features of the medications were preserved in the best formula, indicating chemical stability and absence of interaction. There were some minor variations suggesting possible physical interactions (Figure 5).

**Figure 3:** Scanning electron microscopy of the optimized formula

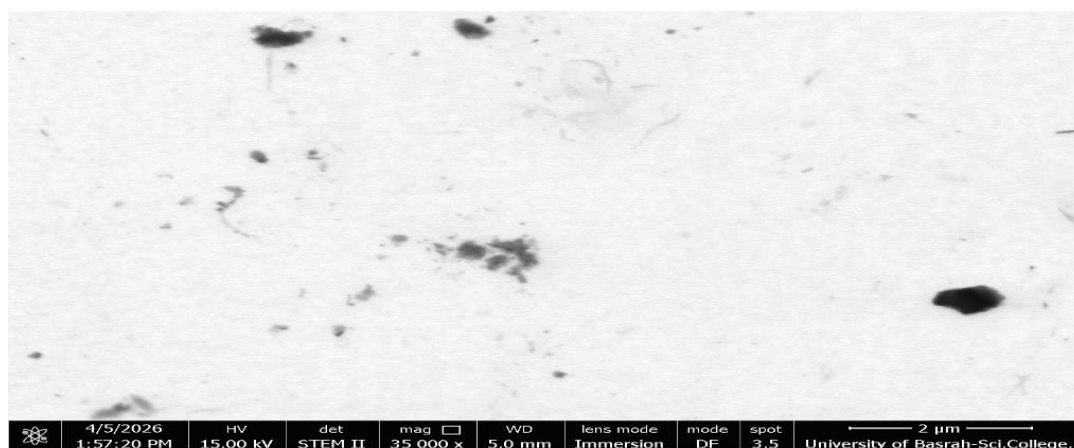


Figure 4: Transmission electron microscopy of the selected formula

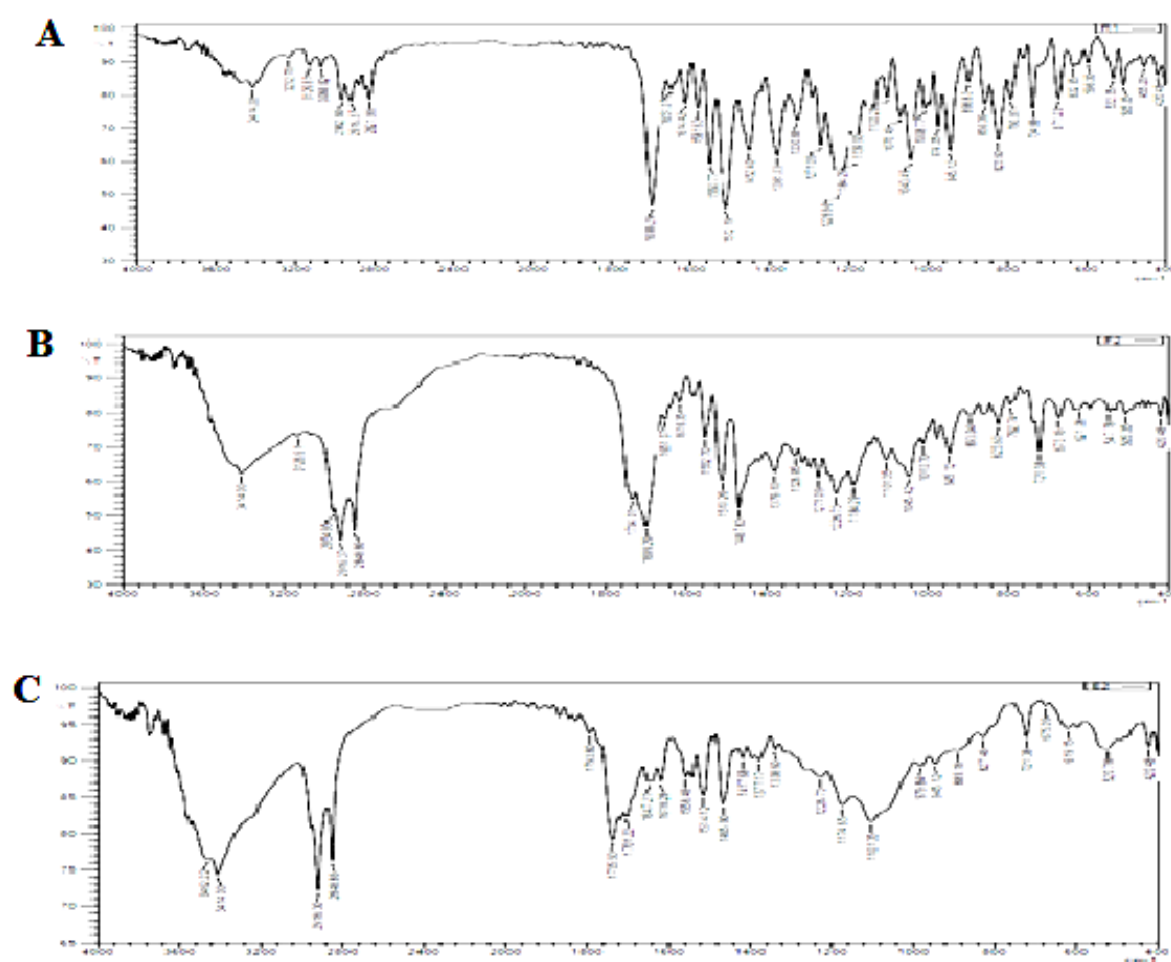


Figure 5: The FTIR spectra of the pure itraconazole (A), physical mixture (B), and the novasome optimized formula (C)

Assessment of biological activity

Antifungal

The agar well diffusion method against *Candida albicans* and *Candida tropicalis* was used to evaluate the antifungal activity of the selected formula (S1). Pure itraconazole and the

marketed capsule demonstrated improved antifungal activity. The formula produced a 10 mm inhibition zone diameter against *Candida tropicalis*, while the pure drug exhibited no detectable inhibition zone (0 mm) under same conditions (Figure 6).

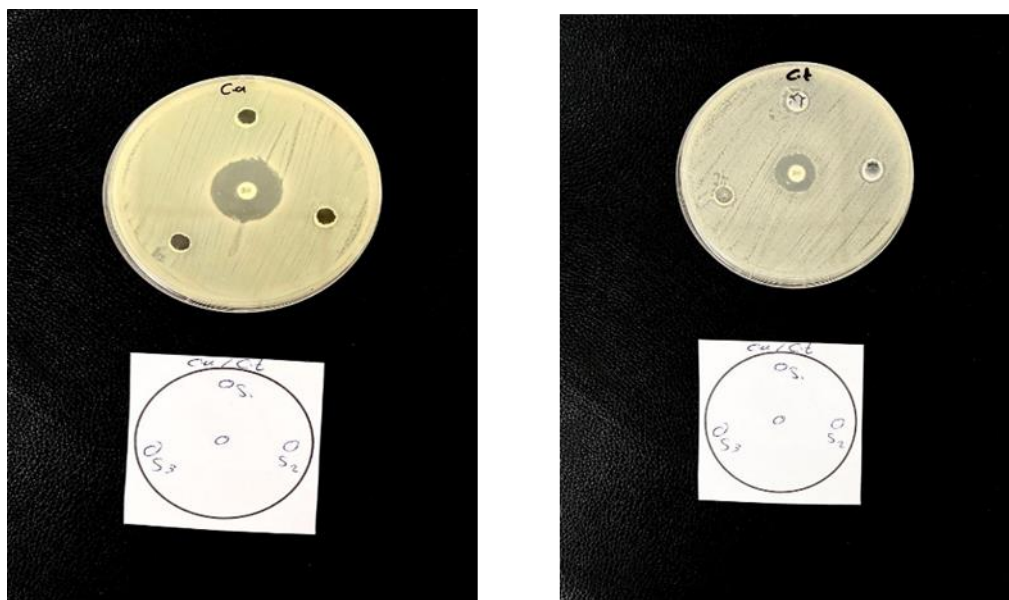


Figure 6: The agar well diffusion technique for the selected formula, marketed capsule, and pure drug against *Candida albicans* and *Candida tropicalis*



Figure 7: Inhibitory effect against bacteria (*Staphylococcus aureus* and *Acinetobacter baumannii*)

Antibacterial activity

The antibacterial activity assays indicated that the optimized novasome formula (S1) exhibited significantly greater inhibitory activity (12 mm) compared to ITZ and the marketed capsule (9 mm), particularly against *Acinetobacter baumannii* ($p < 0.05$; Figure 7).

DISCUSSION

The response factors examined were particle size, entrapment efficiency, and polydispersity, while the independent variables were free fatty acid, span 60, and probe sonication time. Based on the desirability value, the optimized formulation was composed of palmitic acid (27.647 mg), span 60 (123.502 mg) and probe time (8 min) yielding a predicted vesicle size of

289.504 nm, PDI of 0.199, and encapsulation efficiency of 99.915 %, which produced 299.1 nm, 0.2815, and 99 % particle size, PDI and EE %, respectively in the laboratory. These results represented good agreement with predicted values, confirming the validity of the optimization model [18]. Solubility testing is essential for poorly soluble medications such as itraconazole because it directly affects bioavailability, dissolution rates, and formulation efficacy. Solubility study was conducted to determine the parameters affecting ITZ solubility, thereby improving the pharmacological effect and application range of novasome nanocarrier formulations.

Solubility of pure ITZ and prepared novasome formulations was evaluated in two media: distilled water with Brij35 and 0.1 N HCl with

Brij35. The pure medication exhibited very low solubility in distilled water with Brij 35. However, the selected formula (S1) showed higher solubility compared to the pure drug in both media. The developed optimized novasomal formula showed a vesicle size of 299.1 nm, indicating a relatively small particle size, which may contribute to increased surface area and facilitated cellular uptake. The dispersity (PDI) of the optimized formula was 0.199, indicating a narrow size distribution and good homogeneity. This suggested overall uniformity and stability of the vesicular system. Furthermore, non-ionic surfactant (span 60) reduced interfacial tension during the vesicle formation, producing small, uniformly sized novasomes with low dispersity. Palmitic acid used in the preparation led to higher bilayer rigidity through tight packing of its C16 chains. This stabilizes the membrane and prevents aggregation which maintains a consistent vesicle size [19].

High encapsulation efficiency resulting from the optimized formula reflected effective incorporation of ITZ within the vesicular bilayer. The lipophilic nature of itraconazole mainly facilitates partitioning into the lipid bilayer. Furthermore, combined effect of constituents of Novasomes (Span 60, cholesterol, and palmitic acid) limited leakage, improved drug retention, forming a well-organized and rigid vesicular membrane [20]. Micrographs of both SEM and TEM confirmed the formation of well-defined, smooth, homogeneous, and spherical vesicle surfaces. Successful incorporation of ITZ within the vesicular structure was indicated by the absence of visible drug crystals, suggesting the integrity and stability of the novasomal system. However, FTIR spectra of pure ITZ exhibited characteristic absorption peaks at 3067 cm^{-1} (C-H aromatic stretch), 2963, 2916 and 2822 cm^{-1} represented aliphatic, C-H asymmetric and symmetric stretch respectively; 1699 cm^{-1} (C = O stretch) represented triazolone carbonyl, 1651 cm^{-1} (C = N triazole ring stretch), 1614 and 1582 cm^{-1} (C = C aromatic ring stretch), 1229 and 1184 cm^{-1} (C-O-C asymmetric stretch) represented dioxolane ring. Two peaks appeared at 1070 and 1043 cm^{-1} (C-O-C symmetric stretch) indicating a dioxolane ring found in the crystalline form of the drug. The above characteristic peaks were also observed in the physical mixtures and the optimized novasome formulation. Based on the FTIR spectra, there was no significant drug – excipient interactions [21].

Biological activities of the optimized formula were carried out. The selected formula (S1) showed superior antifungal activity against *Candida*

tropicalis, compared to pure itraconazole. Also, the S1 formula showed antibacterial activity against *Acinetobacter baumannii* (IZD of 12 mm) compared to the marketed capsule (IZD of 9 mm), and no inhibition zone diameter with the pure drug. These findings suggest improvement in the biological activity of the optimized formula.

CONCLUSION

The vesicles demonstrated high encapsulation efficiency (EE = 99 %), high solubility, aspherical in shape with uniform size distribution, no drug-excipient interactions, and good antifungal and antibacterial property.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

It is stated in the method.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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