

Original Research Article

Topical delivery enhancement of ketoconazole by utilizing novasomal vesicular systems

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Abstract

Purpose: To determine the antifungal activity of ketoconazole-loaded novasomal delivery systems.

Method: Ethanol injection method was used to fabricate ketoconazole-loaded novasomes using stearic acid (SA) and oleic acid (OA) as free fatty acids (FFA), with the surfactant (SAA) being Span 60, Brij 35, or a combination. Also, cholesterol (CHO) was added as a membrane stabilizer.

Results: Cumulative drug release at 8 h for F9 was $87.33 \pm 8.67\%$, compared with $18.6 \pm 4.96\%$ for pure ketoconazole suspension. Korsmeyer-Peppas model best fit the data ($R^2 = 0.987$), and the n -exponent was 0.615, suggesting non-Fickian diffusion (diffusion with membrane bilayer relaxation). Furthermore, F9 showed a high inhibition zone diameter (25 ± 2.6 mm and 23 ± 2.5 mm) on *Candida albicans* and *Aspergillus niger*, respectively, at 800 µg/mL compared to blank novasomes and pure ketoconazole suspension. The stability study demonstrated that F9 was best stored at 2 - 8 °C.

Conclusion: The novasomes showed narrow PDI, potentiating the activity of ketoconazole by improving drug release and diffusion, and enhancing permeation through the fungal cell wall.

Keywords: Antifungal study, Ethanol injection method, Ketoconazole, Novasomes

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INTRODUCTION

Ketoconazole (ket) is a broad-spectrum synthetic imidazole derivative commonly used for the treatment of systemic or superficial mycosis. Ketoconazole has activity against a wide range of fungal classes, including yeast-like candidiasis, dermatophytosis, histoplasmosis, and chromoblastomycosis [1]. It has poor aqueous solubility (0.05 mg/mL at 25 °C), which is pH-dependent (highly soluble at pH < 3 in the stomach media). The alkaline pH of the small intestine promotes precipitation of ketoconazole, necessitating the use of acid-modifying agents to

enhance dissolution and consequently improve oral bioavailability [2]. Due to the hepatotoxicity of Ketoconazole, it was withdrawn from the European and Australian markets in 2013. However, oral formulation of Ketoconazole is only administered for endemic mycosis when necessary [3]. Ketoconazole is a class II BCS drug with low aqueous solubility ($\log P = 4.74$ and molecular weight 530 daltons). Furthermore, it is a weak basic compound with a pKa between 2.9 and 6.5 [4]. The challenge has been to formulate a dosage with solubilized Ketoconazole that performs its activity on target tissue with minimal side effects. The marketed

topical ketoconazole formulations like gel, cream, lotions, and soaps are associated with local side effects such as moderate burning, blisters, itchiness, discomfort, redness, and sometimes severe allergic reactions. Sometimes, fungal infection may be found in deep skin layers, like the dermis or subcutaneous layer, where the topical formulation is less effective. These formulations have less adhesion to the skin layer and short half-life. As a result, repeated applications are required to maintain activity. Nanotechnology has become the cornerstone to overcome the drawbacks associated with conventional formulations. Main advantages of nanocarrier drug delivery systems involve improved drug activity, lower doses, reduced side effects, tissue-targeted delivery depending on the type of selected nanocarrier, improved tissue permeation, and higher residence time, thereby decreasing dosing frequency.

Ketoconazole has been developed in various nanocarrier systems to improve topical delivery, such as niosomes, liposomes, ethosomes, microemulsion, microsponges, nanoemugel, solid lipid nanoparticle, nanostructured lipid carrier, and ultra-deformable vesicles [5]. Novasomes are novel vesicular delivery systems developed by Novavax Biotechnology Laboratory as a patented technology for improving drug performance over conventional liposomes or niosomes. They are multilamellar vesicles with 2-7 bilayers. Furthermore, size is within 200 - 700 nm (narrow vesicle size and high entrapment efficiency), which incorporates 99 – 100 % hydrophobic materials and 78 - 88 % hydrophilic materials [6]. The composition of novasomes mainly depends on niosome modification, where, in addition to non-ionic surfactant and cholesterol, the free fatty acid was incorporated into the structure, making them more permeable through tissues. Charges can be positive, negative, or neutral. Various drug candidates are formulated with novasomes for different organ targeting [6,7]. This study investigated novasomes as a vesicular carrier for ketoconazole delivery.

EXPERIMENTAL

Materials

Ketoconazole was purchased from Rhwan, China. Span 60, stearic acid, and oleic acid were purchased from Biotech Co., Ltd., India. Cholesterol was provided by Sigma-Aldrich, Canada. Brij 35 was bought from Loba Chemie, India. Dipotassium hydrogen orthophosphoric acid, disodium hydrogen orthophosphate, Sabouraud dextrose agar, and sodium

dihydrogen orthophosphate were obtained from Tmmedia, India. Potassium dihydrogen orthophosphate was purchased from Loba Chemie, India. All other solvents and chemicals, such as ethanol and methanol, were of analytical grade.

Formulation of novasomes-loaded Ketoconazole

The ethanol injection method was used to formulate Ketoconazole-loaded novasomes [8] (Table 1). Different weights of Span 60 (Biotech, CO. Ltd, India), stearic acid (Biotech, CO. Ltd, India), cholesterol (Sigma Aldrich, Canada), and ketoconazole (Rhwan, China) were placed in a beaker, dissolved in 10 mL ethanol, and sonicated for 5 min in a bath sonicator for complete dissolution. The clear solution was warmed in a water bath at 60 °C. The sample was filled into a syringe (21 G) and injected slowly into another beaker containing five-fold phosphate buffer (pH 7.4; Tmmedia, India) mounted on a magnetic stirrer (900 rpm and heated to 60 °C. The sample was continuously stirred till the organic solvent evaporated. The presence of turbidity indicated the formation of novasomes. The sample was sealed in a closed container and stored at 4 °C for further testing. Each formula was done in triplicate.

Vesicle size (VS), polydispersity index (PDI), and zeta potential (ZP)

Dynamic light scattering was used to determine the VS, PDI, and ZP of the blank and loaded novasomes using a Zetasizer (Malvern, UK). The sample was diluted with distilled water (1:15) at a detection angle of 173 ° at 25 °C [9]. The molecules' ability to scatter light was recorded. The readings were done in triplicate. The hydrophilic-lipophilic balance (HLB) was calculated using Eq 1, depending on the weight fraction and HLB of each surfactant in the blend.

$$HLB_{AB} = \{HLB_A (A\% + HLB_B) B\% \dots\dots\dots (1)$$

Where HLB_A and HLB_B are the HLB values for surfactants A and B, HLB_{AB} is the overall HLB of the blend, and A% and B% are the weight percentages in the blend.

Entrapment efficiency

A total of 3 novasomes coded F7, F9, and F13 were loaded with 50 mg Ketoconazole. Each novasome dispersion (2 mL) was placed in an Amicon Ultra-4 centrifuge filter MWCO 10 KD, and centrifuged by utilizing a cooling centrifuge (Hermle, England) at 4 °C and 6000 rpm for 2 h.

The specific volume of the supernatant was filtered through a MICROLIT® syringe filter of 0.22 µm, diluted with methanol, and sample absorbance was recorded at 243 nm by a spectrophotometer (Aquarius Cecil Ce 7200, UK). A calibration curve with the equation $y = 0.0406x + 0.0022$ ($R^2 = 0.9996$) was used to calculate the free concentration of Ketoconazole. Equation 2 was used to determine EE % for the selected formulas [9].

$$\text{Entrapment efficiency (EE; \%)} = (E_a/E_i) \times 100 \dots (2)$$

Where E_a is the amount of entrapped drug, and E_i is the initial/estimated amount

Characterization of the best formulation

Surface morphology

A scanning electron microscope (Nova Nano SEM 450) was used to evaluate surface morphology. A drop of the sample was diluted and put on a carbon-coated copper grid mesh 300, and the sample was measured at an accelerated voltage of 15 KV [10].

Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) analysis was done to study the compatibility and the absence of drug-excipient interaction, for the pure Ketoconazole, physical mixture, and the lyophilized selected formula. Infrared Affinity-1 (Shimadzu, Japan) was employed with a temperature-controlled DLATGS detector and a resolution of 0.5 cm^{-1} . The internal reflection element was an ATR crystal with an incident angle of 45° and scanned at 32 resolutions. The scanning region was between 600 and 4000 cm^{-1} using the KBr pellet method [11].

Powder X-ray diffraction (PXRD)

The diffractometer X Pert PRO (Panalytical, Netherlands) was used to evaluate PXRD of pure Ketoconazole, physical mixture, and lyophilized ketoconazole-loaded Novasomes under certain operating conditions (detection angle range of 5 – 60° (2θ), 40 kV voltage, 30 mA current at 25 °C) [11].

Differential scanning calorimetry (DSC)

The thermotropic properties, such as melting point and glass transition temperature T_g of pure Ketoconazole, physical mixture, and selected lyophilized Ketoconazole-loaded novasomes

were assessed using DSC (DSC-60, Shimadzu, Japan). The samples (10 mg) were briefly placed in aluminum pans and heated at 10 °C/min, with a temperature range of 25 - 250 °C. The used gas was nitrogen [12].

Release study

A dialysis bag with a molecular weight cut-off size (MWCO of 8000-14000 Da, and 34 mm width) was previously soaked overnight in the release medium. Thereafter, 1.5 mg/1.5 mL of both pure Ketoconazole suspension and Ketoconazole-loaded novasomes were placed in the dialysis bag and tightly closed at both ends. Subsequently, the bag was placed in a 200 mL release medium of sodium phosphate buffer pH 5.5 pre-filled in the jar of apparatus II-paddle type (CALEVA) [13]. Volume and type of buffer were selected to improve sink conditions in which the saturated solubility is 0.025 ± 0.0046 mg/mL, mimicking skin pH. The study lasted for 8 h at 32 ± 0.5 °C [14], at 50 rpm. At specific intervals (0, 1, 2, 4, 6, and 8 h), an aliquot sample was withdrawn and diluted with 30 % methanol, and absorbance was recorded at 243 nm using UV spectroscopy. The calibration curve equation of Ketoconazole in 30 % methanol in buffer pH 5.5 ($y = 0.0376x + 0.0087$ ($R^2 = 0.9995$)) was used to calculate drug concentration. Each run was performed in triplicate.

Antifungal sensitivity study

The selected fungi for this study were *Candida albicans* ATCC 10231 and *Aspergillus niger* MTCC 8652 [10]. The antifungal study was done using the agar diffusion test (Cup plate technique). Sabouraud dextrose agar (SDA) 65 g/L powder was dissolved in hot distilled water. The solution was sterilized in an autoclave at 121 °C and 14.5 psi for 20 min [9]. Thereafter, it was allowed to cool to 45 - 50 °C. Chloramphenicol (0.5 g/L) was added to the SDA solution to prevent bacterial contamination [15]. This solution was poured into the plastic Petri dishes and allowed to solidify overnight. One isolated colony of the fungi was transferred to the Sabouraud dextrose broth (28 g/L) and further diluted to obtain 108 colony-forming suspension units/mL [16]. *Candida albicans* (1 mL) and *Aspergillus niger* suspension were streaked over the Petri dishes. Four holes (5 mm) were made in each Petri dish using a metallic borer. Ketoconazole suspension, Ketoconazole-loaded novasomal dispersion, blank novasomal dispersion, and blank ketoconazole medium (phosphate buffer and 1 % ethanol) were poured into the holes of each Petri dish at 200 µg/mL,

400 µg/mL, and 800 µg/mL. The plates were then incubated at 37 °C for 48 h.

Short-term stability for Ketoconazole-novasomal (Ket-NS) dispersion

To investigate the impact of storage conditions on short-term stability of liquid Ket-NS, the optimal formulation was split between two amber-colored glass containers with secure lids, and each container was kept at a temperature of $2-8 \pm 2$ °C and 25 ± 2 °C [17,18] for 90 days. Average particle size, polydispersity index, and zeta potential after 0, 30, 60, and 90 days were recorded in triplicate.

Statistical analysis

Data were analyzed using Microsoft Excel (Microsoft Office Professional Plus 2021, Windows). Results were presented as mean $\pm$ standard deviation (SD), and compared using one-way analysis of variance (ANOVA) single-factor. $P < 0.05$ was considered statistically significant.

RESULTS

Vesicle size, polydispersity index, zeta potential

The vesicle size (VS), polydispersity index (PDI), and zeta potential (ZP) of the formulations revealed that the outcomes were significantly affected by surfactant type (Brij 35, Span 60, and a co-mixture of Brij 35 and Span 60 as in F4, F5, and F6 respectively; $p < 0.0001$) in the order Span 60 > Span 60-Brij 35 > Brij 35 (Table 1).

Entrapment efficiency

The measured EE % was 71.66 ± 4.7 , 93.3 ± 1.8 and 80.63 ± 7.2 for F7, F9 and F13 respectively. The effect of FAA and SAA type on EE % was also investigated (Figure 1). The formula F9 was selected as the best formula, based on the resulting parameters (size of 159.1 ± 3.9 nm, PDI of 0.201 ± 0.02 , zeta potential of -51.0 ± 1.11 mV, and EE % of 93.3 ± 1.868).

Table 1: Vesicle size, polydispersity index, zeta potential of the formulations

Formula	SAA: FFA: CHO w/w	SAA type	HLB	FFA type	VS (nm)	PDI	ZP (mV)
F1	1:1:1	Brij 35	16.9	SA	122.30 $\pm$ 2.64	0.36 $\pm$ 0.11	-39.40 $\pm$ 3.41
F2	1:1:1	Span 60	4.7	SA	213.30 $\pm$ 1.10	0.16 $\pm$ 0.01	-64.50 $\pm$ 0.69
F3	1:1:1	Sp60-Br 35	8.7	SA	74.86 $\pm$ 19.00	0.23 $\pm$ 0.65	-35.30 $\pm$ 2.32
F4	2:1:1	Brij 35	16.9	SA	92.13 $\pm$ 2.09	0.18 $\pm$ 0.03	-39.60 $\pm$ 1.36
F5	2:1:1	Span 60	4.7	SA	435.00 $\pm$ 17.96	0.45 $\pm$ 0.03	-56.7 $\pm$ 1.79
F6	2:1:1	Sp60-Br 35	8.7	SA	112.8 $\pm$ 13.49	0.19 $\pm$ 0.02	-50.9 $\pm$ 2.97
F7	3:1:1	Brij 35	16.9	SA	79.50 $\pm$ 6.96	0.20 $\pm$ 0.04	-33.7 $\pm$ 1.35
F8	3:1:1	Span 60	4.7	SA	957.60 $\pm$ 159.40	0.72 $\pm$ 0.48	-55.4 $\pm$ 1.27
F9	3:1:1	Sp60-Br 35	8.7	SA	159.10 $\pm$ 3.96	0.20 $\pm$ 0.06	-51.0 $\pm$ 1.11
F10	1:1:1	Sp60-Br 35	8.7	SA	182.50 $\pm$ 2.54	0.23 $\pm$ 0.01	-50.7 $\pm$ 0.69
F11	1:1:1	Sp60-Br 35	8.7	SA	150.90 $\pm$ 3.68	0.46 $\pm$ 0.06	-46.0 $\pm$ 0.76
F12	1:1:1	Brij 35	16.9	OA	103.10 $\pm$ 0.81	0.23 $\pm$ 0.02	-37.6 $\pm$ 2.9
F13	3:1:1	Sp60-Br 35	8.7	OA	141.80 $\pm$ 13.05	0.20 $\pm$ 0.01	-76.4 $\pm$ 10.3

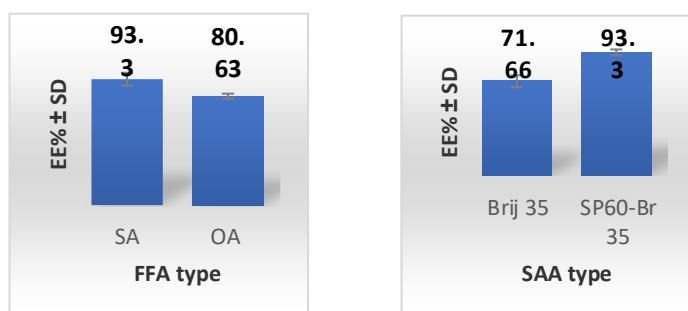


Figure 1: Factors affecting EE %

Surface morphology

The vesicles were scanned at two magnifications (100 k and 150 k; Figure 2). The images showed spherical, discrete vesicles with no aggregations.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectrum showed no significant interaction between the drug and excipients, indicating compatibility (Figure 3).

Powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC)

The z-average of the vesicles was within the nanosize range (Figure 4).

Release studies

The cumulative drug release with four kinetic profiles, namely zero-order, first-order, Higuchi, and Korsmeyer-Peppas models are presented in Figure 5.

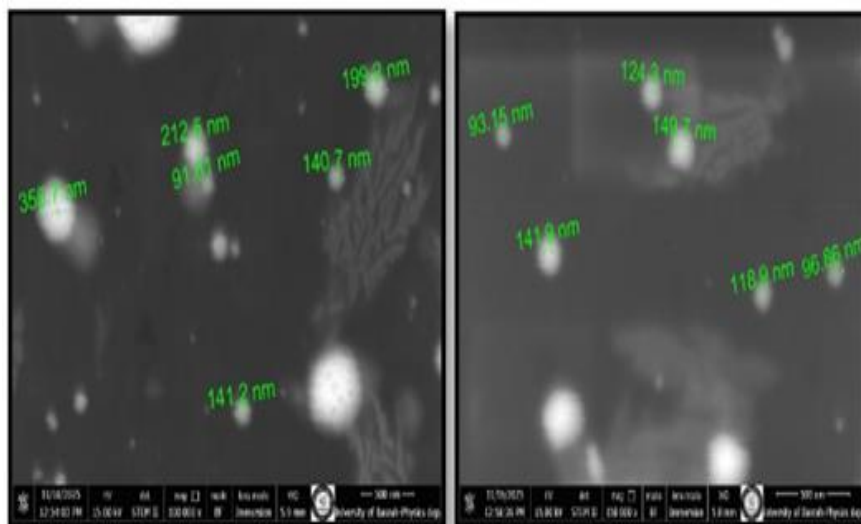


Figure 2: Surface electron microscopy (SEM) of the loaded novosomes

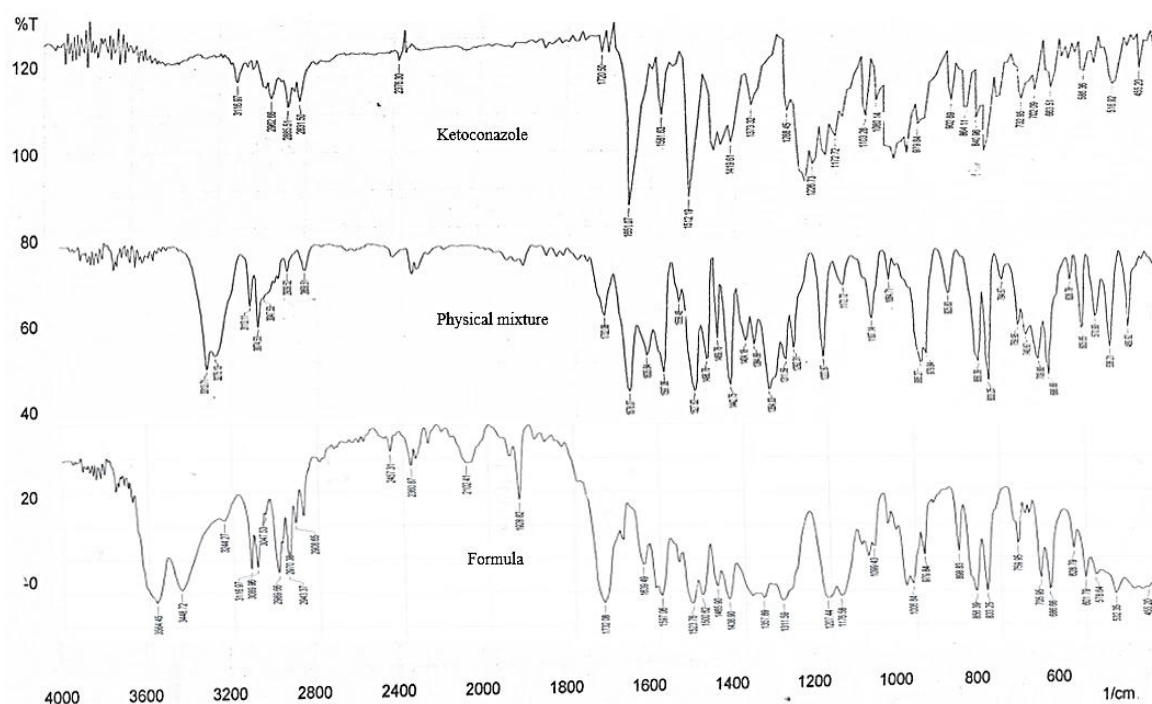


Figure 3: FTIR spectrum for Ket, the physical mixture, and the formula

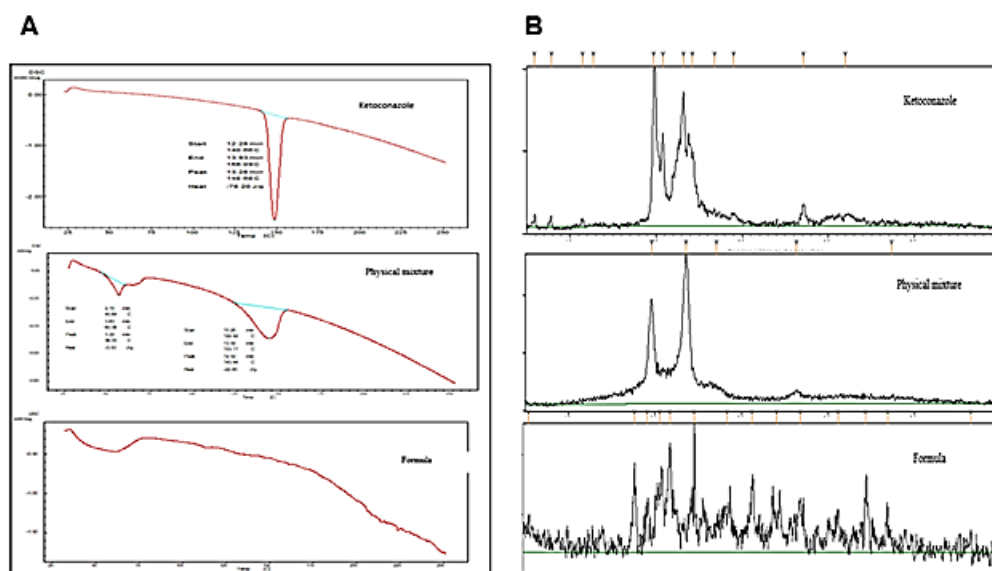


Figure 4: DSC (A) and PXRD (B) for Ketoconazole, physical mixture, and F9

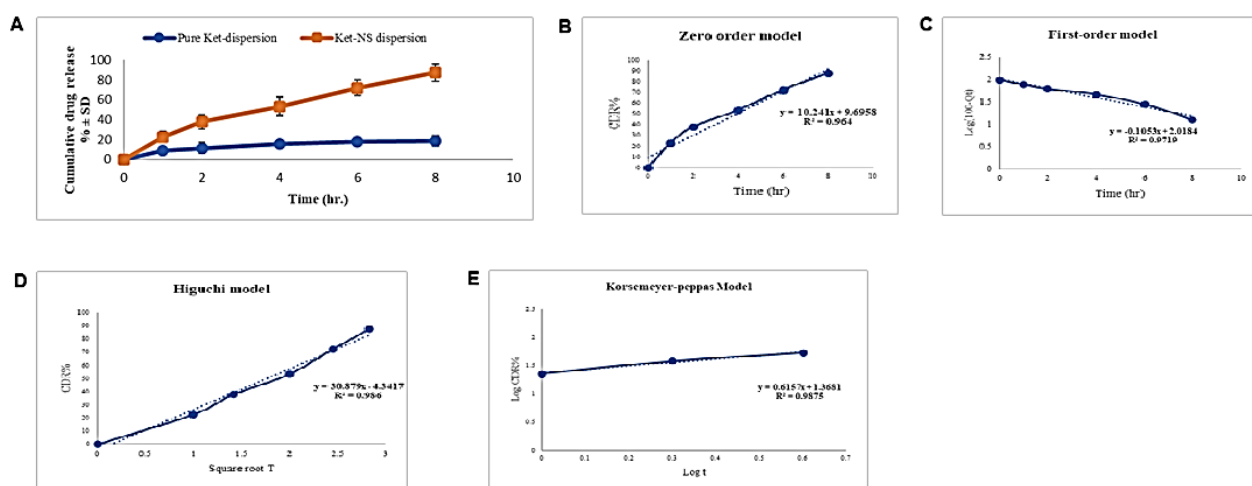


Figure 5: Cumulative drug release with the four kinetic profiles of release

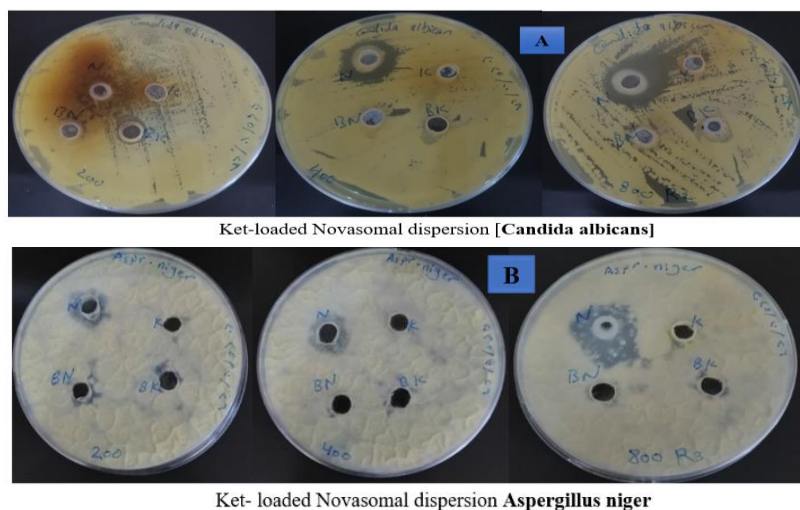


Figure 6: Antifungal sensitivity of the formula on *Candida albicans* and *Aspergillus niger*

Table 2: VS, PDI, and ZP in different storage temperatures for three months

Time (Days)	VS (nm)	PDI	ZP (mV)	Organoleptic
2 - 8 °C				
1	159.1±3.955	0.201±0.062	-51.0±1.11	Stable-milky
30	160.3±6.830	0.245±0.158	-52.9±2.6	Stable-milky
60	163.8±25.93	0.331±0.161	-50.5±5.52	Stable-milky
90	170.1±15.07	0.303±0.156	49.8±6.32	Stable-milky
25 °C				
1	159.1±3.955	0.201±0.062	-51.0±1.11	Stable-milky
30	163.3±14.38	0.540±0.078	-48.1±4.1	Stable-milky
60	186.0±24.97	0.472±0.206	-46.1±2.08	Stable-milky
90	212.9±32.8	0.575±0.186	-40.4±2.89	Stable-milky

Antifungal sensitivity study

Antifungal activity of Ketoconazole-loaded novasomes (F9) was assessed at 200 µg/mL, 400 µg/mL, and 800 µg/mL on selected microorganisms (Figure 6).

Stability study

The obtained data are summarized in Table 2 below.

DISCUSSION

Novasomes are nano-vesicular systems consisting of surfactant, free fatty acids, and cholesterol. The presence of free fatty acids makes these vesicles more skin-permeable. Span 60 and Brij 35 were selected as surfactants, stearic acid and oleic acid were utilized as free fatty acids, and cholesterol was used as a membrane stabilizer. For the topical antifungal delivery system, the vesicle size is a critical parameter, as smaller vesicles produce more permeation and superior activity. The bilayered vesicles formed by Span 60 were more rigid, less flexible, and larger in size [19]. Brij 35 showed a higher HLB value of 16.9. A mixture of Brij 35 and Span 60 resulted in an HLB value of 8.7. The resultant HLB of the blend produced a small vesicle size compared to Span 60 alone. As the HLB decreased for Span 60, the size increased, and vice versa for Brij 35 with a high HLB value [20]. Brij 35 modifies the membrane rigidity of Span 60 and reduces intermolecular aggregation, thus improving vesicle size [21]. Stearic and oleic acids were employed in the study, as in F9 and F13, respectively. The vesicle size for stearic acid was larger than that of oleic acid-containing vesicles. Stearic acid is a saturated fatty acid with straight hydrocarbon chains (C-18) that produces a less flexible, more rigid bilayer with little membrane curvature and large vesicles. Furthermore, the high transition temperature of SA (69 °C) was compared to the unsaturated long C-18 carbon chain of oleic acid, with a low transition temperature (13 °C), making

the vesicle that was formed by oleic acid smaller compared to stearic acid [22]. Also, three SAA concentrations were used (100, 200, and 300 mg), and different vesicle sizes were observed depending on the type of SAA. The effect was studied for F1 to F9. For Span 60, a progressive increase in vesicle size was observed. The lipophilic nature of Span 60, with an HLB value of 4.7 and a long hydrocarbon chain, promotes the formation of a rigid bilayer membrane. Higher concentrations of Span 60 increased membrane thickness and encouraged vesicle fusion, leading to larger vesicles [23]. In contrast, as the concentration of Brij 35 increases, vesicle size significantly decreases. This effect was due to the hydrophilic nature of the surfactant with a higher HLB value, making the bilayer more flexible and fluidized. Furthermore, higher concentration of Brij 35 decreased interfacial tension and prevented aggregation, leading to smaller vesicles.

Combination of Span 60 and Brij 35 (2:1) significantly affected vesicle size. Higher concentrations significantly increased vesicle size. Although Brij 35 usually produced more flexible and fluidized membrane, the presence of Span 60 in higher concentrations increased membrane thickness, subsequently promoting hydrophobic interactions of bilayers and increasing vesicle size. Polydispersity index is a critical quality attribute as it affects physical stability, possibility of aggregation during storage, and vesicle uniformity. For maximum stability and less aggregation, the goal is to obtain PDI close to 0. The PDI of the novasomal dispersion was within 0.163 ± 0.011 to 0.72 ± 0.48 . The results agreed with earlier studies on terconazole-loaded novasomes [24]. The main factor that affected PDI was the type of SAA. The SAA type significantly affected PDI of the vesicles for F4, F5, and F6. The PDI increased in the order Span 60 > Co-surfactant mixture > Brij 35. The PDI was highly correlated with vesicle size, as smaller vesicles usually possessed narrow PDI and a more uniform distribution, which is

associated with Brij 35 compared to Span 60 [25].

Formulation of vesicular dispersion with acceptable zeta potential ± 30 was the goal of this study. When the zeta potential falls within acceptable range, electrostatic repulsion between the vesicles increases, thereby enhancing stability and preventing vesicle aggregation. The study showed zeta potential of -33.7 ± 1.35 to -76.4 ± 10.3 , consistent with findings from a previous study [26]. Type of SAA and FFA were factors that affected zeta potential. The type of surfactant significantly influenced the formulation, with the observed effect decreasing in the following order: Span 60 > Sp60-Br 35 > Brij 35 for formulations F4, F5, and F6, which may be due to the HLB value. As the HLB increases, zeta potential decreases. Brij 35, which has a high HLB value of 16.9, is highly hydrophilic and possesses a large polyoxyethylene (POE 23) head group that may shield or mask the surface charge of the vesicles. In contrast, Span 60 has a relatively low HLB value and lacks a large hydrophilic head group, resulting in less surface-charge masking. Instead, it contains free hydroxyl groups, which increase the overall zeta potential of the vesicle surface [24]. Two types of FFAs, stearic and oleic acid, were used to demonstrate the effect on zeta potential for F9 and F13. The formulated novasomes with oleic acid had higher zeta potential compared to those fabricated with stearic acid. The chemical structure of the FFAs was a long-chain hydrocarbon, C18. The main difference between them was the presence of a double bond in oleic acid, creating a loose structure and increasing the negative charge, compared to the saturated, packed chain of SA with less zeta potential.

The effect of FFA type, such as stearic and oleic acid, was also investigated. The stearic acid-containing novasomes showed higher EE % compared to oleic acid-containing novasomes. This is because SA is a saturated FFA and highly hydrophobic with a C-18 long chain, reflecting high phase transition temperature ($T_c = 69$), resulting in fewer leaky vesicles [26]. On the other hand, oleic acid also has a C-18 alkyl chain but is an unsaturated fatty acid with a double bond that affects the T_c (13) [25]. Another factor that affected EE % is the type of surfactant. A mixture of Brij 35 and Span 60-containing vesicles had higher entrapment compared to Brij 35-containing vesicle. This may be attributed to the blend having an HLB value of 8.7, which enhanced lipophilicity and reduced membrane fluidization and leakage, thereby improving entrapment of ketoconazole. Furthermore, Span

60 had a high phase transition temperature of 53 °C and a packed membrane that decreases leakage [26]. Conversely, Brij 35 alone (HLB value of 16.9) imparts greater hydrophilicity to vesicles and enhances membrane hydration. This increased hydration may facilitate drug diffusion through the membrane, thereby promoting drug leakage and ultimately leading to a lower entrapment efficiency [23].

A scanning electron microscope was utilized for evaluating the size and shape of the vesicles. Some vesicles were smaller than the predicted vesicles, which is due to the method used. During the preparation, the sample was thermally dried on copper grid mesh 300, inducing size reduction [11]. The FTIR spectra of pure ketoconazole exhibited characteristic absorption according to functional groups that confirm the chemical structure of the drug, which is in tandem with earlier reports [11,27]. On the other hand, FTIR spectra of the physical mixture (Ketoconazole with excipients in the same ratio as the best selected formula) exhibited a broad O-H stretching vibration at 3313.7 cm^{-1} and 3275.13 cm^{-1} , which were related to hydroxyl groups of the excipients. The broad nature suggests an intermolecular hydrogen bond, very common in physical mixtures, but it didn't indicate a new chemical interaction. The FTIR spectrum exhibited several characteristic absorption bands attributable to the components of the formulation. The bands at 2939.52 and 2858.51 cm^{-1} were assigned to C-H stretching vibrations of aliphatic CH_3 and CH_2 groups present in long hydrocarbon chains. The peak at 1732.08 cm^{-1} corresponded to the ester carbonyl (C=O) stretching of Span 60, while the sharp band at 1678.07 cm^{-1} was attributed to the carbonyl group of ketoconazole. The absorption band at 1527.62 cm^{-1} was associated with C=C stretching in ketoconazole. Furthermore, the band at 1442.75 cm^{-1} was assigned to C-H bending vibrations associated with Brij 35 and Span 60, whereas the peak at 1404.18 cm^{-1} was attributed to C-H bending of ketoconazole. The absorption at 1222.87 cm^{-1} corresponded to C-O-C stretching of the dioxolane ether linkage in ketoconazole, while the band at 1107.14 cm^{-1} was assigned to C-N stretching of the imidazole ring. The peak observed at 995.27 cm^{-1} was attributed to out-of-plane bending of aromatic C-H bonds in ketoconazole, while the band at 536.21 cm^{-1} was associated with C-Cl stretching vibrations of ketoconazole. Overall, the physical mixture contained all characteristic Ketoconazole peaks, in addition to the absence of a chemical reaction or appearance of a new peak that improved compatibility of the mixture. On the other hand, spectrum of the best selected

lyophilized formula (F9) showed characteristic peaks at 3564.45 cm^{-1} and 3448.72 cm^{-1} related to O-H groups. The absence of characteristic peaks of Ketoconazole indicated that the drug was entrapped in the novasome [10].

To confirm the crystallinity of Ketoconazole and the effect of formulation materials on crystal behavior, PXRD was carried out. Presence of characteristic peaks of Ketoconazole with high intensities in the physical mixture indicated compatibility between the materials, and the mixture didn't affect crystallinity of the drug. Furthermore, the characteristic peaks of Ketoconazole showed that there was no interaction with the excipients, as it was compatible with other components in the physical mixture. Absence of characteristic peaks of ketoconazole indicated that the drug was solubilized and entrapped in the novasomes, and the untrapped concentration was very low and undetectable, consistent with previous report [27].

Thermal behavior of Ketoconazole, the physical mixture, and lyophilized formula was evaluated by DSC to demonstrate the purity of Ketoconazole, identify drug-excipient effect on crystallinity, and detect whether Ketoconazole was entrapped or not. The sharp endothermic peak at $148.68\text{ }^{\circ}\text{C}$ with fusion heat of -78.20 refers to the identity and purity of Ketoconazole [28]. Thermogram of the solid blend (Span 60, Brij 35, stearic acid, cholesterol, and Ket in a ratio 2:1:1:1:0.5) displayed two main phase transitions. The first peak was at $56\text{ }^{\circ}\text{C}$ with an enthalpy of -3 J/g , and the second peak was at $143.44\text{ }^{\circ}\text{C}$ with an enthalpy of -22.80 J/g . In the physical mixture thermogram, the first peak was approximately $56\text{ }^{\circ}\text{C}$ (broadened with low enthalpy). The presence of a mixture of materials that differ in melting point may result in an eutectic mixture that lowers the melting point of the excipients, shifts the usual peaks, and minimizes fusion heat. The second peak was $143.44\text{ }^{\circ}\text{C}$ (low enthalpy) and belongs to ketoconazole. This observation may similarly be attributed to the presence of low-melting-point components, such as Brij 35 and Span 60, which undergo solid-liquid transition around $53\text{ }^{\circ}\text{C}$. The interaction may promote the formation of a eutectic mixture, resulting in reduction and broadening of the overall melting point [22]. Furthermore, the Ketoconazole fraction of the total formula was only 10 % w/w, so sample dilution may interfere with the readings. In contrast, in the DSC of the Ketoconazole-loaded lyophilized novasomes, the characteristic endothermic peak of Ketoconazole was absent. It indicated that Ketoconazole was encapsulated in

the novasomes and became amorphous, consistent with earlier report [11].

The release profile revealed that about $87.33 \pm 8.67\%$, and $18.6 \pm 4.96\%$ were released from the novasomes dispersion and pure Ketoconazole, respectively. This is explained by the Ketoconazole-loaded novasomes, which were nano-sized vesicles 159.1 ± 3.9 with lower surface tension. Two phases were seen in the release profile of Ketoconazole-loaded novasomes. The first phase released $37.8 \pm 0.346\%$ in 2 h, which was considered a burst release. This phase accounted for 25 - 50 % of the total release, due to the rapid release of ketoconazole adsorbed on the vesicle surface or found in bilayers. Furthermore, the highly ordered bilayer structure may have limited capacity to accommodate high drug loads, resulting in an initial burst release of the drug into the pH 5.5 medium during the first few hours, followed by a slower and more sustained diffusion phase, reaching approximately 87 % drug release over 8 h, depending on the extent of membrane relaxation [19]. That is supported by the kinetics of drug release. Among the four models, the Korsmeyer-Peppas model showed the highest correlation coefficient ($R^2 = 0.9875$) compared to the other models. The Korsmeyer-Peppas model was especially suitable for vesicular and matrix-type systems, where the release mechanism includes more than simple diffusion. The (n) exponent value of the model equation specifies the release type. In the study, the n-exponent was 0.615 ($0.45 < n < 0.89$), suggesting non-Fickian diffusion. As a result, drug release from the novasomes occurred by diffusion through the membrane, in addition to structural relaxation of the bilayers. The Higuchi model showed good linearity ($R^2 = 0.986$), suggesting that drug release occurred via diffusion.

The pure Ketoconazole suspension (Ket in phosphate buffer pH 7.4 and 1 % ethanol) showed an inhibition zone diameter (IZD) of $4 \pm 0.2\text{ mm}$ and $2 \pm 0.3\text{ mm}$ on *Candida albicans* and *Aspergillus niger*, respectively. The blank novasomes showed no inhibition zone. *Candida albicans* showed characteristic IZD of $3 \pm 0.9\text{ mm}$, $15 \pm 1.2\text{ mm}$, and $25 \pm 2.6\text{ mm}$ for 200, 400, and 800 $\mu\text{g/mL}$, respectively. Approximate results were investigated with *Aspergillus niger*, where the IZD were $6 \pm 0.2\text{ mm}$, $10 \pm 2.3\text{ mm}$, and $23 \pm 2.5\text{ mm}$ for 200, 400, and 800 $\mu\text{g/mL}$, respectively, similar to an earlier report [18]. Incorporation of Ket in the novasomes potentiated its activity by controlling diffusion through the agar medium and increasing permeation through the fungal cell wall [27].

Presence of stearic acid (acting as a permeation enhancer) may improve the penetration of Ket through the fungal cell wall.

The results of the vesicle size distribution showed a significant increase in vesicle size at 25 °C storage temperature at the end of the study, compared to day 1, consistent with an earlier study [29]. The rate of vesicle growth was higher at 25 °C than at 2 - 8 °C, indicating the minimum change in vesicle size. The high temperature (25 °C) increases energy of the vesicles, leading to an increased collision rate and possible aggregation, compared to low temperature (2 - 8 °C). Therefore, Ket-NS dispersion was best stored at low temperatures (2 - 8 °C) since it showed good stability indicator with relatively lower vesicle size growth. On the other hand, the effect of storage temperature on the polydispersity index was significant at both temperatures. This may be attributed to vesicle growth resulting from increased vesicle – vesicle collisions, particularly at room temperature, which promotes aggregation of nanosized vesicles and consequently increases PDI [24].

Storage temperature had a significant effect on the zeta potential at 25 °C, whereas no significant effect at 4 °C. The presence of stearic acid contributes a negative surface charge to the vesicles, thereby enhancing electrostatic repulsion and maintaining vesicular stability during storage at 4 °C. In contrast, storage at 25 °C resulted in reduced zeta potential, although the values remained within a range that could permit greater tendency for vesicle aggregation [29].

CONCLUSION

The novasomes show narrow PDI, thus potentiating the activity of ketoconazole by improving drug release and diffusion, and enhancing permeation through the fungal cell wall. This action is strongly associated with the synergetic effect of Brij 35 with Span 60.

DECLARATIONS

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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

The authors declare no conflict of interest.

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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