Development of Ketoconazole-Loaded Solid Lipid Nanoparticles and Incorporation into a Shampoo Base for Enhanced Antifungal Activity

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ABSTRACT:

Dandruff and fungal scalp infections are common dermatological problems caused mainly by the overgrowth of *Malassezia* species. Conventional ketoconazole shampoos are widely used for treatment, but their effectiveness is often limited by poor scalp retention, short contact time, and rapid removal during washing. To improve therapeutic performance, the present study was designed to formulate and optimize ketoconazole-loaded solid lipid nanoparticles (SLNs) incorporated into a shampoo base for enhanced antifungal activity and better patient compliance.

Ketoconazole-loaded SLNs were prepared using the hot homogenization followed by ultrasonication method. The formulation was optimized by varying drug, lipid, and surfactant concentrations using a Box-Behnken design. The optimized SLN formulation showed desirable physicochemical properties, including nanometer-sized particles, acceptable polydispersity, suitable zeta potential, and high entrapment efficiency. The optimized nanoparticles were then incorporated into a shampoo base and evaluated for physical appearance, pH, viscosity, foamability, spreadability, drug content, and in-vitro antifungal activity.

The prepared shampoo was found to be clear, homogeneous, smooth, and cosmetically acceptable. Its pH and viscosity were within the desirable range for scalp application. The formulation also exhibited good foam stability and uniform drug distribution. Antifungal testing against *Malassezia* showed that the SLN-loaded shampoo produced a higher zone of inhibition than the conventional ketoconazole shampoo, indicating improved antifungal efficacy.

Overall, the study demonstrates that ketoconazole-loaded SLNs incorporated into a shampoo base can serve as an effective, stable, and patient-friendly approach for the management of dandruff and fungal scalp disorders.

Keywords: Solid Lipid Nanoparticles, Ketoconazole, Optimization, antifungal, Box-Behnken Design (BBD)

How to cite this article: Raj R, Kumar A, Singh U, Singh S, Kumar R, Ali MA. Development of Ketoconazole-Loaded Solid Lipid Nanoparticles and Incorporation into a Shampoo Base for Enhanced Antifungal Activity. Int J Drug Deliv Technol. 2026;16(57s): 49-57. DOI: 10.25258/ijddt.16.57s.7

Source of support: Nil.

Conflict of interest: None.

. INTRODUCTION

Dandruff and other fungal scalp disorders continue to be common dermatological concerns affecting millions of people worldwide [11–15]. These conditions are mainly associated with the excessive growth of lipophilic fungi, particularly *Malassezia furfur*, which can lead to itching, flaking, redness, irritation, inflammation, and persistent scaling of the scalp [14, 15]. Although conventional ketoconazole shampoos are widely used for treatment, they often suffer from limitations such as poor scalp retention, short contact time, inadequate follicular penetration, and rapid removal during rinsing [11, 17]. As a result, their therapeutic effectiveness may be reduced, and frequent application becomes necessary, which can affect patient compliance [11, 17]. To overcome these limitations, novel drug delivery systems have gained significant attention in recent years [1–5, 21–23, 46-50].

Solid lipid nanoparticles have emerged as a promising carrier for topical formulations because they can improve drug stability, enhance penetration, and provide sustained release at the site of application [1–8, 22, 23, 46-50]. Their lipid-based structure also helps in increasing contact with the scalp and hair follicles, thereby improving the localized action of the incorporated drug [3, 4, 7, 25, 28]. Ketoconazole-loaded solid lipid nanoparticles may therefore offer an effective strategy for enhancing antifungal therapy while reducing the drawbacks of conventional formulations [20, 27–29,46-50]. In addition, shampoo bases are preferred because they offer soothing, conditioning, and scalp-friendly properties while improving cosmetic acceptability [18, 19, 42].

Box Behnken Design (BBD) optimization approach were used to optimized the formulation by considering the drug, lipid and surfactant content as a independent variables and mean particle size, polydispersity index, zeta potential, and percentage entrapment efficiency as a dependent variables [31-34].

The present study focuses on the formulation and optimization of ketoconazole-entrapped solid lipid nanoparticles incorporated into a shampoo base. The aim is to develop a stable, effective, and cosmetically acceptable antidandruff formulation with improved physicochemical characteristics, enhanced antifungal activity, and better patient acceptability for long-term management of dandruff and fungal scalp infections.

2. MATERIALS AND METHODS

2.1 Materials

Ketoconazole (KCZ) was obtained from Luex Pharmaceuticals Pvt, Ltd. (Mumbai).

Glyceryl monostearate and Tween 80 was obtained from Himedia. Every additional chemical and reagent that was employed was of analytical quality.

2.2 Methods

2.2.1 Preformulation Studies

Preformulation studies of ketoconazole were carried out to determine its physicochemical properties and compatibility with excipients [36,38–41,43]. The drug was evaluated for organoleptic characteristics such as color, odor, and appearance [36,38,40]. Melting point determination was performed using a digital melting point apparatus [36,38,43]. Solubility studies were conducted in various solvents including distilled water, ethanol, methanol, chloroform, and dimethyl sulfoxide [36,37,38]. UV spectrophotometric analysis was carried out at 269 nm for preparation of the standard calibration curve [36,37,38,43]. Fourier Transform Infrared Spectroscopy (FTIR) studies were performed to identify possible interactions between the drug and excipients [22,38,39,43].

Table 1: Independent and Dependent Variables Used for Optimization of SLN-Loaded Shampoo Formulation

Independent variables	Unit	Levels		
		Low (-1)	Medium (0)	High (+1)
A: Drug	mg	10	15	20
B: Lipid	mg	20	50	80
C: Surfactant	ml	5	22.5	50
Dependent variables	**Desired Constraint**			
R1: Mean particle size	Minimum			
R2: PDI	Minimum			
R3: Zeta potential	In range			
R4: % Entrapment efficiency	Maximum			

2.2.2 Preparation of Ketoconazole-Loaded Solid Lipid Nanoparticles

Ketoconazole-loaded SLNs were prepared by the hot homogenization followed by ultrasonication method [1,2,5,8,20,22,23]. The required quantity of lipid was melted at approximately 70–75°C, and ketoconazole was dispersed uniformly in the molten lipid phase [1,2,20,22]. The aqueous phase containing surfactant and stabilizer was heated to the same temperature and slowly added to the lipid phase under continuous stirring using a high-speed homogenizer [1,5,20,23]. The resulting hot emulsion was homogenized at high speed for a specified duration and then subjected to ultrasonication to reduce particle size and obtain a stable nano-dispersion [1,2,5,20,21]. The prepared SLN dispersion was cooled to room temperature to solidify the lipid nanoparticles [1,2,8,22]. Different formulations were prepared by varying lipid and surfactant concentrations to optimize the formulation [20,21,31–34].

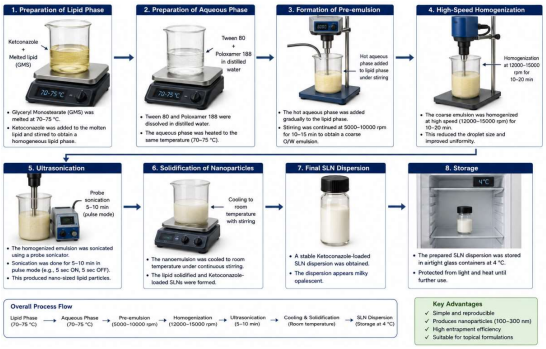


Fig.1: Hot homogenization followed by ultrasonication method for the preparation of SLN

2.2.3 Optimization of SLN Formulation

Optimization of ketoconazole-loaded SLNs was carried out by evaluating various formulation parameters such as drug concentration, lipid concentration and surfactant concentration as independent variables [31–34]. The prepared SLNs were evaluated for particle size, polydispersity index (PDI), zeta potential and entrapment efficiency as dependent variables [1,2,8,20,22–24]. The optimized formulation was selected based on minimum particle size and maximum entrapment efficiency, which are considered critical quality attributes for achieving enhanced stability, drug loading, and topical delivery performance [1,2,20–24,31–34].

2.2.4. Evaluation of Ketoconazole-Loaded SLNs

2.2.4.1 Particle Size and Polydispersity Index

Particle size and polydispersity index (PDI) of the prepared SLNs were determined using dynamic light scattering (DLS) technique [1,2,8,20,22–24]. Samples were diluted appropriately with distilled water before analysis to avoid multiple scattering effects and ensure accurate measurement of nanoparticle size distribution [2,8,23,24].

2.2.4.2 Zeta Potential

Zeta potential of the optimized formulation was measured to determine the surface charge and stability of nanoparticles[24].

2.2.4.3 Entrapment Efficiency

Entrapment efficiency was determined by centrifugation method. The SLN dispersion was centrifuged, and the amount of free drug present in the supernatant was analyzed spectrophotometrically [20,22,27].

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

2.2.5 Preparation of optimized SLN formulation

After statistical analysis of obtained response data and consideration of desired constraints set for each response variables, i.e., minimum particle size and PDI, in range zeta potential, and maximum % EE in optimized formulation. Design expert software suggested a composition of optimized formulation based on criteria of highest desirability value. The maximum desirability (0.643) was predicted in optimized SLN formulation composition of: drug content 20 mg, lipid content 74.39 mg, and surfactant (32.78 ml). The 2D and 3D contour plots depicting highest desirability value in optimized formulation are shown in figure 2.

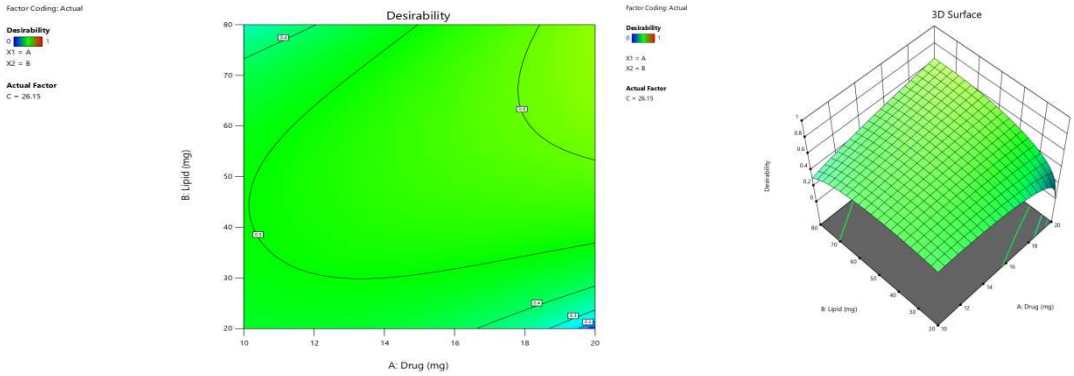


Fig.2: 2D and 3D –contour plot showing maximum desirability of optimized formulation of ketoconazole-loaded SLN

2.2.6 Preparation of Shampoo Base

The shampoo base was prepared using selected shampoo ingredients. Sodium Lauryl Sulphate, cocamidopropyl betaine, glycerin, citric acid, methyl paraben were mixed with distilled water under continuous stirring. Sodium lauryl sulfate was added slowly to avoid excessive foam formation. Preservatives and other additives were incorporated with constant stirring until a homogeneous solution was obtained. The pH of the shampoo was adjusted to 5.5 to 7.00 using citric acid [18,19,42].

2.2.7 Incorporation of Optimized SLNs into Shampoo Base

The optimized ketoconazole-loaded SLN dispersion was slowly incorporated into the prepared shampoo base under gentle stirring to ensure uniform distribution of nanoparticles throughout the formulation. The final formulation was mixed thoroughly until a smooth and homogeneous shampoo was obtained. The prepared SLN-incorporated shampoo was stored in airtight containers for further evaluation.

2.2.8 Evaluation of Shampoo Formulation

2.2.8.1 Physical Appearance

The prepared shampoo was evaluated visually for color, clarity, consistency, and homogeneity.

2.2.8.2 pH Determination

The pH of the shampoo formulation was measured using a digital pH meter at room temperature.

2.2.8.3 Viscosity

Viscosity of the shampoo was determined using a Brookfield viscometer.

2.2.8.4 Foamability and Foam Stability

Foamability and foam stability studies were carried out using cylinder shake method.

2.2.8.5 Spreadability

Spreadability of the formulation was evaluated by determining the ease of spreading on a glass surface.

2.2.8.6 Drug Content

Drug content was determined by dissolving a measured quantity of shampoo in suitable solvent followed by spectrophotometric analysis.

2.2.8.7 In vitro Antifungal Activity

The antifungal activity of the optimized Ketoconazole-loaded solid lipid nanoparticle (SLN) shampoo formulation was evaluated against *Malassezia* species using the agar well diffusion method [11,14,15,17,27]. Briefly, Sabouraud Dextrose Agar (SDA) plates supplemented with olive oil were inoculated with a standardized fungal suspension (approximately 1×10^6 CFU/mL) using a sterile cotton swab [14–16]. Wells of 6 mm diameter were aseptically punched into the agar medium, and equal volumes of the Ketoconazole-loaded SLN shampoo (Treatment group) and 2% w/v ketoconazole solution (standard control) were introduced into the respective wells [11,17,27]. The plates were allowed to stand for 1 h to facilitate diffusion of the samples and were subsequently incubated at 32–35°C for 48–72 h [14–16]. Following incubation, the antifungal activity was assessed by measuring the diameter of the zone of inhibition (mm) surrounding each well using a digital Vernier caliper [15,17,27]. All experiments were performed in triplicate, and the results after 48 hours are presented in Table 8 [27]. The antifungal efficacy of the ketoconazole-loaded SLN shampoo formulation was compared with that of the 2% w/v ketoconazole formulation based on the observed zones of inhibition [11,17,27–29].

3. RESULTS

3.1 Preformulation Studies

3.1.1 Organoleptic Evaluation

Table 2: Organoleptic Properties of Ketoconazole

S. No.	Test	Observation
1	Colour	White to off-white crystalline powder
2	Odour	Characteristic odor
3	Texture	Smooth texture

3.1.2 Melting Point Determination

Table 3: Melting Point of Ketoconazole

Drug	Reported Value	Observed Value
Ketoconazole	148–152°C	150°C

3.1.3 Solubility Studies

Table 4: Solubility Profile of Ketoconazole

S. No.	Solvent	Solubility
1	Ethanol	Soluble
2	Water	Insoluble
3	Chloroform	Soluble
4	Dimethyl sulfoxide	Freely soluble

3.1.4 UV Spectrophotometric Analysis

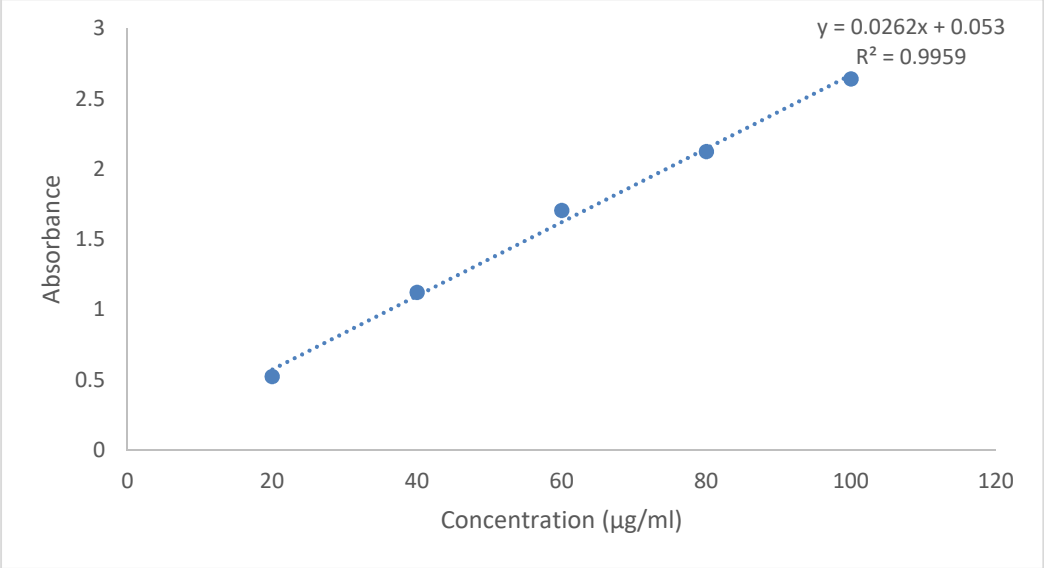


Fig. 3: Calibration Curve of Ketoconazole at 269 nm

3.1.5 FTIR Studies

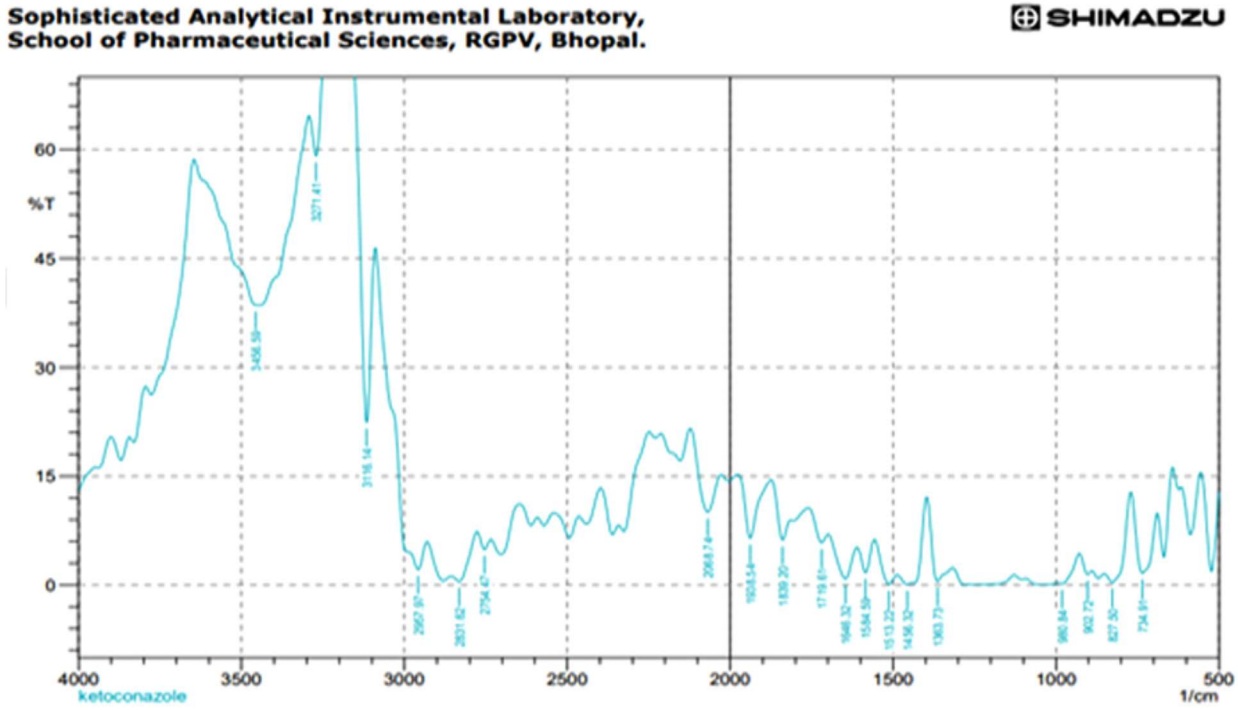


Fig. 4: FTIR of Ketoconazole

3.2 Evaluation of Ketoconazole loaded SLN

3.2.1 Particle Size

Particle size ranged from 149.8 nm to 201 nm. Increase in surfactant concentration and mixing speed significantly influenced vesicle size. The particle size of formulation is essential parameter that was investigated and listed in table 5, while the 3D response surface plots are depicted in fig.5.

3.2.2 Polydispersity Index

PDI values indicated acceptable homogeneity of the prepared formulations as shown in table 5 and the 3D response surface plots are depicted in fig.5.

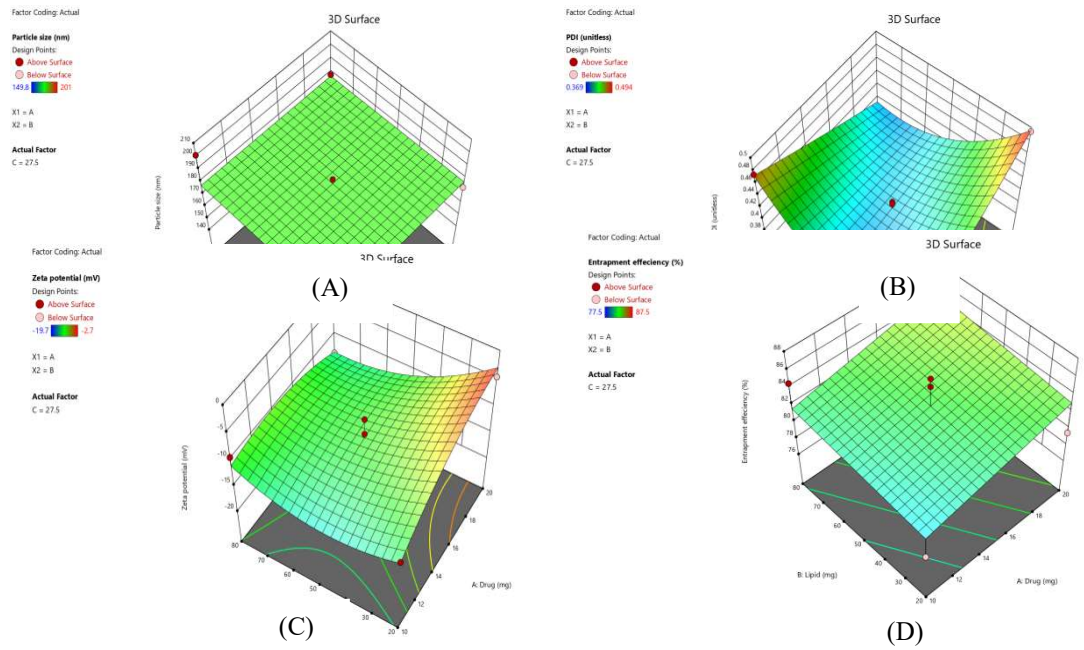
3.2.3 Zeta Potential

Zeta potential ranged from -19.7 mV to -2.7 mV, indicating moderate physical stability of vesicles. As shown in table 5 and the 3D response surface plots are depicted in fig.5.

3.2.4 Entrapment Efficiency

The % Entrapment efficiency ranged between 77.5% and 87.5%, demonstrating high drug encapsulation ability of the SLN and the results are listed in table 5 and the 3D response surface plots are depicted in fig.5.

Table 5: Box- Behnken experimental design of SLN formulation and their measured responses



Run	A:Drug (mg)	B:Lipid (mg)	C:Surfactant (ml)	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
1	15	20	50	150.2	0.458	-2.7	86.4
2	10	50	50	162.3	0.386	-11.7	79.1
3	10	50	5	149.8	0.438	-19.7	78.5
4	15	80	5	187.4	0.478	-8.4	80.5
5	15	50	27.5	177.4	0.405	-7.5	82.3
6	15	50	27.5	167.2	0.378	-10.2	84.5
7	20	20	27.5	173.1	0.494	-4.6	79.5
8	15	20	5	185.4	0.464	-7.2	78.6
9	10	20	27.5	191.5	0.399	-12.5	77.5
10	15	50	27.5	181	0.401	-15	85.4
11	20	50	5	174.6	0.466	-11.5	86.4
12	10	80	27.5	201	0.474	-9.4	84.5
13	20	50	50	196.7	0.452	-8.7	87.5
14	20	80	27.5	178.5	0.369	-13.4	79
15	15	80	50	188	0.487	-12.9	84.9

Fig.5: 3D response surface plot of (A) particle size, (B) PDI, (C) zeta potential, and (D) Entrapment efficiency

3.3 Evaluation of Ketoconazole SLN-Loaded Shampoo

3.3.1 Physical Appearance

The formulated shampoo was clear, homogeneous, smooth in texture, and had a pleasant odor. No phase separation or visible particles were observed.

3.3.2 pH Determination

The pH of the optimized shampoo formulation was found to be 6.02, which is considered suitable for scalp and hair application. The formulation was mild and non-irritating to the scalp.

3.3.3 Viscosity

The viscosity of the formulated shampoo was found to be 1500 Cp, which falls within the acceptable range for shampoo formulations and indicates suitable flow properties.

3.3.4 Foamability and Foam Stability

The prepared shampoo showed satisfactory foamability with a form volume of 180 ml. The foam remained stable with a final form volume of 160 ml after 5 minutes, indicating good foam stability.

3.3.5 Drug Content

Drug content analysis confirmed uniform distribution of ketoconazole throughout the shampoo formulation. The optimized formulation exhibited a drug content of 95.7 ± 0.6%, indicating efficient incorporation of SLNs into the shampoo base.

3.3.6 In vitro Antifungal Activity

The in-vitro antifungal assay for the zone of inhibition was performed on the fungal strain *Malassezia*. The maximum zone of inhibition was 21.07 mm for the treatment group. The zone of inhibition was seen for the standard group was 18.3 mm depicted in table 8. The SLN shampoo exhibited significant antifungal activity against *Malassezia*. Enhanced zone of inhibition indicated improved antifungal efficacy compared to conventional formulation.

Table 6: Zone of Inhibition (Day 1)

S. No.	Groups	Zone 1	Zone 2	Zone 3	Zone 4	Average
1	Control group	No fungal growth observed				
2	Negative group	Fungal growth was uniform; no zone of inhibition was seen.				
3	Standard group (2% w/v KCZ)	12.8	11	11.5	12.5	11.95
4	Treatment group (KCZ loaded SLN shampoo)	14.5	13.7	12.9	14	13.77

Table 7: Zone of Inhibition (Day 2)

S. No.	Groups	Zone 1	Zone 2	Zone 3	Zone 4	Average
1	Control group	No fungal growth observed				
2	Negative group	Fungal growth was uniform; no zone of inhibition was seen.				
3	Standard group (2% w/v KCZ)	15.5	15.8	14.9	16	15.55
4	Treatment group (KCZ loaded SLN shampoo)	16.2	17.9	16.8	18	17.22

Table 8: Zone of Inhibition (Day 3)

S. No.	Groups	Zone 1	Zone 2	Zone 3	Zone 4	Average
1	Control group	No fungal growth observed				
2	Negative group	Fungal growth was uniform; no zone of inhibition was seen.				
3	Standard group (2% w/v KCZ)	19.3	18	17.5	18.4	18.3
4	Treatment group (KCZ loaded SLN shampoo)	20.2	21.4	20.8	21.9	21.07

3.4 Conclusion

The study successfully developed and optimized a ketoconazole-loaded solid lipid nanoparticle (SLN) formulation incorporated into a shampoo base for improved antidandruff therapy.

The optimized SLN formulation was obtained through independent variation of drug, lipid, and

surfactant concentrations, with the best composition showing a desirability value of 0.643 at 20 mg drug, 74.39 mg lipid, and 32.78 ml surfactant. The prepared SLNs demonstrated favorable physicochemical properties, including particle sizes, acceptable polydispersity, moderate negative zeta potential, and high entrapment efficiency, indicating stable and efficient encapsulation of ketoconazole.

When incorporated into the shampoo base, the final formulation remained clear, homogeneous, smooth, and cosmetically acceptable. Its pH of 6.02 suggested suitability for scalp application, while the viscosity of 1500 cP supported good handling and application properties. The shampoo also showed satisfactory foamability, stable foam retention, and uniform drug distribution, confirming that the SLNs were successfully integrated into the formulation without compromising shampoo quality.

Most importantly, the SLN shampoo exhibited stronger in-vitro antifungal activity against *Malassezia* than the conventional ketoconazole shampoo, with a larger zone of inhibition of 21.07 mm compared with 18.3 mm for the standard formulation. This improved antifungal activity for better drug localization at the site of action.

Overall, the study concludes that ketoconazole-loaded SLNs incorporated into a shampoo base represent a promising, stable, effective, and patient-friendly antidandruff formulation with enhanced antifungal performance and good cosmetic acceptability.

Conflict of interest

The authors declare that they have no competing interests.

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