FORMULATION EVALUATION OF POSACONAZOLE NIOSOMES FOR THE EFFECTIVE TREATMENT OF FUNGAL INFECTION OF SKIN

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ABSTRACT

This study explores the formulation, evaluation of noisomes-based delivery systems for effective treatment of fungal skin infections. Utilizing a central composite design computational model, various noisomes formulations were developed to encapsulate antifungal agents. Characterization of the formulations revealed an average particle size of 1525 nm, a zeta potential of 25.6 mV and a polydispersity index of 1.000, indicating favorable stability and uniformity. The formulations demonstrated an impressive entrapment efficiency of 75 %, ensuring a significant amount of drug is retained within the noisome carriers. *In-vitro* drug release studies showed a remarkable release rate of 93.48% and for F3 is 85.98% over 120 min, indicating sustained release profiles that are beneficial for prolonged antifungal activity. Additionally, spreadability tests confirmed the ease of application on the skin, enhancing user compliance. Overall, the noisome-based systems significantly improved drug delivery and efficacy against fungal pathogens, surpassing conventional formulations. These findings suggest that noisomes represent a possible substitute for the successful therapy of dermatophytic infections, with the potential to improve patient outcomes. Future work should focus on clinical validation to further assess the therapeutic benefits of these innovative formulations.

Keywords: Niosomes, Anti-fungal, Posaconazole, Formulation, Evaluation.

INTRODUCTION

FUNGAL INFECTION

Microbiologists are very interested in the fungus since they are eukaryotic. As heterotrophic organisms, fungi depend on organic substances for sustenance of the approximately 1.5 million fungi in the cosmos, Hawks-worth claims that only around 100 cause illness in humans and other animals. In extreme circumstances, fungal pathogenic infection can lead to invasive mycosis, allergies, and superficial infections **[1]**.

Estimated total no. of fungal species in globe has increased dramatically over time. According on morphological, physiological and molecular traits, this estimate of the no. of fungal species surpassed 100,000 in 2015 [Echavarria & Robinson, 2015]. ICN said in that same year that between 1000 and 1500 fungal species were described and recognized annually. Only by 2020 had experts recognized 140,000 fungal species, which is just 12% to 1% of the total believed to be on Earth **[2].**

They represent a broad variety of diseases caused by different fungi, including yeasts, Molds, and dimorphic fungi. While some of them are superficial and relatively easily manageable, others can be rather severe, invasive, and fatal in patients whose immune system is still depressed or who have pre-existing serious conditions such as diabetes, cancer, transplantation of organs and infection with the human immunodeficiency virus. Increasing trend of fungal infections has been due to several factors that will be discussed in ensuing sections, including the growing use of immunosuppression treatments, aging populations, healthcare-associated infections, and environmental changes **[3].**

NIOSOMES

Noisomes are non-ionic surfactant vesicles self-associated into a microscopic lamellar bilayer structure. This structure is created through the self-assembly of monomers of hydrated surfactants. Niosomes are characterised by their multilamellar or unilamellar structure, which is created by combining cholesterol, diethyl ether, and non-ionic surfactant, followed by hydration in aqueous medium **[4].** There is major three type of Niosomes and they are as follows:

I: MLV

II: SUV

III: LUV

MLV result in increased trapped volume and equilibrium solute provider involve hand shaken MLV with adaptation in lipids composition hydration from organic solvent dehydration-rehydration procedure freeze-drying. SUV is normally produced by sonication and French pass procedures. Solvent technique can be used to prepare SUV. The better and most accepted method that is used to prepare LUV is reverse-phase evaporation and can also be shaped by using solubilizing lipid in saturated buffer that contain soap **[5].**

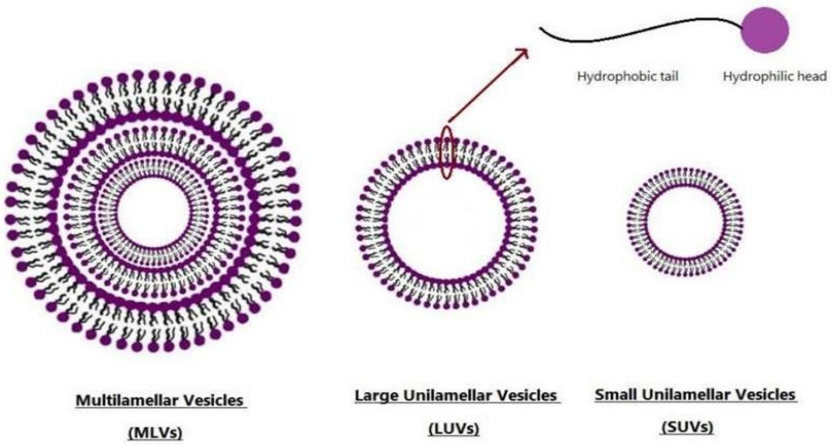

Figure 1. Types of Niosomes.

Features of Noisomes

Noisomes can trap solid particles in a manner comparable to Liposome. Niosomes are osmotically active. Niosomes are incredibly adaptable, with a variety of structural features such as their composition, clarity, and size. They can be tailored to fit specific needs, making them versatile tools for effective drug delivery. Niosomes enhances performance of drug molecules. Niosomes provide improved accessibility to targeted site, by defending the drug from biological surrounding **[6].**

Structure of Noisome [7]

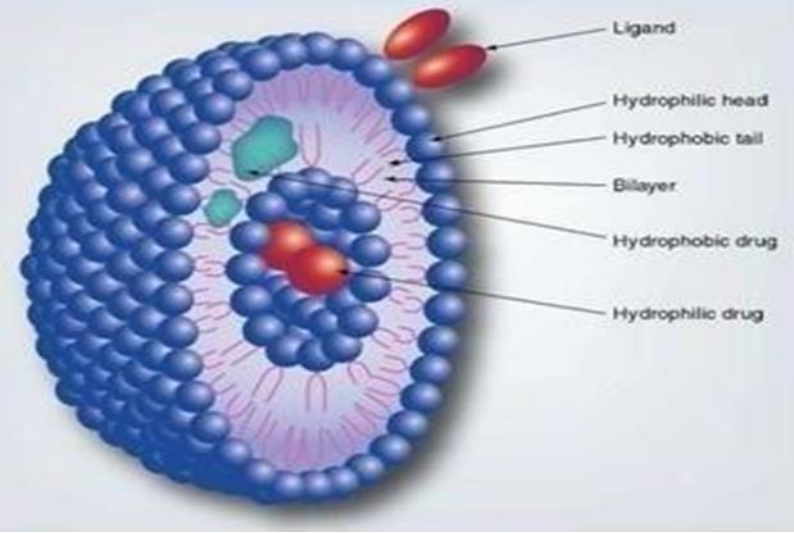

Figure 2. Niosomes.

PSORIASIS

Psoriasis is a persistent immunological skin infestation that has been linked to depression and other conditions include antipathy, psychiatric, cardiovascular, and hepatic disorders. 2014 saw the WHO classify psoriasis as a non-communicable illness and highlight the distress associated with misdiagnosed, inappropriate treatment, and accusations of this condition [HO, 2016]. According to the Global Burden of Disease Examination, at least 5.6 million people worldwide with debilitating conditions had psoriasis in 2016 triadic inflammatory bowel disease **[8].** The preference of psoriasis deviates age, geographical area and genetic background **[9].**

Psoriasis affects both male and female, but it shows early outbreak in females & with family tree. In female it starts at age of 30-39 years and in male 60-69 years but it shows 10 years earlier in females. It is belief that 60 million people suffer from psoriasis. It ordinary in wealthy areas and those with senior demographic in United Kingdom, it influences about 1.52% of society **[10].**

The morbid process of psoriasis is polyfactorial, but the genetic component appears to be the primary contributor especially in those whose plaque psoriasis began early (<40 years of age) **[11].**

POSACONAZOLE

Posaconazole is a chemical derivative of itraconazole, classed as a triazol antifungal. It has been synthesized with a view to improving the chemical structure of itraconazole, specifically by fluorinating the phenyl ring and hydroxylating the main triazolone side chain, in such a way as to make it more potent and increase the spectrum of antifungal activity. The action of posaconazole on fungi can be either fungicidal or fungistatic, based on the fungus's kind and surroundings **[12].**

DRUG PROFILE

Brand Name: Noxafil, Posanol.

Generic Name: Posaconazole.

Chemical Formula: $C_{37}H_{42}F_2N_8O_4$.

Structure of Posaconazole

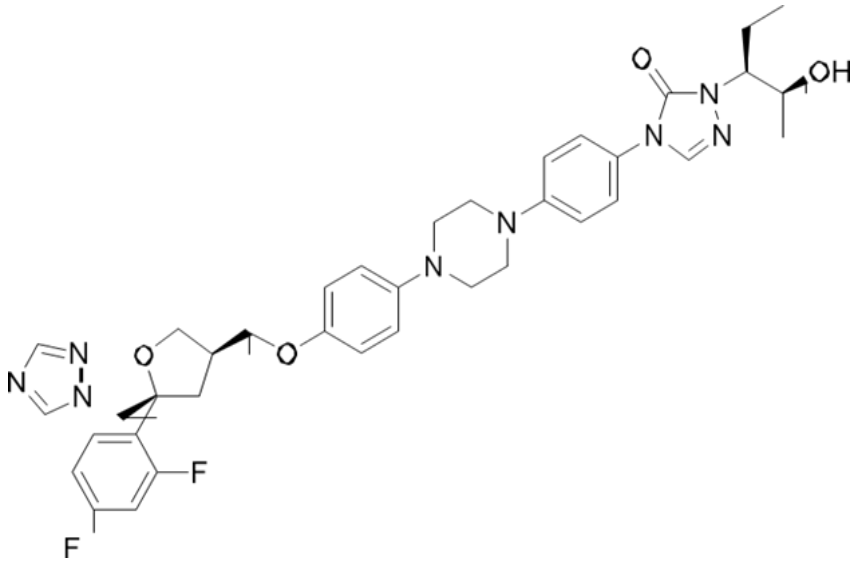

Figure 3. Posaconazole.

Pharmacology

Posaconazole is used to protect people against invasive Aspergillus and Candida infections because they have severe immunocompromised conditions. This elevated risk is frequently linked to procedures such as hematopoietic stem cell transplants (HSCT), particularly in recipients suffering from graft-versus-host disease (GVHD), or in individuals whose haematologic cancers result in chemotherapy-induced persistent neutropenia. In addition to its preventive applications, oropharyngeal candidiasis, including those that are resistant to itraconazole and/or fluconazole, is also treated with posaconazole. Furthermore, it acts as an alternative treatment option for invasive aspergillosis, Fusarium infections, and zygomycotic in patients who are intolerant to or have infections that do not respond to other antifungal therapies **[13].**

MATERIALS AND METHODS

Preparation of Niosomes

Niosomes created using THF involving a lipid blend of non-ionic surfactant (Tween 80) and cholesterol. First, Tween 80 and cholesterol, dissolved in 9 ml of chloroform. In another tube, the drug 10 mg was solubilized in 1 ml of DMSO. Afterwards, the solution in both tubes was mixed in a single flask of 100 ml round-bottom and put on a rotary evaporator, where solvent removed at temp. of 55-65°C until a thin lipid film appeared. After that, the film hydrated with 10 mL of phosphate buffered saline at pH 7.4. Hydration carried out for one hr in a flask rotated at 55- 65°C by rotary evaporator **[14].**

Screening of best formulation

The optimal formulation was found after the posaconazole-loaded phytosomes had been prepared by checking two vital parameters: percentage of drug entrapment efficiency and percentage of drug release. This is assessment was done by measuring the amount of posaconazole effectively incorporated into the phytosomes and the amount of drug released over some time. These metrics therefore provided the basis for ascertaining the effectiveness and suitability of the formulation for the intended therapeutic purpose.

Evaluation of niosomes

Encapsulation efficiency

To estimate the entrapment efficiency of the posaconazole-loaded niosomal formulations, niosomal vesicles were disrupted with Triton-X. Solution centrifuged for 30 min at temp. of 4°C in refrigerated centrifuge at 10,000× g to separate the components. The concentration of unencapsulated drug in supernatant quantified via spectrophotometric analysis, taking absorbance 208 nm utilizing a UV-visible spectrophotometer, subsequent to centrifugation. Subsequently, the EE of drug within the niosomes was determined employing a standardized calculation formula **[15].**

$$\%Drug\ Entraped = \frac{(Total\ drug - Drug\ in\ supernatant)}{Total\ Drug} \times 100$$

In-vitro release of formulations

A dialysis bag with mol. Wt. cut-off range of 12,000-14,000 employed as the donor compartment. After centrifugation, the entrapped drug within the niosomal formulation was re-suspended (1 ml of phosphate buffered saline) at pH 7.4 for the release determination. The dialysis membrane was treated with hot water for 10 minutes before use and sealed at one end. The dialysis bag filled with niosomal formulation was sealed well and kept for sampling, then in 100 ml of PBS at pH 7.4, kept at a temp. of 37°C ± 2°C, and agitated continuously at 100 rpm. Samples of 5 ml from the receptor compartment withdrawn hourly, replaced with fresh buffer. Results shown here were the mean of three independent experiments.

Physiochemical Properties

Particle size & zeta potential

Particle Size: It was identified by Malvern Zetasizer Nano-S90, whose mode of operation is photon correlation spectroscopy. For the measurement, about 3ml of the niosomes suspension was filled into a quartz cuvette. Then, the sample was treated with laser light at 90° to determine the particle size distribution.

Zeta Potential: Value of zeta potential was determined by diluting the niosomal solution and then injecting it into a folded capillary containing platinum electrodes. It was then put in the sample holder of the Zetasizer from Malvern Instrument for analysis.

Polydispersity Index (PDI)

Dynamic light scattering principle used to determine polydispersity index value of the prepared Posaconazole-loaded niosomal formulations in a Zetasizer TM Nano ZS. Measurements performed at fixed angle 180°, with controlled environmental conditions maintained at 25 °C, and sample properties of 0.8872 mPa·s viscosity and 1.330 refractive index, all these obtained values of PDI were calculated by the software itself; each measurement was done thrice for accuracy.

Spreading analysis

In spreadability apparatus, the lower slide was covered with a 1.0 g sample of the gel and an upper slide placed on top of the sample. Spreadability was calculated by the following formula.

S = (m * l) /t

Where, S = Spreadability, m = weight tied to upper slide, l = length travelled by upper slide, t = time taken by slide to travel

Stability study

Stability of niosome formulations assessed using dispersions stored in 20 mL sealed glass vials at 4°C in a refrigerator. Size, polydispersity index, zeta potential, and % entrapment efficiency measured at intervals of 0, 1-, 2-, 3-, and 4-weeks following preparation. Besides, visual observations were conducted in order to prove whether any physical changes had occurred in these formulations.

Antifungal evaluation of the formulation

Organisms used for the study: C. albicans and A. niger.

Method Used: Disc-diffusion method.

Procedure

The antifungal efficacy of the test sample (F3) was evaluated utilizing the disc diffusion assay. Target fungal strains were cultivated in Potato Dextrose Broth and incubated for a period of 24 hrs. Petri dishes with PDA medium, inoculated with diluted fungal suspension. Sterile discs, impregnated with the test sample at concentrations of 500, 1000, and 2000 µg, were placed on the agar surface. Inoculated plates incubated at 28 °C for 24 hrs. Antifungal potency determined measuring the ZOI around disc, expressed in mm.

RESULT AND DISCUSSIONS

Niosomes preparation

Niosomes were formulated utilizing a Central Composite Design (CCD) model that incorporated two factors: cholesterol and tween 80, along with one response variable, the percentage of entrapment efficiency. The formulations consisted of tween 80, cholesterol, the drug Posaconazole, and DMSO in combination with chloroform as the solvent. A detailed composition of all nine formulations is provided in the accompanying table.

Table 1. CCD design and observed responses for different batches of niosomes.

S. No.	Code	Factor 1 A: Cholesterol (mg)	Factor 2 B: Tween 80 (ml)	Response 1 Entrapment Efficiency (%)
1.	F1	25	140	71.17
2.	F2	20	100	67.35
3.	F3	30	200	75.00
4.	F4	25	150	69.41
5.	F5	30	100	65.32
6.	F6	20	200	71.83
7.	F7	25	170	70.84
8.	F8	17.1	150	62.74
9.	F9	30.2	150	72.92

Screening of best formulation

Optimization of formulation via CCD was effectively employed to identify the compositions of niosomes that would yield optimal results for the chosen dependent variable, specifically the maximum encapsulation efficiency. Formulations based on these identified compositions were created, and they exhibited the desired characteristics.

Table 1 provides evidence for the experimental conditions related to the percentage of encapsulation efficiency (% EE) of Posaconazole-loaded niosomes based on CCD. The highest entrapment efficiency recorded was 84.73%, indicating that formulation F3 was optimal for this study. The percentage of encapsulation efficiency is a critical parameter for evaluating the quantity of drug incorporated into any drug delivery system. It plays a vital role in determining the effectiveness of a drug delivery system in encapsulating the specific drug.

Statistical analysis and model fitting

Design-Expert software, the obtained results were fitted into several mathematical models, like the linear and quadratic models. The high value of the multiple correlation coefficient served as the basis for choosing the most appropriate model (R^2).

Table 2. Fit Summary of EE

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9531**	**0.9168**	**Suggested**
2FI	0.8670	0.9441	0.8696	
Quadratic	0.6879	0.9274		
Cubic	0.7990	0.8610		**Aliased**

Table 3. Sequential Model sum of Square [Type I] of EE

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	51030.81	1	51030.81			
Linear vs Mean	**483.83**	**2**	**241.92**	**82.34**	**< 0.0001**	**Suggested**
2FI vs Linear	0.1089	1	0.1089	0.0311	0.8670	
Quadratic vs 2FI	3.87	2	1.93	0.4248	0.6879	
Cubic vs Quadratic	4.94	2	2.47	0.2832	0.7990	Aliased
Residual	8.72	1	8.72			
Total	51532.27	9	5725.81			

Table 4. Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	**1.71**	**0.9648**	**0.9531**	**0.9168**	**41.73**	**Suggested**
2FI	1.87	0.9651	0.9441	0.8696	65.37	
Quadratic	2.13	0.9728	0.9274		*	
Cubic	2.95	0.9826	0.8610		*	Aliased

Table 5. ANOVA for linear model of EE.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	483.83	2	241.92	82.34	< 0.0001	significant
A-Cholesterol	456.22	1	456.22	155.28	< 0.0001	
B-Tween 80	27.62	1	27.62	9.40	0.0221	
Residual	17.63	6	2.94			
Cor Total	501.46	8				

The calculated Model F-value of 82.34 demonstrates the model's substantial statistical significance, corresponding to a minute 0.01% probability of chance occurrence. Term significance is confirmed by P-values below the 0.0500 threshold, with factors A and B exhibiting notable influence. In contrast, P-values exceeding 0.1000 suggest term irrelevance. Model optimization may be achieved through judicious reduction of non-essential terms, supplementing hierarchical requirements.

Table 6. Fit Statistics of EE.

Std. Dev.	1.71		R^2	0.9648
Mean	75.30		Adjusted R^2	0.9531
C.V. %	2.28		Predicted R^2	0.9168
			Adeq Precision	21.5834

Adequate Precision assesses signal-to-noise ratio, with a value greater than 4 being desirable. Your ratio of 21.583 demonstrates an apt signal, indicating that this model is suitable for navigating design space.

Table 7. Coefficients in terms of coded factor of EE.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	75.30	1	0.5714	73.90	76.70	
A-Cholesterol	7.55	1	0.6060	6.07	9.03	1.0000
B-Tween 80	1.86	1	0.6060	0.3752	3.34	1.0000

The coefficient estimate quantifies the anticipated change in the response variable for a single-unit modification in the factor level, ceteris paribus. Within an orthogonal design framework, the intercept represents the overall response average across all experimental runs, whereas the coefficients modify this average in response to specific factor configurations. Notably, orthogonal factors exhibit variance inflation factors (VIFs) equal to 1, indicating independence. VIF values exceeding 1 signify multicollinearity, with escalating values denoting stronger inter-factor correlations. Conventionally, VIFs below the threshold of 10 are deemed acceptable.

Table 8. Final equation in terms of coded factor of EE

Entrapment Efficiency	=
+75.30	
+7.55	A
+1.86	B

The empirical model expressed in terms of coded factors allows for response prediction corresponding to predefined component quantities. Utilizing a standardized coding scheme (+1 for high levels, -1 for low levels), the equation enables quantitative evaluation of component contributions via coefficients magnitude comparison.

Table 9. Final equation in terms of actual factor of EE

Entrapment Efficiency	=
+31.96778%	
+1.51033	Cholesterol
+0.037160	Tween 80

This equation can be utilized to predict the reaction for specific levels of each element. Each of the factor levels should be described in their natural units. This equation should not be used to determine how much effect each element has in proportion because coefficients have been scaled to fit units for each factor, and intercept is not centered at design space centre.

Table 10. Different responses of optimization experiment for niosome preparation of EE by using CCD.

Run Order	Actual Value	Predicted Value
1	71.17	72.67
2	67.35	65.89
3	84.73	84.71
4	76.41	75.30
5	82.91	80.99
6	69.83	69.61
7	78.64	77.93
8	62.74	64.62
9	83.92	85.98

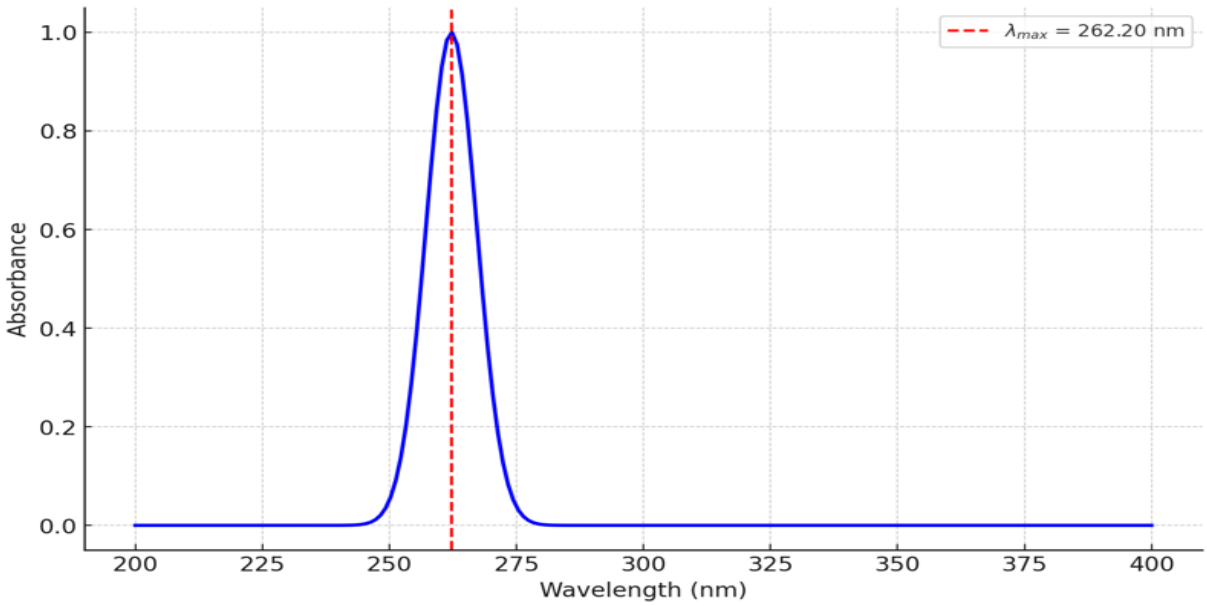

Figure 3. Effect of entrapment efficiency of tween 80 versus cholesterol.

Evaluation of niosomes

Encapsulation efficiency

The niosomes were subjected to estimated encapsulation efficiency using cooling micro centrifuge (Rami Elektrotechnik Ltd. India). The maximum encapsulation efficiency was found to be 84.73 % of formulation F3 shown in Table 10. The result indicated that the niosomes prepared by using 10 mg/mL drug concentration with 30 mg cholesterol and 200 mg tween 80 at 100 rpm have better encapsulation efficiency.

Formulation *In-vitro* release

Percentage drug release of optimized formulation of posaconazole was studied by dialysis method. Drug release study was carried out for 120 mins with phosphate buffer of pH 7.4. After 120 mins % drug release of pure drug was 93.48 % while the formulation F3 (85.98 %) showed maximum drug release. The phytosomes preparations enhanced the solubility of Posaconazole and showed sustained release.

Table 11. Percentage drug release of optimized formulation (F3) of posaconazole loaded phytosomes and drug in PBS pH 7.4.

S. No.	Time (min)	Pure Drug	F3
1.	5	20.6	35.54
2.	10	32.52	48.97
3.	15	38.75	59.76
4.	30	53.64	69.83
5.	45	64.4	78.4
6.	60	73.98	81.4
7.	90	84.5	84.11
8.	120	93.48	85.98

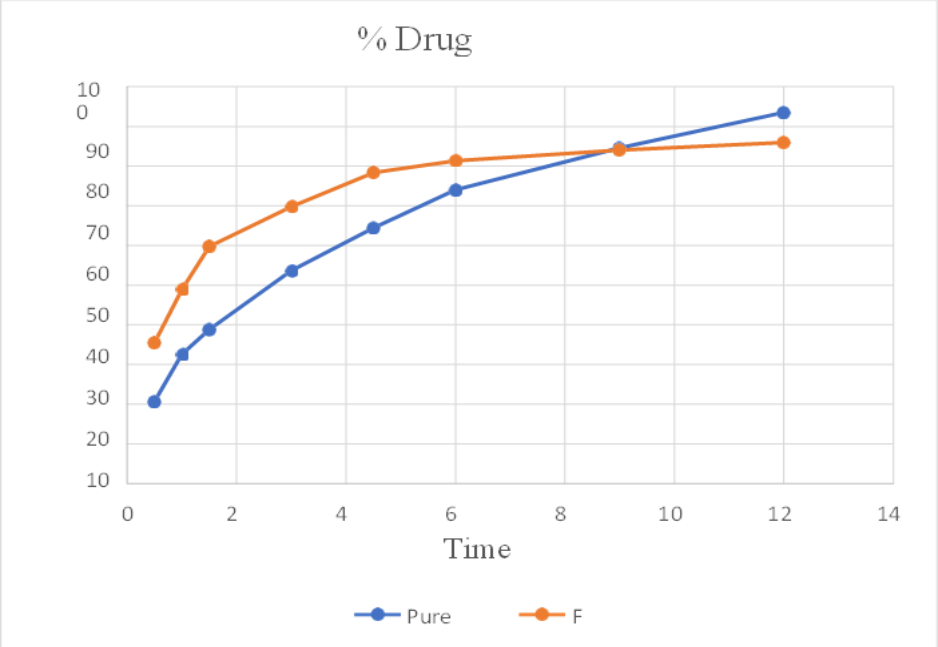

Figure 4. In vitro release of F3 and pure drug (Posaconazole).

Physiochemical Properties

Particle size & PDI

The optimized phytosomes formulation was characterized for vesicle size and PDI. Average size of vesicle optimized niosomal formulation (F3), encountered to be 1525 nm and PDI was found to be 1.000.

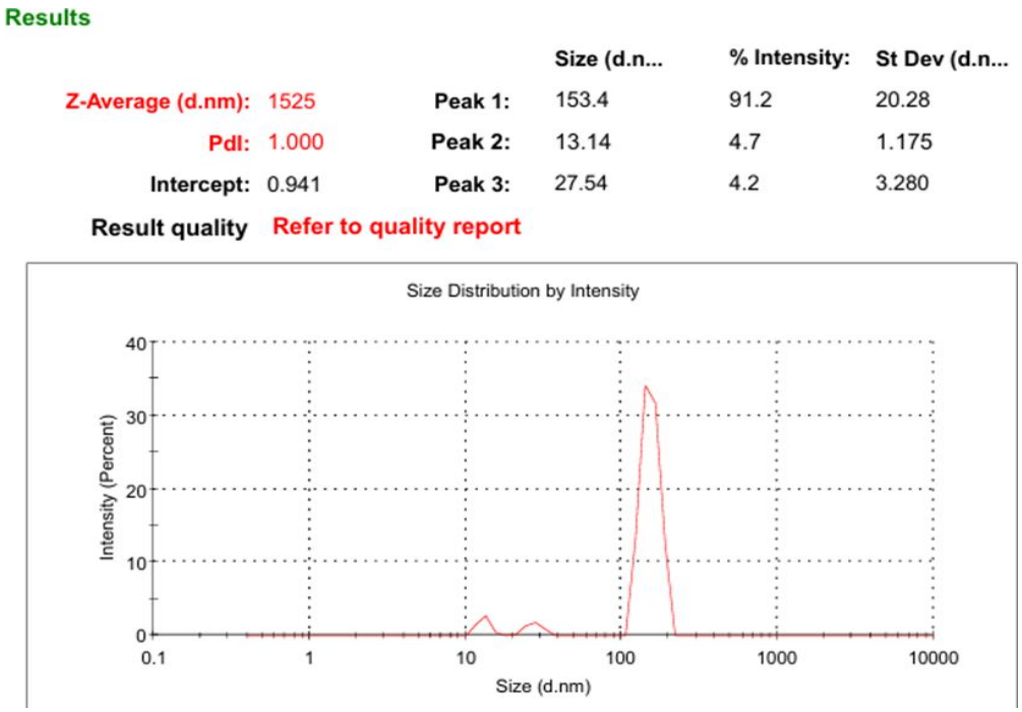

Figure 5. Vesicle size and PDI of F3.

Zeta potential

Zeta potential of optimized micellar formulation (F3) was found to be -28.8.

Result	Mean(mV)	Area (%)	St. Dev (mV)
Zeta potential(mV)	**Peak 1**: 25.6	100.0	4.79
Zeta Deviation(mS/cm)	**Peak 2**: 0.00	0.0	0.00
Conductivity(mS/cm)	**Peak 3**: 0.00	0.0	0.00

Result quality: Good.

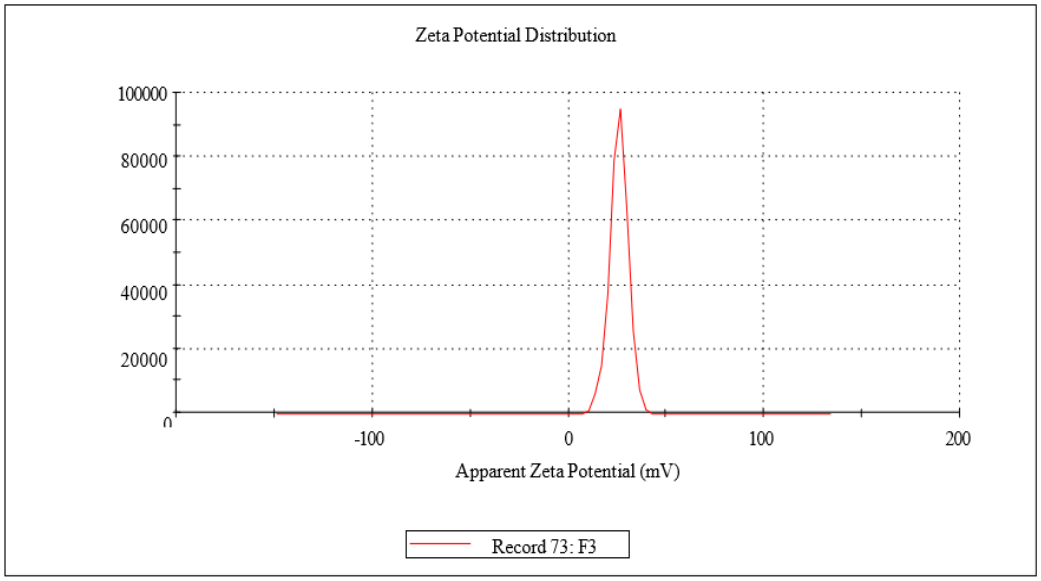

Figure 6. Zeta Potential- F3.

Spreading analysis

The spreadability increases with the weight applied, indicating that higher weights result in a larger diameter of spread, thus demonstrating better spreadability of the niosomes formulation.

Table 12. Spreadability of data of optimized formulation (F3)

t applied(g)	er of spread(cm)	adability (S)[g.cm/s]
50	3.2	50x3.2/60=2.67
100	4.5	100x3.2/60=7.50
150	5.7	150x5.7/60=14.25
200	6.8	200x6.8/60=22.67

Stability study

Storage stability (4°C) of optimized niosomes formulations [F3] analyzed for period of 30 days. Post 1 week storage, resulted in slightly reduced niosomes size, a typicality of niosome. Post sonication stress e.g. 1 week storage, niosomes relaxed, fully matured leading particle size stable final value.

Table 13. Stability study of optimized formulation (F3)

Time [week]	Parameters	Optimized formulation [F3]
0	Size (nm)	1525
	PDI	1.000
	Charge (mV)	-28.8
	EE (%)	84.73
1	Size (nm)	1427
	PDI	0.872
	Charge (mV)	-28.1
	EE (%)	83.72
2	Size (nm)	1347
	PDI	0.865
	Charge (mV)	-26.7
	EE (%)	82.83
3	Size (nm)	1232
	PDI	0.852
	Charge (mV)	-26.1
	EE (%)	80.42
4	Size (nm)	1173
	PDI	0.837
	Charge (mV)	-25.6
	EE (%)	78.98

PDI = Polydispersity Index; EE = Entrapment Efficiency.

Antifungal activity

Antifungal potential of formulation F3, investigated against 2 fungal strains: C. albicans and A. niger, which represent significant opportunistic pathogens in humans. The evaluation was conducted using the disc diffusion method, and Posaconazole (20 µg) was employed as the standard reference drug for comparison. The formulation tested at varying concentrations (500 µg, 1000 µg, and 2000 µg), and the inhibition zones were measured to assess the antifungal efficacy.

The results indicated that formulation exhibited antifungal activity to varying extents. F3 demonstrated very potent activity for C. albicans and A. niger, with ZOI of 12 mm, 14 mm and 16 mm at 500 µg, 1000 µg and 2000 µg respectively, approaching the efficacy of the standard Posaconazole (20 mm). F3 was consistently more effective across all tested concentrations against both fungal strains highlighting their significant antifungal potential.

Table 14. Antifungal activity of F3 on C. alibicans and A. niger.

Formulation	**ZOI (mm)**					
	C. albicans			***A. niger***		
Concentration	**500µg**	**1000µg**	**2000µg**	**500µg**	**1000µg**	**2000µg**
F3	12	14	16	12	14	16
Posaconazole	**20**			**18**		

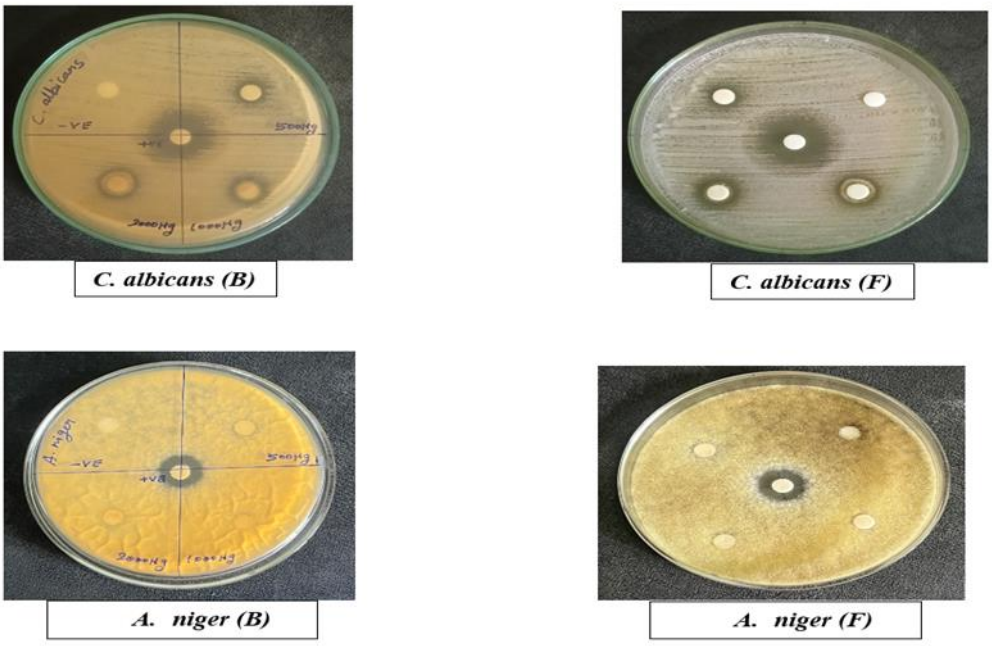

Figure 7. ZOI images for F3.

SUMMARY AND CONCLUSION

The formulation and evaluation of noisomes-based delivery systems present a promising approach for effectively treating fungal infections of the skin. Noisomes, which are vesicular carriers composed of phospholipids and surfactants, enhance the solubility and stability of antifungal agents. This study investigates the preparation of noisome formulations encapsulating antifungal drugs, focusing on Size, charge, and entrapment efficiency which are some of its physicochemical features. *In-vitro* release studies demonstrate that noisomes improve drug bioavailability and penetration through the stratum corneum, facilitating targeted treatment. Moreover, antifungal efficacy may be assessed through microbiological evaluations, confirming the enhanced therapeutic potential of the noisome formulations compared to conventional formulations.

The development of noisomes-based systems for antifungal treatment highlights their effectiveness in addressing skin infections caused by fungi. The improved drug delivery capabilities, coupled with enhanced skin permeation and sustained release, position noisomes as a superior alternative to traditional antifungal therapies.

Further clinical studies are warranted to validate these findings and explore the long-term effectiveness and safety of noisome formulations in real-world applications. This innovative approach could significantly improve patient outcomes in the management of fungal skin infections, providing a targeted and effective treatment strategy.

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Conflict of Interest
Authors declare no conflict of interest.

Ethical approval
Not applicable.

Informed consent
Not applicable

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